

**รายงานวิจัยฉบับสมบูรณ์**

โครงการ

# การสังเคราะห์วงแลคโทนและแลคแทมของสารที่มีฤทธิ์ทางชีวภาพโดยใช้ "เคมีสีเขียว"

# “Green Chemistry" for the Synthesis of Bioactive Lactone and Lactam Derivatives in Drug Development

**RMU5380021**


โดย

**ดร.นพพร ทัศนา**


มิถุนายน 2556

สัญญาเลขที่ **RMU5380021**

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ผู้วิจัย

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สังกัด

**สถาบันบัณฑิตศึกษาจุฬาภรณ์**

**สถาบันวิจัยจุฬาภรณ์**



สนับสนุนโดยสถาบันบัณฑิตศึกษาจุฬาภรณ์ สำนักงานคณะกรรมการการอุดมศึกษาและสำนักงานกองทุนสนับสนุนการวิจัย

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกอ.และสกว.ไม่จำเป็นต้องเห็นด้วยเสมอไป)

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Nopporn Thasana

# Executive Summary

## การสังเคราะห์วงแลคโทนและแลคแทมของสารที่มีฤทธิ์ทางชีวภาพโดยใช้ "เคมีสีเขียว" (RMU5380021)

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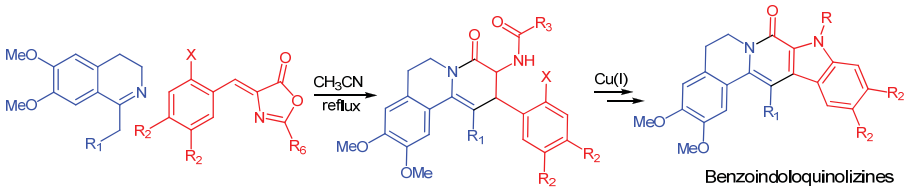
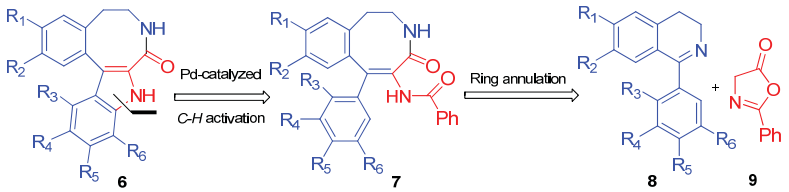
**โครงการย่อยที่ 1:** Copper(I) mediated and Palladium(II) catalyzed reaction: Synthesis of benzoquinolizine derivatives




**Abstract:** ค้นพบกระบวนการใช้เกลือของโลหะทองแดงและพลังงานไมโครเวฟ ในการสังเคราะห์สารประกอบเบนโซอินโดโลควิโนลิซิดีน โดยการสร้างพันธะคาร์บอนกับไนโตรเจนของวงอินโดล จากสารตั้งต้นเตตระไฮโดรไพริโดไอโซควิโนลีน ซึ่งสังเคราะห์มาจากปฏิกิริยาการรวมตัวและสร้างงของสาร 3,4-ไดไฮโดรไอโซควิโนลีน กับอาซแลคโตน นอกจากนี้พบว่าในสภาวะด่าง $Cs_2CO_3$ สามารถเกิดปฏิกิริยา deamidation ให้สารไดไฮโดรเบนโซควิโนลีน อีกด้วย

**Keywords:** benzoquinolizinone • benzoindoloquinolizine • Copper-mediated reaction • Pd(II)-catalyzed reaction • ring annulation

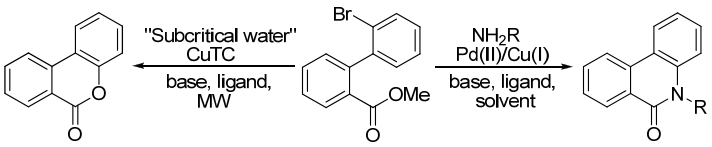
**โครงการย่อยที่ 2:** Palladium-catalyzed intramolecular C-H amidation: Synthesis and biological activities of indolobenzazocin-8-ones




**Abstract:** การใช้โลหะพัลลาเดียมเป็นตัวเร่งปฏิกิริยา C-H activation และ amidation ของสารเบนโซเอโซซิโนน สามารถสังเคราะห์อนุพันธ์สารอินโดโลเบนซาโซซิโนนที่แสดงฤทธิ์ยับยั้งเซลล์มะเร็ง HUCAA-1 A-549 HepG2 และ MOLT-3 โดยแสดงความเป็นพิษต่อระดับนาโนโมลาร์ต่อเซลล์ HepG2 และ MOLT-3

**Keywords:** indolobenzazocinone • benzo[*d*]azocinone • palladium-catalyzed C-H activation • intramolecular amidation

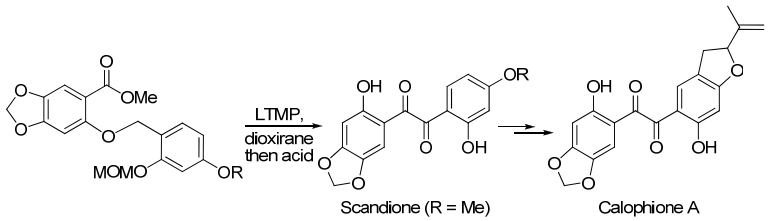
**โครงการย่อยที่ 3:** Cu(I)-mediated/subcritical water and Pd(II)-catalyzed C-O/C-N bond formation: Synthesis of benzopyranones and phenanthridinones




**Abstract:** การสร้างวงเลคโตนและวงเลคแทมโดยใช้เกลือโลหะทองแดงถูกศึกษาในขั้นตอนเดียวภายใต้สภาวะที่เป็นมิตรต่อสิ่งแวดล้อมจากสารตั้งต้นเดียวกันคือ 2-เฮโลไบเอริลคาร์บอกซิเลต ซึ่งการสร้างพันธะคาร์บอนกับออกซิเจนในปฏิกิริยา intramolecular lactone formation จะให้สาร เบนโซไพแรนโนน และการสร้างพันธะคาร์บอนกับไนโตรเจนในปฏิกิริยา intermolecular lactam formation จะให้สารฟีแนนทริดีโนน

**Keywords:** Dibenzopyranones • Phenanthredinones • Subcritical water • Microwave • C-O Bond formation • C-N Bond formation • Pd(II) catalyst • Cu(I) catalyst • Green chemistry

**โครงการย่อยที่ 4:** Synthesis of scandione and calophione A




**Abstract:** การศึกษาการสังเคราะห์สารผลิตภัณฑ์ธรรมชาติกลุ่มไดเอริลอีเทน1,2-ไดคีโตน หรือ เบนซิล ชนิดไม่สมมาตร (unsymmetrical benzil) ได้แก่ scandione และ calophione A ถูกศึกษาโดยใช้ปฏิกิริยาการปิดวงแบบ intramolecular cyclization ของแอนไอออนของสารตั้งต้น O-benzylsalicylate และต่อเนื่องด้วยปฏิกิริยาออกซิเดชัน ให้ได้โครงสร้างหลักของเบนซิล ในขณะที่การสร้างวงไดไฮโดรเบนโซฟิวแรนของสาร calophione A ศึกษาโดยการใช้ปฏิกิริยา palladium(II)-catalyzed oxidative cyclization

**Keywords:** benzil • scandione • calophione A • intramolecular cyclization • Pd(II)-catalyzed cyclization • biological activity

# Executive Summary

## "Green Chemistry" for the Synthesis of Bioactive Lactone and Lactam Derivatives in Drug Development (RMU5380021)

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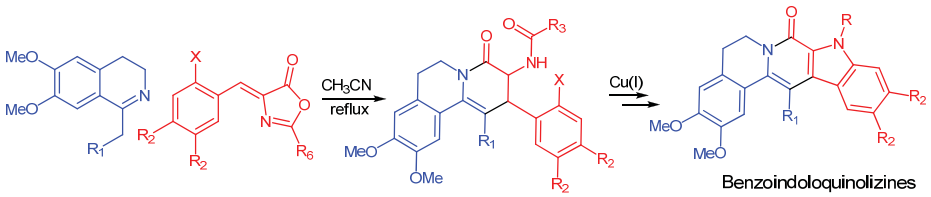
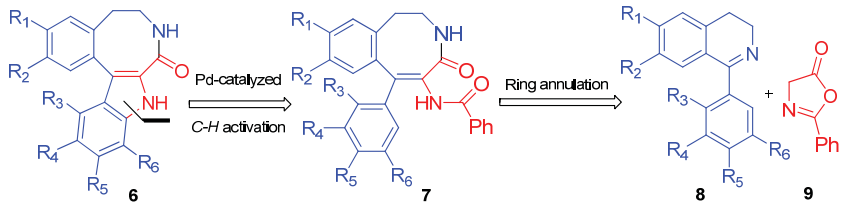
**Subproject I:** Copper(I) mediated and Palladium(II) catalyzed reaction: Synthesis of benzoquinolizine derivatives




**Abstract:** An effective synthesis of the multi ring-fused benzoindoloquinolizines has been accomplished by Cu(I)-mediated and MW-assisted C-$N_{amide}$ bond formation of benzo[*a*]quinolizin-4-ones. The deamination of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines was also studied and was found to give benzoquinolizines. The benzo[*a*]quinolizin-4-ones were prepared based on the annulations of C-1 substituted 3,4-dihydroisoquinolines and azlactones.

**Keywords:** benzoquinolizinone • benzoindoloquinolizine • Copper-mediated reaction • Pd(II)-catalyzed reaction • ring annulation

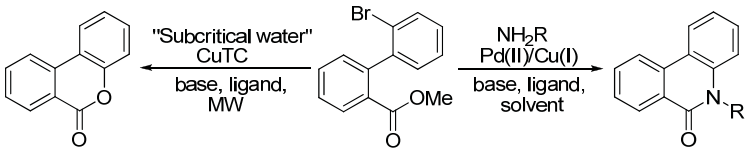
**Subproject II:** Palladium-catalyzed intramolecular C-H amidation: Synthesis and biological activities of indolobenzazocin-8-ones




**Abstract:** The synthesis of multi ring-fused indolobenzazocinone derivatives, an antimitotic agent, has been carried out using palladium-catalyzed C-H activation/intramolecular amidation of benzo[*d*]azocinones which were synthesized by the ring annulations of dihydroisoquinolines and azlactone in refluxing acetonitrile. The target compounds, indolobenzazocin-8-one derivatives, were evaluated for their cytotoxicity against the cancer cell lines HUCCA-1, A-549, HepG2, and MOLT-3. The results showed that an unsubstituted indolobenzazocin-8-one **1e** exhibited very good activities in the nanomolar $IC_{50}$ value range (HepG2 and MOLT-3).

**Keywords:** indolobenzazocinone • benzo[*d*]azocinone • palladium-catalyzed C-H activation • intramolecular amidation

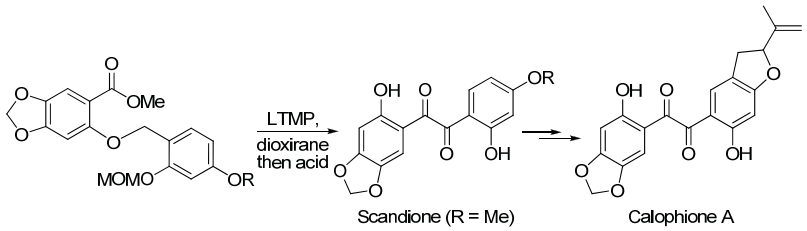
**Subproject III:** Cu(I)-mediated/subcritical water and Pd(II)-catalyzed C-O/C-N bond formation: Synthesis of benzopyranones and phenanthridinones




**Abstract:** Lactone and lactam formation was examined with copper(I)-mediated coupling reaction. The C-O bond formation of benzopyranone and C-N bond formation of phenanthridinone were selectively synthesized with specific conditions. Cu(I)-mediated and subcritical water were found to be a suitable reaction under the benign condition for the synthesis of dibenzopyranone derivatives from the intramolecular lactone formation of various 2-halobiarylcarboxylates. The one-pot synthesis of phenanthridinone was achieved by Pd(II)-mediated coupling reaction. The variation of N-substitute on phenanthridinone was archived in satisfied yield.

**Keywords:** Dibenzopyranones • Phenanthredinones • Subcritical water • Microwave • C-O Bond formation • C-N Bond formation • Pd(II) catalyst • Cu(I) catalyst • Green chemistry

**Subproject IV:** Synthesis of scandione and calophione A




**Abstract:** Described is the construction of unsymmetrical benzils, scandione and calophione A, involving the intramolecular cyclization of anionic benzylic ester of the aryl benzyl ether followed by the oxidation. The palladium(II)-catalyzed oxidative cyclization was also studied to establish the benzofuran unit of calophione A.

**Keywords:** benzil • scandione • calophione A • intramolecular cyclization • Pd(II)-catalyzed cyclization • biological activity

# "Green Chemistry" for the Synthesis of Bioactive Lactone and Lactam Derivatives in Drug Development (RMU5380021)

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## Objectives

1. To perform and develop synthetic methodologies in *"Green Chemistry"* and to apply such methodologies in devising new synthetic routes for some biologically active natural products,

2. To study and develop the coupling reaction of $C_{aryl}$-Y (Y = O, N) bond formation of benzopyranones and phenanthridinones using the copper(I)-mediated and microwave-assisted reaction base on *"Green Chemistry"* conditions,

3. To study the intramolecular and intermolecular cyclizations of azlactone for C-O and C-N bond formation in the synthesis of 3-substituted coumarin derivatives,

4. Develop our method to synthesize complex molecules such as azalamellarin, benzoindoloquinolizine, and benzoisoquinolinonapthyridinone derivatives, and

5. To prepare azalamellarin, benzoindoloquinolizine, and benzoisoquinolino-napthyridinone analogs which possess other desirable pharmacological properties

## SUBPROJECT I

## Copper(I) mediated and Palladium(II) catalyzed reaction: Synthesis of benzoquinolizine derivatives

### Introduction

The benzo[*a*]quinolizine ring system **1** is an important heterocyclic framework that can be found in numerous biologically active compounds[1] including alangiumkaloid A **2**, an oxoprotoberberine alkaloid and isoalangioside **3**, isolated from a Thai folk medicinal plant, *Alangium salviifolium*.[2,3] Schulzeine A **4** and its analogues Schulzeines B-C, new α-glucosidase inhibitors, isolated from the marine sponge *Penares schulzei*, were the first three benzo[*a*]quinolizin-4-ones containing an amide moiety at the C-3 position.[4] The synthetic benzo[*a*]chinolizinone (Ro 41-3696) **5** was reported as an effective non-sedative hypnotic for the induction and maintenance of sleep.[5] 2-Amidobenzo[*a*]quinolizine **6** was synthesized as a novel dipeptidyl peptidase IV (DPP-IV) inhibitors.[6]



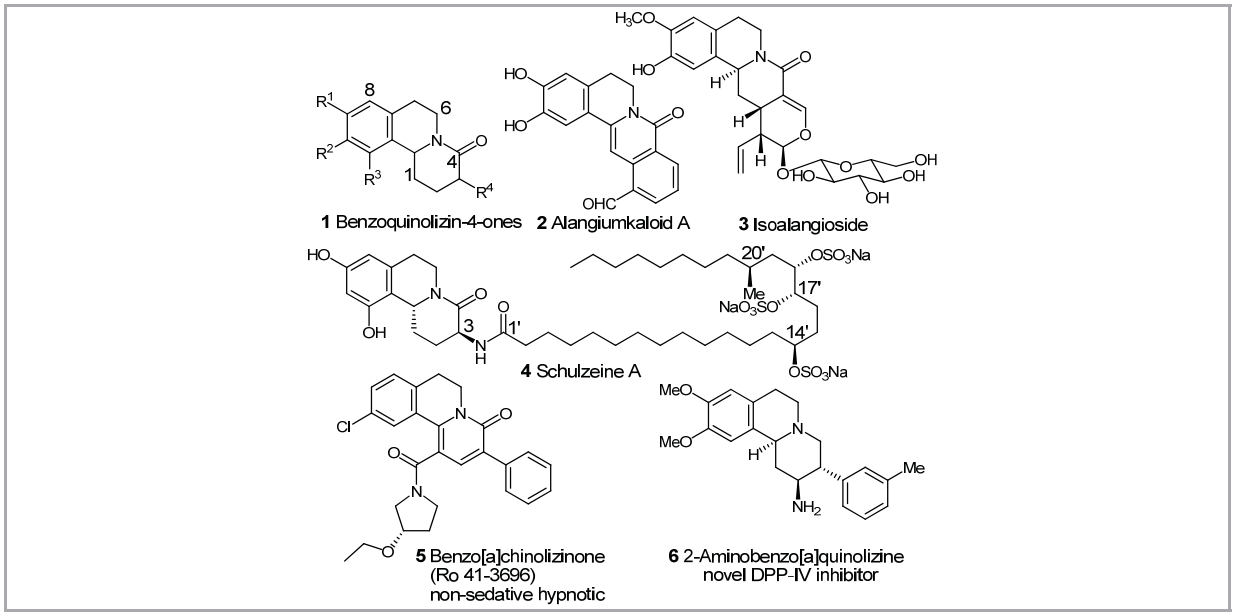
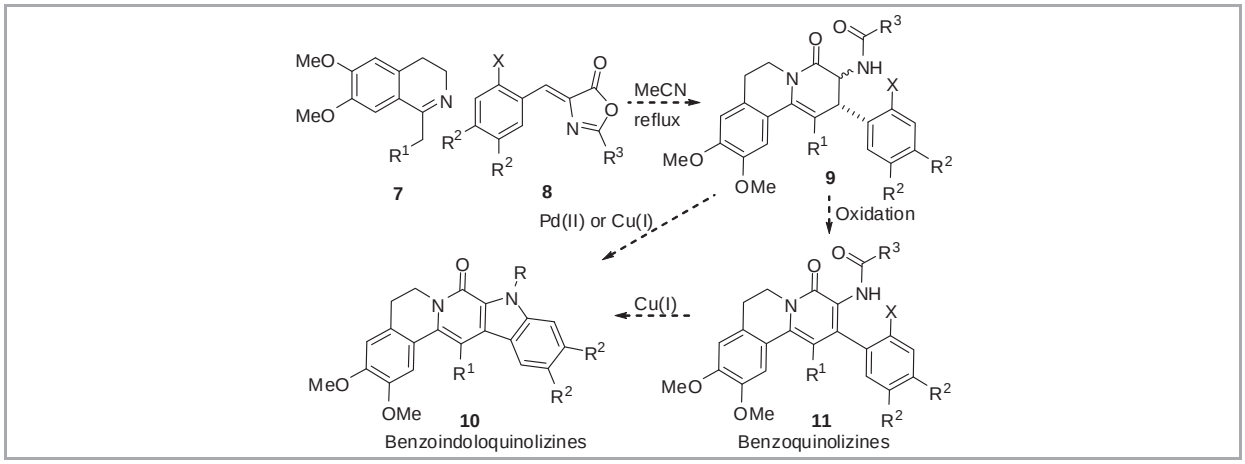



**Figure 1.** Example of natural product alkaloids and biologically synthetic compounds containing benzoquinolizine system.

The classical approaches for the synthesis of the benzo[*a*]quinolizine ring system involved the Dieckmann condensation of 1,2-dialkylesters of dihydroisoquinolines, the Bischler-Napieralski cyclization of arylethylpyridinones, and the reaction of 3,4-dihydroisoquinolines with α,β-unsaturated ketones.[7] Other new methods have been reported in the literature.[8] We have also reported a facile and direct synthetic entry to tricyclic imidazoloisoquinolinones and benzo[*a*]quinolizin-4-ones based on the annulations of 1-unsubstituted and 1-substituted dihydroisoquinolines **7** with azlactones **8** under neutral conditions.[9]

In continuation of our interests in the palladium- and copper-mediated formation of O- and N-aryl bonds[10,11] in general and particularly the C-N bond formation of indole alkaloids,[12] we have studied, in this work, the Pd(II)-catalyzed and Cu(I)-mediated C-$N_{amide}$ bond formations of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9** and benzo[*a*]quinolizin-4-ones **11** in order to obtain the multi ring-fused benzoindoloquinolizines **10** as shown in Scheme 1.




**Scheme 1.** Synthesis of multi ring-fused benzoindoloquinolizinone.

## Results and Discussion

### Synthesis of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines 9

The reaction of azlactones **8** with various 1-substituted 3,4-dihydroisoquinolines **7** in refluxing acetonitrile gave the corresponding 3,4-dihydropyridin-2(*1H*)-ones as a mixture of *cis/trans* tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9** in moderate to good yields as shown in Table 1. It was found that when the substituent $R^3$ on the azlactones **8** was a phenyl group, the corresponding products **9** were obtained in higher yields than in the cases where $R^3$ was a methyl group. In the cases where the substituent $R^1$ on the 3,4-dihydroisoquinolines **7** was a proton ($R^1$ = H) the *cis*-products predominated, however, increasing the steric bulk of the substituents where $R^1$ = $CH_3$ and Ph derivatives, the *trans* isomers became the major products. The cyclocondensation of azlactones **8** ($R^3$ = $CH_3$) with hindered 1-benzyl-3,4-dihydroisoquinolines **7** ($R^1$ = Ph, 3,4-$(OCH_3)_2C_6H_3$) gave cyclocondensation products **9** in poor yield (entries 5 to 7 and 9 to 11). However, the yields could be improved with longer reaction times.

The structure of *cis*-tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9a** was assigned by interpretation of spectral data. The IR spectrum exhibited absorptions at 1683 and 1654 $cm^{-1}$ indicating the presence of two amide carbonyl groups which corresponded to two peaks of carbonyl groups in the $^{13}C$ NMR spectrum at δ 165.3 and 170.0. In addition, the IR spectrum exhibited a secondary amide absorption at 3396 $cm^{-1}$. The $^1H$ NMR showed a coupling constant of 7.6 Hz for *H*-2 and *H*-3 inferring the *cis*-relationship. $^1H$ NMR of the *trans*-**9a** exhibited *trans*-relationship of *H*-2 and *H*-3 with larger coupling constant of 13.4 Hz.

The $^1H$ NMR chemical shift assignment for *cis*-**9a** was confirmed by a detailed observation of NOE effects (Figure 2). In particular, irradiation at the frequency of the signal at δ 5.06 (*H*-3) enhanced the signal at δ 4.40 (*H*-2, 9%) and δ 6.84 (N*H*, 3%). In addition, irradiation at the frequency of the signal at δ 4.40 (*H*-2) enhanced the signal at δ 6.02 (*H*-1, 4%), thus confirming the chemical shifts and the stereochemical assignment between *H*-3 and *H*-2 as having a *cis*-relationship. Similarly, the $^1H$ NMR chemical shift assignment for *trans*-**9a** was confirmed by a detailed observation of NOE effects. It was found that irradiation of the signal at δ 5.16 (*H*-3) did not enhance the signal at δ 4.04 (*H*-2), thus confirming the stereochemical assignment between *H*-2 and *H*-3 as having a *trans*-relationship.

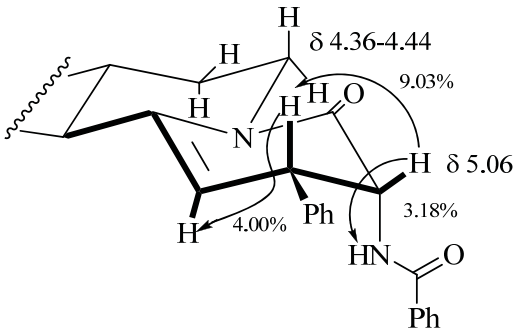
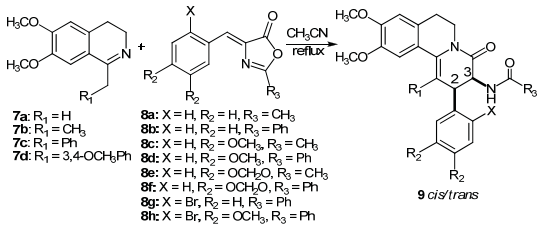



**Figure 2.** NOE's effects observed for *cis*-**9a** ($CDCl_3$, 400 MHz).

**Table 1.** Synthesis of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9**.[a]




| Entry | X | $R_1$ | $R_2$ | $R_3$ | Yield % **9**[b] *(cis:trans)* |
|---|---|---|---|---|---|
| 1 | H | H | H | Ph | **a** 89 (74:26) |
| 2 | H | H | $OCH_3$ | Ph | **b** 79 (84:16) |
| 3 | H | $CH_3$ | $OCH_3$ | $CH_3$ | **c** 65 (40:60) |
| 4 | H | $CH_3$ | $OCH_3$ | Ph | **d** 86 (40:60) |
| 5 | H | Ph | H | $CH_3$ | **e** 42 (17:83) |
| 6 | H | Ph | $OCH_3$ | $CH_3$ | **f** 38[c] (0:100) |
| 7 | H | Ph | $OCH_2O$ | $CH_3$ | **g** 45[d] (20:80) |
| 8 | H | Ph | $OCH_3$ | Ph | **h** 80 (36:64) |
| 9 | H | 3,4-$(OMe)_2$Ph | H | $CH_3$ | **i** 34 (27:73) |
| 10 | H | 3,4-$(OMe)_2$Ph | $OCH_3$ | $CH_3$ | **j** 45[e] (16:84) |
| 11 | H | 3,4-$(OMe)_2$Ph | $OCH_2O$ | $CH_3$ | **k** 39[f] (15:85) |
| 12 | H | 3,4-$(OMe)_2$Ph | H | Ph | **l** 86 (35:65) |
| 13 | H | 3,4-$(OMe)_2$Ph | $OCH_3$ | Ph | **m** 85 (32:68) |
| 14 | H | 3,4-$(OMe)_2$Ph | $OCH_2O$ | Ph | **n** 84 (38:62) |
| 15 | Br | H | H | Ph | **o** 98 (78:22) |
| 16 | Br | H | $OCH_3$ | Ph | **p** 80 (76:24) |
| 17 | Br | $CH_3$ | H | Ph | **q** 70 (71:29) |
| 18 | Br | $CH_3$ | $OCH_3$ | Ph | **r** 77 (56:44) |

[a] All reaction times were 2 h.

[b] Isolated yields of pure product after column chromatography on silica.

[c] Reaction time was 7 hrs, **9f**, 41% (17:83).

[d] Reaction time was 7 hrs, **9g**, 68% (18:82).

[e] Reaction time was 12 hrs, **9j**, 62% (21:79).

[f] Reaction time was 12 hrs, **9k**, 80% (19:81).

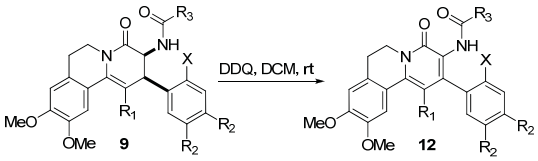
## Survey of C-N bond formation of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines 9

Various approaches for the synthesis of indole ring systems have been reported in the literature.[12a,13] We sought to develop the synthesis of benzoindoloquinolizines **10** via the C-N bond formation of the corresponding tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9**. Initially, our approach to synthesize the indole ring was inspired by the recent findings of palladium-catalyzed C-H activation/C-N bond formation.[13,14] First, we examined the possibility of C-H activation of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9a** using $Pd(OCOCF_3)_2$ as the catalyst in the presence of $Cu(OAc)_2$ and $AgOCOCF_3$ as reoxidant at 80-85 $^oC$ under an argon atmosphere in DMSO.[13c] The oxidation product benzoquinolizine **11a** was obtained in moderate yield (35%) instead of the expected indole product (Table 2, entry 1). Other palladium-catalyzed intramolecular amination conditions were also studied using $PdCl_2$ and $Pd(dba)_2$ hoping to construct the indole system from various tetrahydro-*2H*-pyrido[2,1-

*a*]isoquinolines **9**. In all cases, deamidation led to the formation of benzoquinolizines **12** which were obtained in moderate to good yields as shown in Table 2 (Entries 2 to 8).

As shown in Table 2, a number of benzo[*a*]quinolizinones **12** were synthesized by the oxidation of selected *cis*-benzo[*a*]quinolizinones **9** with DDQ in dichloromethane at room temperature for 0.5 to 2 h. It was found that when the substituent $R_1$ on the *cis*-benzo[*a*]quinolizinones **9** was a proton ($R_1$ = H), the corresponding products **12** were obtained in higher yields than a methyl group ($R_1$ = $CH_3$) of $R_1$ (entries 1 to 4). In the case where the substituent X on the *cis*-benzo[*a*]quinolizinones **9** was a proton (X = H) and $R_1$ was a methyl group ($R_1$ = $CH_3$), the product yields were poor (entries 3 and 4). Moreover, increasing the steric bulk of the substituents (X = Br and $R_1$ = $CH_3$) resulted in no reaction (entries 7 and 8).

**Table 2.** Synthesis of benzo[*a*]quinolizin-4-ones **12**.




| Entry | X | $R_1$ | $R_2$ | $R_3$ | Time (h) | Yield % **12**[a] |
|---|---|---|---|---|---|---|
| 1 | H | H | H | Ph | 0.5 | **a** 56 |
| 2 | H | H | $OCH_3$ | Ph | 0.5 | **b** 67 |
| 3[9] | H | $CH_3$ | H | Ph | 16 | **c** 15 |
| 4 | H | $CH_3$ | $OCH_3$ | Ph | 16 | **d** 8[b] |
| 5 | Br | H | H | Ph | 1 | **e** 57[c] |
| 6 | Br | H | $OCH_3$ | Ph | 1.5 | **f** 53 |
| 7 | Br | $CH_3$ | H | Ph | 16 | N/A[d] |
| 8 | Br | $CH_3$ | $OCH_3$ | Ph | 16 | N/A[d] |

[a] Isolated yields of pure product after column chromatography on silica.

[b] Recovered starting material 47%.

[c] Recovered starting material 41%.

[d] Recovered starting material >99%.

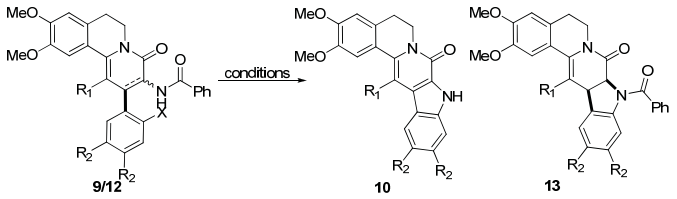
Having established the various approach for the synthesis of indole ring,[12a,13] we sought to develop the synthesis of benzoindoloquinolizines **10** and related derivatives **13** via the C-N bond formation of the corresponding benzoquinolizin-4-ones **12**. Our approach to synthesize the indole ring was inspired by the C-H activation chemistry.[13,14] We began by studying the conversion of benzoquinolizine **12b** using a combination of 10 mol% $Pd(OAc)_2$ and $CuCl_2$ or $Cu(OAc)_2$ as an oxidant reported by Yu and Buchwald (Table 3, entries 1 and 2).[13] However, no reaction was observed. Various C-H activation conditions were also studied with benzoquinolizinones **12**, however they failed to provide the C-N bond formation adducts (Table 3, entries 3 to 6).[14]

Recently, we reported the synthesis of azalamellarin derivatives using CuTC-mediated/MW-assisted lactam formation.[11] By using our developed method, benzoindoloquinolizines **10a** and **10b** were prepared in moderate yields from the corresponding benzoquinolizin-4-ones **12e** and **12f**, respectively (Table 3, entries 7 and 8). 3,4-Dihydropyridin-2(*1H*)-ones **9q** and **9r** were also studied following our procedure and



gave a mixture of benzoindoloquinolizines **10** and *N*-benzoyl benzodihydroindoloquinolizine derivatives **13** (Table 3, entries 9 and 10).

**Table 3.** Synthesis of benzoindoloquinolizines **10** and related derivatives **13**.



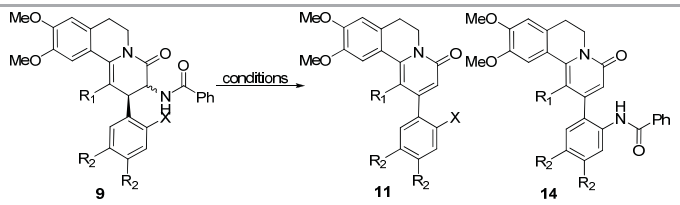

| Entry | SM **9/12** | condition | Time (h) | Yield % **10**[a] | Yield % **13**[a] |
|---|---|---|---|---|---|
| 1 | **12b** | A | 24 | N/A | N/A |
| 2 | **12b** | B | 24 | N/A | N/A |
| 3 | **12e** | C | 72 | N/A | N/A |
| 4 | **12e** | D | 16 | N/A | N/A |
| 5 | **12e** | E | 48 | N/A | N/A |
| 6 | **12f** | E | 24 | N/A | N/A |
| 7 | **12e** | F | 0.25 | **a**, 38 | - |
| 8 | **12f** | F | 0.25 | **b**, 62 | - |
| 9 | **9q** | F | 0.25 | **c**, 20 | **a**, 42 |
| 10 | **9r** | F | 0.5 | **d**, 16 | **b**, 57 |

Condition A: 10 mol% $Pd(OAc)_2$, 1.5 equiv $CuCl_2$, 2 equiv AgOAc, DCE, 100 °C. Condition B: 10 mol% $Pd(OAc)_2$, 1 equiv $Cu(OAc)_2$, 2 equiv $AgOCOCF_3$, DMSO, 50 °C. Condition C: 5 mol% $PdCl_2$, 10 mol% Xantphos, 2 equiv $Cs_2CO_3$, dioxane, reflux. Condition D: 2 mol% $Pd_2(dba)_3$, 4 mol% SPhos, 4 equiv *t*-BuOK, dioxane, reflux. Condition E: 1 mol% $Pd_2(dba)_3$, 3 mol% Xantphos, 1.4 equiv $Cs_2CO_3$, dioxane, 100-120 °C. Condition F: CuTC, DMF, MW, 150 °C. N/A = no reaction and recovered starting material.

[a] Isolated yields of pure product after column chromatography on silica.

Then, we examined the 3,4-dihydropyridin-2(*1H*)-ones **9** under palladium(II) catalysis.[13,14] for the synthesis of benzoindoloquinolizine derivatives **10** and/or **13**. As shown in Table 4, neither compound **10** nor **13** was obtained from the catalytic Pd-mediated C-N bond formation. On the other hand, the unexpected oxidative deamidation leading to benzoquinolizines **11** were obtained in moderate to good yields.

**Table 4.** Synthesis of benzoquinolizines **11** and **14**.




| Entry | SM **9** | condition | Time (h) | Yield % **11**[a] | Yield % **14**[a] |
|---|---|---|---|---|---|
| 1 | *cis*-**9o** | A | 72 | 32 | 21 |
| 2 | *trans*-**9o** | A | 72 | 50 | 0 |
| 3 | *cis*-**9p** | A | 72 | 80 | 0 |
| 4 | *cis*-**9q** | A | 72 | 72 | 0 |
| 5 | *cis*-**9r** | A | 72 | 52[b] | 0 |
| 6 | *cis*-**9p** | B | 24 | 89 | 0 |
| 7 | *cis*-**9q** | B | 56 | 0[b,c] | 0 |
| 8 | *cis*-**9r** | B | 56 | 0[b,c] | 0 |

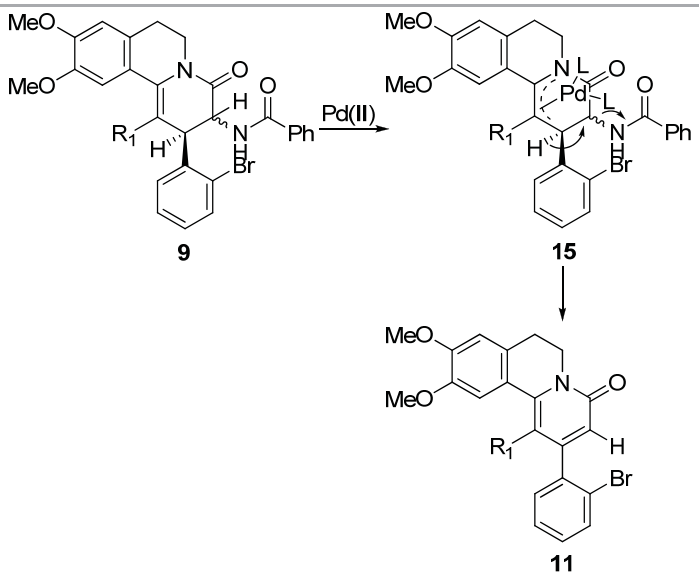
Condition A: 5 mol% $PdCl_2$, 1 mol% Xantphos, 2 equiv $Cs_2CO_3$, 1,4-dioxane, reflux. Condition B: 10 mol% $Pd(dba)_2$, 15 mol% Xantphos, 1.4 equiv $Cs_2CO_3$, 1,4-dioxane, 100 °C.

[a] Isolated yields of pure product after column chromatography on silica.

[b] Recovered starting materials in 25% (for **9r**), 64% (for **9q**) and 66% (for **9r**), respectively.

[c] Debromination products were isolated in 20% (for **9q**) and 12% (for **9r**), respectively.

We proposed a plausible reaction pathway as shown in Scheme 2. Complexation of the enamide moiety of benzoquinolizinones **9** via π-system to Pd(II)- catalysts facilitated the formation of the palladation complex **15**. Oxidative deamidation subsequently led to the benzoquinolizine **11** and release of the benzoyl amide.




**Scheme 2.** Possible reaction pathway for conversion of benzoquinolizines **9** to **11**.

## Conclusion

In summary, we have reported a facile pathway to synthesize multi ring-fused benzoindoloquinolizines **10** and benzoquinolizines **11**. The annulations of 1-substituted dihydroisoquinolines **7** with azlactones **8** under neutral conditions followed by Cu(I)-mediated/MW-assisted C-N bond formation of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9** gave benzoindoloquinolizines **10** and *N*-benzoyl benzodihydroquinolizines **14**. Under basic conditions tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9** were converted into benzoquinolizines **11** in moderate to good yields.

## Experimental Section

### 1. General methods

Microwave reactions were performed with CEM Discover (250 Watt, 100 psi). Melting points were determined on electrothermal melting point apparatus and reported without correction. $^{1}$H-Nuclear magnetic resonance ($^{1}$H NMR) spectra were recorded on 200 MHz, 300 MHz and 400 MHz instruments at 200, 300 and at 400 MHz, respectively. $^{13}$C-Nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded on 200 MHz, 300 MHz and 400 MHz instruments at 50, 75 and at 100 MHz, respectively. FTIR spectra were recorded using Universal Attenuated Total Reflectance (UATR). Low resolution mass spectra were obtained on a LC/MS instrument using Electron Impact Ionization (EI). High resolution mass spectra were obtained on a MicroTOF instrument using atmospheric pressure chemical ionization (APCI) in positive or negative mode. Column chromatography was carried out using aluminum oxide (100-125 mesh) or Merck silica gel (70-230 mesh). Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were carried out on silica. All reagents were purified and dried according to the standard procedures.

### 2. General procedure: Tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines (9)

A solution of azlactones[9] **8** (1.00 mmol) and 6,7-dimethoxy-1-alkyl-3,4-dihydroisoquinolines **7** (1.50 mmol) in acetonitrile (10 mL) was heated at reflux for 2 h. The solvent was evaporated to dryness *in vacuo*. The crude product was purified by PTLC on silica using 50-90% ethyl acetate in hexane as an eluent to give a mixture of diastereoisomers of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9** *cis:trans* in moderate to good yields.

*2.1 N-[2-phenyl-9,10,-dimethoxy-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9a**).* The general procedure was used with azlactone **8b** (249 mg, 1.00 mmol) and 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline **7a** (308 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9a** *cis:trans* (74:26) (405 mg, 89%): **9a**-*cis* as a bright yellow solid. $R_f$ (50% EtOAc:hexane) 0.38; Mp: 156-158 $^{o}$C; IR (cm$^{-1}$): 3396, 1683, 1654, 1604, 1581, 1509; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.63-7.59 (m, 2H), 7.36-7.30 (m, 3H), 7.19-7.14 (m, 3H), 7.08 (s, 1H), 7.05-7.00 (m, 2H), 6.84 (d, $J$ = 5.5 Hz, 1H), 6.61 (s, 1H), 6.02 (d, $J$ = 7.2 Hz, 1H), 5.06 (dd, $J$ = 7.6, 5.5 Hz, 1H), 4.40 (m, 2H), 3.85 (s, 3H), 3.83 (s, 3H), 3.49 (ddd, $J$ = 12.9, 10.2, 4.0 Hz, 1H), 2.92 (ddd, $J$ = 15.7, 10.2, 4.9 Hz, 1H), 2.79 (dt, $J$ = 15.7, 4.0 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 167.4, 166.9, 149.7, 148.3, 136.3, 135.2, 134.2, 131.6, 128.6 (2C), 128.5 (4C), 127.6 , 127.1, 127.0 (2C), 121.2, 110.7, 106.7, 103.1, 56.0, 55.9, 54.3, 39.7, 39.5, 28.6; EI-MS: *m/z* 454 ($M^+$, 5), 349 (26), 334 (24), 333 (100), 332 (33), 105 (2), 77 (3); HRMS-FAB *m/z* [M + H]$^+$ calcd for $C_{28}H_{27}N_2O_4$: 455.1971, found: 455.1970; Anal. Calcd. for $C_{28}H_{26}N_2O_4$: C, 73.99; H, 5.77; N, 6.16. Found: C, 73.67; H, 5.92; N, 6.16: **9a**-*trans* as a white solid. $R_f$ (50% EtOAc:hexane) 0.19; Mp: 143-144 $^{o}$C; IR (cm$^{-1}$): 3292, 1669, 1634, 1549, 1514; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.64-7.59 (m, 2H), 7.43-7.23 (m, 8H), 7.04 (s, 1H), 6.78-6.69 (m, 1H), 6.65 (s, 1H), 5.76 (d, $J$ = 2.8 Hz, 1H), 5.16 (dd, $J$ = 14.3, 8.8 Hz, 1H), 4.53 (ddd, $J$ = 12.7, 4.7, 4.2 Hz, 1H), 4.04 (dd, $J$ = 14.3, 2.8 Hz, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.45 (ddd, $J$ = 12.7, 10.4, 4.2 Hz, 1H), 2.94 (ddd, $J$ = 15.7, 10.4, 4.7 Hz, 1H), 2.79 (dt, $J$ = 15.7, 4.2 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 168.4, 167.8, 149.6, 148.2, 140.6, 134.6, 134.1, 131.3, 128.7 (2C), 128.3 (2C), 128.2 (2C), 127.4, 127.0 (3C), 121.1, 110.7, 106.5, 104.7, 56.0, 55.9, 54.7, 44.3, 39.6, 28.7; EIMS: *m/z* 455 ($M^+$+1, 0.33), 349 (6), 334 (26), 333 (100), 332 (42), 105 (2), 77 (3); HRMS-FAB *m/z* [M + H]$^+$ calcd for $C_{28}H_{27}N_2O_4$: 455.1971, found: 455.1978; Anal. Calcd. for $C_{28}H_{26}N_2O_4$: C, 73.99; H, 5.77; N, 6.16, Found: C, 74.23; H, 5.94; N, 6.29.

*2.2. N-[2-(3′,4′-dimethoxyphenyl)-9,10,-dimethoxy-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9b**).* The general procedure was used with azlactone **8d** (309 mg, 1.00 mmol) and 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline **7a** (308 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9b** *cis:trans* (84:16) (408 mg, 79%): **9b**-*cis* as a bright yellow solid. $R_f$ (70% EtOAc:hexane) 0.19; Mp: 160-161 $^{o}$C; IR (cm$^{-1}$): 3302, 1678, 1635, 1513; $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.75-7.71 (m, 2H),

7.54-7.48 (m, 1H), 7.45-7.40 (m, 2H), 7.17 (s, 1H), 6.98 (d, *J* = 5.4 Hz, 1H), 6.76 (d, *J* = 8.3 Hz, 1H), 6.70 (s, 1H), 6.67 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.61 (d, *J* = 2.0 Hz, 1H), 6.10 (d, *J* = 7.4 Hz, 1H), 5.09 (dd, *J* = 7.4, 5.4 Hz, 1H), 4.50 (ddd, *J* = 14.0, 4.9, 4.1 Hz, 1H), 4.45 (t, *J* = 7.4 Hz, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 3.84 (s, 3H), 3.61 (s, 3H), 3.55 (ddd, *J* = 14.0, 10.2, 4.1 Hz, 1H), 3.01 (ddd, *J* = 15.7, 10.2, 4.9 Hz, 1H), 2.87 (dt, *J* = 15.7, 4.1 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 167.3, 166.9, 149.7, 148.7, 148.3, 148.2, 135.1, 134.0, 131.7, 128.6 (2C), 128.4, 127.0, 126.9 (2C), 121.1, 120.0, 111.8, 111.2, 110.7, 106.7, 103.3, 56.0, 55.9, 55.8, 55.5, 54.5, 39.5, 39.1, 28.6; EIMS: *m/z* 514 ($M^+$, 29), 409 (16), 394 (27), 393 (100), 378 (20); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{30}H_{31}N_2O_6$: 515.2182, found 515.2183; Anal. Calcd. for $C_{30}H_{30}N_2O_6$: C, 70.02; H, 5.88; N, 5.44. Found: C, 69.89; H, 5.88; N, 5.58: **9b**-*trans* as a white solid. $R_f$ (70% EtOAc:hexane) 0.06; Mp: 165-166 °C; IR ($cm^{-1}$): 3322, 1702, 1635, 1606, 1513; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.66-7.61 (m, 2H), 7.44-7.39 (m, 1H), 7.35-7.29 (m, 2H), 7.03 (s, 1H), 6.95-6.91 (m, 2H), 6.83 (d, *J* = 8.7 Hz, 1H), 6.71 (d, *J* = 8.9 Hz, 1H), 6.65 (s, 1H), 5.73 (d, *J* = 2.7 Hz, 1H), 5.16 (dd, *J* = 14.4, 8.9 Hz, 1H), 4.55 (ddd, *J* = 12.5, 4.6, 4.3 Hz, 1H), 4.00 (dd, *J* = 14.4, 2.7 Hz, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.46-3.38 (m, 1H), 2.93 (ddd, *J* = 15.6, 10.6, 4.6 Hz, 1H), 2.78 (dt, *J* = 15.6, 4.3 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 168.5, 167.8, 149.7, 148.9, 148.2 (2C), 134.4, 134.2, 133.0, 131.4, 128.3 (2C), 127.5, 127.0 (2C), 121.0, 120.2, 111.2, 111.1, 110.7, 106.5, 105.1, 56.1, 55.9 (2C), 55.8, 54.5, 43.7, 39.5, 28.8; EIMS: *m/z* 514 ($M^+$, 2), 409 (4), 394 (26), 393 (100), 378 (25), 77 (3); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{30}H_{31}N_2O_6$: 515.2182, found 515.2180; Anal. Calcd. for $C_{30}H_{30}N_2O_6$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.16; H, 5.73; N, 5.59.

*2.3. N-[2-(3′,4′-dimethoxyphenyl)-9,10-dimethoxy-1-methyl-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]acetamide (**9c**).* The general procedure was used with azlactone **8c** (247 mg, 1.00 mmol) and 6,7-dimethoxy-1-ethyl-3,4-dihydroisoquinoline **7b** (329 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9c** *cis:trans* (40:60) (303 mg, 65%): **9c**-*cis* as a white solid. $R_f$ (70% EtOAc:hexane) 0.18; Mp: 171-172 °C; IR ($cm^{-1}$): 3276, 1675, 1664, 1635, 1547, 1515; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.01 (s, 1H), 6.78 (d, *J* = 8.1 Hz, 1H), 6.66 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.77 (s, 1H), 6.62 (d, *J* = 1.9 Hz, 1H), 6.31 (d, *J* = 5.5 Hz, 1H), 4.89 (brt, *J* = 6.7 Hz, 1H), 4.57 (ddd, *J* = 12.4, 4.5, 2.4 Hz, 1H), 4.04 (d, *J* = 6.7 Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H), 3.11 (ddd, *J* = 12.4, 12.3, 4.5 Hz, 1H), 2.98-2.85 (m, 2H), 2.04 (s, 3H), 2.01 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 170.1, 165.6, 148.9, 148.8, 148.4, 146.9, 129.8, 129.6, 127.4, 123.3, 120.6, 117.1, 112.2, 111.8, 111.1, 110.0, 56.1, 56.0, 55.8, 55.6, 53.6, 45.6, 40.6, 28.6, 23.2, 19.1; EIMS: *m/z* 466 ($M^+$, 8), 423 (4), 409 (7), 408 (27), 407 (100), 393 (10), 392 (36); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{26}H_{31}N_2O_6$: 467.2182, found 467.2189; Anal. Calcd. for $C_{26}H_{30}N_2O_6$: C, 66.94; H, 6.48; N, 6.00, Found: C, 67.05; H, 6.36; N, 6.26: **9c**-*trans* as a white solid. $R_f$ (70% EtOAc:hexane) 0.06; Mp: 192-193 °C; IR ($cm^{-1}$): 3254, 1669, 1648, 1558, 1513; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.97 (s, 1H), 6.88 (d, *J* = 1.7 Hz, 1H), 6.84 (d, *J* = 8.2 Hz, 1H), 6.81 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.75 (s, 1H), 6.24 (d, *J* = 6.2 Hz, 1H), 5.01 (brt, *J* = 9.7 Hz, 1H), 3.95-3.86 (m, 13H), 3.75 (d, *J* = 9.7 Hz, 1H), 3.57 (ddd, *J* = 12.9, 6.5, 6.2 Hz, 1H), 2.78 (t, *J* = 6.2 Hz, 2H), 1.93 (s, 3H), 1.81 (d, *J* = 0.8 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 170.3, 167.2, 149.0, 148.9, 148.2, 146.8, 130.3, 130.2, 127.5, 123.3, 121.0, 115.3, 112.6, 111.8, 111.0, 110.1, 56.3, 56.0 (2C), 55.8, 54.0, 49.3, 41.0, 28.6, 23.2, 19.5; EIMS: *m/z* 467 ($(M + 1)^+$, 1), 409 (6), 408 (33), 407 (100), 392 (63); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{26}H_{31}N_2O_6$: 467.2182, found 467.2183.

*2.4. N-[2-(3′,4′-dimethoxyphenyl)-9,10-dimethoxy-1-methyl-4-oxo-3,4,6,7,-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9d**).* The general procedure was used with azlactone **8d** (309 mg, 1.00 mmol) and 6,7-dimethoxy-1-ethyl-3,4-dihydroisoquinoline **7b** (329 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9d** *cis:trans* (40:60) (457 mg, 86%): **9d**-*cis* as a white solid. $R_f$ (70% EtOAc:hexane) 0.34; Mp: 168-169 °C; IR ($cm^{-1}$): 3369, 1672, 1652, 1605, 1578, 1513; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.78-7.74 (m, 2H), 7.54-7.49 (m, 1H), 7.46-7.40 (m, 2H), 7.06 (s, 1H), 7.00 (d, *J* = 5.4 Hz, 1H), 6.80 (s, 1H), 6.76 (d, *J* = 8.3 Hz, 1H), 6.68 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.62 (d, *J* = 1.9 Hz, 1H), 5.07 (dd, *J* = 6.9, 5.4 Hz, 1H), 4.60 (ddd, *J* = 12.7, 4.8, 2.1 Hz, 1H), 4.24 (d, *J* = 6.9 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 3.84 (s, 3H), 3.62 (s, 3H), 3.15 (ddd, *J* = 12.7, 12.7, 3.6 Hz, 1H), 2.98 (ddd, *J* = 13.7, 12.7, 4.8 Hz, 1H), 2.94-2.87 (m, 1H), 2.06 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 167.3, 165.6, 149.0, 148.7, 148.4, 147.0, 134.0, 131.7, 130.1, 129.7, 128.6 (2C), 127.2, 127.0 (2C), 123.3, 120.3, 117.0, 112.3, 112.2, 111.1, 110.1, 56.2, 56.0, 55.7, 55.4, 54.0, 45.4, 40.7, 28.6, 19.2; EIMS: *m/z* 528 ($M^+$, 5), 423 (3), 408 (31), 407 (100), 392 (35), 77 (4); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{31}H_{33}N_2O_6$: 529.2339, found 529.2341; Anal. Calcd. for $C_{31}H_{32}N_2O_6$: C, 70.44; H, 6.10; N, 5.30. Found: C, 70.78; H, 6.09; N, 5.60: **9d**-*trans* as a white solid. $R_f$ (70% EtOAc:hexane) 0.18; Mp: 204-205 °C; IR ($cm^{-1}$): 3284, 1685, 1674, 1636, 1509; $^1$H NMR (400 MHz, $CDCl_3$+$C_6D_6$ (8:1)) $\delta$ 7.61-7.57 (m, 2H), 7.34-7.29 (m, 1H), 7.24-7.18 (m, 2H), 6.97 (d, *J* = 8.8 Hz, 1H), 6.94 (s, 1H), 6.93 (d, *J* = 2.0 Hz, 1H), 6.85 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.73 (d, *J* = 8.2 Hz, 1H), 6.63 (s, 1H), 5.19 (dd, *J* = 10.0, 8.8 Hz, 1H), 3.94-3.86 (m, 2H), 3.83 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.72 (s, 3H), 3.56-3.47 (m, 1H), 2.65 (t, *J* = 5.9 Hz, 2H), 1.79 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$+$C_6D_6$ (8:1)) $\delta$ 167.5, 167.4, 149.0, 148.9, 148.2, 146.9, 134.0, 131.3, 130.3, 130.2, 130.1, 128.2



(2C), 127.0 (2C), 123.4, 121.1, 115.5, 112.7, 111.9, 111.0, 110.1, 56.1, 55.9, 55.8, 55.6, 54.6, 49.3, 41.1, 28.6, 19.5; EIMS: *m/z* 528 ($M^+$, 0.31), 408 (27), 409 (100), 392 (44), 77 (4); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{31}H_{33}N_2O_6$: 529.2339, found 529.2339.

*2.5. N-(9,10-dimethoxy-4-oxo-1,2-diphenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl)acetamide (**9e**).* The general procedure was used with azlactone **8a** (88.2 mg, 0.472 mmol) and 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline **7c** (154 mg, 0.707 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9e** *cis:trans* (17:83) (93.0 mg, 42%): **9e**-*cis* as a yellow-brown solid. $R_f$ (70% EtOAc:hexane) 0.17; Mp: 267-268 °C; IR ($cm^{-1}$): 3328, 2936, 2833, 1651, 1606, 1510, 1403; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.28-7.04 (m, 10H), 6.67 (s, 1H), 6.32 (s, 1H), 6.27 (d, *J* = 6.3 Hz, 1H), 5.19 (t, *J* = 6.3 Hz, 1H), 4.58 (d, *J* = 6.3 Hz, 1H), 4.48 (dt, *J* = 12.1, 4.4 Hz, 1H), 3.87 (s, 3H), 3.36 (td, *J* = 12.1, 4.4 Hz, 1H), 3.19 (s, 3H), 3.16-2.98 (m, 2H), 2.05 (s, 3H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 170.2, 166.2, 148.9, 146.6, 139.6, 134.7, 131.5, 130.2 (2C), 129.4, 128.7 (2C), 128.5 (3C), 127.5, 127.1, 122.7, 121.1, 113.3 (2C), 109.6, 55.8, 55.2, 54.1, 46.9, 40.7, 28.6, 23.2; EIMS: *m/z* 468 ($M^+$, 4), 409 (100), 394 (25); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{29}H_{29}N_2O_4$: 469.2122, found 469.2126: **9e**-*trans* as yellow solid. $R_f$ (70% EtOAc:hexane) 0.08; Mp: 257-258 °C; IR ($cm^{-1}$): 3315, 2924, 2853, 1642, 1515, 1448, 1393; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.34-6.98 (m, 10H), 6.64 (s, 1H), 6.39 (d, *J* = 8.1 Hz, 1H), 6.31 (s, 1H), 4.97 (dd, *J* = 8.1, 3.3 Hz, 1H), 4.38-4.23 (m, 1H), 4.22 (d, *J* = 3.3 Hz, 1H), 3.88 (s, 3H), 3.42-3.24 (m, 1H), 3.17 (s, 3H), 3.08-2.76 (m, 2H), 2.11 (s, 3H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 169.9, 165.6, 148.7, 146.3 , 140.1, 136.4, 129.9 (3C), 128.7 (2C), 128.6 (2C), 127.9 (3C), 127.3, 126.9, 122.1, 117.6, 113.4, 109.6, 55.8, 55.5, 55.1, 50.8, 40.5, 28.7, 23.4; EIMS: *m/z* 468 ($M^+$, 1), 409 (100), 394 (37); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{29}H_{29}N_2O_4$: 469.2122, found 469.2126.

*2.6. N-[2-(3′′,4′′-Dimethoxyphenyl)-9,10-dimethoxy-4-oxo-1-phenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]acetamide (**9f**).* The general procedure was used with azlactone **8c** (247 mg, 1.00 mmol) and 1-benzyl-6,7-dimethoxy-3,4-dihydroisoquinoline **7c** (423 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9f** *cis:trans* (0:100) (203 mg, 35%): **9f**-*cis* as a white solid. $R_f$ (80% EtOAc:hexane) 0.15; Mp: 229 °C; IR ($cm^{-1}$): 3317, 1646, 1516; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.17-7.02 (m, 5H), 6.69-6.58 (m, 3H), 6.53 (brs, 1H), 6.28 (d, *J* = 5.7 Hz, 1H), 6.25 (s, 1H), 5.07 (dd, *J* = 6.8, 5.7 Hz, 1H), 4.47 (d, *J* = 6.8 Hz, 1H), 4.45-4.35 (m, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 3.69 (s, 3H), 3.30 (ddd, *J* = 12.2, 12.2, 4.0 Hz, 1H), 3.12 (s, 3H), 3.02-2.80 (m, 2H), 1.99 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.1, 166.2, 148.8, 148.7, 148.3, 146.6, 139.5, 131.1, 130.2 (2C), 129.2, 128.5 (2C), 127.1, 126.9, 122.7, 121.4, 120.5, 113.2, 111.9, 111.1, 109.5, 55.8, 55.7, 55.6, 55.1, 54.2, 46.3, 40.5, 28.5, 23.2; EIMS: *m/z* 528 ($M^+$, 8), 471 (6), 470 (31), 469 (100), 455 (10), 454 (36); HRMS-FAB *m/z* $[M + H]^+$ calcd. for $C_{31}H_{33}N_2O_6$: 529.2339, found 529.2339: **9f**-*trans* as a white solid. $R_f$ (80% EtOAc:hexane) 0.06; Mp: 170-171 °C; IR ($cm^{-1}$): 3356, 1670, 1637, 1611, 1513; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.24-7.10 (m, 3H), 7.08-7.00 (m, 2H), 6.82 (brd, *J* = 7.9 Hz, 1H), 6.81 (brs, 1H), 6.72 (d, *J* = 7.9 Hz, 1H), 6.62 (s, 1H), 6.59 (d, *J* = 7.9 Hz, 1H), 6.30 (s, 1H), 4.97 (dd, *J* = 7.9, 3.2 Hz, 1H), 4.30-4.20 (m, 1H), 4.17 (d, *J* = 3.2 Hz, 1H), 3.86 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.35-3.22 (m, 1H), 3.17 (s, 3H), 2.98-2.75 (m, 2H), 2.12 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.2, 165.7, 149.0, 148.7, 148.1, 146.3, 140.1, 131.3, 129.9 (2C), 129.8, 128.7, 128.6 (2C), 127.0, 122.1, 119.7, 118.0, 113.4, 111.3, 111.2, 109.6, 55.8, 55.7, 55.6, 55.5, 55.1, 50.2, 40.5, 28.6, 23.2; EIMS: *m/z* 528 ($M^+$, 1), 513 (9), 471 (6), 470 (29), 469 (100), 454 (44); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{31}H_{33}N_2O_6$: 529.2339, found 529.2338.

*2.7. N-[9,10-dimethoxy-2-(3′′,4′′-methylenedioxyphenyl)-4-oxo-1-phenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]acetamide (**9g**).* The general procedure was used with azlactone **8e** (231 mg, 1.00 mmol) and 1-benzyl-6,7-dimethoxy-3,4-dihydroisoquinoline **7c** (423 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9g** *cis:trans* (20:80) (230 mg, 45%): **9g**-*cis* as a pale yellow solid. $R_f$ (80% EtOAc:hexane) 0.27; Mp: 229-230 °C; IR ($cm^{-1}$): 3325, 1645, 1614, 1516; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.23-7.12 (m, 5H), 6.67 (s, 1H), 6.66 (d, *J* = 8.2 Hz, 1H), 6.61 (d, *J* = 1.7 Hz, 1H), 6.56 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.32 (s, 1H), 6.31 (brd, *J* = 4.2 Hz, 1H), 5.89 (d, *J* = 7.5 Hz, 1H), 5.88 (d, *J* = 7.5 Hz, 1H), 5.13 (brt, *J* = 6.5 Hz, 1H), 4.53-4.46 (m, 2H), 3.88 (s, 3H), 3.37 (ddd, *J* = 12.2, 11.9, 3.6 Hz, 1H), 3.20 (s, 3H), 3.06 (ddd, *J* = 15.3, 11.9, 4.4 Hz, 1H), 2.92 (dt, *J* = 15.3, 3.6 Hz, 1H), 2.07 (s, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 170.2, 166.2, 148.9, 147.9, 147.0, 146.6, 139.5, 131.4, 130.2 (2C), 129.5, 128.6 (2C), 128.2, 127.1, 122.6, 122.2, 121.2, 113.3, 109.6, 108.7, 108.3, 100.9, 55.8, 55.1, 54.2, 46.4, 40.6, 28.6, 23.3; EIMS: *m/z* 512 ($M^+$, 9), 470 (31), 469 (100), 455 (14), 454 (71), 453 (91), 438 (26); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{30}H_{29}N_2O_6$: 513.2026, found 513.2026: **9g**-*trans* as a yellow amorphous solid. $R_f$ (80% EtOAc:hexane) 0.11; IR ($cm^{-1}$): 3347, 1669, 1608, 1512; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.24-7.18 (m, 2H), 7.17-7.12 (m, 1H), 7.04 (brd, *J* = 7.0 Hz, 2H), 6.79 (d, *J* = 1.7 Hz, 1H), 6.78 (dd, *J* = 7.2, 1.7 Hz, 1H), 6.68 (d, *J* = 7.2 Hz, 1H), 6.63 (s, 1H), 6.39 (d, *J* = 8.0 Hz, 1H), 6.29 (s, 1H), 5.88 (d, *J* = 3.4 Hz, 1H), 5.87 (d, *J* = 3.4 Hz, 1H), 4.89 (dd, *J* = 8.0, 3.0 Hz, 1H), 4.30 (ddd, *J* = 12.4, 4.2, 4.0 Hz, 1H), 4.13 (d, *J* = 3.0 Hz, 1H), 3.86 (s, 3H), 3.31 (ddd, *J* = 12.4, 11.6, 4.0 Hz, 1H), 3.17 (s, 3H), 2.96 (ddd, *J* = 15.2, 11.6, 4.2



Hz, 1H), 2.82 (dt, *J* = 15.2, 4.0 Hz, 1H), 2.11 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 170.0, 165.5, 148.8, 147.9, 146.8, 146.4, 140.1, 131.5, 130.1, 130.0, 129.9 (2C), 128.7 (2C), 127.1, 122.0, 121.3, 117.7, 113.4, 109.6, 108.6, 108.3, 100.9, 55.8, 55.7, 55.1, 50.5, 40.5, 28.7, 23.3; EIMS: *m/z* 513 ($M^+$+1, 4), 475 (6), 454 (37), 453 (100), 452 (9), 439 (20), 438 (59); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{30}H_{29}N_2O_6$: 513.2026, found 513.2027.

*2.8. N-[2-(3′′,4′′-dimethoxyphenyl)-9,10-dimethoxy-4-oxo-1-phenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9h**).* The general procedure was used with azlactone **8d** (309 mg, 1.00 mmol) and 1-benzyl-6,7-dimethoxy-3,4-dihydroisoquinoline **7c** (423 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9h** *cis:trans* (36:64) (470 mg, 80%): **9h**-*cis* as a white solid. $R_f$ (60% EtOAc:hexane) 0.27; Mp: 237 °C; IR ($cm^{-1}$): 3289, 1672, 1632, 1526, 1514; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.82-7.75 (m, 2H), 7.56-7.39 (m, 3H), 7.27-7.11 (m, 5H), 7.05 (d, *J* = 5.2 Hz, 1H), 6.77-6.66 (m, 3H), 6.59 (brs, 1H), 6.37 (s, 1H), 5.32 (brt, *J* = 6.6 Hz, 1H), 4.76 (d, *J* = 6.6 Hz, 1H), 4.56-4.46 (m, 1H), 3.88 (s, 3H), 3.79 (s, 3H), 3.56 (s, 3H), 3.42 (ddd, *J* = 12.1, 12.1, 3.8 Hz, 1H), 3.22 (s, 3H), 3.14-2.90 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.3, 166.2, 148.9, 148.6, 148.3, 146.6, 139.6, 133.9, 131.8, 131.4, 130.2 (2C), 129.3, 128.6 (2C), 128.5 (2C), 127.2, 127.0 (2C), 126.7, 122.7, 121.3, 120.2, 113.3, 112.3, 111.1, 109.6, 55.8, 55.7, 55.4, 55.1, 54.7, 46.1, 40.6, 28.6; EIMS: *m/z* 590 ($M^+$, 8), 470 (33), 469 (100), 454 (28); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{36}H_{35}N_2O_6$: 591.2495, found 591.2502; Anal. Calcd. for $C_{36}H_{34}N_2O_6$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.52; H, 5.80; N, 4.63: **9h**-*trans* as yellow amorphous solid. $R_f$ (60% EtOAc:hexane) 0.13; IR ($cm^{-1}$): 3336, 1668, 1604, 1579, 1513; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.85-7.77 (m, 2H), 7.56-7.48 (m, 1H), 7.48-7.39 (m, 2H), 7.21-7.08 (m, 3H), 7.07-7.00 (m, 2H), 6.94-6.83 (m, 3H), 6.75 (d, *J* = 8.8 Hz, 1H), 6.67 (s, 1H), 6.33 (s, 1H), 5.15 (dd, *J* = 7.6, 3.6 Hz, 1H), 4.37 (d, *J* = 3.6 Hz, 1H), 4.34-4.25 (m, 1H), 3.88 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.49-3.37 (m, 1H), 3.15 (s, 3H), 3.04-2.82 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.6, 165.8, 149.1, 148.8, 148.2, 146.5, 140.0, 134.0, 131.8, 131.5, 129.9 (2C), 129.8, 128.6 (5C), 127.1 (2C), 127.0, 122.2, 119.8, 118.3, 113.3, 111.4, 111.2, 109.7, 56.0, 55.8, 55.7, 55.1 (2C), 50.0, 40.6, 28.7; EIMS: *m/z* 590 ($M^+$, 0.83), 470 (32), 469 (100), 454 (21); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{36}H_{35}N_2O_6$: 591.2495, found 591.2502.

*2.9. N-(1-(3,4-dimethoxyphenyl)-9,10-dimethoxy-4-oxo-2-phenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl)acetamide (**9i**).* The general procedure was used with azlactone **8a** (190 mg, 1.01 mmol) and 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline **7d** (494 mg, 1.52 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9i** *cis:trans* (27:73) (184 mg, 34%): **9i**-*cis* as a yellow solid. $R_f$ (90% EtOAc:hexane) 0.26; Mp: 203-204 °C; IR ($cm^{-1}$) 3329, 2935, 2835, 1654, 1606, 1508, 1405; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.28-7.02 (m, 5H), 6.68 (d, *J* = 8.3 Hz, 1H), 6.64 (s, 1H), 6.57 (dd, *J* = 8.3, 2 Hz, 1H), 6.46 (d, *J* = 2 Hz, 1H), 6.41 (s, 1H), 6.37 (d, *J* = 8.4 Hz, 1H), 5.02 (dd, *J* = 8.4, 4.8 Hz, 1H), 4.22 (d, *J* = 4.8 Hz, 1H), 4.18 (dt, *J* = 11.0, 4.4 Hz, 1H), 3.87 (s, 3H), 3.78 (s, 3H), 3.62 (s, 3H), 3.54-3.34 (m, 1H), 3.26 (s, 3H), 3.04-2.74 (m, 2H), 2.08 (s, 3H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 170.2, 166.1, 148.9, 148.8, 148.3, 146.7, 134.8, 131.9, 131.1, 129.4 (2C), 128.6 (2C), 128.5 (3C), 127.4, 122.9, 120.9, 113.4, 113.1, 111.3, 109.6, 56.0, 55.8, 55.3, 54.1, 46.9, 40.7, 28.6, 23.2; EIMS: *m/z* 528 ($M^+$, 12), 469 (100), 455 (9); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{31}H_{33}N_2O_6$: 529.2333, found 529.2335: **9i**-*trans* as a brown solid. $R_f$ (90% EtOAc:hexane) 0.08; Mp: 145-146 °C; IR ($cm^{-1}$) 3329, 2935, 2835, 1654, 1606, 1508, 1405; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.32-7.15 (m, 5H), 6.68 (d, *J* = 8.3 Hz, 1H), 6.65 (s, 1H), 6.58 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.46 (s, 1H), 6.43 (s, 1H), 6.13 (d, *J* = 8.2 Hz, 1H), 5.03 (dd, *J* = 8.2, 4.6 Hz, 1H), 4.22 (d, *J* = 4.6 Hz, 1H), 4.19 (m, 1H), 3.88 (s, 3H), 3.78 (s, 3H), 3.62 (s, 3H), 3.53-3.44 (m, 1H), 3.26 (s, 3H), 2.96 (ddd, *J* = 15.0, 10.3, 4.5 Hz, 1H), 2.85 (dt, *J* = 15.0, 4.4 Hz, 1H), 2.09 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 170.0, 165.9, 148.9, 148.8, 148.2, 146.5, 136.6, 132.4, 131.3, 130.0, 128.7 (2C), 128.2 (2C), 127.3, 122.7, 122.4, 117.7, 113.5, 113.2, 111.3, 109.7, 56.0, 55.9 (2C), 55.3, 55.1, 50.6, 40.7, 28.8, 23.4; EIMS: *m/z* 528 ($M^+$, 2), 469 (100), 455 (9); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{31}H_{33}N_2O_6$: 529.2333, found 529.2337.

*2.10. N-[1,2-bis-(3′,4′-dimethoxyphenyl)-9,10-dimethoxy-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]acetamide (**9j**).* The general procedure was used with azlactone **8c** (247 mg, 1.00 mmol) and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline **7d** (512 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9j** *cis:trans* (16:84) (265 mg, 45%): **9j**-*cis* as a yellow oil. $R_f$ (70% EtOAc:hexane; developed twice) 0.11; IR ($cm^{-1}$): 3329, 1656, 1606, 1509; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 6.74 (d, *J* = 8.2 Hz, 1H), 6.73 (s, 1H), 6.70-6.63 (m, 4H), 6.61 (d, *J* = 1.6 Hz, 1H), 6.42 (s, 1H), 6.36 (d, *J* = 5.6 Hz, 1H), 5.13 (brt, *J* = 6.8 Hz, 1H), 4.53 (d, *J* = 6.8 Hz, 1H), 4.47 (ddd, *J* = 12.3, 4.3, 4.0 Hz, 1H), 3.88 (s, 3H), 3.81 (s, 3H), 3.78 (s, 6H), 3.72 (s, 3H), 3.38 (ddd, *J* = 12.3, 12.0, 4.3 Hz, 1H), 3.29 (s, 3H), 3.09-2.88 (m, 2H), 2.07 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 170.2, 166.1, 148.9, 148.8, 148.7, 148.3, 148.3, 146.6, 131.8, 130.8, 129.1, 127.0, 123.0, 122.9, 121.2, 120.5, 113.3, 113.0, 111.9, 111.2, 111.1, 109.5, 56.0, 55.8, 55.7 (2C), 55.6, 55.2, 54.2, 46.3, 40.6, 28.5, 23.2; EIMS: *m/z* 588 ($M^+$, 10), 531 (7), 530 (33), 529 (100), 515 (16), 514 (20); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{33}H_{37}N_2O_8$: 589.2550, found 589.2551: **9j**-*trans* as a yellow amorphous



solid. $R_f$ (70% EtOAc:hexane; developed twice) 0.05; IR ($cm^{-1}$): 1668, 1606, 1513; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 6.78-6.50 (m, 6H), 6.45 (d, $J$ = 8.2 Hz, 1H), 6.40 (brs, 1H), 6.32 (s, 1H), 4.93 (dd, $J$ = 8.0, 4.7 Hz, 1H), 4.18-4.05 (m, 2H), 3.79 (s, 3H), 3.73 (s, 3H), 3.72 (s, 3H), 3.71 (s, 3H), 3.55 (s, 3H), 3.41-3.26 (m, 1H), 3.19 (s, 3H), 2.90-2.66 (m, 2H), 2.01 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.1, 166.1, 149.0, 148.9, 148.7, 148.2, 148.1, 146.4, 132.3, 131.0, 129.8, 128.9, 122.6, 122.4, 119.9, 118.2, 113.5, 113.2, 111.4, 111.3, 111.1, 109.6, 55.9, 55.8 (2C), 55.7 (2C), 55.3, 55.1, 50.0, 40.6, 28.7, 23.3; EIMS: *m/z* 588 ($M^+$, 3), 545 (4), 531 (9), 530 (42), 529 (100), 515 (11), 514 (30); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{33}H_{37}N_2O_8$: 589.2550, found 589.2552.

*2.11. N-[9,10-dimethoxy-1-(3′,4′-dimethoxyphenyl)-2-(3″,4″-methylenedioxyphenyl)-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]acetamide (**9k**).* The general procedure was used with azlactone **8e** (231 mg, 1.00 mmol) and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline **7d** (512 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9k** *cis:trans* (15:85) (225 mg, 39%): **9k**-*cis* as a pale yellow oil. $R_f$ (70% EtOAc:hexane; developed twice) 0.20; IR ($cm^{-1}$): 3326, 1660, 1608, 1580, 1509; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 6.73-6.63 (m, 5H), 6.59 (d, $J$ = 1.5 Hz, 1H), 6.55 (dd, $J$ = 8.0, 1.5 Hz, 1H), 6.41 (s, 1H), 6.35 (d, $J$ = 6.1 Hz, 1H), 5.89 (d, $J$ = 5.6 Hz, 1H), 5.88 (d, $J$ = 5.6 Hz, 1H), 5.11 (brt, $J$ = 6.1 Hz, 1H), 4.53-4.43 (m, 2H), 3.88 (s, 3H), 3.79 (s, 3H), 3.71 (s, 3H), 3.36 (ddd, $J$ = 12.4, 12.1, 3.9 Hz, 1H), 3.29 (s, 3H), 3.05 (ddd, $J$ = 13.4, 12.1, 4.7 Hz, 1H), 2.97-2.87 (m, 1H), 2.07 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.3, 166.1, 148.9, 148.8, 148.3, 147.8, 146.9, 146.6, 131.7, 131.0, 129.4, 128.3, 122.9 (2C), 122.2, 121.0, 113.3, 113.0, 111.1, 109.5, 108.6, 108.3, 100.9, 56.0, 55.8, 55.7, 55.2, 54.1, 46.4, 40.6, 28.5, 23.2; EIMS: *m/z* 572 ($M^+$, 8), 515 (6), 514 (34), 513 (100), 498 (39); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{32}H_{33}N_2O_8$: 573.2237, found 573.2242: **9k**-*trans* as a white solid. $R_f$ (70% EtOAc:hexane; developed twice) 0.09; Mp: 167-169 °C; IR ($cm^{-1}$): 3384, 1664, 1578, 1509; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 6.80-6.62 (m, 5H), 6.62-6.55 (m, 1H), 6.49 (brs, 1H), 6.40 (s, 1H), 6.21 (d, $J$ = 8.0 Hz, 1H), 5.88 (s, 2H), 4.92 (dd, $J$ = 8.0, 3.9 Hz, 1H), 4.28-4.18 (m, 1H), 4.14 (d, $J$ = 3.9 Hz, 1H), 3.87 (s, 3H), 3.79 (s, 3H), 3.65 (s, 3H), 3.46-3.36 (m, 1H), 3.27 (s, 3H), 3.03-2.89 (m, 1H), 2.89-2.78 (m, 1H), 2.09 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.0, 165.7, 149.0, 148.8, 148.3, 147.9, 146.8, 146.5, 132.3, 131.2, 130.3, 130.0, 122.6, 122.3, 121.4, 117.8, 113.4, 113.2, 111.4, 109.7, 108.5, 108.4, 100.9, 56.0, 55.9 (2C), 55.5, 55.3, 50.3, 40.6, 28.7, 23.4; EIMS: *m/z* 572 ($M^+$, 7), 529 (2), 515 (7), 514 (36), 513 (100), 498 (11); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{32}H_{33}N_2O_8$: 573.2237, found 573.2236.

*2.12. N-[1-(3′,4′-dimethoxyphenyl)-9,10-dimethoxy-4-oxo-2-phenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9l**).* The general procedure was used with azlactone **8b** (249 mg, 1.00 mmol) and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline **7d** (512 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9l** *cis:trans* (35:65) (513 mg, 86%): **9l**-*cis* as a white solid. $R_f$ (60% EtOAc:hexane) 0.26; Mp: 146-148 °C; IR ($cm^{-1}$): 3371, 1645, 1603, 1579, 1515; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.86-7.82 (m, 2H), 7.63-7.57 (m, 1H), 7.55-7.49 (m, 2H), 7.33-7.20 (m, 5H), 7.07 (d, $J$ = 5.6 Hz, 1H), 6.86 (s, 1H), 6.82 (d, $J$ = 8.4 Hz, 1H), 6.79 (s, 1H), 6.75 (d, $J$ = 8.4 Hz, 1H), 6.56 (s, 1H), 5.44 (dd, $J$ = 7.0, 5.6 Hz, 1H), 4.83 (d, $J$ = 7.0 Hz, 1H), 4.60 (ddd, $J$ = 12.4, 4.6, 3.5 Hz, 1H), 3.98 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 3.50 (ddd, $J$ = 12.4, 12.0, 3.5 Hz, 1H), 3.40 (s, 3H), 3.20 (ddd, $J$ = 15.2, 12.0, 4.6 Hz, 1H), 3.04 (dt, $J$ = 15.2, 3.5 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 167.6, 166.2, 149.0, 148.8, 148.3, 146.7, 134.6, 134.1, 131.9, 131.7, 131.3, 129.4, 128.8 (2C), 128.6 (4C), 127.5, 127.1 (2C), 122.9 (2C), 120.9, 113.3, 113.0, 111.2, 109.6, 56.1, 55.9, 55.8, 55.3, 54.4, 46.7, 40.7, 28.6; EIMS: *m/z* 590 ($M^+$, 17), 513 (8), 470 (33), 469 (100), 454 (26), 105 (1), 77 (1); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{36}H_{35}N_2O_6$: 591.2495, found 591.2496; Anal. Calcd. for $C_{36}H_{34}N_2O_6$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.31; H, 5.80; N, 4.68: **9l**-*trans* as a white solid. $R_f$ (60% EtOAc:hexane) 0.20; Mp: 183 °C; IR ($cm^{-1}$): 3285, 1667, 1634, 1601, 1579, 1532, 1513; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.82-7.77 (m, 2H), 7.56-7.51 (m, 1H), 7.47-7.42 (m, 2H), 7.39-7.34 (m, 2H), 7.25-7.31 (m, 2H), 7.23-7.17 (m, 1H), 6.76 (d, $J$ = 7.8 Hz, 1H), 6.69 (s, 1H), 6.66 (d, $J$ = 8.3 Hz, 1H), 6.59 (dd, $J$ = 8.3, 1.8 Hz, 1H), 6.48 (d, $J$ = 1.8 Hz, 1H), 6.44 (s, 1H), 5.19 (dd, $J$ = 7.8, 4.5 Hz, 1H), 4.43 (d, $J$ = 4.5 Hz, 1H), 4.25 (ddd, $J$ = 12.8, 4.6, 4.5 Hz, 1H), 3.90 (s, 3H), 3.78 (s, 3H), 3.55 (s, 3H), 3.58-3.48 (m, 1H), 3.26 (s, 3H), 3.01 (ddd, $J$ = 15.2, 10.6, 4.6 Hz, 1H), 2.89 (dt, $J$ = 15.2, 4.5 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 167.6, 165.8, 149.0, 148.8, 148.2, 146.5, 136.5, 134.1, 132.3, 131.8, 131.4, 130.0, 128.8 (2C), 128.7 (2C), 128.2 (2C), 127.4, 127.0 (2C), 122.5 (2C), 118.0, 113.2, 113.0, 111.3, 109.7, 55.9 (2C), 55.8 (2C), 55.3, 50.4, 40.8, 28.8; EIMS: *m/z* 590 ($M^+$, 0.91), 470 (32), 469 (100), 454 (35); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{36}H_{35}N_2O_6$: 591.2495, found 591.2492; Anal. Calcd. for $C_{36}H_{34}N_2O_6$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.04; H, 5.97; N, 4.45.

*2.13. N-[1,2-bis-(3′,4′-dimethoxyphenyl)-9,10-dimethoxy-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9m**).* The general procedure was used with azlactone **8d** (309 mg, 1.00 mmol) and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline **7d** (512 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9m** *cis:trans* (32:68) (553 mg, 85%): **9m**-*cis* as a pale brown oil. $R_f$ (70% EtOAc:hexane) 0.49; IR ($cm^{-1}$): 1652, 1604, 1509; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.82-7.74 (m, 2H),



7.56- 7.48 (m, 1H), 7.48-7.39 (m, 2H), 7.05 (d, *J* = 5.2 Hz, 1H), 6.82-6.65 (m, 6H), 6.60 (s, 1H), 6.46 (s, 1H), 5.30 (brt, *J* = 6.5 Hz, 1H), 4.73 (d, *J* = 6.5 Hz, 1H), 4.55-4.45 (m, 1H), 3.89 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.74 (s, 3H), 3.58 (s, 3H), 3.42 (ddd, *J* = 12.1, 11.9, 4.0 Hz, 1H), 3.31 (s, 3H), 3.06 (ddd, *J* = 13.4, 11.9, 4.8 Hz, 1H), 3.01-2.91 (m, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.4, 166.2, 149.1, 148.9, 148.7, 148.5, 148.4, 146.8, 134.0, 132.0, 131.8, 131.1, 129.2, 128.6 (2C), 127.0 (2C), 126.9, 123.1, 123.0, 121.2, 120.3, 113.6, 113.2, 112.3, 111.4, 111.3, 109.7, 56.1, 55.9 (2C), 55.7, 55.4, 55.3, 54.7, 46.3, 40.7, 28.6; EIMS: *m/z* 650 ($M^+$, 6), 531 (7), 530 (33), 529 (100), 515 (9), 514 (25), 470 (30), 469 (88), 455 (12), 454 (27); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{38}H_{39}N_2O_8$: 651.2706, found 651.2709: **9m**-*trans* as a pale brown oil. $R_f$ (70% EtOAc:hexane) 0.40; IR ($cm^{-1}$): 3336, 1668, 1604, 1579, 1513; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.81-7.74 (m, 2H), 7.55-7.46 (m, 1H), 7.46-7.36 (m, 2H), 7.02-6.83 (m, 3H), 6.74 (d, *J* = 8.2 Hz, 1H), 6.66 (s, 1H), 6.65 (d, *J* = 7.5 Hz, 1H), 6.56 (dd, *J* = 8.2, 1.5 Hz, 1H), 6.48 (brs, 1H), 6.41 (s, 1H), 5.16 (dd, *J* = 7.4, 4.7 Hz, 1H), 4.39 (d, *J* = 4.7 Hz, 1H), 4.25-4.14 (m, 1H), 3.88 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.76 (s, 3H), 3.54 (s, 3H), 3.50-3.37 (m, 1H), 3.24 (s, 3H), 3.01-2.79 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.6, 166.1, 149.0, 148.9, 148.7, 148.1 (2C), 146.5, 134.0, 132.3, 131.7, 131.1, 129.7, 128.7, 128.5 (2C), 127.0 (2C), 122.5 (2C), 120.0, 118.5, 113.2, 113.0, 111.4, 111.1, 111.2, 109.7, 55.8 (2C), 55.7 (4C), 55.3, 49.7, 40.7, 28.7; EIMS: *m/z* 650 ($M^+$, 11), 531 (7), 530 (35), 529 (100), 514 (25), 105 (2), 77 (3); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{38}H_{39}N_2O_8$: 651.2706, found 651.2712.

*2.14. N-[9,10-dimethoxy-1-(3′,4′-dimethoxyphenyl)-2-(3″,4″-methylenedioxyphenyl)-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]-benzamide (**9n**).* The general procedure was used with azlactone **8f** (293 mg, 1.00 mmol) and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline **7d** (512 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9n** *cis:trans* (38:62) (532 mg, 84%): **9n**-*cis* as a white solid. $R_f$ (60% EtOAc:hexane) 0.23; Mp: 134-135 °C; IR ($cm^{-1}$): 3392, 1677, 1651, 1607, 1580, 1515; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.80-7.72 (m, 2H), 7.55-7.50 (m, 1H), 7.47-7.42 (m, 2H), 7.03 (d, *J* = 5.3 Hz, 1H), 6.76 (brs, 1H), 6.73 (brd *J* = 8.5 Hz, 1H), 6.69 (s, 1H), 6.68 (d, *J* = 8.5 Hz, 1H), 6.65 (d, *J* = 1.6 Hz, 1H), 6.63 (dd, *J* = 8.0 Hz, 1H), 6.57 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.45 (s, 1H), 5.88 (d, *J* = 4.2 Hz, 1H), 5.87 (d, *J* = 4.2 Hz, 1H), 5.28 (brt, *J* = 6.8 Hz, 1H), 4.68 (d, *J* = 6.8 Hz, 1H), 4.52 (ddd, *J* = 12.5, 4.7, 3.5 Hz, 1H), 3.89 (s, 3H), 3.80 (s, 3H), 3.74 (s, 3H), 3.40 (ddd, *J* = 12.5, 12.3, 3.5 Hz, 1H), 3.31 (s, 3H), 3.14-3.04 (m, 1H), 2.94 (dt, *J* = 15.0, 3.5 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 167.6, 166.2, 149.0, 148.9, 148.3, 147.9, 147.0, 146.7, 134.1, 131.8, 131.7, 131.2, 129.4, 128.6 (2C), 128.2, 127.1 (2C), 123.0, 122.9, 122.5, 121.0, 113.3, 113.0, 111.2, 109.6, 108.5, 108.3, 100.9, 56.1, 55.9, 55.8, 55.3, 54.6, 46.4, 40.7, 28.6; EIMS: *m/z* 634 ($M^+$, 20), 515 (8), 514 (28), 513 (100), 498 (17), 77 (6); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{37}H_{35}N_2O_8$: 635.2393, found 635.2405; Anal. Calcd. for $C_{37}H_{34}N_2O_8$: C, 70.02; H, 5.40; N, 4.41. Found: C, 69.72; H, 5.69; N, 4.32: **9n**-*trans* as a white solid. $R_f$ (60% EtOAc:hexane) 0.17; Mp: 221-222 °C; IR ($cm^{-1}$): 3279, 1669, 1634, 1601, 1578, 1509; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.82-7.74 (m, 2H), 7.55-7.50 (m, 1H), 7.46-7.41 (m, 2H), 6.88-6.84 (m, 2H), 6.81 (d, *J* = 7.7 Hz, 1H), 6.71 (d, *J* = 8.6 Hz, 1H), 6.67 (s, 1H), 6.66 (d, *J* = 8.2 Hz, 1H), 6.58 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.49 (d, *J* = 1.4 Hz, 1H), 6.40 (s, 1H), 5.90 (d, *J* = 3.1 Hz, 1H), 5.89 (d, *J* = 3.1 Hz, 1H), 5.08 (dd, *J* = 7.7, 4.0 Hz, 1H), 4.35 (d, *J* = 4.0 Hz, 1H), 4.27 (ddd, *J* = 12.3, 4.4, 3.9 Hz, 1H), 3.88 (s, 3H), 3.77 (s, 3H), 3.56 (s, 3H), 3.45 (ddd, *J* = 12.3, 11.4, 3.9 Hz, 1H), 3.24 (s, 3H), 2.99 (ddd, *J* = 15.1, 11.4, 4.4 Hz, 1H), 2.86 (dt, *J* = 15.1, 3.9 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 167.5, 165.7, 149.0, 148.9, 148.2, 148.0, 146.8, 146.5, 134.0, 132.3, 131.9, 131.3, 130.1, 130.0, 128.7 (2C), 127.0 (2C), 122.5, 122.4, 121.5, 118.0, 113.1, 113.0, 111.3, 109.7, 108.6, 108.4, 101.0, 56.2, 55.9, 55.8 (2C), 55.3, 50.0, 40.7, 28.7; EIMS: *m/z* 634 ($M^+$, 22), 529 (3), 515 (7), 514 (33), 513 (100), 498 (18); HRMS-FAB *m/z* $[M + H]^+$ calcd for $C_{37}H_{35}N_2O_8$: 635.2393, found 635.2390; Anal. Calcd. for $C_{37}H_{34}N_2O_8$: C, 70.02; H, 5.40; N, 4.41. Found: C, 70.12; H, 5.45; N, 4.45.

*2.15. N-2-(2”-bromophenyl)-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]-benzamide (**9o**).* The general procedure was used with azlactone **8g** (327 mg, 1.00 mmol) and 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline **7a** (308 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9o** *cis:trans* (78:22) (520 mg, 98%): **9o**-*cis* as a pale green solid. $R_f$ (60% EtOAc:hexane) 0.32; Mp: 179-180 °C; IR ($cm^{-1}$): 3410, 3058, 2938, 1656, 1609, 1511; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.75-7.65 (m, 2H), 7.60-7.35 (m, 4H), 7.30-7.05 (m, 4H), 6.74 (d, *J* = 6.8 Hz, 1H), 6.67 (s, 1H), 6.08 (d, *J* = 6.8 Hz, 1H), 5.37 (t, *J* = 6.8 Hz, 1H), 4.90 (t, *J* = 6.8 Hz, 1H), 4.47 (dt, *J* = 12.7, 4.0 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.58 (ddd, *J* = 12.7, 9.6, 4.0 Hz, 1H), 3.08-2.76 (m, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 167.8, 167.1, 149.8, 148.2, 136.8, 135.4, 134.3, 133.3, 131.4, 128.9, 128.6, 128.3 (3C), 128.0, 127.1 (2C), 125.7, 121.0, 110.7, 106.7, 102.7, 56.0, 55.9, 53.1, 39.6, 39.3, 28.6; EIMS: *m/z* 534 ($M^+$+2, 5), 532 ($M^+$, 6), 429 (29), 427 (31), 413 (98), 411 (100), 332 (63), 105 (53), 77 (36); HRMS (microTOF) *m/z* $[M + H]^+$ calcd for $C_{28}H_{26}BrN_2O_4$: 533.1071, 535.1054, found 533.1082, 535.1083: **9o**-*trans* as a pale yellow sticky gum. $R_f$ (60% EtOAc:hexane) 0.22; IR ($cm^{-1}$): 3325, 3053, 2938, 1645, 1513; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.72-7.65 (m, 2H), 7.62 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.50-7.30 (m, 4H), 7.16-7.06 (m, 1H), 7.03 (s, 1H), 6.68 (d, *J* = 9.5 Hz, 1H), 6.66 (s, 1H), 5.67 (d, *J* = 2.1 Hz, 1H), 5.28 (dd, *J* = 14.7, 9.5 Hz, 1H), 4.66 (dd, *J* = 14.7, 2.1 Hz, 1H), 4.55 (dt, *J* = 12.6, 4.5 Hz, 1H), 3.91 (s, 3H), 3.88 (s,



3H), 3.55-3.35 (m, 1H), 3.05-2.70 (m, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 167.9, 167.7, 149.7, 148.1, 139.7, 135.1, 133.9, 132.6, 131.4, 129.6, 128.9, 128.3 (2C), 128.2, 127.5, 127.1 (2C), 124.9, 120.9, 110.7, 106.5, 103.8, 56.1, 55.9, 54.6, 42.9, 39.6, 28.8; EIMS: *m/z* 534 ($M^+$+2, 2), 532 ($M^+$, 10), 453 (30), 413 (23), 411 (24), 105 (100), 77 (23); HRMS (microTOF) *m/z* $[M + H]^+$ calcd for $C_{28}H_{26}BrN_2O_4$: 533.1071, 535.1054, found 533.1054, 535.1062.

*2.16. N-2-(2"-bromo-4",5"-dimethoxyphenyl)-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]-benzamide (**9p**).* The general procedure was used with azlactone **8h** (388 mg, 1.00 mmol) and 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline **7a** (308 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9p** *cis:trans* (76:24) (479 mg, 80%): **9p**-*cis* as a pale yellow sticky gum. $R_f$ (55% EtOAc:hexane) 0.21; IR ($cm^{-1}$): 3413, 3053, 2937, 1657, 1508; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.77-7.68 (m, 2H), 7.55-7.36 (m, 3H), 7.13 (s, 1H), 6.99 (s, 1H), 6.74-6.64 (m, 3H), 6.09 (d, *J* = 7.4 Hz, 1H), 5.33 (t, *J* = 7.4 Hz, 1H), 4.77 (t, *J* = 7.4 Hz, 1H), 4.50 (dt, *J* = 12.6, 4.9 Hz, 1H), 3.92 (s, 6H), 3.85 (s, 3H), 3.75 (s, 3H), 3.62-3.46 (m, 1H), 3.12-2.76 (m, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 167.7, 167.3, 149.8, 148.8, 148.6, 148.3, 135.3, 134.4, 131.4, 128.4 (2C), 128.3, 127.1 (2C), 127.0, 121.0, 115.7, 115.6, 110.9, 110.7, 106.8, 103.4, 56.1 (2C), 56.0, 55.8, 53.3, 39.6, 39.2, 28.6; EIMS: *m/z* 594 ($M^+$+2, 12), 592 ($M^+$, 13), 511 (35), 473 (98), 471 (100), 392 (57), 363 (37), 105 (21); HRMS (microTOF) *m/z* $[M + H]^+$ calcd for $C_{30}H_{30}BrN_2O_6$: 593.1282, 595.1267, found 593.1272, 595.1266: **9p**-*trans* as a pale yellow sticky gum. $R_f$ (55% EtOAc:hexane) 0.16; IR ($cm^{-1}$): 3344, 3053, 2937, 1650, 1603, 1508; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.69 (d, *J* = 7.0 Hz, 2H), 7.50-7.28 (m, 3H), 7.08 (s, 1H), 7.03 (s, 1H), 6.99 (s, 1H), 6.79 (brd, *J* = 8.8 Hz, 1H), 6.66 (s, 1H), 5.61 (d, *J* = 2.7 Hz, 1H), 5.36 (dd, *J* = 14.9, 8.8 Hz, 1H), 4.60 (m, 1H), 4.54 (dd, *J* = 14.9, 2.7 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 6H), 3.82 (s, 3H), 3.50-3.32 (m, 1H), 3.04-2.72 (m, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 168.3, 167.8, 149.7, 148.8, 148.6, 148.1, 134.8, 133.9, 131.4, 131.1, 128.3 (2C), 127.5, 127.0 (2C), 120.9, 115.1, 114.5, 111.5, 110.7, 106.6, 104.1, 56.3, 56.0 (2C), 55.9, 53.7, 42.7, 39.6, 28.8; EIMS: *m/z* 594 ($M^+$+2, 6), 592 ($M^+$, 5), 473 (93), 471 (100), 392 (31), 282 (37), 207 (66), 178 (36), 105 (84), 77 (20); HRMS (microTOF) *m/z* $[M + H]^+$ calcd for $C_{30}H_{30}BrN_2O_6$: 593.1282, 595.1267, found 593.1278, 595.1260.

*2.17. N-2-(2"-bromophenyl)-1-methyl-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]-benzamide (**9q**).* The general procedure was used with azlactone **8g** (327 mg, 1.00 mmol) and 6,7-dimethoxy-1-ethyl-3,4-dihydroisoquinoline **7b** (329 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9q** *cis:trans* (71:29) (384 mg, 70%): **9q**-*cis* as a white solid. $R_f$ (60% EtOAc:hexane) 0.34; Mp: 135-136 $^o$C; IR ($cm^{-1}$): 3407, 3324, 3053, 2935, 1652, 1509, 1405; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.77-7.65 (m, 2H), 7.60-7.35 (m, 4H), 7.30-7.05 (m, 3H), 7.04 (s, 1H), 6.78 (s, 1H), 6.66 (d, *J* = 7.3 Hz, 1H), 5.38 (t, *J* = 7.3 Hz, 1H), 4.70 (d, *J* = 7.3 Hz, 1H), 4.65 (m, 1H), 3.94 (s, 6H), 3.20-2.80 (m, 3H), 1.99 (s, 3H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 167.9, 165.5, 149.0, 146.9, 135.7, 134.4, 133.1, 131.3, 130.6, 129.7, 129.1, 128.5, 128.3 (2C), 128.1, 127.1 (2C), 126.9, 123.0, 117.0, 112.3, 110.1, 56.1, 55.9, 52.9, 45.5, 40.5, 28.5, 19.0; EIMS: *m/z* 548 ($M^+$+2, 8), 546 ($M^+$, 6), 425 (100), 391 (43), 373 (36), 346 (64), 149 (84), 105 (60); HRMS (microTOF) *m/z* $[M + H]^+$ calcd for $C_{29}H_{28}BrN_2O_4$: 547.1227, 549.1211 found 547.1222, 549.1217: **9q**-*trans* as a pale orange solid. $R_f$ (60% EtOAc:hexane) 0.19; Mp: 148-149 $^o$C; IR ($cm^{-1}$): 3324, 3053, 2936, 1647, 1510, 1390; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.66-7.50 (m, 4H), 7.48-7.28 (m, 4H), 7.09 (td, *J* = 7.5, 1.5 Hz, 1H), 6.94 (s, 1H), 6.74 (s, 1H), 6.55 (d, *J* = 9.2 Hz, 1H), 5.37 (dd, *J* = 13.8, 9.2 Hz, 1H), 4.70-4.45 (m, 2H), 3.93 (s, 3H), 3.90 (s, 3H), 3.19 (ddd, *J* = 11.8, 11.8, 5.2 Hz, 1H), 3.00-2.70 (m, 2H), 1.74 (d, *J* = 1.6 Hz, 3H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 167.6, 167.1, 148.9, 146.7, 137.8, 134.2, 132.7, 131.5, 131.3, 130.5, 128.8, 128.3 (3C), 127.8, 127.0 (2C), 126.0, 123.2, 113.9, 112.7, 110.1, 56.3, 56.0, 53.5, 48.7, 41.0, 28.7, 20.0; EIMS: *m/z* 548 ($M^+$+2, 1), 546 ($M^+$, 3), 427 (100), 425 (99), 410 (45), 105 (47); HRMS (microTOF) *m/z* $[M + H]^+$ calcd for $C_{29}H_{28}BrN_2O_4$: 547.1227, 549.1211, found 547.1234, 549.1213.

*2.18. N-2-(2"-bromo-4",5"-dimethoxyphenyl)-1-methyl-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]-benzamide (**9r**).* The general procedure was used with azlactone **8h** (388 mg, 1.00 mmol) and 6,7-dimethoxy-1-ethyl-3,4-dihydroisoquinoline **7b** (329 mg, 1.50 mmol) to give tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9r** *cis:trans* (56:44) (470 mg, 77%): **9r**-*cis* as a pale yellow sticky gum. $R_f$ (55% EtOAc:hexane) 0.17; IR ($cm^{-1}$): 3413, 3320, 2934, 1655, 1507; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.78-7.70 (m, 2H), 7.56-7.36 (m, 3H), 7.04 (s, 1H), 7.00 (s, 1H), 6.77 (s, 1H), 6.73 (s, 1H), 6.67 (d, *J* = 6.9 Hz, 1H), 5.34 (dd, *J* = 7.5, 6.9 Hz, 1H), 4.72-4.60 (m, 1H), 4.58 (d, *J* = 7.5 Hz, 1H), 3.94 (s, 6H), 3.85 (s, 3H), 3.80 (s, 3H), 3.26-3.08 (m, 1H), 2.98-2.84 (m, 2H), 2.02 (s, 3H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 167.9, 165.9, 149.1, 148.9, 148.6, 147.1, 134.5, 131.4, 130.2, 129.4, 128.4 (2C), 127.4, 127.2 (2C), 123.2, 117.7, 116.9, 115.6, 112.4, 110.7, 110.1, 56.2, 56.0 (2C), 55.7, 53.0, 45.5, 40.5, 28.6, 19.1; EIMS: *m/z* 608 ($M^+$+2, 6), 606 ($M^+$, 6), 487 (100), 485 (99), 406 (65); HRMS (microTOF) *m/z* $[M + H]^+$ calcd for $C_{31}H_{32}BrN_2O_6$: 607.1438, 609.1423, found 607.1447, 609.1339: **9r**-*trans* as a pale yellow sticky gum. $R_f$ (55% EtOAc:hexane) 0.11; IR ($cm^{-1}$) 3333, 3053, 2936, 1651, 1508; $^1$H

NMR (200 MHz, $CDCl_3$) $\delta$ 7.68-7.60 (m, 2H), 7.50-7.33 (m, 3H), 7.04 (s, 1H), 6.98 (s, 1H), 6.93 (s, 1H), 6.74 (s, 1H), 6.59 (d, $J$ = 9.5 Hz, 1H), 5.42 (dd, $J$ = 14.0, 9.5 Hz, 1H), 4.60-4.45 (m, 2H), 3.93 (s, 6H), 3.89 (s, 3H), 3.83 (s, 3H), 3.30-3.12 (m, 1H), 3.00-2.72 (m, 2H), 1.74 (d, $J$ = 1.4 Hz, 3H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 167.7 (2C), 148.9, 148.6 (2C), 146.7, 134.2, 131.4, 130.8, 130.4, 129.2, 128.3 (2C), 127.0 (2C), 123.3, 116.1, 115.1, 115.0, 112.7, 112.4, 110.1, 56.4, 56.3, 56.0 (2C), 52.6, 48.2, 41.1, 28.7, 19.2; EIMS: *m/z* 608 ($M^+$+2, 5), 606 ($M^+$, 5), 487 (72), 485 (100), 472 (49), 420 (35), 105 (35); HRMS (microTOF) *m/z* $[M + H]^+$ calcd for $C_{31}H_{32}BrN_2O_6$: 607.1438, 609.1423, found 607.1459, 609.1446.

**3. General procedure: Synthesis of benzoquinolizinones (12)**

**Condition B:** A suspension of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9** (0.20 mmol), 5 mol% $PdCl_2$ (1.70 mg, 0.010 mmol), 10 mol% Xantphos (11.6 mg, 0.020 mmol), 2 equiv. $Cs_2CO_3$ (130 mg, 0.40 mmol), and 1.25 M 1,4-dioxane (1.6 mL) in vessel was stirred under reflux for 3 day. The suspension was filtered through celite and washed with dichloromethane (3×5 mL). The solvent was evaporated to dryness *in vacuo*. The crude product was purified by PTLC on silica using 90:10:1 $CH_2Cl_2$:hexane:MeOH as an eluent to give benzoquinolizin-4-ones **12** in moderate to good yield.

**Condition C:** A suspension of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9** (0.10 mmol), 10 mol% $Pd(dba)_2$ (5.80 mg, 0.010 mmol), 15 mol% Xantphos (8.70 mg, 0.015 mmol), 1.4 equiv. $Cs_2CO_3$ (45.6 mg, 0.14 mmol) and 1.25 M 1,4-dioxane (0.8 mL) in vessel was stirred at 100 $^oC$ for 24-56 h. The suspension was filtered through celite and washed with dichloromethane (3×5 mL). The solvent was evaporated to dryness *in vacuo*. The crude product was purified by PTLC on silica using 90:10:1 $CH_2Cl_2$:hexane:MeOH as an eluent to give benzoquinolizin-4-ones **12** in moderate yield.

**Condition D:** A suspension of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9** (0.10 mmol) and 2 equiv. $Cs_2CO_3$ (65.0 mg, 0.20 mmol), and 1.25 M 1,4-dioxane (0.8 mL) in vessel was stirred at 100 $^oC$ for 16-96 h. The suspension was filtered through celite and washed with dichloromethane (3×5 mL). The solvent was evaporated to dryness *in vacuo*. The crude product was purified by PTLC on silica using 90:10:1 $CH_2Cl_2$:hexane:MeOH as an eluent to give benzoquinolizine-4-ones **12** in moderate to good yield.

*3.1. Benzoindoloquinolizinones **(12a)**.* Using the general procedure (Condition B) with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9a**) (90.8 mg, 0.20 mmol), 5 mol% $PdCl_2$ (1.70 mg, 0.010 mmol), 10 mol% Xantphos (11.6 mg, 0.020 mmol), 2 equiv. $Cs_2CO_3$ (130 mg, 0.40 mmol), and 1.25 M 1,4-dioxane (1.6 mL) gave benzoquinolizine-4-ones **12a** (45.6 mg, 68%) as a pale yellow solid. $R_f$ (90:10:1 $CH_2Cl_2$:hexane:MeOH; developed twice) 0.31; Mp: 182-183 $^oC$; IR ($cm^{-1}$): 2936, 2847, 1652, 1564, 1506; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.68-7.60 (m, 2H), 7.53-7.41 (m, 3H), 7.23 (s, 1H), 6.80 (d, $J$ = 1.8 Hz, 1H), 6.77 (s, 1H), 6.74 (d, $J$ = 1.8 Hz, 1H), 4.30 (t, $J$ = 6.4 Hz, 2H), 3.96 (s, 3H), 3.95 (s, 3H), 2.95 (t, $J$ = 6.4 Hz, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 162.7, 151.1, 151.0, 148.4, 143.2, 138.2, 129.2, 129.1, 128.9 (2C), 126.7 (2C), 121.5, 114.3, 110.4, 108.1, 101.6, 56.2, 56.0, 39.2, 27.5; EIMS: *m/z* 333 ($M^+$, 100), 318 (54), 166 (30), 152 (24), 105 (60), 77 (32); HRMS (microTOF) *m/z* $[M + H]^+$ calcd. for $C_{21}H_{20}NO_3$: 334.1438, found 334.1434.

*3.2. Benzoindoloquinolizinones **(12b and 13)**.* Using the general procedure (Condition B) with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9o**) (106 mg, 0.20 mmol) gave a mixture of benzoquinolizine-4-ones **12b** (26.2 mg, 32%) and **13** (18.9 mg, 21%). Employing the general procedure (Condition B) with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines (*trans*-**9o**) (53.2 mg, 0.10 mmol), 5 mol% $PdCl_2$ (0.85 mg, 0.005 mmol), 10 mol% Xantphos (5.80 mg, 0.010 mmol), 2 equiv. $Cs_2CO_3$ (65.0 mg, 0.20 mmol), and 1.25 M 1,4-dioxane (0.8 mL) gave benzoquinolizine-4-ones **12b** (20.7 mg, 50%) as a pale yellow solid. $R_f$ (90:10:1 $CH_2Cl_2$:hexane:MeOH; developed twice) 0.27; Mp: 193-194 $^oC$; IR ($cm^{-1}$): 3427, 3053, 2933, 1647, 1588, 1505; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.69 (dd, $J$ = 7.6, 1.2 Hz, 1H), 7.46-7.22 (m, 3H), 7.16 (s, 1H), 6.76 (s, 1H), 6.64 (d, $J$ = 1.8 Hz, 1H), 6.54 (d, $J$ = 1.8 Hz, 1H), 4.32 (t, $J$ = 6.5 Hz, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 2.97 (t, $J$ = 6.5 Hz, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 162.4, 151.5, 151.0, 148.5, 142.5, 140.1, 133.4, 130.2, 129.9, 129.0, 127.6, 121.5 (2C), 117.3, 110.4, 108.1, 103.9, 56.2, 56.1, 39.3, 27.6; EIMS: *m/z* 413 ($M^+$+2, 62), 411 ($M^+$, 64), 396 (43), 191 (33), 149 (63), 114 (72), 104 (75), 95 (88), 83 (75), 71 (86), 57 (100), 55 (91); HRMS (microTOF) *m/z* $[M + H]^+$ calcd. for $C_{21}H_{19}BrNO_3$: 412.0543, 414.0525, found 412.0541, 414.0536: **13** as a pale yellow solid. $R_f$ (90:10:1 $CH_2Cl_2$:hexane:MeOH; developed twice) 0.16; Mp: 261-263 $^oC$; IR ($cm^{-1}$): 3236, 3053, 2938, 1646, 1567, 1506; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.48 (brs, 1H), 8.31 (d, $J$ = 8.1 Hz, 1H), 7.86-7.83 (m, 2H), 7.51-7.46 (m, 2H), 7.42-7.34 (m, 3H), 7.30-7.25 (m, 1H), 6.98 (s, 1H), 6.71 (s, 1H), 6.67 (d, $J$ = 1.4 Hz, 1H), 6.54 (d, $J$ = 1.4 Hz, 1H), 4.22 (t, $J$ = 6.4 Hz, 2H), 3.92 (s, 3H), 3.64 (s, 3H), 2.90 (t, $J$ = 6.4 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 165.6, 162.4, 151.2, 149.6, 148.5, 143.7, 134.4, 134.3, 132.0, 131.1, 129.7, 129.1, 128.9, 128.8 (2C), 127.1

(2C), 125.3, 123.6, 120.9, 116.6, 110.3, 107.7, 103.1, 56.0, 55.9, 39.2, 27.4; EIMS: *m/z* 452 ($M^+$, 9), 347 (33), 105 (100), 77 (64); HRMS (microTOF) *m/z* $[M + H]^+$ calcd for $C_{28}H_{25}N_2O_4$: 453.1809, found 453.1818.

*3.3. Benzoindoloquinolizinone (**12c**).* Using the general procedure (Condition B) with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9p**) (119 mg, 0.20 mmol) gave benzoquinolizine-4-ones **12c** (75.5 mg, 81%). Employing the general procedure (Condition C) with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9p**) (59.3 mg, 0.10 mmol) gave benzoquinolizine-4-ones **12c** (17.5 mg, 37%) as a pale yellow solid. $R_f$ (90:10:1 $CH_2Cl_2$:hexane:MeOH; developed twice) 0.21; Mp: 104-106 °C; IR ($cm^{-1}$): 2933, 2893, 1720, 1651, 1587, 1502; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.18 (s, 1H), 7.14 (s, 1H), 6.87 (s, 1H), 6.77 (s, 1H), 6.68 (d, *J* = 1.7 Hz, 1H), 6.54 (d, *J* = 1.7 Hz, 1H), 4.32 (t, *J* = 6.4 Hz, 2H), 3.95 (s, 3H), 3.93 (s, 6H), 3.89 (s, 3H), 2.97 (t, *J* = 6.4 Hz, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 162.4, 151.5, 151.0, 149.6, 148.5, 148.4, 142.4, 132.0, 129.0, 121.5, 117.3, 115.9, 112.8, 111.5, 110.4, 108.1, 104.1, 56.3, 56.2, 56.1, 56.0, 39.2, 27.5; EIMS: *m/z* 473 ($M^+$+2, 98), 471 ($M^+$, 100), 458 (31), 456 (39); HRMS (microTOF) *m/z* $[M + H]^+$ calcd. for $C_{23}H_{23}BrNO_5$: 472.0754, 474.0737, found 472.0767, 474.0750.

*3.4. Benzoindoloquinolizinone (**12d**).* Using the general procedure (Condition B) with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9q**) (109 mg, 0.20 mmol) gave benzoquinolizine-4-ones **12d** (61.4 mg, 72%): **12d** as a pale yellow sticky gum. $R_f$ (90:10:1 $CH_2Cl_2$:hexane:MeOH; developed twice) 0.22; IR ($cm^{-1}$): 3047, 2932, 1650, 1583, 1494; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.66 (d, *J* = 8.0 Hz, 1H), 7.46-7.20 (m, 3H), 7.17 (s, 1H), 6.82 (s, 1H), 6.43 (s, 1H), 4.55-4.40 (m, 1H), 4.00 (m, 1H), 3.96 (s, 3H), 3.89 (s, 3H), 2.89 (t, *J* = 6.0 Hz, 2H), 2.11 (s, 3H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 161.2, 154.5, 149.9, 146.6, 141.0, 140.5, 132.6, 132.2, 129.6, 129.4, 127.4, 122.1, 122.0, 117.9, 113.3, 111.3, 110.0, 56.2, 55.9, 40.8, 28.6, 18.4; EIMS: *m/z* 428 ($M^+$+2, 2), 427 ($M^+$, 9), 412 (10), 410 (11), 149 (33), 111 (34), 109(52), 97 (55), 95 (65), 83 (82), 69 (99), 55 (100); HRMS (microTOF) *m/z* $[M + H]^+$ calcd. for $C_{22}H_{21}BrNO_3$: 426.0699, 428.0682, found 426.0696, 428.0676.

*3.5. Benzoindoloquinolizinone (**12e**).* Using the general procedure (Condition B) with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9r**) (121 mg, 0.20 mmol) gave benzoquinolizine-4-ones **12e** (57.6 mg, 52%) and recovered starting material *cis*-**9r** (30.1 mg, 25%): **12e** as a pale yellow sticky gum. $R_f$ (90:10:1 $CH_2Cl_2$:hexane:MeOH; developed twice) 0.19; IR ($cm^{-1}$): 3053, 2936, 1647, 1583, 1495; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.18 (s, 1H), 7.11 (s, 1H), 6.81 (s, 1H), 6.73 (s, 1H), 6.44 (s, 1H), 4.60-4.44 (m, 1H), 4.00 (m, 1H), 3.96 (s, 3H), 3.93 (s, 3H), 3.89 (s, 3H), 3.87 (s, 3H), 2.88 (t, *J* = 6.0 Hz, 2H), 2.14 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 161.4, 154.6, 149.9, 149.2, 148.4, 146.7, 141.1, 132.6, 132.2, 122.3, 118.3, 115.3, 113.4, 112.2, 112.1, 111.8, 110.0, 56.2 (2C), 56.1, 56.0, 40.8, 28.7, 18.4; EIMS: *m/z* 487 ($M^+$+2, 97), 485 ($M^+$, 100), 472 (83), 188 (66); HRMS (microTOF) *m/z* $[M + H]^+$ calcd. for $C_{24}H_{25}BrNO_5$: 486.0911, 488.0894, found 486.0915, 488.0894.

**4. General procedure: Synthesis of benzoquinolizin-4-ones (11)**

A solution of tetrahydro-*2H*-pyrido[2,1-*a*]isoquinolines **9** (1.00 mmol) and DDQ (1.00 mmol) in dichlorometane (20 mL) was stirred at room temperature for 0.5-16 h. The solvent was evaporated to dryness *in vacuo*. The crude product was purified by PTLC on silica using 4% MeOH in $CH_2Cl_2$ as an eluent to give benzoquinolizin-4-ones **11** in moderate yield.

*4.1. Benzoquinolizin-4-one (**11a**).* The general procedure was used with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9a** (45.4 mg, 0.10 mmol) and DDQ (22.7 mg, 0.10 mmol) to give benzoquinolizinone **11a** (25.5 mg, 56%) as a pale yellow oil. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.53; IR ($cm^{-1}$): 3253, 3053, 2936, 1636, 1590, 1508, 1476; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 8.27 (s, 1H), 7.82-7.72 (m, 2H), 7.64-7.54 (m, 2H), 7.52-7.28 (m, 6H), 7.17 (s, 1H), 6.76 (s, 1H), 6.71 (s, 1H), 4.33 (t, *J* = 6.3 Hz, 2H), 3.94 (s, 3H), 3.93 (s, 3H), 2.96 (t, *J* = 6.3 Hz, 2H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 165.6, 159.6, 150.8, 148.6, 143.6, 139.2, 139.0, 134.4, 131.6, 128.5 (2C), 128.4 (2C), 128.2 (2C), 127.4 (2C), 127.0 (2C), 121.7, 121.3, 110.4, 107.8, 104.3, 56.3, 56.0, 40.3, 27.5; EIMS: *m/z* 453 ($M^+$+1, 29), 452 ($M^+$, 100), 347 (89), 331 (35); HRMS (microTOF) *m/z* $[M + H]^+$ calcd for $C_{28}H_{25}N_2O_4$: 453.1809, found 453.1821.

*4.2. Benzoquinolizin-4-one (**11b**).* The general procedure was used with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9b** (207 mg, 0.40 mmol) and DDQ (110 mg, 0.44 mmol) to give benzoquinolizinone **11b** (137 mg, 67%): as a pale yellow sticky gum. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.49; IR ($cm^{-1}$): 3248, 3000, 2936, 2836, 1635, 1592, 1507; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 8.22 (s, 1H), 7.81-7.78 (m, 2H), 7.51-7.46 (m, 1H), 7.42-7.37 (m, 2H), 7.21-7.14 (m, 3H), 6.93 (d, *J* = 8.3 Hz, 1H), 6.76 (s, 1H), 6.71 (s, 1H), 4.32 (t, *J* = 6.4 Hz, 2H), 3.95 (s, 3H), 3.93 (s, 3H), 3.87 (s, 3H), 3.78 (s, 3H), 2.95 (t, *J* = 6.4 Hz, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 165.9, 159.7, 150.9, 149.0, 148.7, 148.6, 143.5, 139.3,

134.4, 131.8, 131.4, 128.5 (2C), 128.4, 127.4 (2C), 121.4, 121.3, 119.8, 111.2, 110.5, 110.2, 107.8, 104.3, 56.3, 56.0, 55.8, 55.7, 40.2, 27.5; EIMS: *m/z* 513 ($M^+$+1, 33), 512 ($M^+$, 100), 407 (82), 105 (55), 77 (36); HRMS (microTOF) *m/z* [M + H]$^+$ calcd for $C_{30}H_{29}N_2O_6$: 513.2020, found 513.2017.

*4.3. Benzoquinolizin-4-one* ***(11c)***. The general procedure was used with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9**[9] (46.8 mg, 0.10 mmol) and DDQ (22.7 mg, 0.10 mmol) to give benzoquinolizinone **11c** (7.10 mg, 15%) as a pale yellow oil. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.43; IR (cm$^{-1}$): 2920, 1631, 1580, 1501; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.73 (s, 1H), 7.63-7.60 (m, 2H), 7.45-7.25 (m, 8H), 7.17 (s, 1H), 6.80 (s, 1H), 4.26 (brt, *J* = 5.8 Hz, 2H), 3.96 (s, 3H), 3.89 (s, 3H), 2.90 (t, *J* = 5.8 Hz, 2H), 2.15 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 166.1, 158.7, 149.9, 149.2, 146.9, 138.8, 137.4, 134.6, 131.7, 131.4, 128.4 (2C), 128.3 (2C), 128.1 (2C), 127.7, 127.3 (2C), 122.8, 122.3, 113.6, 111.8, 110.1, 56.2, 56.0, 41.4, 28.5, 20.0; EIMS: *m/z* 467 ($M^+$+1, 27), 466 ($M^+$, 100), 361 (89), 346 (38), 345 (42), 105 (100), 77 (64); HRMS (microTOF) *m/z* [M + H]$^+$ calcd for $C_{29}H_{27}N_2O_4$: 467.1965, found 467.1963.

*4.4. Benzoquinolizin-4-one* ***(11d)***. The general procedure was used with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9d** (128 mg, 0.242 mmol) and DDQ (55.0 mg, 0.242 mmol) to give benzoquinolizinone **11d** (10.0 mg, 8%) as a pale yellow oil. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.26; IR (cm$^{-1}$): 3233, 2919, 2840, 1630, 1584, 1501; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.68-7.64 (m, 3H), 7.47-7.42 (m, 1H), 7.38-7.32 (m, 2H), 7.18 (s, 1H), 6.96-6.89 (m, 3H), 6.80 (s, 1H), 4.42 (s, 1H), 4.17-4.05 (m, 1H), 3.96 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 2.90 (t, *J* = 5.7 Hz, 2H), 2.18 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 166.4, 158.7, 149.9, 149.3, 148.6, 148.4, 146.8, 139.0, 134.6, 131.8, 131.5, 129.7, 128.4 (2C), 127.4 (2C), 123.0, 122.3, 121.1, 113.5, 112.1, 111.8, 110.9, 110.1, 56.2, 56.0 (2C), 55.7, 41.4, 28.6, 20.1; EIMS: *m/z* 527 ($M^+$+1, 23), 526 ($M^+$, 92), 421 (63), 105 (100), 77 (51); HRMS (microTOF) *m/z* [M + H]$^+$ calcd for $C_{31}H_{31}N_2O_6$: 527.2177, found 527.2172.

*4.5. Benzoquinolizin-4-one* ***(11e)***. The general procedure was used with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9o** (48.7 mg, 0.092 mmol) and DDQ (21.0 mg, 0.092 mmol) to give benzoquinolizinone **11e** (27.8 mg, 57%) as a pale yellow sticky gum. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.56; IR (cm$^{-1}$): 3233, 3056, 1637, 1595, 1509, 1477; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.30 (s, 1H), 7.75-7.71 (m, 2H), 7.62 (dd, *J* = 8.1, 1.0 Hz, 1H), 7.49 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.46-7.41 (m, 1H), 7.38-7.32 (m, 3H), 7.18-7.12 (m, 2H), 6.75 (s, 1H), 6.63 (s, 1H), 4.47-4.39 (m, 1H), 4.29-4.20 (m, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 2.97 (t, *J* = 6.4 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 165.5, 159.3, 150.7, 148.4, 142.7, 139.4, 139.0, 134.3, 132.8, 131.5, 130.0, 129.3, 128.3 (2C), 128.2, 127.4 (3C), 122.7, 121.2, 121.1, 110.3, 107.6, 104.3, 56.1, 55.9, 40.1, 27.3; EIMS: *m/z* 532 ($M^+$+2, 61), 530 ($M^+$, 61), 451 (100), 346 (46); HRMS (microTOF) *m/z* [M + H]$^+$ calcd for $C_{28}H_{24}BrN_2O_4$: 531.0914, 533.0898, found 531.0926, 533.0905.

*4.6. Benzoquinolizin-4-one* ***(11f)***. The general procedure was used with tetrahydro-*2H*-pyrido[2,1-*a*]isoquinoline **9p** (119 mg, 0.20 mmol) and DDQ (46.0 mg, 0.20 mmol) to give benzoquinolizinone **11f** (62.2 mg, 53%) as a pale yellow solid. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.52; Mp: 137-139 $^o$C; IR (cm$^{-1}$): 3233, 3053, 2935, 1638, 1591, 1505; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 8.18 (s, 1H), 7.82-7.72 (m, 2H), 7.52-7.32 (m, 3H), 7.17 (s, 1H), 7.10 (s, 1H), 6.98 (s, 1H), 6.76 (s, 1H), 6.66 (s, 1H), 4.56-4.36 (m, 1H), 4.32-4.12 (m, 1H), 3.94 (s, 3H), 3.93 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H), 2.97 (t, *J* = 6.4 Hz, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 165.7, 159.4, 150.8, 149.1, 148.5, 148.2, 143.1, 139.0, 134.4, 131.6, 131.2, 128.3 (2C), 128.2, 127.4 (2C), 122.7, 121.2, 115.4, 112.4, 111.5, 110.4, 107.8, 104.7, 56.2, 56.1, 56.0 (2C), 40.2, 27.4; EIMS: *m/z* 592 ($M^+$+2, 5), 590 ($M^+$, 5), 511 (100), 406 (52); HRMS (microTOF) *m/z* [M + H]$^+$ calcd for $C_{30}H_{28}BrN_2O_6$: 591.1125, 593.1110, found 591.1130, 593.1121.

**5. General procedure: Synthesis of benzoindolo-quinolizinone derivatives (10 and 14)**

A solution of benzoquinolizin-4-ones **9** or **11** (0.10 mmol) and CuTC (0.11 mmol) in DMF (1 mL) was irradiated in a microwave reactor (250 watt, 100 psi) at 150 $^o$C for 15-30 min. The crude product was purified by PTLC on silica using 4% MeOH in $CH_2Cl_2$ as an eluent to give a mixture of benzoindoloquinolizin-4-ones **10** and/or *N*-benzoyl benzoindoloquinolizin-4-ones **14** in moderate yield.



*5.1 Benzoindoloquinolizinone (**10a**).* The general procedure was used with benzoquinolizin-4-one **11e** (48.3 mg, 0.090 mmol) and CuTC (19.0 mg, 0.099 mmol) to give benzoindoloquinolizinone **10a** (11.9 mg, 38%): as a pale yellow solid. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.52; Mp: >300 $^oC$; IR ($cm^{-1}$): 3159, 1635, 1557, 1510, 1264; $^1H$ NMR (400 MHz, $CDCl_3$+$CD_3OD$(2 drops)) δ 8.08 (d, J = 8.2 Hz, 1H), 7.60 (d, J = 8.2 Hz, 1H), 7.53-7.47 (m, 1H), 7.46 (s, 1H), 7.37 (s, 1H), 7.30-7.25 (m, 1H), 6.81 (s, 1H), 4.46 (t, J = 6.1 Hz, 2H), 4.04 (s, 3H), 3.96 (s, 3H), 3.00 (t, J = 6.1 Hz, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$+$CD_3OD$(2 drops)) δ 155.0, 149.5, 148.3, 139.7, 134.1, 127.5, 127.0, 126.0, 124.9, 122.9, 122.0, 121.0, 119.8, 112.2, 110.3, 107.7, 97.7, 56.0, 55.7, 39.8, 27.8; EIMS: m/z 347 ($M^+$+1, 30), 346 ($M^+$, 100); HRMS (microTOF) m/z $[M + H]^+$ calcd for $C_{21}H_{19}N_2O_3$: 347.1390, found 347.1388.

*5.2 Benzoindoloquinolizinone (**10b**).* The general procedure was used with benzoquinolizin-4-one **11f** (39.5 mg, 0.067 mmol) and CuTC (14.0 mg, 0.74 mmol) to give benzoindoloquinolizinone **10b** (16.9 mg, 62%) as a pale brown solid. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.20; Mp: >300 $^oC$; IR ($cm^{-1}$): 3173, 2927, 1635, 1586, 1557, 1512; $^1H$ NMR (400 MHz, $CDCl_3$+$CD_3OD$(2 drops)) δ 7.43 (s, 1H), 7.36 (s, 1H), 7.34 (s, 1H), 7.07 (s, 1H), 6.79 (s, 1H), 4.44 (t, J = 6.0 Hz, 2H), 4.04 (s, 3H), 4.02 (s, 3H), 4.00 (s, 3H), 3.95 (s, 3H), 2.98 (t, J = 6.0 Hz, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$+$CD_3OD$(2 drops)) δ 154.8, 150.9, 149.6, 148.3, 145.2, 135.2, 133.9, 127.6, 125.3, 125.2, 123.2, 114.4, 110.4, 107.8, 101.9, 97.5, 94.6, 56.2, 56.18, 55.9 (2C), 39.8, 28.0; EIMS: m/z 406 ($M^+$, 35), 203 (13), 149 (35), 104 (39), 97 (59), 85 (71), 71 (82), 57 (100); HRMS (microTOF) m/z $[M + H]^+$ calcd for $C_{23}H_{23}N_2O_5$: 407.1602, found 407.1597.

*5.3 Benzoindoloquinolizinone (**10c** and **14a**).* The general procedure was used with tetrahydro-2H-pyrido[2,1-a]isoquinoline **9q** (50.4 mg, 0.092 mmol) and CuTC (19.0 mg, 0.101 mmol) to give a mixture of benzoindoloquinolizinone **10c** (6.50 mg, 20%) and benzoindoloquinolizinone **14a** (17.9 mg, 42%):**10c** as a pale yellow solid. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.24; Mp: 279-280 $^oC$; IR ($cm^{-1}$): 3138, 2951, 1635, 1578, 1543, 1510; $^1H$ NMR (400 MHz, $CDCl_3$) δ 10.88 (s, 1H), 8.19 (d, J = 8.1 Hz, 1H), 7.65 (d, J = 8.1 Hz, 1H), 7.46 (dd, J = 8.1, 8.1 Hz, 1H), 7.27-7.22 (m, 1H), 7.17 (s, 1H), 6.83 (s, 1H), 4.45 (brs, 2H), 3.97 (s, 3H), 3.96 (s, 3H), 2.97 (s, 3H), 2.96-2.88 (m, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 154.4, 148.8, 146.7, 139.8, 131.4, 131.3 (2C), 126.4, 126.3, 125.4, 123.4, 123.2, 120.0 113.6, 112.7, 110.6, 110.2, 56.3, 56.0, 41.5, 29.3, 18.6. EIMS: m/z 361 ($M^+$+1, 25), 360 ($M^+$, 100), 345 (59). HRMS (microTOF) m/z $[M + H]^+$ calcd for $C_{22}H_{21}N_2O_3$: 361.1547, found 361.1552. **14a**: as a white solid. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.48; Mp: 205-207 $^oC$; IR ($cm^{-1}$): 3053, 2934, 1667, 1511, 1472, 1385; $^1H$ NMR (200 MHz, $CDCl_3$) δ 8.06 (brs, 1H), 7.84-7.72 (m, 2H), 7.48-7.18 (m, 5H), 7.12-7.02 (m, 1H), 6.96 (s, 1H), 6.68 (s, 1H), 4.97 (d, J = 8.5 Hz, 1H), 4.31(dt, J = 11.7, 4.5 Hz, 1H), 4.19 (d, J = 8.5 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 2.93 (ddd, J = 11.7, 11.3, 4.5 Hz, 1H), 2.70-2.55 (m, 2H), 2.38 (s, 3H); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 170.4, 164.9, 148.9, 146.6, 142.1, 137.0, 132.2, 131.1, 130.0, 129.6, 128.4, 128.3 (2C), 127.7 (3C) 124.6, 122.5, 118.2, 112.4, 110.1, 108.5, 62.8, 56.2, 55.9, 44.7, 41.1, 28.8, 21.4; EIMS: m/z 467 ($M^+$+1, 2), 466 ($M^+$, 7), 361 (28), 105 (100), 77 (58); HRMS (microTOF) m/z $[M + H]^+$ calcd for $C_{29}H_{27}N_2O_4$: 467.1965, found 467.1970.

*5.4 Benzoindoloquinolizinone (**10d** and **14b**).* The general procedure was used with tetrahydro-2H-pyrido[2,1-a]isoquinoline **9r** (52.9 mg, 0.087 mmol) and CuTC (18.0 mg, 0.096 mmol) to give a mixture of benzoindoloquinolizinone **10d** (5.80 mg, 16%) and benzoindoloquinolizinone **14b** (26.3 mg, 57%):**10d** as a pale yellow solid. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.18; Mp: >300 $^oC$; IR ($cm^{-1}$): 3173, 2931, 1632, 1582, 1509; $^1H$ NMR (200 MHz, $CDCl_3$) δ 11.32 (brs, 1H), 7.55 (s, 1H), 7.18 (s, 1H), 7.15 (s, 1H), 6.84 (s, 1H), 4.47 (brs, 2H), 4.00 (s, 3H), 3.97 (s, 6H), 3.96 (s, 3H), 2.93 (brs, 5H); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 154.0, 150.3, 148.7, 146.7, 145.0, 135.6, 131.1, 130.9, 125.7, 125.5, 123.3, 115.4, 113.5, 110.1 (2C), 104.3, 94.8, 56.5, 56.2, 56.0, 55.8, 41.3, 29.4, 18.6; EIMS: m/z 421 ($M^+$+1, 27), 420 ($M^+$, 100), 405 (57), 361 (17), 210 (32), 105 (46); HRMS (microTOF) m/z $[M + H]^+$ calcd for $C_{24}H_{25}N_2O_5$: 421.1758, found 421.1755: **14b** as a pale yellow oil. $R_f$ (4% MeOH:$CH_2Cl_2$) 0.42; IR ($cm^{-1}$): 2935, 2833, 1662, 1495, 1444, 1395; $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.01 (brs, 1H), 7.83-7.73 (m, 2H), 7.47-7.36 (m, 3H), 6.97 (s, 1H), 6.72 (s, 1H), 6.70 (s, 1H), 4.92 (brs, 1H), 4.41-4.31 (m, 1H), 4.16 (d, J = 8.1 Hz, 1H), 3.90 (s, 9H), 3.82 (s, 3H), 2.99-2.84 (m, 1H), 2.72-2.69 (m, 2H), 2.37 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 170.1, 165.1, 148.9, 148.8, 146.6, 146.4, 137.1, 136.2, 131.0, 129.9, 129.6, 128.2 (2C), 127.7 (2C), 122.8, 122.6, 112.3, 110.1, 108.3, 106.2, 103.0, 63.2, 56.4, 56.2, 56.0, 55.9, 44.5, 41.0, 28.7, 21.4; EIMS: m/z 527 ($M^+$+1, 7), 526 ($M^+$, 29), 421 (50), 282 (26), 105 (100), 77 (41); HRMS (microTOF) m/z $[M + H]^+$ calcd for $C_{31}H_{31}N_2O_6$: 527.2177, found 527.2176.

**References and notes**

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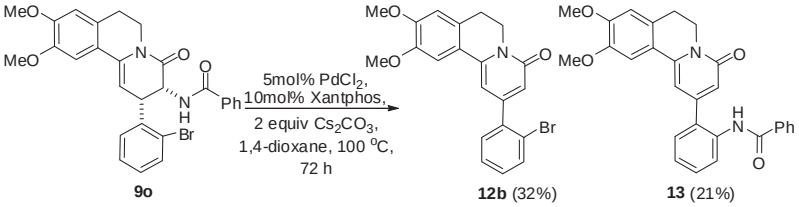
15. The reaction of *cis*-**9o** gave a mixture of **12b** (32% yield) and **13** (21% yield).



## SUBPROJECT II

# Palladium-catalyzed intramolecular C-H amidation: Synthesis and biological activities of indolobenzazocin-8-ones

## Introduction

Benzazocinone belongs to a class of alkaloid displaying a wide range of biological activities including hepatoprotective activity against chemical toxin and antiamnesia as well as acting as inhibitors of tubulin polymerization.[1] Recently our group reported a facile and convenient protocol based on ring annulations of dihydroisoquinoline with azlactone to synthesize benzo[*d*]azocin-4-ones **1**.[2] This protocol served as a useful template for the synthesis of eight-membered ring molecules. In our continuing interest concerning the application of this protocol, we focused our attention to the synthesis of indolobenzazocinone analogues from benzo[*d*]azocin-4-one derivatives. Indolobenzazocinone **2c** is closely related to some natural products and other biologically active compounds such as indolobenzazepinones (with C5 substituted group) **3**, an antimitotic agent,[3] latonduine **4**, a cytotoxic agent,[4] and their regioisomers, paullones **5**, which act as cyclin-dependent kinase inhibitors (Figure 1).[5]

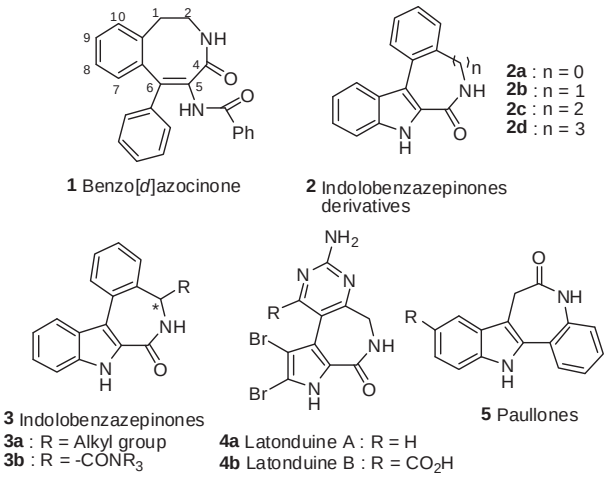


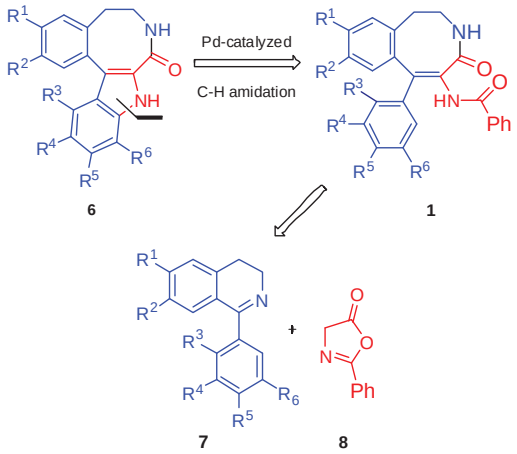

**Figure 1.** Indolobenzazepinone derivatives and some related natural compounds.

In 2007, Joseph reported the synthesis of indolobenzazepinones **2b** using the intramolecular Heck reaction.[6] Similarly, Dodd revealed that the C5-alkylated indolobenzazepinones (**3a**) showed high cytotoxicity against various cancer cell lines and were categorized as antimitotic agents.[7] This methodology involved Suzuki coupling and lactamization steps. They also synthesized compounds with different substitution pattern **3b** at C5 position by using the application of an isocyanide-based multicomponent reactions (MCRs).[3]

Recently, Joseph and colleagues reported the synthesis and SAR studies involving six-, seven-, eight- and nine-membered ring derivatives of indolobenzazepinones. The result showed that almost all of them exhibited good potency in both cell-based and target-based assays, especially, 5,6,7,9-tetrahydro-8*H*-indolo[2,3-*e*][3]benzazocin-8-one **2c**.[8] Moreover, the molecular modeling of indolobenzazepinones was also studied in comparison with tubulin polymerization inhibitor colchicine.[8] To accomplish these target indolobenzazocinones **6**, we

have elaborated not only the condensation of various dihydroisoquinolines with azlactone but also investigated the C-N bond formation involving palladium-catalyzed intramolecular C-H amidation as depicted in Scheme 1.

During the past decade, Pd-catalyzed C-H activation[9] has played a significant role in construction of the desired C-C, C-O, C-S, C-X bond formation and particularly the formation of C-N bond, as pioneered by Buchwald and co-workers.[10] The advantage of this approach is the support of green chemistry because reducing the number of steps as well as improving atom economy led to an increase in overall efficiency.[11] Accordingly, Pd-catalyzed C-N bond formation has attracted attention from many research groups.[9-10, 12] Herein, this chemistry was used as the first time application to establish biologically active indolobenzazocinone derivatives.



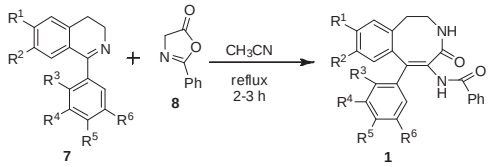

**Scheme 1.** Retrosynthetic plan for the synthesis of indolobenzazocin-8-one derivatives.

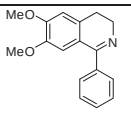
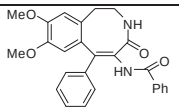
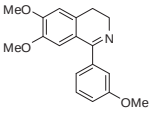
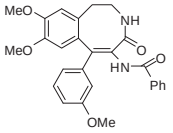
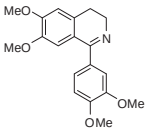
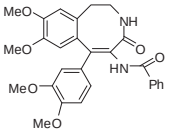
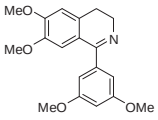
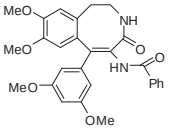
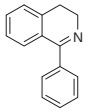
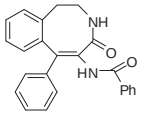
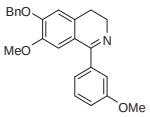
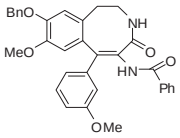
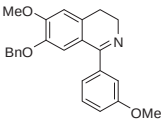
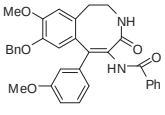
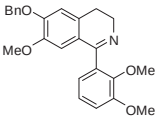
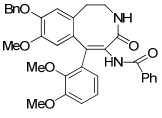
## Results and Discussion

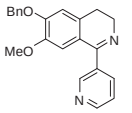
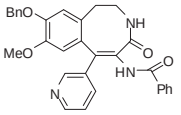
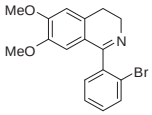
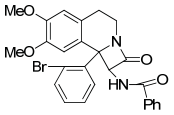
Ten analogues of dihydroisoquinolines **7a-j** were first investigated to expand our developed protocol.[2, 13] Various dihydroisoquinolines **7** were prepared from different arylethylamines and various benzoic acids and nicotinic acid to generate amides followed by Bischler-Napieralski reaction.[2] Only dihydroisoquinoline **7e** was prepared using the Movassaghi's method.[14] Azlactone **8** was obtained from hippuric acid, as previously reported.[2, 15] With both key starting materials in hand, we studied the ring annulations of dihydroisoquinolines **7a-j** with azlactone **8** in refluxing acetonitrile under dry conditions to afford compounds **1a-i** in moderate yields (30 to 74%) as shown in Table 1.

Notably, beta-lactam intermediate **9j** was isolated in 45% yield (Table 1, entry 10).[16] This supported our previously proposed mechanism that beta-lactams are intermediates in the formation of benzo[*d*]azocinones.[2] Furthermore, the isolation of beta-lactam **9j** shed some lights on the mechanism of ring expansion. Possible pathway might involve ring expansion via the carbocation **10** and the carbocation must be stabilized by both adjacent phenyl groups. In the case of beta-lactam **9j** ring expansion was not possible because the ortho bromo substituent would interfere with the stability of carbocation via steric inhibition to resonance owing to the loss of co-planarity as shown in Scheme 2. Under refluxing toluene, a higher boiling point solvent, compound **9j** underwent retro-ring annulation reaction and imine **7j** was isolated.

**Table 1.** Synthesis of 5-amido-8,9-dimethoxy-6-aryl-2,3-dihydrobenzo[*d*]azocin-4-ones **1**.[a]

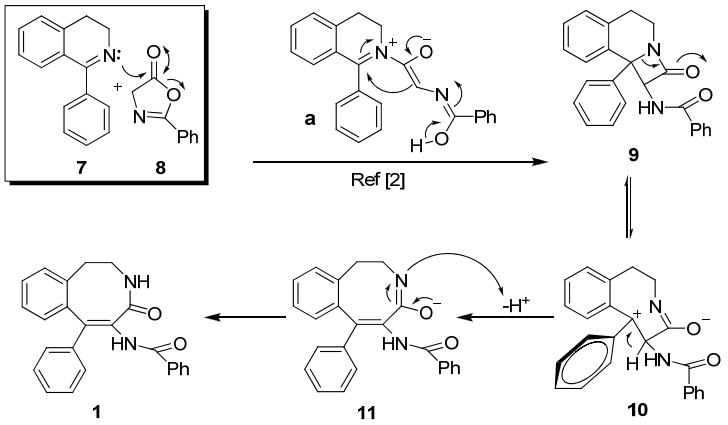



| Entry | Dihydroisoquinolines **7** | Products **1** | Yield [%] |
|---|---|---|---|
| 1 | **7a**  |  | **1a**, 74[a] |
| 2 | **7b**  |  | **1b**, 39[a] |
| 3 | **7c**  |  | **1c**, 40[a] |
| 4 | **7d**  |  | **1d**, 48[a] |
| 5 | **7e**  |  | **1e**, 37[a] |
| 6 | **7f**  |  | **1f**, 40[b] |
| 7 | **7g**  |  | **1g**, 37[b] |
| 8 | **7h**  |  | **1h**, 30[a] |

| | | | | |
|---|---|---|---|---|
| 9 | **7i** |  |  | **1i**, 58[a] |
| 10 | **7j** |  |  | **9j**, 45[a] |

[a] Products were purified by crystallization.

[b] Products were purified by column chromatography.




**Scheme 2.** Proposed mechanism for the formation of compounds **1** and **9**.

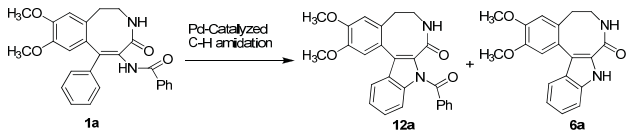
Moreover, the presence of electron-donating group $R^1$ of dihydroisoquinoline **7** may facilitate the ring expansion step but it is not an absolute requirement since we could prepare unsubstituted benzo[*d*]azocinone **1e** in moderate yield (Table 1, entry 5).

Benzo[*d*]azocinones **1a-i** thus obtained were further investigated as precursors for the indolobenzazocinones **6**. Palladium-catalyzed C-H activation chemistry and hydrolysis were studied with the aim to form the C-N bond followed by debenzoylation. We began to study the formation of the C-N bond by screening the catalytic system using benzo[*d*]azocinone **1a** as the substrate.

Hiroya[12b] and co-workers reported the synthesis of indazole derivatives using the palladium-catalyzed C-H activation and C-N bond formation. We initially attempted to modify Hiroya's conditions using 0.1 equiv of $Pd(OAc)_2$, 1.0 equiv of $Cu(OAc)_2$ and 2.0 equiv of $AgOCOCF_3$ in 0.05 M DMSO at 50 $^oC$ under argon atmosphere and obtained the desired indolobenzazocinone **12a** in 21% yield (Table 2, entry 1). Increasing the amount of catalyst and longer reaction time gave no significant increase in the yield of product **12a** (Table 2, entry 2). A mixture of indolobenzazocinone **12a** and hydrolyzed product **6a** was obtained when the reaction temperature was increased to 100 $^oC$ (Table 2, entry 3). When the palladium catalyst was changed to $Pd(OCOCF_3)_2$ more promising results were obtained (Table 2, entries 4 to 5). Increasing the reaction temperature to 80 $^oC$ gave a better yield in a shorter reaction time (Table 2, entry 6). We then optimized the reaction condition by screening other palladium catalysts and achieved the target product in the range of 60 to 71% yield (Table 2, entries 7 to 9). We also studied the re-oxidant and the use of atmospheric oxygen for oxidation, as reported by Buchwald.[10] Interestingly, using an oxygen atmosphere

could eliminate the use of $AgOCOCF_3$ and gave the product in 68% yield (Table 2, entry 10) while using only $AgOCOCF_3$ without co-oxidant $Cu(OAc)_2$ under an $O_2$ atmosphere gave a very poor yield of **12a** (Table 2, entry 11). The catalytic amount of co-oxidant was also studied and found that 0.3 equiv of $Cu(OAc)_2$ was enough to mediate the reaction whereas 0.1 equiv of $Cu(OAc)_2$ was insufficient to complete the reaction (Table 2, entries 12 to 14).

**Table 2.** Pd-Catalyzed C-H activation.[a]

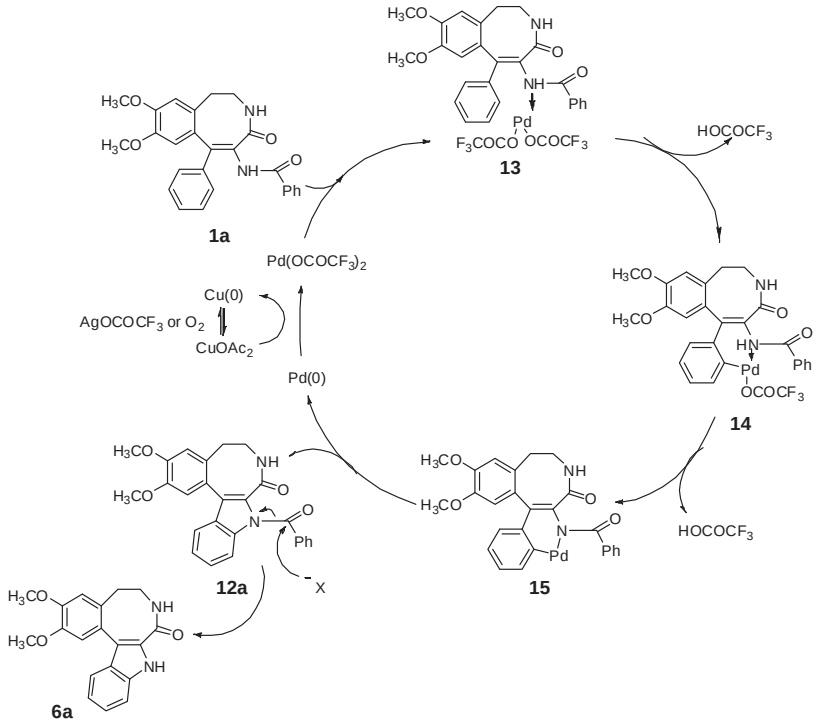



| Entry | Pd(II) | Re-oxidant (eq) | Temp. (°C) | Time (h) | yield [%] **12a** | **6a** | SM [%] |
|---|---|---|---|---|---|---|---|
| 1 | $Pd(OAc)_2$[b] | $Cu(OAc)_2$(1) / $AgOCOCF_3$(2) | 50 | 24 | 21 | - | 67 |
| 2 | $Pd(OAc)_2$ | $Cu(OAc)_2$(3) / $AgOCOCF_3$(6) | 50 | 144 | 37 | - | - |
| 3 | $Pd(OAc)_2$ | $Cu(OAc)_2$(3) / $AgOCOCF_3$(6) | 100 | 24 | 13 | 33 | - |
| 4 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$(3) / $AgOCOCF_3$(6) | 50 | 24 | 42 | - | 20 |
| 5 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$(3) /$AgOCOCF_3$(6) | 50 | 44 | 54 | 26 | - |
| 6 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$(3) / $AgOCOCF_3$(6) | 80 | 20 | 72 | 12 | - |
| 7 | $PdCl_2$ | $Cu(OAc)_2$(3) /$AgOCOCF_3$(6) | 80 | 16 | 71 | 7 | 4 |
| 8 | $PdCl_2(PPh_3)_2$ | $Cu(OAc)_2$(3) / $AgOCOCF_3$(6) | 80 | 16 | 60 | 8 | 19 |
| 9 | $Pd_2(dba)_3$ | $Cu(OAc)_2$(3) / $AgOCOCF_3$(6) | 80 | 16 | 71 | 8 | 19 |
| 10 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$ (3) | 80 | 54 | 68 | 7 | - |
| 11 | $Pd(OCOCF_3)_2$ | $AgOCOCF_3$(3) | 80 | 54 | 17 | - | 64 |
| 12 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$ (1) | 80 | 5 | 73 | 2 | - |
| 13 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$ (0.3) | 80 | 42 | 68 | 6 | - |
| 14 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$ (0.1) | 80 | 89 | 21 | - | 72 |

[a]All reactions were performed with 0.3 equiv of palladium catalyst in 0.05 M DMSO under argon atmosphere except entries 10-14, the reactions were done under $O_2$ atmosphere.

[b] 0.1 equiv of $Pd(OAc)_2$ was required. SM = Starting material.

The postulated reaction pathway of Pd catalyzed C-H amidation for benzo[*d*]azocinones cores is depicted in Scheme 3.[12b] The amide nitrogen atom could complex to $Pd(OCOCF_3)_2$ and release trifluoroacetic acid (TFA) followed by the formation of palladacycle **15**. Subsequent reductive elimination could generate the product **12**. The debenzoylated product **6a** which is sometimes, obtained in low yield, likely to be formed via simple benzoyl cleavage as depicted.



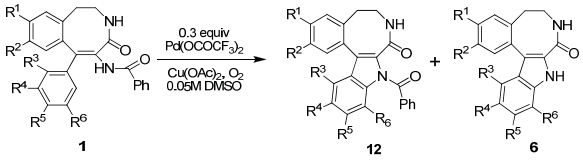

**Scheme 3.** Proposed mechanism for the formation of **12a** and **6a**.

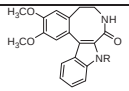
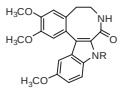
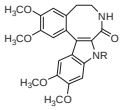
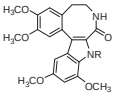
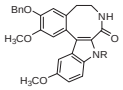
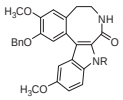
With effective conditions for the synthesis of indolobenzazocinone **1a**, we then directed our attention to the synthesis of a small library of indolobenzazocinone analogues using $Pd(OCOCF_3)_2$ as the catalyst and $Cu(OAc)_2$ as the re-oxidant under the oxygen atmosphere. An increased amount of $Cu(OAc)_2$ was necessary for the higher oxygenated analogues **1b-i** in the context of decreasing reaction time and obtaining optimal yield (Table 3). In addition, in some cases, increasing the amount of palladium catalyst to 0.5 equivalent was required to obtain higher yield (Table 3, entry 7). We reasoned that the steric hindrance makes it difficult to incorporate the palladium species in the six-membered palladacycle complexes as shown in the postulated mechanism (Scheme 3).

Moreover, we found that steric effect played an important role in the regioselectivity. Only the regioselective products were obtained from unsymmetrical materials **1b-c** and **1f-g** which were confirmed by spectroscopic data. Unfortunately, the pyridine derivative **1i** gave no reaction even after increasing the amount of $Pd(OCOCF_3)_2$ or $Cu(OAc)_2$ (Table 3, entries 11 to 12). This could be due to the complexation of pyridine nitrogen with the transition metal rendering the catalyst inactive.[17]

With the success of Pd(II)-catalyzed C-H amidation of compounds **1**, we then turned our attention to find a way to effect the transformation of compounds **1** to indolobenzazocinones **6** in one pot. To effect such transformation, 4 N NaOH in $H_2O$ was added to the crude products to hydrolyse the amide group to give compounds **6** in moderate to good yields as shown in Table 4.

**Table 3.** Pd-Catalyzed C-H activation.[a]




| Entry | SM | $Cu(OAc)_2$ (eq) | Time (h) | Products | yield [%] | |
|---|---|---|---|---|---|---|
| | | | | | **12** (R = Bz) | **6** (R = H) |
| 1 | **1a** | 0.3 | 42 |  | **a**, 68 | **a**, 6 |
| 2 | **1b**[d] | 0.3 | 50 |  | **b**, 25 | **b**, 5 |
| 3 | **1b** | 0.6 | 40 | | **b**, 59 | **b**, 6 |
| 4 | **1c**[d] | 0.3 | 50 |  | **c**, 31 | - |
| 5 | **1c** | 0.6 | 16 | | **c**, 88 | **c**, 7 |
| 6 | **1d**[d] | 0.6 | 40 |  | **d**, 14 | **d**, 5 |
| 7 | **1d**[b,d] | 1 | 47 | | **d**, 21 | **d**, 7 |
| 8 | **1e** | 0.6 | 18 |  | **e**, 91 | - |
| 9 | **1f** | 0.6 | 15 |  | **f**, 49 | **f**, 9 |
| 10 | **1g** | 0.6 | 23 |  | **g**, 50 | **g**, 3 |
| 11 | **1i** | 0.6 | 20 | - | NA[c] | - |
| 12 | **1i** | 1 | 20 | - | NA[c] | - |

[a] All reactions were performed with 0.3 equiv $Pd(OCOCF_3)_2$ in 0.05 M DMSO at 80 °C under $O_2$ atmosphere and were monitored by TLC.

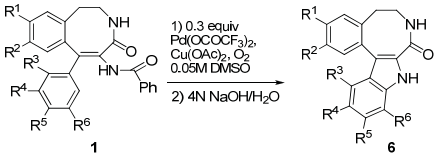
[b] 0.5 equiv of $Pd(OCOCF_3)_2$ was required.

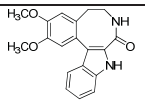
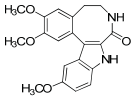
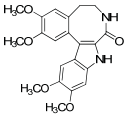
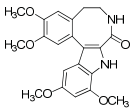
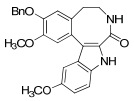
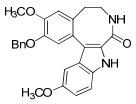
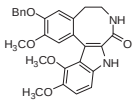
[c] Complex mixture of products were detected.

[d] Starting materials were recovered as follow entry 2 (33%), entry 4 (39%), entry 6 (44%) and entry 7 (6%). SM = Starting material.



**Table 4.** Pd-Catalyzed C-H activation and hydrolysis.[a]



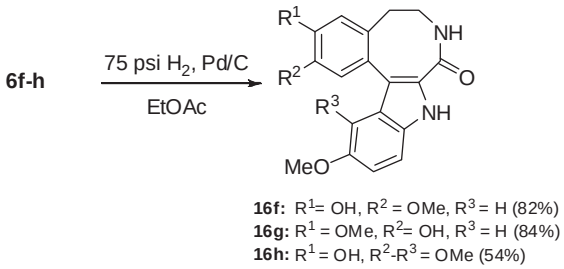

| Entry | SM | $Cu(OAc)_2$ (eq) | Time (h) | Products | yield [%] **6** |
|---|---|---|---|---|---|
| 1 | **1a** | 0.3 | 22 |  | **a**, 83 |
| 2 | **1b** | 0.6 | 20 |  | **b**, 60 |
| 3 | **1c** | 0.6 | 22 |  | **c**, 60 |
| 4 | **1d**[b] | 1 | 40 |  | **d**, 10 |
| 5 | **1e** | 0.6 | 42 |  | **e**, 66 |
| 6 | **1f** | 0.6 | 15 |  | **f**, 67 |
| 7 | **1g** | 0.6 | 20 |  | **g**, 54 |
| 8 | **1h** | 0.6 | 22 |  | **h**, 54 |

[a] All reactions were performed with 0.3 equiv $Pd(OCOCF_3)_2$ in 0.05 M DMSO at 80 °C under $O_2$ atmosphere and were monitored by TLC.

[b] 0.5 equiv of $Pd(OCOCF_3)_2$ was required.

Compounds **6f-h** required an additional step involving debenzylation in order to furnish indolobenzazocinone derivatives **16f-h**. The hydrogenolysis of compounds **6f-h** was conducted using palladium on activated charcoal in ethyl acetate at 75 psi in the Parr apparatus at room temperature to give the debenzylated products **16f-h** in moderate to good yield (54 to 84%, Scheme 4).






**16f:** $R^1$= OH, $R^2$ = OMe, $R^3$ = H (82%)
**16g:** $R^1$ = OMe, $R^2$= OH, $R^3$ = H (84%)
**16h:** $R^1$ = OH, $R^2$-$R^3$ = OMe (54%)

**Scheme 4.** Debenzylation.

Interestingly, all $^1$H NMR spectra of indolobenzazocinone derivatives **6** and **16** showed the absence of two sets of methylene protons at room temperature (Figure 2, bottom). By decreasing the operating temperature to –20 $^o$C, $^1$H NMR spectra of four methylene protons appeared separately in the range of 2.6 to 4.0 ppm (Figure 2, top). This phenomenon possibly resulted from the restricted rotation of the secondary cyclic amide in this indolobenzazocin-8-one system.[18]

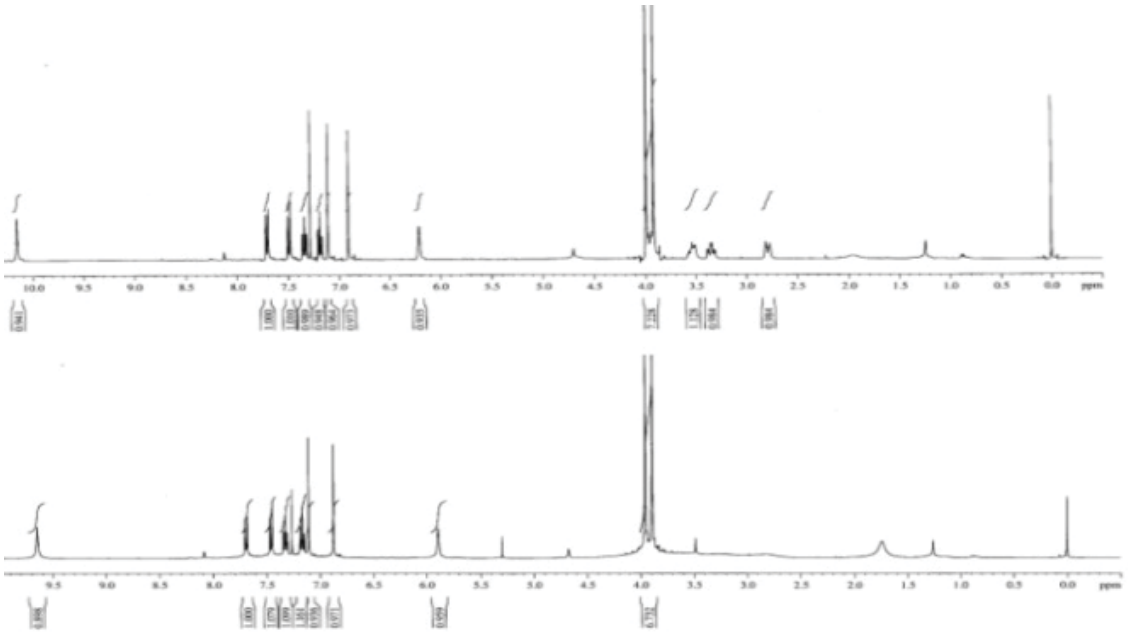


**Figure 2.** Selected $^1$H NMR spectra of compound **6a** at –20 $^o$C (top) and $^1$H NMR spectra of compound **6a** at room temperature (bottom).

**Biological activity**

As reported by Joseph *et al.*, indolobenzazocin-8-one **6e** showed the most potent antiproliferative activity inhibiting cell growth in several cancer cell lines in the nanomolar $IC_{50}$ value range.[6] Our synthetic indolobenzazocinones **6a-d** and **16f-h** were evaluated for cytotoxicity as compared with unsubstituted compound **6e** as cytotoxic agents on a panel of four human tumor cell lines; cholangiocarcinoma HUCCA-1, lung carcinoma A549, hepatoblastoma HepG2, and T-lymphoblast (acute lymphoblastic leukemia) MOLT-3 using MTT and XTT assays depending on the cell-line types as shown in Table 5.

**Table 5.** Biological activities of synthetic indolobenzazocinone derivatives **6a-e** and **16f-h** in MTT and XTT assays.[a]

| Entry | Compounds | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|---|
| | | HuCCA-1[b,f] | A549[c,f] | HepG2[d,f] | MOLT-3[e,g] |
| 1 | **6a** | Inactive | Inactive | 96.23 | 44.50 |
| 2 | **6b** | 83.8 | 71.0 | 39.8 | 17.12 |
| 3 | **6c** | Inactive | Inactive | 122.1 | 71.7 |
| 4 | **6d** | Inactive | Inactive | 11.04 | 3.98 |
| 5 | **6e** | 48.1 | 12.6 | 0.46 | 0.009 |
| 6 | **16f** | 102.8 | 66.4 | 69.80 | 26.6 |
| 7 | **16g** | Inactive | Inactive | 84.79 | 80.8 |
| 8 | **16h** | Inactive | 124.26 | 99.65 | 25.98 |
| 9 | Etoposide | ND | ND | 29.8 | 0.047 |
| 10 | Doxorubicin | 0.83 | 0.63 | 0.57 | ND |

[a] DMSO solution (10 mg/mL).
[b] HuCCA-1: Cholangiocarcinoma.
[c] A549: Lung Carcinoma.
[d] HepG2: Hepatoblastoma.
[e] MOLT-3: T-lymphoblast (acute lymphoblastic leukemia).
[f] MTT assay.
[g] XTT assay.
ND = Not determined.

The results showed that unsubstituted indolobenzazocin-8-one **6e** showed the highest $IC_{50}$ values for all cancer cell lines tested as compared to other substituted compounds. Compound **6e** exhibited hepatoblastoma HepG2 in the lower micromolar range ($IC_{50}$ = 0.46 μM) and T-lymphoblast (acute lymphoblastic leukemia) MOLT-3 in nanomolar range ($IC_{50}$ = 9 nM) (Table 5, entry 5). The presence of either methoxy or hydroxy groups at the C-4, C-5 or C-6 position of the indole ring and C-8 or C-9 of benzoazocinone ring led to decreasing cytotoxic activity or becoming inactive, whereas the presence of C-7 methoxy group of the indole ring in compound **6d** led to modest cytotoxicity in both MOLT-3 and HepG-2 ($IC_{50}$ = 3.98 to 11.04 μM) (Table 5, entry 4). This study provided crucial information about the role of the oxygenated substitution on indolobenzazocin-8-one core skeleton in support of the previous reports by Dodd and Joseph groups.[8]

## Conclusion



In summary, we have reported the synthesis and biological activities of indolobenzazocin-8-ones using ring annulations of various dihydroisoquinolines with azlactone and Pd-catalyzed C-H amidation. The method we have developed and reported here should be applicable to the synthesis of other biologically active indolobenzazocin-8-ones in just two steps and also capable of constructing a wide range of related compounds with different substitution patterns. Indolobenzazocin-8-one **6e** exhibited very good activities in the nanomolar $IC_{50}$ value range as compared to the oxygenated substitution on this core system. This study also delineated the structure-activity relationship studies involving substitution patterns of the indolobenzazocin-8-ones.

## Experimental Section

### 1. General Methods

Melting points were measured with a Thermo Fisher Scientific IA920 digital melting point apparatus and reported without correction. $^1$H-Nuclear magnetic resonance ($^1$H NMR) spectra were recorded on Varion Germini2000, Bruker AV-300, and Bruker AV-400 NMR instruments at 200, 300, and 400 MHz, respectively, using deuterochloroform as solvents with tetramethylsilane as an internal standard. $^{13}$C-Nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded on Varion Germini2000, Bruker AV-300, and Bruker AV-400 NMR instruments at 50, 75, and 100 MHz, respectively, using deuterochloroform with tetramethylsilane as an internal standard and dimethylsulfoxide-D6 for some compound. FTIR spectra were obtained on the Spectrum One FTIR spectrometer, Perkin Elmer System with the universal ATR (UATR) accessory. Mass spectra were performed with an AEI-MS-902. High-resolution mass spectra were performed with a MicroTOF$_{LC}$, Bruker Daltonics. Column chromatography was carried out using Fluka aluminum oxide (type 507 C neutral; 100-125 mesh) and Merck silica gel (70-230 mesh ASTM). Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were carried out on silica gel (E. Merck PF 254). All reagents were purified and dried according to the standard procedures. Solvents were removed by using Eyela Aspirator A-2S and Büchi Rotavapor R110. All products were evacuated by a Christ Freeze Dryer Unit Alpha l/6, to remove the last traces of solvents.

### 2. General Procedure for the Preparation of Benzo[*d*]azocin-4-ones (1)

A solution of azlactone **8** (1 equiv) and 3,4-dihydroisoquinolines **7a-j** (1 equiv) in acetonitrile (6 mL/mmol of starting material) was heated at reflux for 2 h. The resulting reaction was allowed cool to room temperature and was then concentrated under reduced pressure. The crude product was further purified by recrystallization to furnish the desired product (37-74%).

*2.1. 5-Amido-6-(3'-methoxylphenyl)-8,9-dimethoxy-1,2-dihydrobenzo[d]azocin-4-one* (**1b**). Pale yellow solid (1.15 g, 39% yield). $R_f$ 0.33 (90% EtOAc : Hexane, 2 times). mp: 191.2 $^o$C. IR (UATR): $\nu_{max}$ 3231, 1655, 1653 cm$^{-1}$. $^1$H NMR (200 MHz, $CDCl_3$): δ 2.88 – 2.96 (m, 1H), 3.36 – 3.48 (m, 2H), 3.86 – 4.07(m, 1H), 3.72 (s, 3H), 3.76 (s, 3H), 3.87 (s, 3H), 4.06 (m, 1H), 5.99 (s, 1H), 6.63 (s, 1H), 6.68 (s, 1H), 6.83 – 6.96 (m, 3H). $^{13}$C NMR (50 MHz, $CDCl_3$): δ 32.5, 41.7, 55.2, 55.9 (2C), 112.1, 113.0, 113.5, 114.0, 120.2, 127.2 (2C), 128.6 (2C), 129.2 (2C), 130.3 (2C), 131.1, 132.0, 132.8, 139.6, 147.6, 148.8, 160.2, 164.5, 169.2; EI-MS: *m/z* (%) 458 ($M^+$, 12), 298 (23), 105 (100); TOF-HRMS calcd for $C_{27}H_{27}N_2O_5$: 459.1915; found 459.1925.

*2.2. 5-Amido-6-(3',4'-dimethoxylphenyl)-8,9-dimethoxy-1,2-dihydrobenzo[d]azocin-4-one* (**1c**). Pale yellow solid(1.84 g, 40% yield). $R_f$ 0.25 (90% EtOAc : Hexane, 2 times). Mp: 214 $^o$C. IR (UATR): $\nu_{max}$ 3320, 3227, 1652, 1649 cm$^{-1}$. $^1$H NMR (200 MHz, $CDCl_3$): δ 2.87 – 2.98 (m, 1H), 3.39 – 3.48 (m, 2H), 3.72 (s, 3H), 3.80 (s, 3H), 3.87 (s, 6H), 3.93 – 4.05 (m, 1H), 6.08 (br s, 1H), 6.63 (s, 1H), 6.69 (s, 1H), 6.84 (s, 1H), 6.89 (s, 2H) 7.35 – 7.72 (m, 5H), 8.54 (br s, 1H). $^{13}$C NMR (50 MHz, $CDCl_3$): δ 32.4, 41.7, 55.8, 55.9 (2C), 56.0, 111.2, 111.6, 112.0, 113.0, 121.0, 127.1 (3C), 127.8, 128.5, 128.5, 128.9, 129.2, 130.6, 131.4, 132.0, 132.8, 147.5, 148.7, 149.3, 164.5, 169.5. EI-MS: *m/z* (%) 488 ($M^+$, 1), 105 (77), 77 (44), 69 (26), 57 (26). TOF-HRMS calcd for $C_{28}H_{29}N_2O_6$: 489.2020; found 489.1994.

*2.3. 5-Amido-6-(3',5'-dimethoxylphenyl)-8,9-dimethoxy-1,2-dihydrobenzo[d]azocin-4-one* (**1d**). Pale yellow solid (0.64 g, 48% yield). $R_f$ 0.33 (90% EtOAc : Hexane, 2 times). mp: 214 $^o$C. IR (UATR): $\nu_{max}$ 3336, 3233, 1654, 1602 cm$^{-1}$. $^1$H NMR (200 MHz, $CDCl_3$): δ 2.85 – 2.95 (m, 1H), 3.31 – 3.50 (m, 2H), 3.73 (s, 3H), 3.77 (s, 3H), 3.88 (s, 3H), 3.96 – 4.10 (m, 1H), 6.15 (br s, 1H), 6.41 (d, $J$ = 2.2 Hz, 1H), 6.48 (d, $J$ = 2.2 Hz, 2H), 6.61 (s, 1H), 6.70 (s, 1H), 7.37 – 7.51 (m, 3H), 7.71 (dd, $J$ = 8.6, 2.8 Hz, 2H). $^{13}$C NMR (50 MHz, $CDCl_3$): δ 32.3, 41.5, 55.3 (2C), 55.8, 55.8, 99.9, 106.1 (2C), 112.0, 112.8, 127.1 (3C), 128.5 (2C), 129.1, 130.9, 132.0

(2C), 132.7, 140.1, 147.5, 148.8, 161.4 (2C), 164.6, 169.4. EI-MS: *m/z* (%) 489 ($M+H^+$, 1), 488 ($M^+$, 3) 328 (10), 105 (100), 77 (35). TOF-HRMS calcd for $C_{28}H_{29}N_2O_6$: 489.2020; found 489.2042.

*2.4. 5-Amido-6-phenyl-1,2-dihydrobenzo[d]azocin-4-one* (**1e**). Pale yellow solid (51.2 mg, 58% yield). $R_f$ 0.51 (90% EtOAc : Hexane, 2 times). mp: 222 °C. IR (UATR): $\nu_{max}$ 3235, 1651 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): δ 3.00 (m, 1H), 3.37 – 3.53 (m, 2H), 4.13 (m, 1H), 5.77 (br s, 1H), 7.12 – 7.69 (m, 14H), 8.30 (s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 32.8, 41.5, 126.8, 127.3 (2C), 128.2 (3C), 128.3, 128.7 (3C), 129.1, 129.3, 129.4 (2C), 130.1, 132.2, 132.7, 136.8, 138.2, 139.1, 164.7, 169.2. EI-MS: *m/z* (%) 369 ($M+H^+$, 4), 368 ($M^+$, 15), 219 (30), 208 (41), 105 (100), 77 (28); TOF-HRMS calcd for $C_{24}H_{21}N_2O_2$: 369.1598; found 369.1600.

*2.5. 5-Amido-6-(3'-methoxylphenyl)-9-benzyloxy-8-methoxy-1,2-dihydrobenzo[d]azocin-4-one* (**1f**). Yellow amorphous (0.92 g, 40% yield). $R_f$ 0.58 (90% EtOAc : Hexane, 2 times). mp: 214 °C. IR (UATR): $\nu_{max}$ 3227, 1655, 1508, 1473 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 2.68 – 2.78 (m, 1H), 3.18 – 3.36 (m, 2H), 3.65 (s, 3H), 3.68 (s, 3H), 3.92 – 3.96 (m, 1H), 5.05 (s, 2H), 5.82 (br s, 1H), 6.57 (s, 1H), 6.62 (s, 1H), 6.75 (s, 1H), 6.77 (d, *J* = 9.0 Hz, 2H), 6.88 (d, *J* = 7.5 Hz, 1H), 7.20 – 7.43 (m, 9H), 7.58 (s, 1H), 7.59 (d, *J* = 7.8 Hz, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 32.4, 41.7, 55.3, 56.1, 71.1, 113.5, 113.6, 114.0, 114.7, 120.3, 127.3 (4C), 127.9, 128.4, 128.6 (3C), 128.7 (2C), 129.2, 130.5, 131.7, 132.2, 132.8, 137.0, 139.6, 148.1, 148.3, 160.3, 164.6, 169.3. EI-MS: *m/z* (%) 535 ($M+H^+$, 3), 534 ($M^+$, 9), 388 (24), 105 (100), 91 (45), 77 (25). TOF-HRMS calcd for $C_{33}H_{31}N_2O_5$: 535.2228; found 535.2226.

*2.6. 5-Amido-6-(3'-methoxylphenyl)-8-benzyloxy-9-methoxy-1,2-dihydrobenzo[d]azocin-4-one* (**1g**). Yellow amorphous (0.51 g, 37% yield). $R_f$ 0.53 (90% EtOAc : Hexane, 2 times). mp: 212 °C. IR (UATR): $\nu_{max}$ 3233, 1652, 1603, 1473 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 2.73 – 2.83 (m, 1H), 3.25 – 3.34 (m, 2H), 3.63 (s, 3H), 3.76 (s, 3H), 3.92 – 3.99 (m, 1H), 4.88 (q, *J* = 12.0 Hz, 2H), 6.06 (s, 1H), 6.60 (s, 2H), 6.67 (s, 1H), 6.71 – 6.79 (m, 2H), 7.15 – 7.39 (m, 9H), 7.59 (d, *J* = 7.8 Hz, 2H), 8.37 (s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 32.5, 41.5, 55.1, 55.9, 71.0, 112.6, 113.4, 113.8, 115.8, 120.2, 127.2 (5C), 127.6, 128.3 (3C), 128.5 (2C), 129.7, 130.2, 130.9, 132.0, 132.7, 136.8, 139.4, 146.6, 149.3, 160.0, 164.6, 169.4. EI-MS: *m/z* (%) 535 ($M+H^+$, 4), 534 ($M^+$, 13), 374 (12), 105 (100), 91 (56), 77 (26). TOF-HRMS calcd for $C_{33}H_{31}N_2O_5$: 535.2228; found 535.2211.

*2.7. 5-Amido-6-(2',3'-dimethoxylphenyl)-9-benzyloxy-8-methoxy-1,2-dihydrobenzo[d]azocin-4-one* (**1h**). Yellow amorphous (0.33 g, 30% yield). $R_f$ 0.43 (100% EtOAc). IR (UATR): $\nu_{max}$ 3231, 1654, 1471 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 2.82 – 2.88 (m, 1H), 3.33 – 3.48 (m, 2H), 3.69 (s, 3H), 3.89 (s, 3H), 3.96 (s, 3H), 4.10 – 4.16 (m, 1H), 5.13 (s, 2H), 5.96 (br s, 1H), 6.49 (s, 1H), 6.75 (s, 1H), 6.86 – 6.99 (m, 2H), 7.29 – 7.47 (m, 9H), 7.81 (d, *J* = 9.6 Hz, 2H), 9.34 (br s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 32.2, 41.3, 55.7, 55.9, 61.9, 70.9, 111.9, 114.0, 114.8, 122.5, 124.7, 127.2 (3C), 127.7 (2C), 128.3 (3C), 128.4 (2C), 129.6, 129.7, 131.8, 132.2, 132.3, 132.8, 136.8, 145.1, 147.9, 148.0, 153.1, 164.9, 169.5. EI-MS: *m/z* (%) 564 ($M^+$, 2), 105 (100), 91 (47), 77 (23). TOF-HRMS calcd for $C_{34}H_{33}N_2O_6$: 565.2333; found 565.2351.

*2.8. 5-Amido-6-(3'-pyridyl)-9-benzyloxy-8-methoxy-1,2-dihydrobenzo[d]azocin-4-on***e** (**1i**). Pale yellow solid (0.29 g, 58% yield). $R_f$ 0.20 (90% EtOAc : Hexane, 2 times). mp: 200 °C. IR (UATR): $\nu_{max}$ 3328, 3221, 1654 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 2.81 – 2.89 (m, 1H), 3.26 – 3.53 (m,2H), 3.72 (s, 3H), 4.01 – 4.16 (m, 1H), 5.14 (s, 2H), 5.95 (bs, 1H, N*H*), 6.61 (s, 1H), 6.73 (s, 1H), 7.23- 7.52 (m, 8H), 7.57 – 7.69 (m, 3H), 8.32 (s, 1H), 8.50 (d, *J* = 3.9 Hz, 1H), 8.70 (s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 32.2, 42.1, 56.1, 71.1, 113.5, 114.8, 123.9, 127.2, 127.3 (4C), 128.0 (2C) 128.6 (2C), 128.7 (2C), 129.3, 129.4, 130.9, 132.3, 132.7, 136.1, 136.9, 148.5, 148.6, 149.0, 149.2, 165.1, 169.3. EI-MS: *m/z* (%) 506 ($M^+$, 0), 105 (100), 91 (53), 77 (34). TOF-HRMS calcd for $C_{31}H_{28}N_3O_4$: 506.2074; found 506.2071.

*2.9. 1-Amido-9b-(2'-bromophenyl)-7,8-dimethoxy-1,4,5,9b-tetrahydro-2H-azeto[2,1-a]isoquinolin-2-one* (**9j**). White solid (1.22 g, 45% yield). $R_f$ 0.48 (90% EtOAc : Hexane). mp: 157 °C. IR (UATR): $\nu_{max}$ 1684, 1247 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 2.52 – 2.79 (m, 2H), 3.59 – 3.65 (m, 2H), 3.92 (s, 1H), 4.08 (s, 1H), 5.85 (d, *J* = 8.4 Hz, 1H), 6.74 (s, 1H), 7.04 – 7.22 (m, 4H), 7.33 – 7.53 (m, 5H), 7.62 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.93 (s, 1H, N*H*). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 26.5, 39.6, 56.0, 56.4, 66.6, 111.5, 111.6, 121.6, 126.7 (2C), 127.2 (2C), 127.7, 128.6 (2C), 129.8, 131.3, 131.9, 132.1, 133.4, 135.4 (2C), 147.8, 148.7, 166.3, 167.6. EI-MS: *m/z* (%) 506 ($M^+$, 0), 508 ($M+2H^+$, 0), 348 (7), 346 (27), 105 (100), 77 (63). TOF-HRMS calcd for $C_{26}H_{24}BrN_2O_4$: 507.0914; found 507.0928.

**3. General Procedure for the Preparation of *N*- Benzoylindolobenzazocinones (12a-g)**

In a round bottle flask, mixture of benzo[*d*]azocinone **1**, $Pd(OCOCF_3)_2$ (0.3 – 0.5 equiv), $Cu(OAc)_2$ (0.3 – 1 equiv) was evacuated and refilled with Ar. Under argon atmosphere, DMSO (0.05 M) was added via syringe and flask was again evacuated and refilled with $O_2$. The reaction mixture was then stirred at 70 – 80 °C. The reaction was monitored via TLC until completion. After being cooled to room temperature, the reaction mixture was filtered through celite and then extracted with EtOAc. Combined organic layer was washed with water and dried over $Na_2SO_4$. The solvent was evaporated and the residue was purified by PTLC (80%EtOAc/Hexane) to give products **12**.



*3.1. 9-Benzoyl-(2,3-dimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one* (**12a**). White solid (26.1 mg, 68% yield). $R_f$ 0.43 (100% EtOAc). mp: 255 °C. IR (UATR): $\nu_{max}$ 1687, 1647 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$): 2.80 – 2.90 (m, 1H), 3.24 – 3.51 (m, 2H), 3.70 – 3.80 (m, 1H), 3.90 (s, 3H), 3.96 (s, 3H), 5.78 (br s, 1H), 6.88 (s, 1H), 7.10 (s, 1H), 7.27 – 7.68 (m, 6H), 7.79 – 7.86 (m, 3H). $^{13}C$ NMR (50 MHz, $CDCl_3$): δ 32.6, 45.0, 56.0, 56.1, 112.4, 113.1, 114.7, 121.2, 123.7, 123.8, 124.3, 126.6, 127.7, 128.3 (2C), 129.3 (2C), 129.8, 131.2, 132.7, 135.8, 138.4, 147.8, 149.0, 165.1, 168.6. EI-MS: *m/z* (%) 427 ($M+H^+$, 15), 426 ($M^+$, 47), 304 (31), 293 (10), 105 (100), 77 (55). HRMS (microTOF) *m/z* calcd for $C_{26}H_{23}N_2O_4$ ($M + H^+$): 427.1652; found: 427.1651.

*3.2. 9-Benzoyl-(2,3,12-trimethoxyl)-5,6,7,9-tetrahydroindolo [2,3-e][3]benzazocin-8-one* (**12b**). White solid (43.7 mg, 59%). $R_f$ 0.45(100% EtOAc). mp: 262 °C. IR (UATR): $\nu_{max}$ 3333, 1683, 1646 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): δ 2.81 – 2.87 (m, 1H), 3.25 – 3.34 (m, 1H), 3.39 – 3.47 (m, 1H), 3.74 – 3.80 (m, 1H), 3.81 (s, 3H), 3.89 (s, 3H), 3.95 (s, 3H), 5.63 (br s, 1H), 6.88 (s, 1H), 7.01 (dd, $J$ = 9.1, 2.6 Hz, 1H), 7.05 (d, $J$ = 2.4 Hz, 1H), 7.09 (s, 1H), 7.46 – 7.60 (m, 3H), 7.73 (d, $J$ = 9.1 Hz, 1H), 7.81 (d, $J$ = 7.0 Hz, 2H). $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 32.6, 44.9, 55.7, 56.0, 56.1, 102.8, 112.5, 112.9, 115.8, 116.0, 123.7, 124.4, 128.3, 128.3, 128.5, 129.3 (2C), 129.8, 131.7, 132.6, 133.3, 136.0, 147.9, 149.1, 156.6, 165.0, 168.4. EI-MS: *m/z* (%) 457 ($M+H^+$, 311), 456 ($M^+$, 38), 352 (7), 105 (100), 77 (31). HRMS (microTOF) *m/z* calcd for $C_{27}H_{25}N_2O_5$ $(M + H)^+$: 457.1758; found: 457.1758.

*3.3. 9-Benzoyl-(2,3,11,12-tetramethoxyl)-5,6,7,9-tetrahydro indolo[2,3-e][3]benzazocin-8-one* (**12c**). White solid (64.1 mg, 88% yield). $R_f$ 0.38 (100% EtOAc). mp: 258 °C. IR (UATR): $\nu_{max}$ 3328, 1681, 1644 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 2.81 – 2.87 (m, 1H), 3.20 – 3.33 (m, 1H), 3.45 – 3.56 (m, 1H), 3.70 – 3.75 (m, 1H), 3.89 (s, 6H), 3.92, (s, 3H), 3.96 (s, 3H), 5.41 (br s, 1H), 6.89 (s, 1H), 7.02 (s, 1H), 7.09 (s, 1H), 7.48 – 7.59 (m, 3H), 7.62 (s, 1H), 7.82 (d, $J$ = 6.9 Hz, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 32.5, 45.3, 56.0, 56.1 (3C), 97.8, 101.6, 112.2, 112.7, 120.0, 124.4, 124.6, 128.2 (2C), 129.1 (2C), 129.6, 130.0, 132.4, 133.6, 136.4, 147.5, 147.8, 149.0, 150.1, 165.0, 168.8. EI-MS: *m/z* (%) 488 ($M+2H^+$, 4), 487 ($M+H^+$, 19), 486 ($M^+$, 63), 364 (12), 105 (100), 77 (17). HRMS (microTOF) *m/z* calcd for $C_{28}H_{26}N_2O_6$ $(M + H)^+$: 487.1864; found: 487.1870.

*3.4. 9-Benzoyl-(2,3,10,12-tetramethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one* (**12d**). White solid (18.6 mg, 21% yield). $R_f$ 0.48 (100% EtOAc). mp: 236 °C; IR (UATR): $\nu_{max}$ 3333, 1703, 1648, 1516 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): δ 2.72 - 2.78 (m, 1H), 3.21 – 3.33 (m, 2H), 3.34 (s, 3H), 3.72 (s, 3H), 3.84 (s, 3H), 3.86 – 3.90 (m, 1H), 3.88 (s, 3H), 5.59 (br s, 1H), 6.32 (d, $J$ = 2.1 Hz, 1H), 6.58 (d, $J$ = 2.1, 1H), 6.78 (s, 1H), 7.06 (s, 1H), 7.35 (d, $J$ = 7.4, 1H), 7.37 (d, $J$ = 7.8 Hz, 1H), 7.48 (dd, $J$ = 7.4, 7.4 Hz, 1H), 7.77 (d, $J$ = 7.1 Hz, 2H). $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 33.0, 44.7, 55.1, 55.7, 56.0, 56.1, 93.7, 98.5, 109.5, 111.6, 112.9, 113.4, 121.4, 124.9, 128.1 (2C), 129.0, 129.6, 129.7 (2C), 132.2, 132.7, 136.0, 147.8, 156.9, 165.8, 169.4 (1C not observed). EI-MS: *m/z* (%) 487 ($M+H^+$, 30), 486 ($M^+$, 100), 381 (2), 105 (11), 77 (3). HRMS (microTOF) *m/z* calcd for $C_{28}H_{26}N_2O_6$ $(M + H)^+$: 487.1864; found: 487.1871.

*3.5. 9-Benzoyl-5,6,7,9-Tetrahydroindolo[2,3-e][3]benzazocin-8-one* (**12e**). White solid (52.9 mg, 91% yield). $R_f$ 0.43 (100% EtOAc). mp: 223 °C; IR (UATR): $\nu_{max}$ 1690, 1651 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): δ 2.85 – 2.90 (m, 1H), 3.20 – 3.30 (m, 1H), 3.37 – 3.45 (m, 1H) 3.69 – 3.74 (m, 1H), 5.91 (br s, 1H), 7.28 – 7.80 (m, 13H). $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 32.8, 44.9, 114.7, 121.3, 123.6, 126.6, 126.9, 127.6, 128.3 (3C), 128.5, 129.3 (2C), 129.6, 130.2, 131.5, 132.2, 132.8, 135.8, 137.4, 138.4, 165.1, 168.6. EI-MS: *m/z* (%) 368 ($M+2H^+$, 6), 367 ($M+H^+$, 22), 366 ($M^+$, 79), 105 (100), 77 (27). HRMS (microTOF) *m/z* calcd for $C_{24}H_{19}N_2O_2$ $(M + H)^+$: 367.1441; found: 367.1435.

*3.6. 9-Benzoyl-(3-benzyloxyl-2,12-dimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one* (**12f**). Yellow amorphous (50 mg, 49% yield). $R_f$ 0.55 (100% EtOAc). IR (UATR): $\nu_{max}$ 3660, 1683, 1647, 1513 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 2.63 – 2.67 (m, 1H), 3.06 – 3.32 (m, 2H), 3.58 – 3.60 (m, 1H), 3.71 (s, 3H), 3.79 (s, 3H), 5.11 (s, 2H), 5.82 (br s, 1H), 6.81 (s, 1H), 6.91 (d, $J$ = 9.0 Hz, 1H), 6.96 (s, 1H), 7.01 (s, 1H), 7.20 – 7.46 (m, 8H), 7.63 – 7.69 (m, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 32.6, 44.9, 55.7, 56.3, 71.1, 102.9, 113.5, 115.1, 115.9, 116.0, 123.6, 124.9, 127.4 (2C), 128.0 (2C), 128.4 (2C), 128.6, 128.7 (2C), 129.3, 129.9, 131.8, 132.7, 133.3, 135.9, 136.9, 148.3, 148.5, 156.7, 165.2, 168.5. EI-MS: *m/z* (%) 532 ($M^+$, 0.3), 270 (100), 166 (96), 105 (11), 91 (61), 77 (21). HRMS (microTOF) *m/z* calcd for $C_{33}H_{29}N_2O_5$ $(M + H)^+$: 533.2071; found: 533.2077.

*3.7. 9-Benzoyl-(2-benzyloxyl-3,12-dimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one* (**12g**). Yellow amorphous (87.8 mg, 50% yield); $R_f$ 0.38 (100% EtOAc). IR (UATR): $\nu_{max}$ 3327, 1684, 1646, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 2.78 – 2.85 (m, 1H), 3.25 – 3.43 (m, 2H), 3.57 – 3.72 (m, 1H), 3.70 (s, 3H), 3.95 (s, 3H), 5.15 (q, $J$ = 12.6 Hz, 2H), 6.11 (br s, 1H), 6.79 (d, $J$ = 2.4 Hz, 1H), 6.90 (s, 1H), 6.96 (dd, $J$ = 9.0, 2.4 Hz, 1H), 7.11 (s, 1H), 7.25 – 7.56 (m, 8H), 7.69 (d, $J$ = 9.0 Hz, 1H), 7.78 (d, $J$ = 7.2 Hz, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 32.6, 45.0, 55.8, 56.1, 71.1, 102.9, 113.0, 115.2, 115.7, 116.0, 123.6, 124.3, 127.2 (3C), 128.0 (2C), 128.4, 128.7 (3C), 129.3, 130.5, 131.7, 132.7, 133.2, 136.0, 137.0, 147.1, 149.7, 156.7, 165.3, 168.5. EI-

MS: *m/z* (%) 534 ($M+2H^+$, 2), 533 ($M+H^+$, 9), 532 ($M^+$, 30), 105 (100), 77 (33). HRMS (microTOF) *m/z* calcd for $C_{33}H_{29}N_2O_5$ $(M + H)^+$: 533.2071; found: 533.2071.

**4. General Procedure for the Preparation of Indolobenzazociones (6a-h)**

In a round bottle flask, mixture of benzo[*d*]azocinone **1**, $Pd(OCOCF_3)_2$ (0.3 – 0.5 equiv), $CuOAc_2$ (0.3 – 1 equiv) was evacuated and refilled with Ar. Under argon atmosphere, DMSO (0.05 M) was added via syringe and the flask was again evacuated and refilled with $O_2$. The reaction mixture was then stirred at 70 – 80 °C. The reaction was monitored via TLC until completion. After being cooled to room temperature, the reaction mixture was filtered through celite and washed with EtOAc. The resulting solution was evaporated to give a brown solution in DMSO which was added with 4M $NaOH/H_2O$. Reaction mixture was stirred at room temperature for 5 minutes and was then added with water and extracted with EtOAc 3 times. An organic layer was washed with water 3 times was then with brine. The organic layer was evaporated to give brown oil which further purified by PTLC (80 % EtOAc:Hexane) to give products **6** (10 – 83%).

*4.1. (2,3-Dimethoxyl)-5,6,7,9-tetrahydroindolo[2,3e][3] benzazocin-8-one* (**6a**). White solid (25 mg, 83% yield). $R_f$ 0.35 (100% EtOAc). mp: 256 °C. IR (UATR): $\nu_{max}$ 3273, 2953 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, at –20 °C): δ 2.77 – 2.81 (m, 1H), 3.31 – 3.39 (m, 1H), 3.51 – 3.57 (m, 1H), 3.91 (s, 3H), 3.92 – 3.97 (m, 1H), 3.99 (s, 3H), 6.21 (br s, 1H), 6.91 (s, 1H), 7.11 (s, 1H), 7.19 (ddd, *J* = 8.0, 7.2, 0.6 Hz, 1H), 7.34 (ddd, *J* = 8.0, 7.3, 0.8 Hz, 1H), 7.49 (d, *J* = 8.3 Hz), 7.70 (d, *J* = 8.1 Hz, 1H), 10.16 (s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 31.9, 48.6, 56.0, 56.1, 111.9, 112.0, 113.3, 118.0, 120.7, 121.2, 124.9, 125.8, 126.8, 128.3. 129.9, 136.8, 147.8, 148.5, 167.5. EI-MS: *m/z* (%) 323 ($M+H^+$), 322 (M+, 61), 293 (25), 278 (24), 250 (13), 69 (100). HRMS (microTOF): *m/z* calcd for $C_{19}H_{19}N_2O_3$: 323.1390; found: 323.1389.

*4.2. (2,3,12-Trimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3] benzazocin-8-one* (**6b**). White solid (39.8 mg, 60% yield). $R_f$ 0.33(100% EtOAc). mp: 206 °C. IR (UATR): $\nu_{max}$ 3330, 3329, 1652 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, at – 20 °C): δ 2.75 – 2.78 (m, 1H), 3.27 – 3.35 (m, 1H), 3.47 – 3.53 (m, 1H), 3.79 (s, 3H), 3.90 (s, 3H), 3.93 (m, 1H), 3.96 (s, 3H), 6.82 (s, 1H), 6.86 (s, 1H), 6.94 (dd, *J* = 8.8, 1.9 Hz, 1H), 7.04 (s, 1H), 7.09 (s, 1H), 7.35 (d, *J* = 8.8 Hz, 1H), 10.86 (s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 32, 48.3, 55.6, 56.0, 56.1, 100.6, 111.5, 112.5, 113.4, 116.1, 117.5, 125.7, 126.6, 128.5, 129.6, 132.3, 147.3, 147.9, 154.4, 168.2. EI-MS: *m/z* (%) 353 ($M+H^+$, 21), 352 ($M^+$, 100), 323 (55), 308 (44). HRMS (microTOF) *m/z* calcd for $C_{20}H_{20}N_2O_4$ $(M + H)^+$: 353.1496 found: 353.1497.

*4.3. (2,3,11,12-Tetramethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3] benzazocin-8-one* (**6c**). Yellow amorphous (27.5 mg, 60% yield). $R_f$ 0.33 (100% EtOAc). IR (UATR): $\nu_{max}$ 3273, 1628, 1540, 1514 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, at –20 °C): δ 2.77 (d, *J* = 14.1 Hz, 1H), 3.34 (td, *J* = 12.9, 4.5 Hz, 1H), 3.54 (t, *J* = 12.6 Hz, 1H), 3.78 (s, 3H), 3.85 (s, 3H), 3.91 (s, 3H), 3.91 (m, 1H), 4.00 (s, 3H), 6.57 (br s, 1H), 6.90 (s, 1H), 6.91 (s, 1H), 7.00 (s, 1H), 7.10 (s, 1H), 11.1 (br s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 32.0, 49.0, 56.0, 56.0, 56, 56.1, 93.9, 100.3, 111.3, 112.1, 118.4, 119.0, 126.0, 126.5, 129.8, 132.2, 145.6, 147.3, 147.9, 149.1, 168.4. EI-MS: *m/z* (%) 383 ($M+H^+$, 23), 382 ($M^+$, 100), 367 (30), 353 (35), 338 (23), 57 (55). HRMS (microTOF) *m/z* calcd for $C_{21}H_{23}N_2O_5$ $(M + H)^+$: 383.16015; found: 383.1591.

*4.4. (2,3,10,12-Tetramethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e] [3]benzazocin-8-one* (**6d**). Pale yellow solid (14.1 mg, 10% yield). $R_f$ 0.35 (100% EtOAc). mp: 243 °C. IR (UATR): $\nu_{max}$ 3284, 1628, 1517, 1455 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, at –20 °C): δ 2.71 (d, *J* = 14.2 Hz, 1H), 3.25 (td, *J* = 13.3, 4.6 Hz, 1H), 3.27 (t, *J* = 12.4 Hz, 1H), 3.72 (s, 3H), 3.83 (s, 3H), 3.85 (m, 1H), 3.86 (s, 3H), 3.90 (s, 3H), 6.18 (s, 1H), 6.37 (d, *J* = 1.2 Hz, 1H), 6.55 (d, *J* = 0.8 Hz, 1H), 6.82 (s, 1H), 7.02 (s, 1H), 9.59 (s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): δ 31.7, 48.3, 55.5, 55.6, 55.8, 55.9, 91.8, 96.5, 111.2, 112.1, 117.6, 123.1, 125.6, 126.7, 128.0, 129.6, 146.9, 147.1, 147.7, 155.2, 167.3. EI-MS: *m/z* (%) 383 ($M+H^+$, 18), 382 ($M^+$, 96), 353 (28), 83 (42), 57 (100), 55 (86). HRMS (microTOF) *m/z* calcd for $C_{21}H_{23}N_2O_5$ $(M + H)^+$: 383.1601 found: 383.1600.

*4.5. 5,6,7,9-Tetrahydro-8H-indolo[2,3-e][3]benzazocin-8-one* (**6e**). Pale yellow solid (7.5 mg, 66% yield). $R_f$ 0.48 (100% EtOAc). mp: 269 °C. IR (UATR): $\nu_{max}$ 3173, 1620 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$+$CD_3OD$, at –20 °C): δ 2.83 (d, *J* = 13.8 Hz, 1H), 3.35 (td, *J* = 13.4, 4.9 Hz, 1H), 3.47 (td, *J* = 12.7, 4.6 Hz, 1H), 3.93 (dd, *J* = 11.8 Hz, 1H), 6.12 (s, 1H), 7.15 (dd, *J* = 7.5, 7.5 Hz, 1H), 7.33 (dd, *J* = 7.8, 7.4 Hz, 1H), 7.37 – 7.42 (m, 3H), 7.47 (d, *J* = 8.2 Hz, 1H), 7.56 (d, *J* = 7.1 Hz, 1H), 7.66 (d, *J* = 8.1 Hz, 1H), 10.16 (s, 1H). $^{13}C$ NMR (100 MHz, $(CD_3)_2SO$): δ 33.3, 45.8, 112.6, 115.3, 120.5, 120.5, 123.9, 126.3, 126.7, 127.5, 130.4, 130.5, 134.1, 137.1, 138.0, 166.6, 1C not observed. EI-MS: *m/z* (%) 263 ($M+H^+$, 14), 262 ($M^+$, 97), 233 (57), 217 (21), 204 (100), 176 (24), 102 (14), 57 (42). HRMS (microTOF) *m/z* calcd for $C_{17}H_{15}N_2O$ $(M + H)^+$: 263.1179; found: 263.1139.

*4.6. (3-Benzyloxyl-2,12-dimethoxyl)-5,6,7,9-tetrahydroindolo [2,3-e][3]benzazocin-8-one* (**6f**). Yellow amorphous (185.1 mg, 53% yield). $R_f$ 0.45 (100% EtOAc). IR (UATR): $\nu_{max}$ 3282, 1652, 1515 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, at –20 °C): δ 2.58 (d, *J* = 14.3 Hz, 1H), 3.16 (td, *J* = 11.9, 4.5 Hz, 1H), 3.32 (t, *J* = 12.1 Hz, 1H), 3.70 (s, 3H), 3.76 – 3.83 (m, 1H), 3.80 (s, 3H), 5.12 (s, 2H), 6.70 (br s, 1H), 6.77 (s, 1H), 6.85 (dd, *J* = 8.9,

2.1 Hz, 1H), 6.96 (d, *J* = 1.6 Hz, 1H), 7.01 (s, 1H), 7.25 – 7.41 (m, 6H), 10.7 (s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 33.1, 45.7, 55.7, 56.1, 70.6, 101.1, 113.5, 114.2, 114.9, 115.2, 115.7, 126.5, 126.6, 128.3 (3C), 128.8 (2C), 129.9, 130.5, 132.2, 137.7, 147.3, 147.9, 154.5, 166.6; EI-MS: *m/z* (%) 429 ($M+H^+$, 7), 428 ($M^+$, 29), 281 (14), 280 (30), 105 (11), 91 (100), 77 (6). HRMS (microTOF) *m/z* calcd for $C_{26}H_{25}N_2O_4$ $(M + H)^+$: 429.1809; found: 429.1815.

*4.7. (2-Benzyloxyl-3,12-dimethoxyl)-5,6,7,9-tetrahydroindolo [2,3-e][3]benzazocin-8-one* (**6g**). Yellow amorphous (185.1 mg, 53% yield). $R_f$ 0.35 (100% EtOAc ). IR (UATR): $\nu_{max}$ 3297, 2925, 1706 $cm^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$, at –20 °C): 2.64 – 2.68 (m, 1H), 3.16 – 3.24 (m, 1H), 3.37 – 3.43 (m, 1H), 3.51 (s, 3H), 3.79 – 3.83 (m, 1H), 3.89 (s, 3H), 5.09 (q, *J* = 12.7 Hz, 2H), 6.61 (br s, 1H), 6.68 (d, *J* = 1.9 Hz, 1H), 6.81 (s, 1H), 6.83 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.01 (s, 1H), 7.23-7.37 (m, 5H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 32.1, 48.3, 55.8, 56.2, 71.1, 101.7, 112.7, 112.9, 115.6, 116.1, 117.4, 126.1, 126.9, 127.1 (2C), 127.8, 128.5 (2C), 128.8, 130.5, 132.3, 137.1, 147.1, 149.1, 154.9, 167.7. EI-MS: *m/z* (%) 429 ($M+H^+$, 18), 428 ($M^+$, 68), 337 (12), 281 (31), 250 (12), 105 (7), 91 (100), 65 (11). HRMS (microTOF) *m/z* calcd for $C_{26}H_{25}N_2O_4$ $(M + H)^+$: 429.1808; found: 429.1818.

*4.8. (3-Benzyloxyl-2,12,13-trimethoxyl)-5,6,7,9-tetrahydro indolo [2,3-e][3]benzazocin-8-one* (**6h**). Yellow amorphous (143.5 mg, 54% yield). $R_f$ 0.42 (100% EtOAc). IR (UATR): $\nu_{max}$ 3277, 1632, 1509, $cm^1$. $^1$H NMR (400 MHz, $CDCl_3$, at –20 °C): 2.71 – 2.74 (m, 1H), 3.26 (s, 3H), 3.31 – 3.40 (m, 2H), 3.84 – 3.87 (m, 1H), 3.90 (s, 3H), 3.92 (s, 3H), 5.24 (s, 2H), 5.98 (br s, 1H), 6.84 (s, 1H), 7.10 (d, *J* = 8.9 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 1H), 7.20 (s, 1H), 7.36 – 7.53 (m, 5H), 9.82 (br s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 31.8, 48.2, 56.1, 58.1, 60.9, 71.4, 107.1, 113.6, 114.5, 116.2, 120.6, 122.6, 124.7, 126.8, 127.4 (2C), 127.8, 128.4 (2C), 128.5, 128.9, 129.5, 133.9, 145.9, 147.5, 147.9, 167.1. EI-MS: *m/z* (%) 459 ($M+H^+$, 8), 428 ($M^+$, 26), 368 (19), 367 (81), 105 (20), 91 (100), 77 (8). HRMS (microTOF) *m/z* calcd for $C_{27}H_{27}N_2O_5$ $(M + H)^+$: 459.1915; found: 459.1906.

**5. General Procedure for the Synthesis of Indolobenzazecinones 16f-h**

A solution of **6f-h** (1 equiv.) in EtOAc (60 mL/mmol) was placed in a high pressure parr apparatus at room temperature. To this solution was added palladium on activated charcoal (*ca*. 100 mg). The resulting mixture was hydrogenated (75 psi) until all starting material was consumed (normally 16 hours) as indicated by TLC. The mixture was then filtered through a plug of Celite and concentrated under reduced pressure to give a white solid. The crude material was further purified by recrystallization (PTLC) to give the desired product.

*5.1. (2,12-Dimethoxyl-3-hydroxyl)-5,6,7,9-tetrahydroindolo [2,3-e][3]benzazocin-8-one* (**16f**). White solid (113.5 mg, 82% yield). $R_f$ 0.40 (100% EtOAc). mp: 238 °C. IR (UATR): $\nu_{max}$ 3280, 2929, 1652, 1513 $cm^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$+$CD_3OD$, at –20 °C): δ 2.65 (ddd, *J* = 14.4, 4.3, 4.3 Hz, 1H), 3.17 (td, *J* = 14.1, 4.9 Hz, 1H), 3.36 (td, *J* = 12.4, 5.0 Hz, 1H), 3.73 (s, 3H), 3.80 (m, 1H), 3.80 (s, 1H), 6.47 (br s, 1H), 6.83 (s, 1H), 6.91 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.96 (s, 1H), 6.98 (d, *J* = 2.1 Hz, 1H), 7.31 (d, *J* = 8.9 Hz, 1H), 8.16 (br s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$+ $CD_3OD$): δ 31.4, 47.9, 55.4, 55.7, 100.5, 112.3, 113.0, 115.2, 115.8, 117.5, 124.6, 126.2, 127.9, 129.9, 131.9, 144.7, 145.7, 154.1, 167.7. EI-MS: *m/z* (%) 339 ($M+H^+$,18), 309 (53), 294 (22), 266 (30), 97 (16), 83 (15). HRMS (microTOF) *m/z* calcd for $C_{19}H_{19}N_2O_4$: 339.1339; found: 339.1343.

*5.2. (3,12-Dimethoxyl-2-hydroxyl)-5,6,7,9-tetrahydroindolo [2,3-e][3]benzazocin-8-one* (**16g**): White solid (68.0 mg, 84% yield). $R_f$ 0.30 (100% EtOAc). mp: 217 °C. IR (UATR): $\nu_{max}$ 3282, 2928, 1623, 1512 $cm^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$ + $CD_3OD$, at –20 °C): 2.69 (dt, *J* = 14.3, 3.6 Hz, 1H), 3.21 (td, *J* = 12.7, 4.9 Hz, 1H), 3.40 (td, *J* = 12.5, 4.9 Hz, 1H), 3.73 (s, 3H), 3.80 – 3.83 (m, 1H), 3.89 (s, 3H), 6.39 (br s, 1H), 6.81 (s, 1H), 6.90 (dd, *J* = 8.9, 2.3 Hz, 1H), 6.98 (d, *J* = 2.0 Hz, 1H), 7.02 (s, 1H), 7.29 (d, *J* = 8.9 Hz, 1H), 8.01 (br s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$ + $CD_3OD$): δ 31.6, 48.0, 55.5, 55.6, 100.6, 111.2, 112.8, 116.0, 116.0, 117.2, 125.8, 126.2, 127.9, 128.7, 131.9, 144.2, 146.3, 154.1, 168.5. EI-MS: *m/z* (%) 338 ($M^+$, 8), 178 (18), 149 (19), 97 (22), 85 (16), 69 (81), 57 (100). HRMS (microTOF) *m/z* calcd for $C_{19}H_{19}N_2O_4$: 339.1333; found: 339.1323.

*5.3. (3-Hydroxyl-2,12,13-trimethoxyl)-5,6,7,9-tetrahydroindolo [2,3-e][3]benzazocin-8-one* (**16h**). White solid (77.0 mg, 54% yield). $R_f$ 0.43 (100% EtOAc). mp: 248 °C. IR (UATR): $\nu_{max}$ 3280, 2929, 1623, 1508, 1455 $cm^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$ + $CD_3OD$, at –20 °C): 2.71 – 2.75 (m, 1H), 3.25 (s, 3H), 3.21-3.42 (m, 2H), 3.83 – 3.87 (m, 1H), 3.87 (s, 3H), 3.93 (s, 3H), 6.61 (br s, 1H), 6.83 (s, 1H), 7.10 (s, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 7.22 (d, *J* = 8.8 Hz, 1H), 8.10 (br s, 1H), 10.6 (br s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$ + $CD_3OD$): δ 31.7, 47.6, 56.1, 58.1, 60.9, 107.4, 113.6, 114.3, 115.3, 116.5, 118.1, 120.3, 125.3, 129.6, 134.1, 143.3, 144.9, 145.0, 145.6, 167.6. EI-MS: *m/z* (%) 368 ($M^+$,10), 57 (100), 55 (60). HRMS (microTOF) *m/z* calcd for $C_{20}H_{21}N_2O_5$: 369.1445; found:369.1455.

**6. Cytotoxicity Test**

All indolobenzazepinone derivatives **6** and **16** were solubilized in DMSO and tested for their cytotoxic activities against HuCCA-1, A-549, HepG2, and MOLT-3 cancer cell lines. The cells suspended in the corresponding culture medium were inoculated in 96-well microtiter plates (Corning Inc., NY, USA) at a



density of 10000–20000 cells per well, and incubated at 37 °C in a humidified atmosphere with 95% air and 5% $CO_2$. After 24 h, an equal volume of additional medium containing either the serial dilutions of the test compounds, positive control (etoposide), or negative control (DMSO) was added to the desired final concentrations, and the microtiter plates were further incubated for an additional 48 h. The number of surviving cells in each well was determined using either MTT assay (for adherent cells) or XTT assay (for suspended cells) in order to determine the $IC_{50}$ which is defined as the concentration that inhibits cell growth by 50% (relative to negative control) after 48 h of continuous exposure to each test compound. Within each experiment, determinations were done in triplicate, and each compound was tested in at least two separate experiments. Any experiments with a variation greater than 10% were excluded from the analysis. The results are expressed as the mean $IC_{50}$ value; standard deviations are omitted for visual clarity.

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## SUBPROJECT III

# Cu(I)-mediated/subcritical water and Pd(II)-catalyzed C-O/C-N bond formation: Synthesis of benzopyranones and phenanthridinones

## 3.1 Synthesis of Dibenzopyranones and Urolithins A-C

### Introduction

Recently, aqueous and microwave conditions have attracted both academic and industrial interests as an economical and environmentally friendly processes.[1-3] Water showed an important role as a reaction medium in organometallic reactions including Suzuki-Miyaura,[4,5] Negishi,[6] Stille,[7] and Sonogashira[8,9] cross-couplings. Because of its beneficial properties such as inexpensiveness, environmentally friendliness, non-inflammability, and safety, the role of water in research works has gained growing interest during the past decade.[10,11] Moreover, water has the dielectric constant ($\acute{\varepsilon}$) higher than other organic solvents at room temperature which can effectively absorb microwave energy and acts as pseudo-organic solvent at high temperature.[12] Apart from the superheated condition of water (>100°C), supercritical water (SCW, >374°C) has been widely studied in several fields[13-15] but some limitations retard its utilization due to its degenerative properties.[16] On the other hands, subcritical water (near-critical water, NCW), generated between 150 and 300°C, is reliable to use under a milder condition and is still able to maintain its pseudo-organic solvent properties.[17,18]

Benzopyranone **1** is the structural motif of various natural oxygen heterocycles which typically consist of dibenzo[*d,b*]pyran-6-one or 6*H*-benzo[*c*]chromen-6-one and these lactone containing natural products as shown in Figure 1 have been isolated from various sources. Urolithins A-C (**2a-c**), the intestinal microbial metabolites produced by *in vitro* fermentation of punicalagins, shows the antioxidant activity.[19,20] They also showed colon cancer chemopreventive activities by inhibiting TCDD-induced CYP1-mediated EROD activity.[21] Alternariol (**3**), a metabolite of toxin-producing *Alternaria* fungi, has been found to be the natural food contaminant in various grains, crops and decayed fruits.[22,23] TMC-264 (**4**), isolated from the fermentation broth of a fungus *Phoma* sp. TC 1674, displays potent inhibitory activity against tyrosine phosphorylation of STAT6.[24]

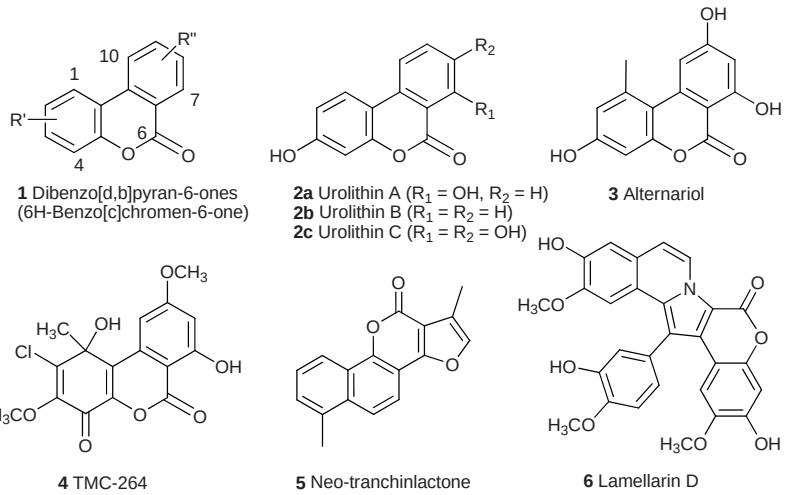



**Figure 1**. Various natural lactone containing heterocycles.

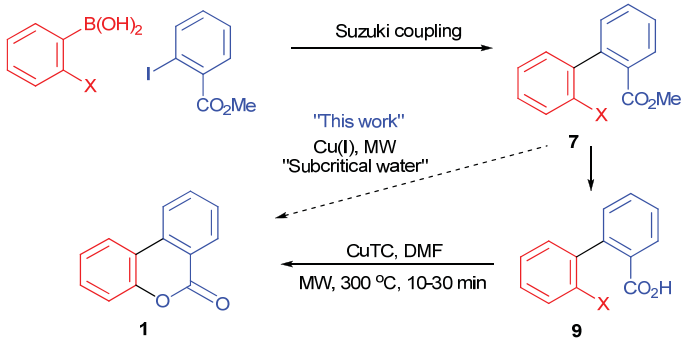
Neo-tranchinlactone (**5**), isolated from *Salvia miltiorrhiza* and first synthesized by Lee,[25] shows potent and selective anti-breast cancer activity.[26] The complex benzopyranone lamellarin D (**6**), isolated from a marine organism,[27] displays potent cytotoxic activity against multidrug-resistant tumour cell lines and is highly cytotoxic to prostate-cancer cell lines.[28-30]

Due to the potential uses as pharmacologically active compounds, benzopyranones have attracted much interest from various groups including ours.[31] Adhering to the economical aspect and "Green Chemistry" concept, in this study we report a short synthesis of benzopyranones using Cu(I)-mediated C-$O_{carboxylate}$ bond formation in subcritical water. Our protocol was also extended to synthesize the antioxidant benzopyranones, urolithins A (**2a**), B (**2b**), and C (**2c**).[19]

## Results and Discussion

### Study of the lactone formation using Cu(I)-mediated/MW-assisted C-$O_{carboxylate}$ coupling under "subcritical water" condition

In a preliminary investigation, the synthesis of dibenzo[*b,d*]pyran-6-one **1** was studied.[18] Synthesis of the required methyl 2-halobiarylcarboxylates **7** were prepared by the Suzuki cross-coupling reaction of methyl 2-iodoarylcarboxylate esters **8** and 2-haloarylboronic acids **9**. Lactonization of acids **10** were carried out in DMF and/or solvent mixtures of DMF either with EtOH or water using a series of copper(I) salts under microwave irradiation. Among the various copper salts examined, CuTC provided the desired product **1** in the best yields using microwave irradiation with ligand- and base-free conditions. Our endeavor to use a single solvent either EtOH or water failed. However the yields were moderate when mixtures of DMF:EtOH or DMF:water were used as solvents.




**Scheme 1**. Scope of previous work and this work

This notwithstanding, to dig deeper and intensify the research in this direction, we next turned our attention to investigate the "*green chemistry*" reaction conditions which require only catalytic amount of Cu(I) salts for lactonization. CuI and CuTC were used to study the Cu-catalyzed/microwave-assisted lactonization of 2-halobiarylcarboxylate **7** by varying the type and amount of ligands **11** under subcritical water condition. A set of bidentate ligands (Figure 2) including *N,N,N',N'*-tetramethylethelene diamine (**8a**, TMEDA, L1), phenanthroline (**8b**, Bip, L2), bipyridine (**8c**, Phen, L3), methyl 2-oxocyclohexanecarboxylate (**8d**, MOCHC, L6), ethyl 2-oxocyclohexanecarboxylate (**8e**, EOCHC, L7), 2-acetylcyclohexanone (**8f**, ACH, L8), and 2-benzoylcyclohexanone (**8g**, BCH, L9) was examined together with substoichiometric amount (50 mol% of Cu(I)) as

catalyst and $Cs_2CO_3$/$K_2CO_3$ as bases on the three-component reaction in the presence of subcritical water using microwave irradiation.

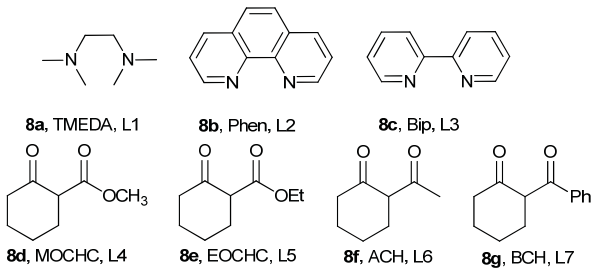
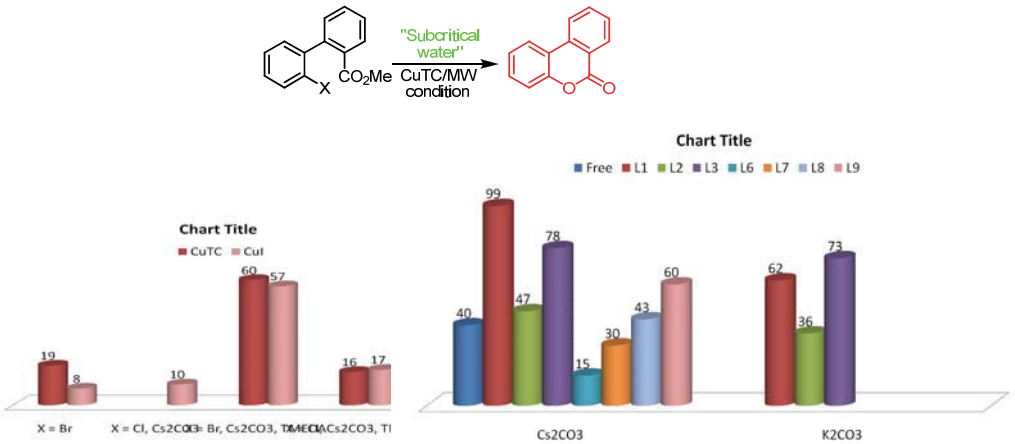



**Figure 2**. Ligands under screening

The synthesis of benzopyranones was studied successfully. The reaction of biaryl carboxylates proceeded under the *"subcritical water"* condition as shown in Figure 3. Without base and/or ligand, the lactone was obtained in poor yields together with recovered starting material using subcritical water at 200-250 $^oC$ (conditions A and B). The most effective condition of the Cu(I)-catalyzed C-O bond formation was found to be 50 mol% CuTC with 0.5 equiv $Cs_2CO_3$ and 1.0 equiv TMEDA using subcritical water at 300 $^oC$.

Accordingly, the lactone compound **1** was satisfyingly synthesized based on the CuTC-catalyzed, TMEDA ligand, and $Cs_2CO_3$ base were also required to promote the excellence yield while best ability of living group was concerned, Br group. The condition C was indicated the condition of approximate subcritical water in this reaction, 300$^oC$ at 200 psi. 2-Bromobiphenylcarboxylate **7a** was used to study the Cu-catalyzed/microwave-assisted lactonization under Green Chemistry condition. Without base and ligand condition, the lactone **1** was obtained in poor yields together with recovered starting material using subcritical water at 200-250 $^oC$. The yield was improved under $Cs_2CO_3$ as base and TMEDA as ligand. With the same condition, the lactonization of 2-bromobiphenylcarboxylate **7a** at 250 $^oC$ for 20 min gave lactone **1** higher than 2-chlorobiphenylcarboxylate **7b**.




Condition A Condition B Condition C

**Figure 3**. Screening of reaction conditions in the synthesis of dibenzo[b,d]pyran-6-one (**7**) using Cu(I)-mediated/microwave-assisted C-$O_{carboxylate}$ coupling under "*subcritical water*" conditions. The reactions were performed in 10 mL microwave vessel, ester (0.2 mmol), Cu(I) salts (0.1 mmol) in water (1 mL). Condition A: 200 $^{o}$C, 100 psi, 20 min; Condition B: base (0.3 mmol), 250 $^{o}$C, 280 psi, 20 min; Condition C: base (0.3 mmol), 300 $^{o}$C, 200 psi, 10 min.

The yield of lactonization was improved when using higher temperature to 300 $^{o}$C for 10 min. Among the various ligands and bases examined, TMEDA provided the desired product **7** in the best yields using $Cs_2CO_3$ as base. The most effective condition was found to be 50 mol% CuTC with 0.5equiv $Cs_2CO_3$ and 1.0 equiv TMEDA. This C-$O_{carboxylic}$ bond formation represents the first reported copper-mediated lactonization using Cu(I) under "subcritical water" condition as Green Chemistry.

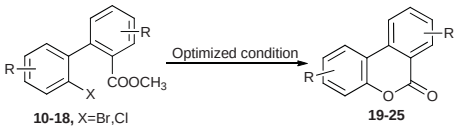
**Synthesis of the benzopyranone derivatives using the optimized condition**

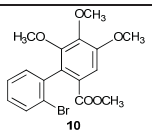
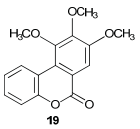
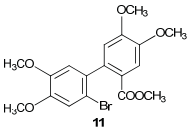
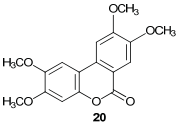
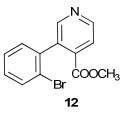
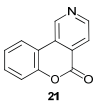
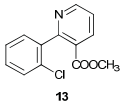
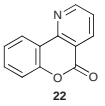
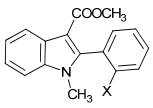
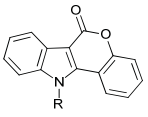
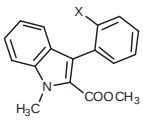
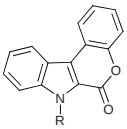
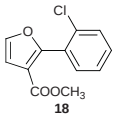
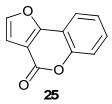
In the above screening, we found that the best conditions to form the C-$O_{carboxylate}$ bond of benzopyranone was the use of TMEDA as ligand in the presence of $Cs_2CO_3$ in subcritical water at 300 $^{o}$C. Various biaryl carboxylates **10-18** were prepared using the Suzuki-Miyaura coupling reaction.[31a] With these starting materials in hands, we then studied the Cu(I)-mediated C-$O_{carboxylate}$ lactone formation of benzopyranones **19-25** as shown in Table 2. The polyoxygenated benzopyranones **19** and **20** were synthesized from tri- and tetra-methoxybiaryl carboxylates **10** and **11**, respectively, in moderate yields (Table 1, entries 1 and 2).[31a] In these reactions, the reaction time was increased to 20 min. The presence of electron-donating groups may play the important role in the C-O bond forming step and gave lower yield of the products.

In order to demonstrate the versatility of our strategy in the synthesis of benzopyranone derivatives, we then directed our attention to synthesize the chromenopyridinones **21** and **22**.[34] 3-Arylisonicotinate **12** and 2-arylnicotinate **13** were prepared from the corresponding pyridine derivatives using Suzuki-Miyaura cross-coupling reaction with 2-haloarylboronic acids as previously reported.[31a] The chromenopyridinones **21** and **22** were obtained in moderate yields (46-66%) (Table 1, entries 3 and 4). Fused heteroaromatic rings, tetracyclic chromenoindolones **23a** and **23b** were prepared from the corresponding 2-arylindole-3-carboxylates **14** (X = Br) or **15** (X = Cl).[31a,35] Compound **24a** and **24b** could also be obtained under the same conditions from 3-arylindole-2-carboxylates **16** (X = Br) or **17** (X = Cl).[31a,35] The reaction of 2-(2-bromophenyl)indole-3-carboxylate **14** gave a mixture of the lactone products, *N*-methyl chromenoindolone **23a** (66%) together with the demethylated chromenoindolone **23b** (10%) (Table 1, entry 5). In contrast, the reaction of 2-(2-chlorophenyl)indole-3-carboxylate **15** gave only *N*-methyl chromenoindolone **23a** in good yield (73%) (Table 1, entry 6). When 3- arylindole-2-carboxylates **16** and **17** were employed, both compounds gave a mixture of *N*-methyl chromenoindolone **24a** and demethylated chromenoindolone **24b** (Table 1, entries 7 and 8). The benzopyranone derivative, furochromenone **25**[36-39] was also successfully prepared from 2-arylfuran-3-carboxylate **18** in 30% yield (Table 1, entry 9).



**Table 1.** Synthesis of the benzopyranone derivatives using the optimized conditions.[a]




| Entry | Substrate | Product | Yield (%)[b] |
|---|---|---|---|
| 1 |  **10** |  **19** | 39[c] |
| 2 |  **11** |  **20** | 31[c] |
| 3 |  **12** |  **21** | 46 |
| 4 |  **13** |  **22** | 66 |
| 5 |  **14**, X=Br **15**, X=Cl |  **23a**, R=Me **23b**, R=H | 66, 10[d] |
| 6 | | | 73[e] |
| 7 |  **16**, X=Br **17**, X=Cl |  **24a**, R=Me **24b**, R=H | 45, 11[f] |
| 8 | | | 47, 4[g] |
| 9 |  **18** |  **25** | 30 |

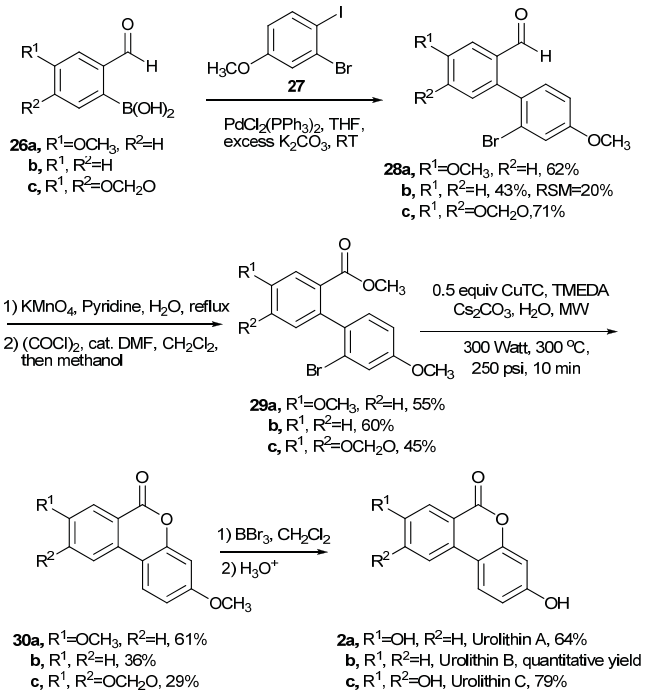
[a] *Reaction conditions*: biaryl ester (0.2 mmol), CuTC (0.5 equiv), $Cs_2CO_3$ (0.5 equiv), TMEDA (1.0 equiv) in water (1 mL), MW (300 Watt), 300 °C, 250 psi, 10 min; [b] Isolated yields; [c] Reaction time 20 min.; [d] %yield of **23a** and **23b** from **14**; [e] % yield of **23a** from **15**; [f] % yield of **24a** and **24b** from **16**; [g] % yield of **24a** and **24b** from **17**.

## Synthesis of urolithins A-C

The utility of this method was further demonstrated with the synthesis of natural benzopyranones urolithins A-C (**2a-c**).[19] Our synthetic route was performed using the same sequence of reactions requiring five steps from commercially available boronic acids **26a-c** as



shown in Scheme 1. The reaction of boronic acids **26a-c** and 2-bromo-1-iodo-4-methoxybenzene **27** was performed using $PdCl_2(PPh_3)_2$ under basic conditions in THF at room temperature to afford the desired 2'-bromo-4'-methoxy-(1,1'-biphenyl)-2-carbaldehydes **28a-c** in moderate to good yields (43-71%). 2-Bromo-1-iodo-4-methoxybenzene **27** was prepared from the selective *para*-iodination of 3-bromoanisole.[40] The transformation of carbaldehydes **28a-c** to biaryl esters **29a-c** was conducted by oxidation followed by esterification. Treatment of biaryl esters **29a-c** with 0.5 equiv of CuTC in the presence of TMEDA and $Cs_2CO_3$ in subcritical water for 10 min furnished the methyl ether of urolithins A-C **(30a-c)** which were further demethylated with $BBr_3$ in dichlomethane at 0 $^oC$. These conditions gave urolithins A-C (**2a-c**) in 15%, 9%, and 7% overall yield respectively over 4 steps.




**Scheme 2.** Synthesis of urolithins A-C

**Biological activities**

The antioxidant radical-scavenging of urolithins A-C and cytotoxic activities are summarized in Table 2. Urolithin C (**2c**) scavenged 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical with $IC_{50}$ value of 12.6±0.9 µM which is better than the reference compound, ascorbic acid at 21.2 µM. Superoxide anion radical formation in the xanthine/xanthine oxidase (XXO) and xanthine oxidase (IXO) assays was not inhibited by urolithins A-C. Compounds **2a-c** inhibited aromatase activity (AIA) with $IC_{50}$ values of 13.2±1.4, 11.9±1.0, and 21.6±0.6 µM, respectively.

They also exhibited the potent antioxidant activity in oxygen radical absorbance capacity (ORAC) assay with 3.0±0.5, 3.0±0.7 and 5.5±0.5 ORAC units respectively (Table 2).[41] The urolithin B can also inhibit the proliferation of leukemia cell with the $IC_{50}$ value of 86.8±13.6 µM.

**Table 2.** Radical scavenging, antioxidant, and aromatase inhibitory activities of synthetic urolithins A-C[a]

| Urolithins | $IC_{50}$ [μM] | | | | unit |
|---|---|---|---|---|---|
| | DPPH | IXO | AIA | HL-60[b] | ORAC[c] |
| A | >250 | 157.6±14.5 | 13.2±1.4 | >100 | 3.0±0.5 |
| B | >250 | 334.3±1.8 | 11.9±1.0 | 86.8±13.6 | 3.0±0.7 |
| C | 12.6±0.9 | 165.0±3.6 | 21.6±0.6 | >100 | 5.5±0.5 |

[a] Positive controls for each assay are as follows: DPPH: ascorbic acid ($IC_{50}$ = 21.2 μM); IXO: allopurinol ($IC_{50}$=3.0 μM); aromatase inhibition (AIA): ketoconazole ($IC_{50}$=2.4 μM). [b] HL-60: Human Promyelocytic Leukemia cells. [c] The results are expressed as ORAC units: 1 ORAC unit equals the net protection of β-phytoerythrin produced by 1 μM of Trolox.

## Conclusion

In summary, we have reported the synthesis of various benzopyranones and urolithins A-C (**2a-c**) based on the green chemistry principle using the one-pot lactone formation from biaryl esters under microwave irradiation with the catalytic amount of CuTC. With the environmental consideration, the successful use of water, the ideal green solvent, in the key lactone formation further adds the merit of the protocol. The biological activity evaluation of urolithins A-C (**2a-c**) showed the potent antioxidant activity in ORAC assay while urolithin C (**2c**) exhibited the radical-scavenging activity.

## Experimental Section

### 1. General Methods

Microwave reaction was performed in CEM Discover. Melting points were measured using a Thermo Fisher Scientific IA920 digital melting point instrument which was reported without correction. $^{1}$H-NMR spectra were recorded on Bruker AV-300 (300 MHz), Bruker AV-400 (400 MHz) and Varian Germini2000 (200 MHz) using the deuterochloroform as solvent with tetramethylsilane as an internal standard and dimethylsulfoxide-$d_6$ for some compounds. $^{13}$C-NMR spectra were recorded on Bruker AV-300 (75 MHz), Bruker AV-400 (100 MHz) and Varian Germini2000 (50 MHz) using the deuterochloroform as solvent with tetramethylsilane as an internal standard and dimethylsulfoxide-$d_6$ for some compounds. Infrared spectra (IR) were obtained on Perkin Elmer System 2000FT-IR and JASCO A-302 spectrometers. Mass Spectrometry was performed with an AEI-MS-902. High Resolution Mass Spectrometry was performed with a MicroTOFLC, Bruker Daltonics. Column chromatography was carried out using Fluka aluminum oxide (type 507 C neutral; 100-125 mesh) and Merck silica gel (70-230 mesh ASTM). Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were carried out on silica gel (E. Merck PF 254). All reagents were purified and dried according to the standard procedures. Solvents were removed using Eyela Aspirator A-2S and Büchi Rotavapor R110. All products were evacuated by a Christ Freeze Dryer Unit Alpha l/6, to remove the last traces of solvents.

### 2. General Procedure for the Preparation of Biarylcarboxylate Esters (7a, 7b, and 10–18)

A solution of 2-halophenylboronic acid (2.0 equiv), 2-halophenylcarboxylate (1.0 equiv) and 10 mol% $Pd(PPh_3)_4$ in three portion of toluene:EtOH:10%$Na_2CO_3$ (5:1:2) solution was refluxed overnight. After the complete reaction was observed by TLC, water was added to quench and partition with EtOAc to obtain the crude product. The crude product was then purified by flash column chromatography or the preparative thin layer chromatography (EtOAc/Hexane) to obtain the product. Compounds **7a, 7b, 10, 11,** and **14–17** were previously reported in the literature.[31a]



*2.1 Methyl 3-(2-bromophenyl)isonicotinate* ***(12).*** Yellow oil (143.0 mg, 68%). $R_f$ 0.43 (30% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2951, 1734, 1434, 1273, 1105, 756 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.59 (s, 1H), 7.89 (d, *J* = 4.5 Hz, 1H), 7.84 (d, *J* = 4.8 Hz, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.41 (t, *J* = 7.5 Hz, 1H), 7.31 – 7.25 (m, 2H), 3.72 (s, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 165.6, 151.5, 149.5, 138.4, 137.1, 135.8, 132.2, 52.4, 130.2, 129.4, 127.1, 123.1, 122.7. EI-MS: *m/z* (%) 291 ($M+H^+$, 0) 212 (100), 197 (38), 126 (20). TOF-HRMS calcd for $C_{13}H_{11}BrNO_2$: 291.9967; found 291.9956, 293.9939.

*2.2 Methyl 2-(2-chlorophenyl)nicotinate* ***(13).*** Yellow oil (792.0 mg, quantitative yield). $R_f$ 0.14 (10% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2951, 1725, 1565, 1424, 1273, 754 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 8.81 (d, *J* = 3.9 Hz, 1H), 8.31 (d, *J* = 8.1 Hz, 1H), 7.43 – 7.34 (m, 5H), 3.70 (s, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 166.2, 157.4, 151.8, 139.5, 138.0, 132.1, 130.0, 129.3, 128.8, 126.8, 126.6, 122.4, 52.3. EI-MS: *m/z* (%) 247 ($M^+$, 0), 212 (100), 197 (32). TOF-HRMS calcd for $C_{13}H_{11}ClNO_2$: 248.0472; found 248.0474, 250.0456.

*2.3 Methyl 2-(2-chlorophenyl)furan-3-carboxylate* ***(18)***. Yellow solid (92.0 mg, 80%). $R_f$ 0.34 (20% EtOAc/Hexane). Mp: 61 °C. IR (UATR): $\nu_{max}$ 2952, 1720, 1619, 1470, 1439, 1299, 755 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 7.40 – 7.38 (m, 2H), 7.48 – 7.46 (m, 3H), 6.85 (s, 1H), 3.72 (s, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 163.3, 155.0, 142.2, 134.1, 131.9, 130.6, 129.6, 129.3, 126.1, 116.2, 111.3, 51.4. EI-MS: *m/z* (%) 236 ($M^+$, 3), 201 (100), 186 (35), 170 (8). TOF-HRMS calcd for $C_{12}H_{10}ClO_3$: 237.0313; found 237.0315, 239.0284.

**3. General Procedure for the Preparation of Benzopyranones (19–25, and 30a–c)**

In a 10 mL microwave vessel, a mixture of methyl 2-halobiarylcarboxylate ester (0.2 mmol, 1.0 equiv), CuTC (0.1 mmol, 0.5 equiv), $Cs_2CO_3$ (0.1 mmol, 0.5 eqiuv) in deionized water (2 mL) was added with TMEDA (0.2 mmol, 1.0 eqiuv) via microsyinge. The mixture was allowed to stir at room temperature for 15 min and then placed into the microwave instrument. The reaction was then irradiated based on conditions appropriate for each reaction (see Table 1.) and reactions followed by TLC. After completion, the suspension was filtered through silica gel and washed with EtOAc (4 x 25 mL). The solvent was removed under reduced pressure to give a pale yellow solid which was then purified by PTLC (EtOAc/Hexane) to give the product. Compounds **1**, **19**, **20**, **23**, and **24** were previously reported in the literature.[34]

*3.1 5H-chromeno[4,3-c]pyridin-5-one* ***(21).*** White solid (15.3 mg, 46%). $R_f$ 0.34 (20% EtOAc/Hexane). Mp: 159 °C. IR (UATR): $\nu_{max}$ 1735, 1609, 1411, 1277, 1240, 1086, 757 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 9.57 (s, 1H), 8.87 (d, *J* = 5.1 Hz, 1H), 8.19 (d, *J* = 9.6 Hz, 1H), 8.17 (d, *J* = 7.2 Hz, 1H), 7.56 (t, *J* = 7.3 Hz, 1H), 7.44 – 7.39 (m, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 159.7, 151.6, 149.4, 145.4, 131.4, 128.5, 126.9, 125.2, 122.4, 122.0, 118.0, 115.7. EI-MS: *m/z* (%) 197 ($M^+$, 100), 169 (42), 142 (13), 114 (20). TOF-HRMS calcd for $C_{12}H_8NO_2$: 198.0549; found 198.0543.

*3.2 5H-chromeno[4,3-b]pyridin-5-one* ***(22)***. Yellow solid (49.3 mg, 62%). $R_f$ 0.40 (20% EtOAc/Hexane). Mp: 146 °C. IR (UATR): $\nu_{max}$ 1736, 1727, 1602, 1449, 764 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 9.02 (dd, *J* = 4.6, 1.95 Hz, 1H), 8.62 (dd, *J* = 8.1, 1.8 Hz, 1H), 8.58 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.59 (td, *J* = 8.2, 1.5 Hz, 1H), 7.52 (dd, *J* = 7.9, 4.5 Hz, 1H), 7.40 (td, *J* = 9.0, 1.2 Hz, 1H), 7.38 (d, *J* = 8.7 Hz, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 161.1, 155.6, 152.5, 151.8, 138.1, 132.2 , 124.9, 124.6, 123.7, 119.2, 117.3,117.1. EI-MS: *m/z* (%) 197 ($M^+$, 100), 169 (39), 140 (17), 114 (15), 70 (11). TOF-HRMS calcd for $C_{12}H_8NO_2$: 198.0549; found 198.0545.

*3.3 4H-furo[3,2-c]chromen-4-one* ***(25)***. Brown solid (20.1 mg, 30%): $R_f$ 0.14 (10% EtOAc/Hexane). Mp: 171 °C. IR (UATR): $\nu_{max}$ 1686, 1483, 1313, 1146, 753 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 7.50 (d, *J* = 1.5 Hz, 1H), 7.48 – 7.29 (m. 4H), 6.87 (d, *J* = 1.5 Hz, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 167.8, 156.3, 142.5, 134.3, 132.1, 130.9, 129.7, 129.2, 128.6, 128.1, 126.2, 111.6. EI-MS: *m/z* (%): 187 ($M+H^+$, 100), 170 (2), 149 (6), 131 (5), 115 (14). TOF-HRMS calcd for $C_{11}H_7O_3$: 187.0389; found 187.0385.

*3.4 3,8-Dimethoxy-6H-benzo[c]chromen-6-one* ***(30a).*** Yellow solid (37.5 mg, 61%). $R_f$ 0.43 (50% $CH_2Cl_2$/Hexane). Mp: 124 °C. IR (UATR): $\nu_{max}$ 2924, 2846, 1732, 1623, 1491, 1319, 1295 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 7.90 (d, *J* = 9.0 Hz, 1H), 7.85 (d, *J* = 8.7 Hz, 1H), 7.75 (d, *J* = 2.7 Hz, 1H), 7.36 (dd, *J* = 8.8,

3.0 Hz, 1H), 6.90 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.85 (d, *J* = 2.4 Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 161.5, 160.6, 159.0, 151.5, 128.5, 124.3, 122.7, 120.9, 112.3, 111.2, 110.9, 101.4, 55.6 (2C). EI-MS: *m/z* (%) 256 ($M^+$, 23), 241 (15), 178 (20), 149 (160). TOF-HRMS calcd for $C_{15}H_{13}O_4$: 257.0808; found 257.0811.

*3.5 3-Methoxy-6H-benzo[c]chromen-6-one* ***(30b).*** Yellow solid (3.3 mg, 36%): $R_f$ 0.46 (10% EtOAc/Hexane). Mp: 107 °C. IR(UATR): $\nu_{max}$ 2924, 2846, 1732, 1623, 1491, 1319, 1295 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.36 (d, *J* = 8.1 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.95 (d, *J* = 8.1 Hz, 1H), 7.79 (t, *J* = 7.8 Hz, 1H), 7.51 (t, *J* = 8.1 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 6.88 (s, 1H), 3.89 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 161.5, 135.1, 134.8, 130.5, 127.7, 124.4, 123.9, 123.7, 121.0, 119.1, 112.4, 111.1, 101.6, 55.6. EI-MS: *m/z* (%) 256 ($M^+$, 23), 241 (14), 178 (20), 149 (16). TOF-HRMS calcd for $C_{13}H_9O_3$: 213.0546; found 213.0554.

*3.6 3-Methoxy-6H-[1,3]dioxolo[4',5':4,5]benzo[1,2-c]chromen-6-one* ***(30c).*** Brown solid (2.3 mg, 29%): $R_f$ 0.43 (50% $CH_2Cl_2$/Hexane). Mp: 118 °C. IR (UATR): $\nu_{max}$ 2921, 2849, 2155, 1716, 1612, 1480, 1270, 1034, 935 cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): δ 8.19 (d, *J* = 8.4 Hz, 1H), 7.91 (s, 1H), 7.53 (s, 1H), 6.989 – 6.952 (m, 2H), 6.24 (s, 2H), 3.84 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ 161.2, 154.6, 151.9, 148.3, 132.9, 130.0, 125.0, 113.4, 112.7, 111.9, 107.4, 103.01, 101.6, 101.5. EI-MS: *m/z* (%) 271 ($M+H^+$, 100), 256 (53), 241 (29), 128 (12). TOF-HRMS calcd for $C_{15}H_{11}O_5$: 271.0610; found 271.0603.

**4. General Procedure for the Preparation of 2-Bromo-1-iodo-4-methoxybenzene (27)**

A mixture of 3-bromophenol (0.53 mmol, 1.0 equiv), $I_2$ (0.53 mmol, 1.0 eqiuv) and $CF_3COOAg$ (0.80 mmol, 1.5 equiv) in $CHCl_3$ (5 mL) was stirred under Ar atmosphere for 3 h. The reaction was quenched with saturated $Na_2S_2O_5$ and extracted with $CH_2Cl_2$ (3 x 25 mL). The solvent was then removed by rotary evaporation to give the brown crude oil. The crude product was purified by flash column chromatography (Hexane 100%) to obtain product as pink oil (57.6 mg, 35%): $R_f$ 0.512 (20% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2975, 1578, 1460, 1283, 1223, 1032, 840 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.69 (d, *J* = 8.7 Hz, 1H), 7.20 (d, *J* = 2.4 Hz, 1H), 6.60 (dd, *J* = 8.7, 2.1 Hz, 1H), 3.78 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ 160.1, 140.1, 129.8, 118.3, 115.3, 89.4, 55.5. EI-MS: *m/z* (%) 312 ($M+H^+$, 87), 297 (17), 172 (30). TOF-HRMS calcd for $C_7H_6BrIO$: 311.8641; found 311.8645, 313.8622.

**5. General Procedure for the Preparation of Biarylcarboxaldehyde (28a–c)**

The mixture of 2-formylphenyl boronic acid **26a–c** (1.0 equiv), 2-bromo-1-iodo-4-methoxybenzene **27** (2.0 equiv) and 5 mol% $PdCl_2(PPh_3)_2$ in solution of THF was stirred under Ar atmosphere at room temperature. The solution of 2N $K_2CO_3$ was then transferred into the reaction via syringe until the reaction turned a brown-red solution. The reaction was allowed to stir at room temperature overnight. After checking by TLC, the reaction was quenched with water and partition with EtOAc to obtain the dark-brown crude oil. The crude product was purified by flash chromatography or preparative thin layer chromatography (EtOAc/Hexane) to obtain the product.

*5.1 2'-Bromo-4,4'-dimethoxy-[1,1'-biphenyl]-2-carbaldehyde* ***(28a).*** Yellow solid (32.0 mg, 62%): $R_f$ 0.40 (5% EtOAc/Hexane). Mp: 104 °C. IR (UATR): $\nu_{max}$ 2916, 2848, 2344, 1688, 1601, 1481 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$): δ 9.75 (s, 1H), 7.50 (d, *J* = 2.5 Hz, 1H), 7.19 – 7.25 (m, 4H), 6.94 (dd, *J* = 8.4, 2.5 Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 191.5, 159.9, 159.4, 137.3, 134.9, 132.4, 132.3, 130.5, 124.6, 121.0, 117.8, 113.4, 109.7, 55.6, 55.5. EI-MS: *m/z* (%) 230 ($M^+$, 1), 241 (100), 198 (28), 121 (14). TOF-HRMS calcd for $C_{15}H_{14}BrO_3$: 321.0120; found 321.0132, 323.0117.

*5.2 2'-Bromo-4'-methoxy-[1,1'-biphenyl]-2-carbaldehyde* ***(28b).*** Pink oil (20.1 mg, 43%): $R_f$ 0.26 (5% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2838, 2933, 1693, 1597, 1221, 1033, 776 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$): δ 9.81 (s, 1H), 8.02 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.64 (td, *J* = 7.5, 1.4 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.32 (dd, *J* = 7.5, 0.6 Hz, 1H), 7.24 (d, *J* = 1.0 Hz, 1H), 7.22 (d, *J* = 4.62, 1H), 6.96 (dd, *J* = 8.4, 2.5 Hz, 1H), 3.86 (s, 3H).



$^{13}$C NMR (100 MHz, $CDCl_3$): δ 191.6, 160.0, 55.6, 144.2, 133.9, 133.4, 132.0, 131.2, 130.8, 128.2, 126.9, 124.0, 117.8, 113.5. EI-MS: *m/z* (%) 290 ($M^+$, 0), 211 (100), 196 (11), 168 (25), 139 (30), 105 (6). TOF-HRMS calcd for $C_{14}H_{12}BrO_2$: 291.0015; found 291.0022, 293.0000.

*5.3 6-(2-Bromo-4-methoxyphenyl)benzo[d][1,3]dioxole-5-carbaldehyde (**28c**):* Brown oil (37.9 mg, 71%): $R_f$ 0.37 (5% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2909, 2849, 1680, 1602, 1476, 1235, 1034 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$): δ 9.55 (s, 1H), 7.44, (s, 1H), 7.21 (d, *J* = 2.4 Hz, 1H), 7.19 (d, *J* = 8.8 Hz, 1H), 6.92 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.20 (s, 2H), 3.85 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 189.9, 160.0, 151.9, 141.6, 132.2, 130.4, 124.3, 117.9, 114.4, 113.4, 110.7, 110.6, 105.9, 102.0, 55.6. EI-MS: *m/z* (%) 334 ($M+H^+$, 1), 255 (100), 212 (23), 197 (10), 154 (10). TOF-HRMS calcd for $C_{15}H_{12}BrO_4$: 334.9913; found 334.9911, 336.9891.

**6. Genaeral Procedure for the Preparation of Biaryl Esters (29a–c)**

To a solution of 2'-bromo-4'-methoxy-(1,1'-biphenyl)-2-carbaldehydes **28a–c** (1.0 equiv), pyridine (5 mL) in water (15 mL) was added $KMnO_4$ (2.0 equiv) at ambient atmosphere. The reaction was then refluxed and monitored by TLC until the starting material was completely oxidized. The reaction was cooled to room temperature and acidified with 2 N HCl to afford white solid precipitation. The white solid was recrystallized with EtOAc/hexane (1:4) to obtain the biaryl carboxylic acid. The obtained product was then placed into the round bottom flask and dissolved with $CH_2Cl_2$. To a precooled (0 $^o$C) solution of carboxylic acid and DMF (3 drops) was added dropwise oxalyl chloride $(COCl)_2$ slowly. The reaction was stirred at the same temperature for 2 h, and then concentrated under reduced pressure to afford a yellow residue. The residue was then esterified with methanol (25 mL) at room temperature. The reaction was quenched with water and extracted with EtOAc (3 x 25 mL). The combined organic layer was washed with brine, dried with anhydrous $Na_2SO_4$, and concentrated under reduced pressure to obtain the residue which was purified by flash column chromatography (5 – 95% EtOAc/Hexane) to give the biaryl esters.

*6.1 Methyl 2'-bromo-4,4'-dimethoxy-[1,1'-biphenyl]-2-carboxylate (**29a**).* Colorless oil (113.3 mg, 55%): $R_f$ 0.29 (20% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2949, 2837, 1727, 1601, 1479 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.53 (d, *J* = 2.4 Hz, 1H), 7.53 – 7.08 (m, 4H), 6.90 (dd, *J* = 8.4, 2.4 Hz, 1H), 3.90 (s, 3H), 3.84 (s, 3H), 3.70 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 167.2, 158.9, 158.7, 134.5, 134.3, 132.5, 130.7, 123.6, 117.8, 117.3, 114.6, 113.0, 55.4 (2C), 52.1. EI-MS: *m/z* (%) 350 ($M^+$, 2), 271 (100), 256 (60), 241 (39), 225 (10), 187 (8). TOF-HRMS calcd for $C_{16}H_{16}BrO_4$: 351.0226; found 351.0230, 353.0212.

*6.2 Methyl 2'-bromo-4'-methoxy-[1,1'-biphenyl]-2-carboxylate (**29b**)* Yellow oil (18.6 mg, 60%): $R_f$ 0.34 (5% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2946, 2927, 1725, 1602, 1475, 1435, 1256, 1033 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.99 (dd, *J* = 9.0, 0.9 Hz, 1H), 7.53 (td, *J* = 7.5, 1.5 Hz, 1H), 7.43 (td, *J* = 7.6, 1.2 Hz, 1H), 7.23 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.17 (d, *J* = 2.7 Hz. 1H), 7.15 (d, *J* = 6.9 Hz, 1H), 7.11 (s, 1H), 6.89 (dd, *J* = 8.4, 2.7 Hz, 1H), 3.81 (s, 3H), 3.68 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 167.2, 159.0, 141.8, 134.7, 131.6, 131.4, 130.4, 129.9, 127.6, 123.0, 117.3, 113.4, 112.9, 55.3, 51.9. EI-MS: *m/z* (%) 320 ($M^+$, 2), 241 (100), 226 (53), 139 (27). TOF-HRMS calcd for $C_{15}H_{14}BrO_3$: 321.0121; found 321.0119, 323.0111.

*6.3 Methyl 6-(2-bromo-4-methoxyphenyl)benzo[d][1,3]dioxole-5-carboxylate (**29c**).* Brown solid (18.2 mg, 45%): $R_f$ 0.31 (20% EtOAc/Hexane). Mp: 85$^o$C. IR (UATR): $\nu_{max}$ 2949, 2901, 1724, 1601, 1478, 1240, 1032, 852 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.48 (s, 1H), 7.17 (d, *J* = 2.7 Hz, 1H), 7.11 (d, *J* = 8.7 Hz, 1H), 6.89 (dd, *J* = 8.4, 2.7 Hz, 1H), 6.67 (s, 1H), 6.09 (s, 2H), 3.84 (s, 3H), 3.67 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 159.0, 150.2, 147.0, 138.1, 134.7, 130.7, 130.4, 123.7, 123.3, 117.3, 113.0, 111.4, 109.9, 102.0, 55.4, 51.9. EI-MS: *m/z* (%) 364 ($M^+$, 1), 285 (100), 270 (70), 255 (29), 143 (21). TOF-HRMS calcd for $C_{16}H_{11}BrO_5$: 365.0019; found 365.0021, 367.0007.

**7. General Procedure for the Preparation of Urolithins a-c (2a–c).**



A solution of methyl ether of urolithins A-C **30a–c** (1.0 equiv) was cooled at 0 °C under Ar atmosphere and added with $BBr_3$ slowly. Until the stating material was completely consumed by checked with TLC, the solution of 2N HCl was added to acidify and partitioned with EtOAc to give the crude product. The crude product was purified by size exclusion chromatography to obtain the product.

*7.1 3,8-Dihydroxy-6H-benzo[c]chromen-6-one (**2a**).* Brown solid (7.0 mg, 64%): $R_f$ 0.06 (20% EtOAc/Hexane). Mp: 331°C. IR (UATR): $\nu_{max}$ 3332, 3140, 1703, 1614, 1458, 1273 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): δ 10.20 (bs, 2H), 8.10 (d, *J* = 9.0 Hz, 1H), 8.01 (d, *J* = 8.7 Hz, 1H), 7.50 (d, *J* = 2.7 Hz, 1H), 7.31 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.79 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.71 (d, *J* = 2.1, 1H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ 160.6, 159.8, 152.1, 135.3, 135.1, 129.7, 127.7, 124.8, 121.6, 118.9, 113.2, 109.4, 102.9. EI-MS: *m/z* (%) 228 ($M^+$, 100), 200 (13), 115 (20). TOF-HRMS calcd for $C_{13}H_9O_4$: 229.0495; found 229.0493.

*7.2 3-Hydroxy-6H-benzo[c]chromen-6-one (**2b**).* Brown solid (10.0 mg, quantitative yield): $R_f$ 0.14 (20% EtOAc/Hexane). Mp 207 °C. IR (UATR): $\nu_{max}$ 3254, 2919, 1691, 1625, 1608, 1315, 1276 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): δ 10.36 (s, 1H), 8.25 (d, *J* = 8.1 Hz, 1H), 8.18 (dd, *J* = 7.3, 1.2 Hz, 1H), 8.15 (d, *J* = 8.7 Hz, 1H), 7.88 (td, *J* = 7.0, 1.5 Hz, 1H), 7.57 (td, *J* = 7.6, 0.9 Hz, 1H), 6.85 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.75 (d, *J* = 2.4 Hz, 1H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ 160.6, 159.8, 152.1, 135.3, 135.1, 129.7, 124.8, 121.6, 118.9, 113.2, 109.4, 102.9. EI-MS: *m/z* (%) 212 ($M^+$, 100), 184 (21), 128 (17), 127 (15). TOF-HRMS calcd for $C_{13}H_9O_3$: 213.0546; found 213.0548.

*7.3 3,8,9-Trihydroxy-6H-benzo[c]chromen-6-one (**2c**).* Olive-green solid (4.0 mg, 79%): $R_f$ 0.028 (10% EtOAc/Hexane). Mp: >333 °C. IR (UATR): $\nu_{max}$ 3230, 1690, 1614, 1461, 1278 $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 10.12 (bs, 3H), 7.85 (d, *J* = 8.7 Hz, 1H), 7.47 (s, 1H), 7.41 (s, 1H), 6.77 (dd, *J* = 8.2, 2.4 Hz, 1H), 6.67 (d, *J* = 2.4 Hz, 1H). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): δ 103.4, 107.2, 110.2, 111.3, 113.2, 114.6, 124.1, 129.6, 146.5, 151.8, 153.8, 159.0, 160.7. EI-MS: *m/z* (%) 224 ($M^+$, 100), 216 (11), 129 (17), 97 (34), 83 (40), 69 (55). TOF-HRMS calcd for $C_{13}H_9O_5$: 245.0444; found 245.0445.

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## 3.2 Synthesis of Phenanthridinones

### Introduction

Phenanthridinone scaffold was widely found in various biologically active compounds.[1-4] In 1893, phenanthridinone was first synthesized by Grabe and Wander using Hofmann reaction on 2,2'-amidobiphenylcarboxylic acid but the unsatisfied yield was obtained.[5] The



Curtius degradation of diphenic monoazide in alcoholic solvent under the acid condition was then developed to lead the phenanthridinone derivative but remained limitation of variation of its analogue.[6-7] The syntheses of phenanthridinones have been further studied to attempt for more effective routes. Since the C-N bond formations have been conducted by Buchwald and Hartwig groups, palladium-catalyzed C-N bond formations using the necessary ligand and base were reported for more efficient conditions to construct the nitrogen containing compounds.[8-14] Moreover, some several evidences reported the obtained phenanthridinone from Pd-catalyzed condition but the unsatisfied yield, uneconomical steps and the limitation *N*-substitution were indicated.[15-17]

Phenanthridinone is a common moiety found in various bioactive alkaloids from various sources as shown in Fig. 1. Oxynitidine (**1**), a phenanthridinone derived from Xanthoxylum, is a potential antitumor and antiviral agent.[18] Pancratistatin (**2**), shows a high level of inhibition of in vivo cancer cell growth.[19] Indenoisoquinoline (**3**) acts as a non-camtothecin topoisomerase I inhibitor.[20] Azalamellarin D (**4**) is an extension of lamellarin D (**5**) investigation in our group.[21] The lactam on the B-ring of an azalamellarin replaces the lactone structure of lamellarin, improving the compound's stability.[21] Lamellarin D (**5**) is an interested compound that has received attention from many research institutions including our group.[22] Biological and synthetic studies of lamellarin D in particular have been reported extensively.[23]

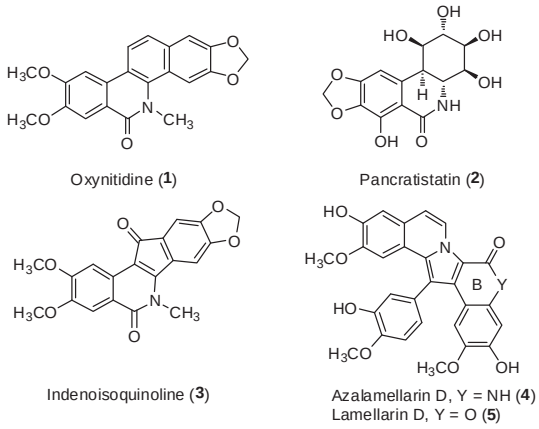



**Figure 1.** Structures of oxynitidine (**1**), pancratistatin (**2**), indenoisoquinoline (**3**), azalamellarin D (**4**), and lamellarin D (**5**).

Reported herein is the methodology for the one-pot synthesis of a small-molecule lactam model that is analogs to the core of many pharmacologically active compounds. Developing an efficient synthesis for this lactam will aid in optimizing reaction conditions for biologically active lactam derivatives.

## Results and Discussion

### Cu(I)-mediated and microwave-assisted lactone and lactam formation: Competition of C-O and C-N bond formation

Methyl-2-bromocarboxylate (**6**) and homoveratylamine (**7**) were used to study the Cu(I)-catalyzed C-N bond formation of the corresponding phenanthridinone **8**.[21,24] Various bases and bidentate ligands (a to e in Fig. 2) were screened with the subcritical water under the benign conditions to perform the green chemistry aspect as our previous work.[25] The

competition of intermolecular C-N bond formation and intramolecular C-O bond formation was observed and afforded a mixture of the target compound **8** and benzopyranone **9** in moderate to good yields as summarized in the Table 1.

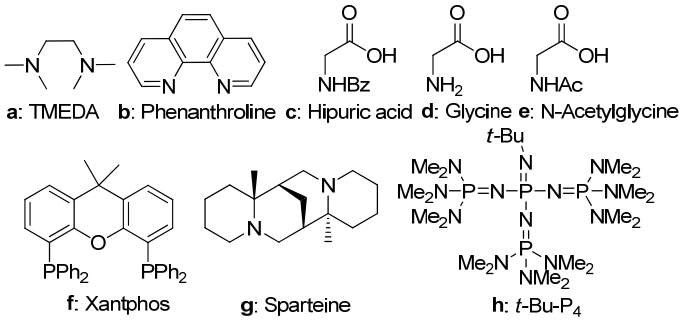
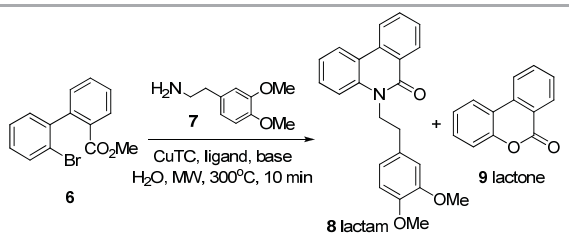



**Figure 2.** Screened ligands **a-g** and phophazine base **h** in this study.

First, we examined the possibility of Cu(I)-catalyzed C-N bond formation of compounds **6** and **7** with subcritical water at 300 $^{o}$C using CuTC as catalyst in presence of $Cs_2CO_3$ as a base.[25] The C-N bond formation product, lactam **8** and C-O bond formation product, lactone **9** were obtained in 23% and 36% yields, respectively (Table 1, entry 1). The bidentate ligands **a-e** were screened and found that TMEDA (ligand **a**) gave the C-N bond formation product **8** in higher yield (Table 1, entry 2) compared with other bidentate ligands **b-e** which gave lactone **9** in higher yield (Table 1, entries 3 to 6). Some bases were then screened in the presence of TMEDA (ligand **a**), and gave lower yield of the corresponding lactam **8** (Table 1, entries 7 and 8). Interestingly, using phosphazine base *t*-Bu-$P_4$ as base in the absent of ligand, the highest overall yield was obtained in the ration 1:2 of lactam **8**: lactone **9** (Table 1, entry 9). Adjustment the amounts of amine **7** (3 to 10 equivalents) in basic condition and TMEDA as ligand, both lactam **8** and lactone **9** yields relatively increased (Table 1, entries 10 to 12).

The results of Table 1 can summarize that the intramolecular C-O bond formation can performed faster than intermolecular C-N bond formation under the subcritical water corresponding with our previous report.[25] We then spent an effort to optimize the condition by screening other solvents with different the dielectric constant for microwave irradiation. The specific double C-N bond formation of methyl-2-bromocarboxylate (**6**) and 5 equiv homoveratylamine (**7**) was turned back to study again using 0.5 equiv CuTC, in presence of $Cs_2CO_3$ and TMEDA as shown in Table 2. Using dioxane as solvent in the optimized condition can improved the ratio of compounds 8:9 to 2:1 but reaction carried out in dioxane gave lower overall yield (Table 2, entry 1). When the solvent was changed to DMF the yield of mixture **8** and **9** was increased but gave disappointed ratio in 1:1 (Table 2, entry 2). Mixture of water and DMF or toluene in ratio 1:1 was observed the decrease in ratio of C-N bond formation product (Table 2, entries 3 to 4). Interestingly reaction carried out in toluene gave C-O bond formation in excellent yield (Table 2, entry 5). Whereas using dichloromethane, methanol or solvent free condition gave no reaction (Table 2, entries 6 to 8). Among different alkyl groups, methyl ester gave the best result (Table 2, entries 9 to 10).

**Table 1.** Reaction of methyl-2-bromocarboxylate and homoveratylamine mediated by CuTC in the subcritical water condition with screening of bases and ligands.[a]

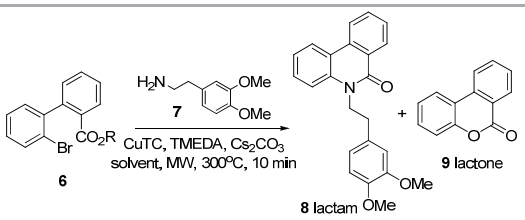



| Entry | **7** (equiv) | Base | Ligand | Yield **8** (%)[b] | Yield **9** (%)[b] |
|---|---|---|---|---|---|
| 1 | 5 | $Cs_2CO_3$ | - | 23 | 36 |
| 2 | 5 | $Cs_2CO_3$ | A | 31 | 18 |
| 3 | 5 | $Cs_2CO_3$ | B | 9 | 41 |
| 4 | 5 | $Cs_2CO_3$ | C | 23 | 50 |
| 5 | 5 | $Cs_2CO_3$ | D | 10 | 37 |
| 6 | 5 | $Cs_2CO_3$ | E | 11 | 38 |
| 7 | 5 | $K_2CO_3$ | A | 26 | 33 |
| 8 | 5 | NaO*t*-Bu | A | 16 | 34 |
| 9 | 5 | *t*-Bu-$P_4$ | - | 31 | 60 |
| 10 | 3 | $Cs_2CO_3$ | A | 20 | 36 |
| 11 | 7 | $Cs_2CO_3$ | A | 34 | 36 |
| 12 | 10 | $Cs_2CO_3$ | A | 34 | 49 |

[a] Unless otherwise noted, the reactions were performed in a 10 mL microwave vessel: 0.5 equiv of CuTC, 300°C, 10 min.

[b] Isolated yields of pure product after PTLC on silica.

**Table 2.** Screening of solvents and alkyl groups.[a]




| Entry | R | Solvent | Yield **8** (%)[b] | Yield **9** (%)[b] |
|---|---|---|---|---|
| 1 | Me | Dioxane | 17 | 10 |
| 2 | Me | DMF | 25 | 24 |
| 3 | Me | $H_2O$:DMF 1:1 | 25 | 51 |
| 4 | Me | $H_2O$:Toluene 1:1 | 18 | 31 |
| 5 | Me | Toluene | trace | 95 |
| 6 | Me | DCM | NR[c] | NR[c] |
| 7 | Me | MeOH | NR[c] | NR[c] |
| 8 | Me | -[d] | NR[c] | NR[c] |
| 9 | Et | $H_2O$ | 23 | 39 |
| 10 | *i*-Pr | $H_2O$ | 19 | 18 |

[a] Unless otherwise noted, the reactions were performed in a 10 mL microwave vessel: 5 equiv of homoveratrylamine, 0.5 equiv of CuTC, 300°C, 10 min.

[b] Isolated yields of pure product after PTLC on silica.

[c] NR: no reaction.

[d] Solvent free reaction.



**Pd(II)-mediated C-N bond formation: Optimized conditions**

The competition of Cu(I)-mediated C-N and C-O bond formation gave a mixture of phenanthridinone **8** and benzopyranone **9** under microwave irradiation. To study the selectivity of C-N bond formation, we then turn our interest to palladium catalyst. The reaction of methyl-2-bromocarboxylate (**6**) and homoveratylamine (**7**) was studied using Pd(II)-catalyzed C-N bond formation in various bases and ligands as shown in Table 3.

We initially attempted to use 5 mol% $Pd(OAc)_2$, 10 mol% xantphos (ligand **f**) and $Cs_2CO_3$ in 1,4-dioxane at 300 $^oC$ under microwave irradiation for 10 min and obtained only the C-O bond formation of benzopyranone **9** in 60% yield (Table 3, entry 1). Dramatically change when using conventional heating at 115 $^oC$ for 23 h, the double C-N bond formation product **8** was obtained in moderate yield (55% yield) together with undesired product, carbazole **10**[32] in 35% yield (Table 3, entry 2). Increasing the amount of catalyst gave no significant increase in the yield of product **8** and carbazole **10** (Table 3, entry 3) as well as using $Pd(TFA)_2$ as a catalyst instead of $Pd(OAc)_2$ (Table 3, entry 4).

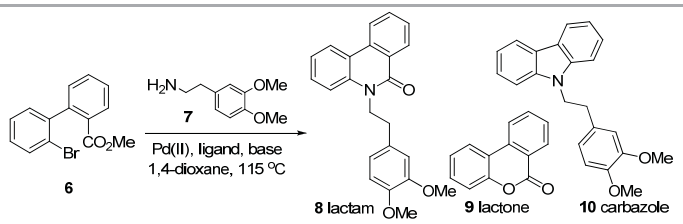
The mechanism of the competition of C-N bond formation was proposed as shown in Scheme 1. The oxidative addition of a palladium complex inserted into the C-X bond and followed by reacting with amine to form the intermediate **B**. The reductive elimination of **B** afforded the C-N bond formation product **C** which was further lactamization to give the target product **8**.[26] The carbazole **10** was obtained by the tandem decarboxylative palladium-catalyzed intramolecular C-N bond formation.[27-28] It may arise from palladium complex **E** produced by the palladium-catalyzed decarboxylation and initial oxidative addition of carboxylate **D** followed by either the second oxidative addition to form palladacycle[29] **F** or palladium complex **G** which underwent reductive elimination to give carbazole **10**.

The yield of **8** was dramatically increased to 72% yield when 5 mol% $PdCl_2$ was used instead in the same protocol using 10 mol% xantphos as a ligand and $Cs_2CO_3$ as a base in refluxing 1,4-dioxane at 115 $^oC$ (Table 3, entry 5). We then paid an effort to optimize the reaction condition by screening other bases (Table 3, entries 6 to 9) and found that $Cs_2CO_3$ gave the best result. Sparteine (ligand **g**) and phosphazine (*t*-$BuP_4$, **h**) were also studied and gave lower yield (Table 3, entries 10 to 12).

With the effective condition for the synthesis of phenanthridinone **8**, we then directed our attention to the synthesis of a small library of phenanthridionone analogues using both 5 mol% $PdCl_2$ and 10 mol% $Pd(TFA)_2$ as the catalyst, xantphos as ligand, and $Cs_2CO_3$ as base in refluxing 1,4-dioxane at 115 $^oC$. The phenanthridionone analogues **11-17** were obtained in 12-86% yields as shown in Table 4. The *N*-benzylated phenanthridinone **11** was obtained in 45% and 86% yields using $PdCl_2$ and $Pd(OAC)_2$ as catalyst, respectively (Table 4, entries 1 to 2). The increasing of the electron donating group such as methoxy group of the benzylamine derivatives affected to the yield of Pd-catalyzed C-N bond formation products (Table 4, entries 3 to 6).



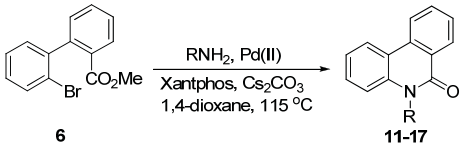
**Table 3.** Screening of palladium (II), bases and ligands.

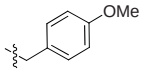
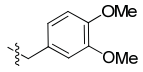
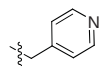
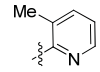



| Entry | Pd(II) (mol%) | Base | Ligand (mol%) | Time (h) | **8**[b] (%) | **9 (10)**[b] (%) |
|---|---|---|---|---|---|---|
| 1[a] | $Pd(OAc)_2$ (5) | $Cs_2CO_3$ | F (10) | 0.16 | - | **9**, 60 |
| 2 | $Pd(OAc)_2$ (5) | $Cs_2CO_3$ | F (10) | 23 | 55 | **10**, 35 |
| 3 | $Pd(OAc)_2$ (10) | $Cs_2CO_3$ | F (20) | 23 | 53 | **10**, 32 |
| 4 | $Pd(TFA)_2$ (10) | $Cs_2CO_3$ | F (20) | 23 | 45 | **10**, 31 |
| 5 | $PdCl_2$ (5) | $Cs_2CO_3$ | F (10) | 23 | 72 | trace |
| 6 | $PdCl_2$ (5) | $Na_2CO_3$ | F (10) | 23 | NR[c] | - |
| 7 | $PdCl_2$ (5) | $K_2CO_3$ | F (10) | 23 | 25 | - |
| 8 | $PdCl_2$ (5) | $Ag_2CO_3$ | F (10) | 23 | NR[c] | - |
| 9 | $PdCl_2$ (5) | $K_3PO_4$ | F (10) | 23 | NR[c] | - |
| 10 | $PdCl_2$ (5) | $Cs_2CO_3$ | G (10) | 23 | 45 | - |
| 11 | $PdCl_2$ (5) | $Cs_2CO_3$ | H (10) | 23 | 32 | - |
| 12 | $PdCl_2$ (5) | $Cs_2CO_3$ | H (10) | 36 | 40 | - |

[a] The reactions were performed with 2.0 equiv of base, 0.05 equiv of palladium except entries 3,4 and 5.0 equiv of amine in 5 mL of 1,4-dioxane under Ar at 115°C. [b] Isolated yields of pure product after PTLC on silica (30% EtOAc:Hexane). [c] NR: no reaction.

**Table 4.** Synthesis of phenanthridin-4-one derivatives.




| Entry | Product (R) | Pd(II) (mol%) | Time (h) | lactam (%)[b] |
|---|---|---|---|---|
| 1[a] |  | $PdCl_2$ (5) | 23 | **11**, 45 |
| 2 | | $Pd(OAc)_2$ (10) | 23 | **11**, 86 |
| 3 |  | $PdCl_2$ (5) | 57 | **12**, 34 |
| 4 | | $Pd(OAc)_2$ (10) | 23 | **12**, 75 |
| 5 |  | $PdCl_2$ (5) | 23 | **13**, 12 |
| 6 | | $Pd(OAc)_2$ (10) | 23 | **13**, 29 |
| 7 |  | $PdCl_2$ (5) | 23 | **14**, 11 |
| 8 | | $Pd(OAc)_2$ (10) | 28 | **14**, 26 |
| 9 |  | $PdCl_2$ (5) | 23 | **15**, 65 |
| 10 | | $Pd(OAc)_2$ (10) | 29 | **15**, 6 |
| 11 |  | $PdCl_2$ (5) | 69 | **16**, 32 |
| 12 | | $Pd(OAc)_2$ (10) | 55 | **16**, 2 |
| 13 |  | $PdCl_2$ (5) | 23 | **17**, 32 |
| 14 | | $Pd(OAc)_2$ (20) | 23 | **17**, 64 |
| 15 | allyl | $PdCl_2$ (5) | 23 | NR[c] |
| 16 | *n*-propyl | $PdCl_2$ (5) | 23 | NR[c] |

[a] The reactions were performed with 2.0 equiv of base, 0.05 or 0.1 equiv of palladium and 5.0 equiv of amine in 5 mL of 1,4-dioxane under Ar at 115°C. [b] Isolated yields of pure product after PTLC on silica (30% EtOAc:Hexane). [c] NR: no reaction.

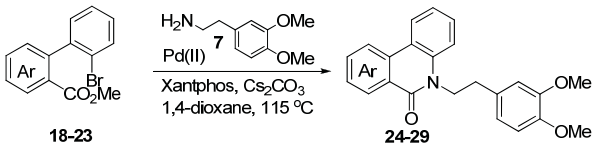


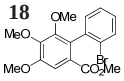
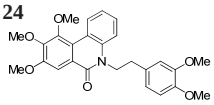
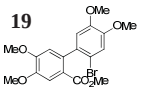
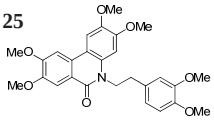
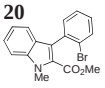
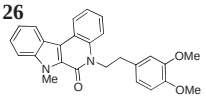
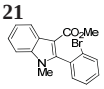
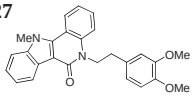
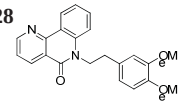
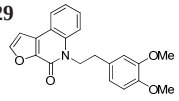
## Pd(II)-mediated C-N bond formation: Synthesis of phenanthridinone derivatives

In order to demonstrate our strategy in the synthesis of heterocyclic benzopyranone derivatives, we then pay our attention to synthesize a small group of phenanthrolines using the optimized condition as shown in Table 5. The polyoxygenated phenanthrolines **24** and **25** were prepared from tri- and tetra-methoxybiaryl carboxylates **18** and **19**, respectively, in poor to moderate yield (Table 5, entries 1-4).

Fused heteroaromatic rings such as tetracyclic 7-methyl-5H-indolo[2,3-*c*]quinolin-6(7H)-one **26** and 11-methyl-5H-indolo[3,2-*c*]quinolin-6(11H)-one **27** were also prepared from the corresponding 2-arylindole-3-carboxylates **20** and 3-arylindole-2-carboxylates **21** in poor to moderate yield (table 5, entries 5-6). Benzo[*h*][1,6]naphthyridin-5(6*H*)-one **28** was synthesized from 2-arylnicotinate **22** in moderate yield (Table 5, entries 7-8). The last heterocyclic compound, furo[2,3-*c*]quinolin-4(5H)-one **29** was also succeeded from 2-arylfuran-3-carboxylate **23** in poor yield (Table 5, entries 9-10).

**Table 5.** Synthesis of various lactam derivatives.




| Entry | Substrate | Product | cond[a] | Time (h) | Yield (%)[b] |
|---|---|---|---|---|---|
| 1 | **18**  | **24**  | A | 12 | 48 |
| 2 | | | B | 30 | 8[c] |
| 3 | **19**  | **25**  | A | 17 | 40 |
| 4 | | | B | 60 | 26 |
| 5 | **20**  | **26**  | A | 17 | 55 |
| 6 | **21**  | **27**  | A | 17 | 13 |
| 7 | **22**  | **28**  | A | 17 | 26 |
| 8 | | | B | 17 | 53 |
| 9 | **23**  | **29**  | A | 85 | 4 |
| 10 | | | B | 40 | 9 |

[a] Condition A: 0.1 equiv of $PdCl_2$, 0.2 equiv of Xantphos, 7.0 equiv of $Cs_2CO_3$ and 5.0 equiv of amine in 1,4-dioxane under Ar at 115°C; Condition B: 0.1 equiv of $Pd(OAc)_2$, 0.1 equiv of Xantphos, 2.0 equiv of $Cs_2CO_3$ and 5.0 equiv of amine in 1,4-dioxane under Ar at 115°C.

[b] Isolated yields of pure product after PTLC on silica (30% EtOAc:Hexane).

[c] Recovered starting material 81%.

## Conclusion

In summary, we have demonstrated a new method based on the Pd(II)-catalyzed domino coupling/lactamization process to construct phenanthridinones from various 2-halobiaryl-carboxylates. The efficiency and functional group effect were studied and applied

to synthesize a number of phenanthridinones and heterocyclic-fused quinolinone derivatives. This approach is particularly efficient route to pharmaceutically heterocyclic and polycyclic quinolinone scaffolds.

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## SUBPROJECT IV

### Synthesis of scandione and calophione A

**Introduction**

Hydroxybenzil **1** is a core structure of a small family of natural products isolated from the Leguminosae family of plants.[1] Their structure typically consist of 1,2-diarylethane-1,2-diones framework which were often occurred concurrently with other flavanoids as common constituents of this plan family (Figure 1). Licoagrodione (**2**), isolated from a Chinese herb, *Glycyrrhiza glabra* (licorice), was found to exhibit antimicrobial activity.[2] Scandione (**3**) was isolated from the stem of a Thai medicinal plant, *Derris scandens*.[3] Calophione A (**4**), isolated from the roots of *Tephrosia calophylla*, was tested with mouse macrophage cells (RAW) and colon cancer cells HT-29 and showed significant cytotoxicity with $IC_{50}$ of 5.00 (RAW) and 2.9 μM (HT-29), respectively.[4] Recently, tenuifodione (**5**) was isolated from the whole plant of *Iris tenuifolia* Pall (Iridaceae), the first plant isn't the congener with previous works.[5] Whereas dimethylethylamino ethanediones, saxoguattine (**6**)[6] and cryptopleurospermine (**7**),[7] are widely distributed in the families, Annonaceae, Lauraceae, Papaveraceae, and have been reviewed as a group of oxoprotopine alkaloids which also showed antibacterial activity.

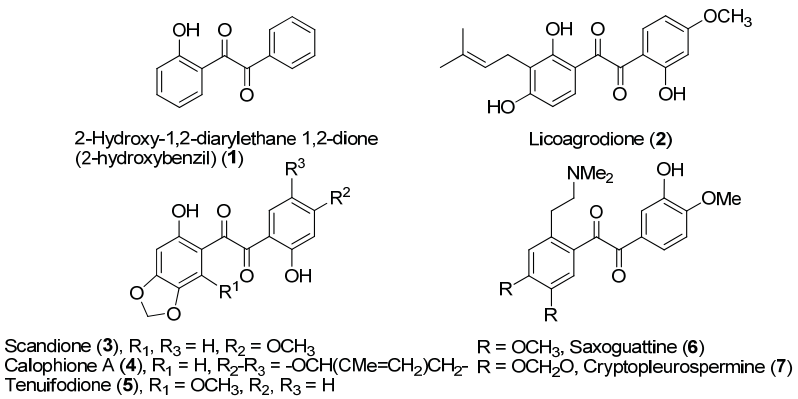


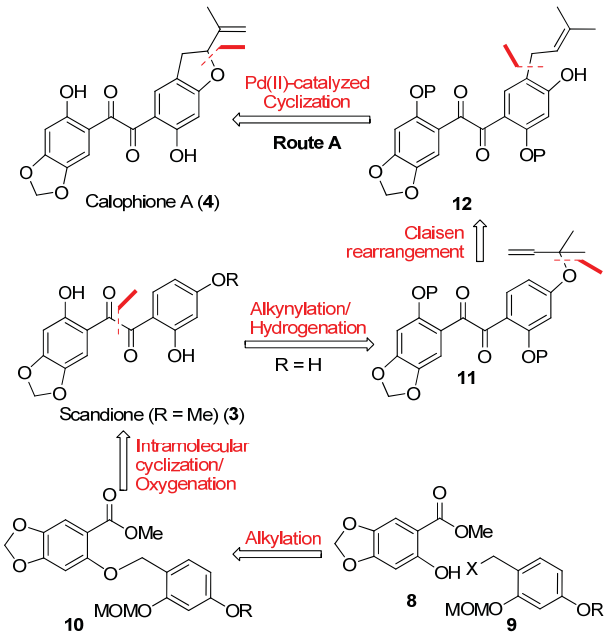

**Figure 1.** Selected unsymmetrical bioactive benzils

In our ongoing research on the organolithiation,[8,9] we previously reported the synthesis of unsymmetrical 2-hydroxybenzils involving the intramolecular cyclization of anionic benzylic ester of the aryl benzyl ether followed by oxidation employing dioxirane.[10] Licoagrodione (**2**) was then synthesized using the Claisen rearrangement of isoprene unit under neutral condition and microwave irradiation.[10] Herein we reported the first synthesis of scandione (**3**) and calophione A (**4**) using our developed method and palladium(II)-catalyzed oxidative cyclization.[11] The antibacterial activity, antioxidant activity and cytotoxicity results of unsymmetrical benzils were also reported.

**Results and Discussion**

The synthetic plan of natural unsymmetrical benzils **3** and **4** could be constructed by the intramolecular cyclization of the anionic benzylic ester of the aryl benzyl ether **10** followed by oxidation employing dioxirane to afford the target compound **3** as shown in Scheme 1. Benzylation of methyl salicylate derivative **8** with benzyl halides **9** could afford the key intermediates, aryl benzyl ethers **10**. The "C-5" isoprene unit could be introduced into the

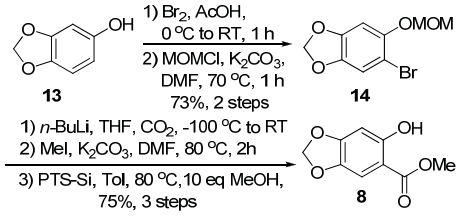
core molecule by alkynylation followed by hydrogenation to afford the alkenyl ether **11**. Claisen rearrangement of **11** will give the prenylated 2-hydroxybenzil **12** which further cyclization using palladium(II)-catalyzed dihydrofuran formation to afford calophione A (**4**).




**Scheme 1.** Retrosynthetic plan for the synthesis of scandione **(3)** and calophione A (**4**).

## Synthesis of Scandione (3)

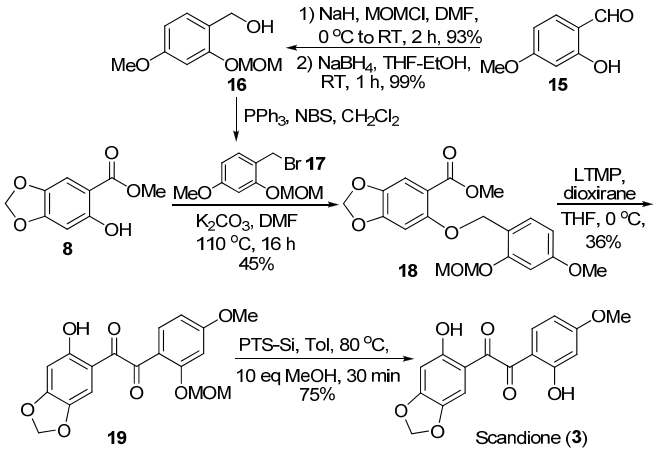
To synthesize scandione (**3**) and calophione A (**4**), the corresponding methyl salicylate **8** was prepared in five steps from sesamol **13**. Bromination and protection gave bromo compound **14** in 73% yield (two steps). The key step was the lithium-halogen exchange/carboxylation of **14** to give carboxylic acid which was further methylation and deprotection with *p*-TsOH immobilized on silica (PTS-Si) in toluene and minute amount of methanol under constant temperature 80 $^{o}$C.[12] Methyl salicylate derivative **8** was obtained in 75% yield (three steps) as shown in Scheme 2.




**Scheme 2.** Preparation of methyl salicylate (**8**).

The benzylation of methyl salicylate **8** with benzyl bromide **17** which was prepared in three steps from 2-hydroxy-4-methoxy benzaldehyde **15** afforded *O*-benzyl ether **18**. The corresponding benzil **19** were obtained in moderate yield using our developed method.[10] The key reaction involved the intramolecular cyclization of the anionic benzylic ester of methyl *O*-benzyl salicylate **18** with 4.5 equiv LTMP in THF followed by the oxygenation with

dioxirane. Deprotection with PTS-Si in toluene[12] and minute amount of methanol under constant temperature 80 °C in 30 min gave scandione (**3**) in 75% yield as shown in Scheme 3. The overall yield of the synthesis of compound **3** is 6% yield in eight steps from two available precursors.




**Scheme 3.** Synthesis of scandione (**3**).

## Synthesis of Calophione A (4) (Route A)

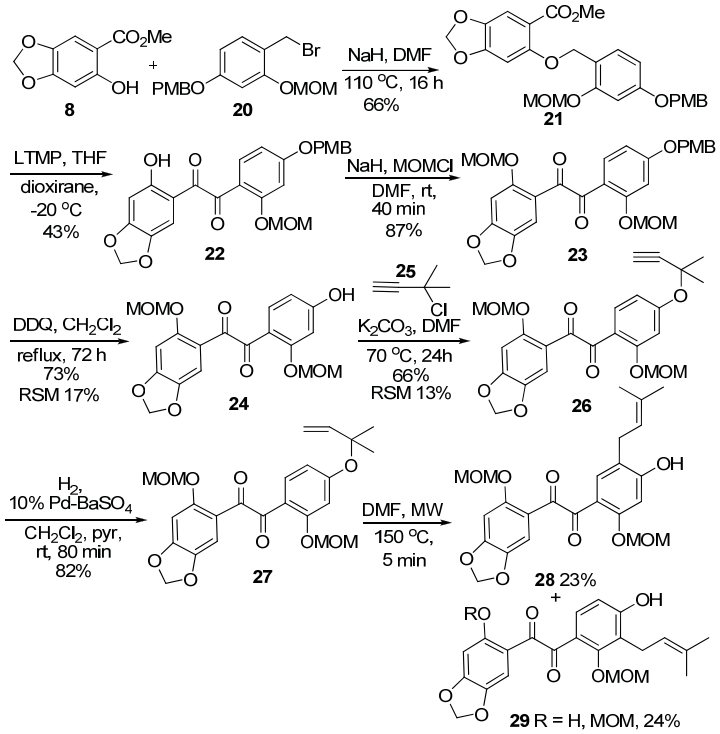
Having established the approach for the synthesis of scandione (**3**), we sought to develop the synthesis of calophione A (**4**) via the same benzil skeleton and further introduction of "C-5" dimethylallyl group equivalent. The palladium(II)-catalyzed oxidative cyclization will be studied to establish the benzofuran of compound **4**.[11]

The benzylation of methyl salicylate **8** with benzyl bromide **20**[13] afforded *O*-benzyl ether **21** in moderate yield (66%) as shown in Scheme 4. The unsymmetrical benzil **22** were obtained in 43% yield using the intramolecular cyclization of the anionic benzylic ester of methyl *O*-benzyl salicylate **21** with 4.5 equiv LTMP in THF followed by the oxygenation with dioxirane.[10] The protection of a hydroxyl group with methoxy methylene chloride (MOMCl) gave **23** in good yield following the deprotection of *p*-methoxybenzyl (PMB) group gave the calophione A core **24** in good yield. "C-5" dimethylallyl group equivalent was introduced into the molecule as the corresponding alkynyl ether of the phenolic group by reacting **24** with 3-chloro-3-methyl-1-butyne **25** in the presence of $K_2CO_3$ in DMF to afford the corresponding alkyne **26** in 66% yield.[14] Lindlar's reduction of alkyne **26** gave alkene **27** in good yield.[15] The Claisen rearrangement was then used in further step to prepare the corresponding C-5 isoprene diketone **28**. However the competition products **28** and **29** were obtained in ratio 1:1.

The dihydrobenzofuran formation of the corresponding isoprene **28** was then studied using 5 mol% $Pd(TFA)_2$ and 20 mol% pyridine as ligand in the presence of $Na_2CO_3$ and MS4Å in toluene at 80 °C.[11d] The mixture was observed and the $^1$H NMR spectra showed the key peak of two isomers, dihydrobenzofuran **30** and chromene **31** moiety. Compound **30** was obtained in poor yield (16%) after chromatography.

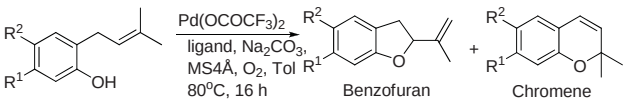
Therefore we next re-examined the palladium(II)-catalyzed oxidative cyclization to construct the dihydrobenzofuran as shown in Table 1. 2'-Hydroxyarylbutenes were studied to form the dihydrobenzofuran system base on the previous condition reported by Stoltz *et*

*al.*[11d] A mixture of competitive cyclization, dihydrobenzofuran and chromene, was obtained in moderate yields. Using the 10 mol% $Pd(TFA)_2$ and 40 mol% pyridine as ligand in the presence of 2 equiv $Na_2CO_3$ and MS4Å (500 mg/mmol) in toluene at 80 °C under oxygen atmosphere gave the dihydrobenzofuran **34** as a major cyclization product. As shown in Table 1, dihydrobenzofurans **34** was succeeded using the palladium(II)-catalyzed oxidative cyclization. It led us move to synthesize the target calophione A (**4**) in the alternative route.




**Scheme 4.** Synthesis of calophione A (**4**) route A.

**Table 1.** Screening of the Pd(II)-catalyzed oxidative cyclization.

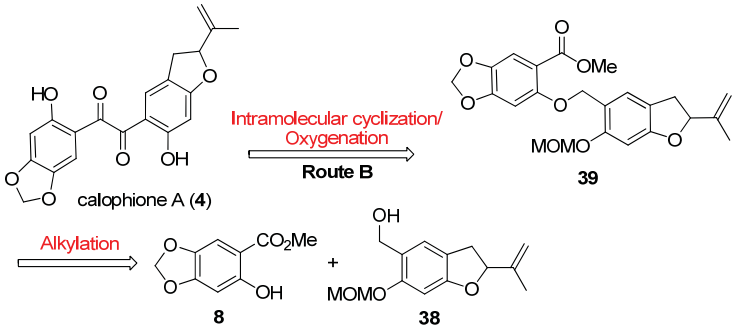



| Entry | $R^1$ | $R^2$ | Condition[b] | yield [%][a] | |
|---|---|---|---|---|---|
| | | | | **benzofuran** | **chromene** |
| 1 | OMOM | COCOAr | A | **30**: 7[c] | **31**: trace |
| 2 | OMOM | COCOAr | B | **30**: 16 | **31**: trace |
| 3 | H | CHO | B | **32**: 47 | **33**: trace |
| 4 | H | COOMe | B | **34**: 23 | **35**: 3 |
| 5 | OMOM | COOMe | B | **36**: 49 | **37**: 11 |

[a] Isolated yields of pure product after PTLC on silica. [b] condition A: Condition A: 5 mol% $Pd(TFA)_2$, 40 mol% sparteine sulfate; condition B: 5-20 mol% $Pd(TFA)_2$, 20-40 mol% pyridine. [c] Recovered starting materials 6%.

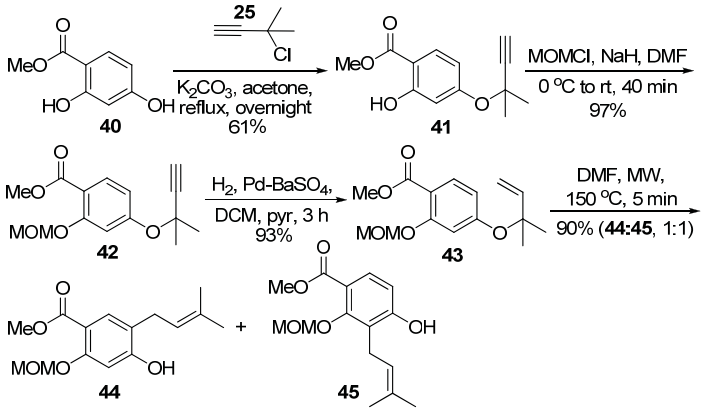
## Synthesis of Calophione A (4) (Route B)

Scheme 5 showed the short synthesis of calophione A (**4**) in route B. Compound **4** could be constructed by two segments, methyl salicyalte derivative **8** and dihydrofuran **38** via the key intermediate, alkylation product **39**. The intramolecular cyclization following the oxygenation could give the target **4**.




**Scheme 5.** Retrosynthetic plan for the synthesis of calophione A (**4**).

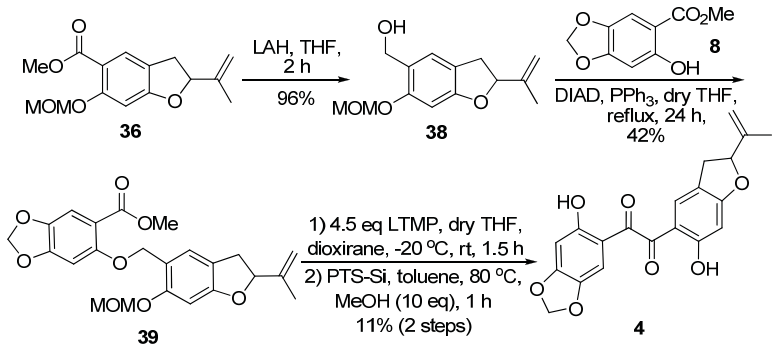
The synthesis of dihydrofuran **36** was succeeded in five steps from methyl 2,4-dihydroxy benzoate **40**. The selective alkynylation at *para*-hydroxyl group of **40** with 3-chloro-3-methyl-1butyne **25** in the presence of $K_2CO_3$ in acetone gave alkyne **41** in 61% yield. The protection at *ortho*-hydroxyl group with MOMCl in the presence of NaH in DMF gave alkyne **42** in quantitative yields. Lindlar's reduction of alkyne **42** gave alkene **43** in 90% yield. Claisen rearrangement of alkene **43** in DMF with microwave irradiation gave a mixture of alkenes **44** and **45** in very good yield in raito 1:1 as shown in Scheme 6. Compound **44** was then cyclized to dihydrobenzofuran **36** using 10 mol% $Pd(TFA)_2$ and 40 mol% pyridine as ligand in the presence of $Na_2CO_3$ and MS4Å in toluene at 80 $^oC$ under oxygen atmosphere overnight (Table 1, entry 5).




**Scheme 6.** Synthesis of compound **44**.

To synthesize the target compound **4**, dihydrobenzofuran **36** was then reduced with LAH in THF to give benzyl alcohol **38** in excellent yield. Mitsunobu reaction of compounds **8** and **38** gave the corresponding methyl *O*-benzyl salicylate **39** in moderate yield. Calophione A (**4**) were successful obtained in moderate yield using the intramolecular cyclization of the anionic benzylic ester of compound **39** with 4.5 equiv LTMP in THF followed by the oxygenation with dioxirane.[10] Deprotection with PTS-Si in toluene and minute amount of methanol under

constant temperature 80 °C in 1 h gave calophione A (**4**) in good yield as shown in Scheme 7. The overall yield of the synthesis of compound **4** is 0.54% yield in nine steps from commercial available compound **40**.




**Scheme 7.** Synthesis of calophione A (**4**).

## Antibacterial Activity

A panel of four bacterial strains was used to evaluate antimicrobial activity of unsymmetrical benzils **1** to **4** using disc diffusion susceptibility test accordance with NCCLS Performance Standards.[16] The test organisms were *Escherichia coli* (TISTR 887, ATCC 25922), *Staphylococcus aureus* (TISTR517, ATCC 25923), *Pseudomonas aeruginosa* (TISTR 1467, ATCC 27853), and *Salmonella typhimurium* (TISTR 292, ATCC 13311). Pure cultures were selected and transferred from an agar plate culture into a tube containing saline broth (3-5 mL). The broth cultures were adjusted to match the turbidity of the 0.5 McFarland standard. Adjusted inocolumns suspension was swabbed uniformly onto dried surface of a Mueller-Hinton agar (MHA) plate.

Compounds **1** to **4**, dissolved in DMSO, were prepared to be 2 µg/mL (40 mg/disc) and loaded on six mm sterile disc. The loaded disc was placed onto the surface of the inoculated agar plate. The diameters of zones of complete inhibition were measured in millimeter after incubation at 35 °C for 18 h. Gentamycin and chloramphenicol were used as the positive control drugs.

Antimicrobial result showed that compounds **1** and **2** have positive result against gram positive bacteria, *S. aureus*, at 40 µg/disc concentration.

## Cancer Chemoprevention Activity[17]

The radical scavenging, antioxidant, and aromatase inhibitory activities of unsymmetrical benzils **1-4** were also evaluated as shown in Table 2. Licoagrodione (**2**) showed cytotoxicity with HL-60.[18] Compound **2** inhibited aromatase activity with $IC_{50}$ value of 5.9 µM.[19] Compound **2** also exhibited potent antioxidant activity in the oxygen radical absorbance capacity (ORAC) assay with 25.2 ORAC units.[20]

**Table 2.** Radical scavenging, antioxidant, and aromatase inhibitory activities of unsymmetrical benzils **1-4**.[a]

| benzil | $IC_{50}$ (μM) | | | | ORAC[b] |
|---|---|---|---|---|---|
| | DPPH | HL-60[18] | XXO | AIA | |
| **1** | inactive | n.d.[c] | inactive | inactive | 0.6 |
| **2** | inactive | 41.3 | inactive | 5.9 | 25.2 |
| **3** | inactive | n.d.[c] | inactive | inactive | n.d.[c] |
| **4** | inactive | n.d.[c] | inactive | inactive | n.d.[c] |

[a] Positive controls for each assay are as follows: DPPH: ascorbic acid ($IC_{50}$ = 21.2 μM); XXO: superoxide dismutase (scavenging 100% of the radical); IXO: allopurinol ($IC_{50}$ = 3.0 μM); aromatase inhibition: ketoconazole ($IC_{50}$ = 2.4 μM). [b] The results are expressed as ORAC units: 1 ORAC unit equals the net protection of β-phycoerythrin produced by 1 μM of Trolox. [c] n.d. = not determined.

**Cytotoxicity Activity**

Only calophione A (**4**) was previously reported the cytotoxicity with mouse macrophage cells (RAW) and colon cancer cells HT-29.[4] Our unsymmetrical benzils **1-4** were then evaluated for cytotoxicity with a panel of four human tumor cell lines; cholangiocarcinoma HUCCA-1, lung carcinoma A549, hepatoblastoma HepG2, and T-lymphoblast (acute lymphoblastic leukemia) MOLT-3 using MTT and XTT assays depending on the cell-line types as shown in Table 3.[21] The results showed that calophione A (**4**) showed the highest $IC_{50}$ values for all cancer cell lines tested as compared to other benzils **1-3**. Compound **4** exhibited T-lymphoblast (acute lymphoblastic leukemia) MOLT-3 in the micromolar range ($IC_{50}$ = 9.25 μM).

**Table 3.** Biological activities of synthetic unsymmetrical benzil derivatives **1-4** in MTT and XTT assays.[a]

| Entry | Compounds | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|---|
| | | HuCCA-1[b,f] | A549[c,f] | HepG2[d,f] | MOLT-3[e,g] |
| 1 | **1** | Inactive | Inactive | 96.23 | 44.50 |
| 2 | **2** | 83.8 | 71.0 | 39.8 | 17.12 |
| 3 | **3** | Inactive | Inactive | 122.1 | 71.7 |
| 4 | **4** | Inactive | 124.26 | 99.65 | 25.98 |
| 5 | Etoposide | ND | ND | 29.8 | 0.047 |
| 6 | Doxorubicin | 0.83 | 0.63 | 0.57 | ND |

[a] DMSO solution (10 mg/mL). [b] HuCCA-1: Cholangiocarcinoma. [c] A549: Lung Carcinoma. [d] HepG2: Hepatoblastoma. [e] MOLT-3: T-lymphoblast (acute lymphoblastic leukemia). [f] MTT assay. [g] XTT assay. ND = Not determined.



## Conclusions

In summary, we have reported the total synthesis of two natural unsymmetrical benzils, scandione (**3**) and calophione A (**4**). The key reaction involved the intramolecular cyclization of the anionic benzylic ester of methyl *O*-benzyl salicylate with LTMP in THF followed by the oxygenation with dioxirane. The Pd(II)-catalyzed oxidative cyclization of dihydrobenzofuran formation was also investigated. Scandione (**3**) was accomplished 6% overall yield in eight steps and calophione A (**4**) was succeeded in 0.54% overall yield in nine steps. Benzils **1** and **2** showed potent antimicrobial activity. Compound **2** also showed cytotoxicity with HL-60 and antioxidant activity.

## Experimental Section

**1. General Methods:** Melting points were measured with a Thermo Fisher Scientific IA920 digital melting point apparatus and reported without correction. $^{1}$H-Nuclear magnetic resonance ($^{1}$H NMR) spectra were recorded on Varion Germini2000, Bruker AV-300, and Bruker AV-400 NMR instruments at 200, 300, and 400 MHz, respectively, using deuterochloroform as solvents with tetramethylsilane as an internal standard. $^{13}$C-Nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded on Varion Germini2000, Bruker AV-300, and Bruker AV-400 NMR instruments at 50, 75, and 100 MHz, respectively, using deuterochloroform with tetramethylsilane as an internal standard and dimethylsulfoxide-D6 for some compound. FTIR spectra were obtained on the Spectrum One FTIR spectrometer, Perkin Elmer System with the universal ATR (UATR) accessory. Mass spectra were performed with an AEI-MS-902. High-resolution mass spectra were performed with a MicroTOF$_{LC}$, Bruker Daltonics. Column chromatography was carried out using Fluka aluminum oxide (type 507 C neutral; 100-125 mesh) and Merck silica gel (70-230 mesh ASTM). Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were carried out on silica gel (E. Merck PF 254). All reagents were purified and dried according to the standard procedures. Solvents were removed by using Eyela Aspirator A-2S and Büchi Rotavapor R110. All products were evacuated by a Christ Freeze Dryer Unit Alpha l/6, to remove the last traces of solvents.

*5-Bromo-6-(methoxymethoxy)benzo[d][1,3]dioxole* ***(14):*** To a solution of bromine (0.87 mL, 23.9 mmol) in 25 mL of glacial acetic acid was added dropwise over 10 min period to a stirred solution of sesamol **13** (3.00 g, 21.7 mmol) in glacial acetic acid (25 mL) at 0 $^{o}$C and stirred at room temperature for 1 h. The reaction mixture was quenched with water (30 mL) and extracted with $CH_2Cl_2$ (3 × 30 mL). Combined organic layers were washed with water, brine, dried over anh. $Na_2SO_4$ and concentrated to give pale yellow solid. The residue was dissolved with DMF (38 mL) then, treated with $K_2CO_3$ (4.50 g, 32.6 mmol), MOMCl (2.48 mL, 32.6 mmol) and heated at 70 $^{o}$C for 1 h. The reaction mixture was quenched with water and extracted with ethyl acetate (3 × 25 mL), washed with water, brine, dried over anh. $Na_2SO_4$ and concentrated to furnish brown oil. The crude product was then purified by column chromatography using 5-10% ethyl acetate in hexane to afford compound **14** (4.15 g, 73%) as a colorless oil. $R_f$ = 0.49 (EtOAc/hexane, 3:7). $^{1}$H NMR (200 MHz, $CDCl_3$): $\delta$ = 6.98 (s, 1 H, Ar), 6.78 (s, 1 H, Ar), 5.95 (s, 2 H, OC$H_2$O), 5.13 (s, 2 H, OC$H_2$), 3.53 (s, 3 H, OC$H_3$) ppm. $^{13}$C NMR (50 MHz, $CDCl_3$): $\delta$ = 148.6, 147.6, 143.1, 112.2, 103.1, 101.8, 100.0, 96.1, 56.3 ppm. IR (UATR): ν = 2953, 2901, 1501, 1473, 1383 cm$^{-1}$. EI-MS: *m/z* (%) = 262 (93) $[C_9H_9{}^{81}BrO_4]^+$, 260 (97) $[C_9H_9{}^{79}BrO_4]^+$, 181 (100). HRMS (microTOF): calcd. for $C_9H_{10}{}^{81}BrO_4$ $[M + H]^+$ 262.9737; found 262.9731, for $C_9H_{10}{}^{79}BrO_4$ $[M + H]^+$ 260.9757; found 260.9750.

*Methyl 6-hydroxybenzo[d][1,3]dioxole-5-carboxylate* ***(8):*** *n*-BuLi (2.86 M, 2.87 mL, 8.22 mmol) was added to a stirred solution of compound **14** (1.95 g, 7.47 mmol) in dry THF (76 mL) at -100 $^{o}$C under argon atmosphere. After stirring at -100 $^{o}$C 15 min, an excess of carbon dioxide gas was flow through the reaction mixture and stirred at this temperature for 1 h. The reaction mixture was allowed to room temperature for 30 min and concentrated to dryness. The residue was dissolved with DMF (33 mL) and treated with $K_2CO_3$ (2.06 g, 14.9 mmol). After methyl iodide (1.40 mL, 22.4 mmol) was added, the reaction mixture was heat at 80 $^{o}$C for 2 h. The reaction mixture was quenched with water, extracted with ethyl acetate (3 × 25 mL), washed with water, brine, dried over anh. $Na_2SO_4$ and concentrated to furnish yellow oil. The crude yellow oil was dissolved in toluene (98 mL) and treated with PTS-Si (5.53 g, 4.48 mmol), MeOH (3.48 mL, 74.7 mmol), then heated at 80 $^{o}$C for 1 h. The reaction mixture was filtered and concentrated to provide a yellow solid which was purified by column chromatography using 5% ethyl acetate in hexane to give compound **8** (1.10 g, 75%) as a white solid. $R_f$



= 0.45 (EtOAc/hexane, 2:8). M.p. 100 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 11.05 (s, 1 H, O*H*), 7.17 (s, 1 H, Ar), 6.45 (s, 1 H, Ar), 5.96 (s, 2 H, O$CH_2$O), 3.90 (s, 3 H, O$CH_3$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 170.4, 160.1, 153.8, 140.6, 106.7, 103.9, 101.7, 98.3, 52.0 ppm. IR (UATR): ν = 3065, 1664, 1636, 1480, 1439 cm$^{-1}$. EI-MS: *m/z* (%) = 196 (100) $[M]^+$, 164 (36). HRMS (microTOF): calcd. for $C_9H_9O_5$ $[M + H]^+$ 197.0445; found 197.0441.

*Methyl 6-(4-methoxy-2-(methoxymethoxy)benzyloxy)-benzo[d][1,3]dioxole-5-carboxylate* ***(18):*** A suspension of compound **8** (517 mg, 2.64 mmol) and $K_2CO_3$ (547 mg, 3.96 mmol) in DMF (15 mL) was treated with a solution of 1-(bromomethyl)-4-methoxy-2-(methoxymethoxy)benzene **17**[13] (1.72 g, 6.60 mmol) in DMF (2 mL) at room temperature and then, was heated at 110 °C 16 h. The reaction mixture was quenched with water, extracted with ethyl acetate (3 × 25 mL), washed with water, brine, dried over anh. $Na_2SO_4$ and concentrated to furnish brown oil which was purified by column chromatography using 25-30% ethyl acetate in hexane to give compound **18** (445 mg, 45%) as a pale yellow solid. $R_f$ = 0.29 (EtOAc/hexane, 3:7). M.p. 115–116 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.51 (d, *J* = 8.5 Hz, 1 H, Ar), 7.32 (s, 1 H, Ar), 6.71 (d, *J* = 2.4 Hz, 1 H, Ar), 6.65 (s, 1 H, Ar), 6.59 (dd, *J* = 8.5, 2.4 Hz, 1 H, Ar), 5.97 (s, 2 H, O$CH_2$O), 5.21 (s, 2 H, O$CH_2$), 5.10 (s, 2 H, O$CH_2$O$CH_3$), 3.85 (s, 3 H, O$CH_3$), 3.80 (s, 3 H, O$CH_3$), 3.48 (s, 3 H, O$CH_3$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 166.0, 160.4, 156.1, 155.4, 151.8, 141.2, 129.6, 118.0, 112.6, 110.4, 106.4, 101.9, 101.1, 97.5, 94.6, 67.0, 56.1, 55.4, 51.8 ppm. IR (UATR): ν = 1723, 1616, 1507 cm$^{-1}$. EI-MS: *m/z* (%) = 361 (5), 181 (100), 151 (72), 121 (17). HRMS (microTOF): calcd. for $C_{19}H_{21}O_8$ $[M + H]^+$ 377.1231; found 377.1246.

*1-(6-Hydroxybenzo[d][1,3]dioxol-5-yl)-2-(4-methoxy-2-(methoxymethoxy)phenyl)ethane-1,2-dione* ***(19):*** A solution of LTMP (4.5 equiv.) was prepared by dropwise addition of *n*-BuLi (2.03 M in hexane) (1.11 mL, 2.25 mmol, 4.5 equiv) to tetramethylpiperidine (0.78 mL, 4.50 mmol) in dry THF (6 mL) under argon atmosphere at -78 °C then, warm to 0 °C and stirred at this temperature for 1 h. A solution of compound **18** (188 mg, 0.50 mmol) in dry THF (3 mL) was added and stirred at this temperature for 40 min. The pale yellow solution turned to brown solution and dimethyldioxirane (5 mL) was added dropwise at -78 °C and stirred at this temperature for 30 min. The reaction mixture was allowed to warm to room temperature for 1.5 h and quenched with saturated $NH_4Cl$, extracted with ethyl acetate (3 × 10 mL), washed with water, brine, dried over anh. $Na_2SO_4$ and concentrated to furnish brown oil. The crude product was purified by PTLC using 20% ethyl acetate in hexane as developing solvent to afford compound **19** (64.5 mg, 36%) as a pale yellow oil which was recrystallized with $CH_2Cl_2$ and hexane to give a pale yellow solid. $R_f$ = 0.24 (EtOAc/hexane, 3:7). M.p. 130–131 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 12.05 (s, 1 H, O*H*), 8.03 (d, *J* = 8.8 Hz, 1 H, Ar), 6.76 (s, 1 H, Ar), 6.70 (dd, *J* = 8.8, 2.2 Hz, 1 H, Ar), 6.61 (d, *J* = 2.2 Hz, 1 H, Ar), 6.52 (s, 1 H, Ar), 5.97 (s, 2 H, O$CH_2$O), 4.97 (s, 2 H, O$CH_2$O$CH_3$), 3.88 (s, 3 H, O$CH_3$), 3.23 (s, 3 H, O$CH_3$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 196.8, 189.9, 166.8, 162.7, 160.0, 154.9, 140.8, 132.7, 116.8, 109.0, 108.5, 107.9, 102.1, 100.0, 98.8, 94.3, 56.5, 55.8 ppm. IR (UATR): ν = 1716, 1628, 1592, 1501, 1479 cm$^{-1}$. EI-MS: *m/z* (%) = 360 (22) $[M]^+$, 343 (3), 299 (9), 298 (8), 195 (100), 165 (95). HRMS (microTOF): calcd. for $C_{18}H_{17}O_8$ $[M + H]^+$ 361.0918; found 361.0916.

*Scandione* ***(3):*** A solution of compound **19** (54.0 mg, 0.150 mmol) in toluene (2 mL) was treated with PTS-Si (185 mg, 0.150 mmol), MeOH (0.07 mL, 1.50 mmol) and heated at 80 °C for 30 min. The reaction mixture was filtered and concentrated to provide a yellow solid. The crude product was recrystallized with $CH_2Cl_2$ and hexane to furnish scandione **3** (35.5 mg, 75%) as a yellow solid. $R_f$ = 0.35 (EtOAc/hexane, 3:7). M.p. 147–148 °C (M.p. 132–133 °C)[3]. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 12.22 (s, 1 H, O*H*), 11.82 (s, 1 H, O*H*), 7.40 (d, *J* = 9.0 Hz, 1 H, Ar), 6.80 (s, 1 H, Ar), 6.53 (s, 1 H, Ar), 6.50 (d, *J* = 2.4 Hz, 1 H, Ar), 6.45 (dd, *J* = 9.0, 2.4 Hz, 1 H, Ar), 6.00 (s, 2 H, O$CH_2$O), 3.88 (s, 3 H, O$CH_3$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 194.2, 193.7, 167.8, 166.9, 164.3, 156.4, 141.3, 134.0, 110.8, 109.4, 109.0, 107.8, 102.4, 101.2, 99.0, 55.8 ppm. IR (UATR): ν = 1625, 1603, 1594, 1476 cm$^{-1}$. EI-MS: *m/z* (%) 316 (20) $[M]^+$, 298 (10), 165 (57), 151 (100). HRMS (microTOF): calcd. for $C_{16}H_{13}O_7$ $[M + H]^+$ 317.0656; found 317.0660.

*Methyl 6-(4-(4-methoxybenzyloxy)-2-(methoxymethoxy)-benzyloxy)benzo[d][1,3]dioxole-5-carboxylate* ***(21):*** A suspension of compound **8** (584 mg, 2.98 mmol) and NaH (260 mg, 5.96 mmol) in DMF (20 mL) was treated



with a solution of 1-(bromomethyl)-4-(4-methoxybenzyloxy)-2-(methoxymethoxy) benzene **20**[13] (2.80 g, 7.63 mmol) in DMF (2 mL) at 0 °C and then, was heated at 110 °C for 16 h. The reaction mixture was quenched with water and extracted with ethyl acetate (3 × 25 mL), washed with water, brine, dried over anh. $Na_2SO_4$ and concentrated to furnish brown solid. The crude mixture was purified by column chromatography using 30% ethyl acetate in hexane to give compound **21** (954 mg, 66%) as a white solid. $R_f$ = 0.27 (EtOAc/hexane, 3:7). M.p. 119–120 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 7.50 (d, $J$ = 8.5 Hz, 1 H, Ar), 7.36 (d, $J$ = 8.7 Hz, 2 H, Ar), 7.33 (s, 1 H, Ar), 6.92 (d, $J$ = 8.7 Hz, 2 H, Ar), 6.79 (d, $J$ = 2.4 Hz, 1 H, Ar), 6.66 (dd, $J$ =8.5, 2.4 Hz, 1 H, Ar), 6.65 (s, 1 H, Ar), 5.97 (s, 2 H, $OCH_2O$), 5.20 (s, 2 H, $OCH_2$), 5.10 (s, 2 H, $OCH_2$), 4.97 (s, 2 H, $OCH_2OCH_3$), 3.85 (s, 3 H, $OCH_3$), 3.82 (s, 3 H, $OCH_3$), 3.48 (s, 3 H, $OCH_3$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 166.0, 159.6, 159.4, 156.2, 155.3, 151.8, 141.2, 129.5, 129.3 (2 C), 128.8, 118.2, 114.0 (2 C), 112.6, 110.4, 107.3, 102.0, 101.9, 97.4, 94.6, 69.9, 67.0, 56.1, 55.3, 51.8 ppm. IR (UATR): ν = 1716, 1611, 1587, 1505 cm$^{-1}$. EI-MS: *m/z* (%) = 482 (0.4) $[M]^+$, 287 (13), 255 (9), 121 (100). HRMS (microTOF): calcd. for $C_{26}H_{27}O_9$ $[M + H]^+$ 483.1650; found 483.1663.

*1-(6-Hydroxybenzo[d][1,3]dioxol-5-yl)-2-(4-(4-methoxybenzyloxy)-2-(methoxymethoxy)phenyl)ethane-1,2-dione (**22**):* A solution of LTMP (4.5 equiv.) was prepared by dropwise addition of *n*-BuLi (2.86 M in hexane) (0.94 mL, 2.70 mmol, 4.5 equiv) to tetramethylpiperidine (0.94 mL, 5.40 mmol) in dry THF (10 mL) under argon atmosphere at -78 °C then, warm to 0 °C. After 1 h, the reaction mixture was placed into -20 °C and added a solution of compound **21** (289 mg, 0.60 mmol) in dry THF (5 mL) and stirred at this temperature for 30 min. The pale yellow solution turned to brown solution and dimethyldioxirane (5 mL) was added dropwise at -78 °C and stirred at this temperature for 30 min. The reaction mixture was allowed to warm to room temperature for 1.5 h and quenched with saturated $NH_4Cl$, extracted with ethyl acetate (3 × 15 mL), washed with water, brine, dried over anh. $Na_2SO_4$ and concentrated to furnish brown oil. The crude product was purified by PLC using 30% ethyl acetate in hexane as developing solvent to afford compound **22** (121 mg, 43%) as a yellow oil which was recrystallized with $CH_2Cl_2$ and hexane to give a yellow solid. $R_f$ = 0.43 (EtOAc/hexane, 3:7, developed twice). M.p. 137–138 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 12.04 (br s, 1 H, O*H*), 8.02 (d, $J$ = 8.8 Hz, 1 H, Ar), 7.35 (d, $J$ = 8.6 Hz, 2 H, Ar), 6.93 (d, $J$ = 8.6 Hz, 2 H, Ar), 6.76 (s, 1 H, Ar), 6.75 (dd, $J$ = 8.8, 2.0 Hz, 1 H, Ar), 6.69 (d, $J$ = 2.0 Hz, 1 H, Ar), 6.52 (s, 1 H, Ar), 5.96 (s, 2 H, $OCH_2O$), 5.05 (s, 2 H, $OCH_2$), 4.94 (s, 2 H, $OCH_2OCH_3$), 3.82 (s, 3 H, $OCH_3$), 3.22 (s, 3 H, $OCH_3$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 196.6 (2 C), 165.8, 162.6, 159.7, 159.6, 154.7, 140.7, 132.5, 129.2 (2 C), 127.4, 116.6, 113.9 (2 C), 109.0, 108.9, 107.6, 101.9, 100.8, 98.6, 94.1, 70.2, 56.3, 55.1 ppm. IR (UATR): ν = 2910, 1628, 1593, 1515, 1479 cm$^{-1}$. EI-MS: *m/z* (%) = 466 (6) $[M]^+$, 301 (30), 121 (100). HRMS (microTOF): calcd. for $C_{25}H_{23}O_9$ $[M + H]^+$ 467.1337; found 467.1358.

*1-(4-(4-Methoxybenzyloxy)-2-(methoxymethoxy)phenyl)-2-(6-(methoxymethoxy)benzo[d][1,3]dioxol-5-yl)ethane-1,2-dione (**23**):* A suspension of compound **22** (121 mg, 0.260 mmol) and NaH (23.0 mg, 0.520 mmol) in dry DMF (2.5 mL) was added dropwise with MOMCl (0.03 mL, 0.390 mmol) at 0 °C under argon atmosphere. The ice-bath was removed and stirred at room temperature for 40 min. The reaction mixture was quenched with water, extracted with ethyl acetate (3 × 5 mL), washed with water, brine, dried over anh. $Na_2SO_4$ and concentrated to furnish pale yellow oil. The crude product was recrystallized with $CH_2Cl_2$ and hexane to afford compound **23** (115 mg, 87%) as a white solid. $R_f$ = 0.30 (EtOAc/hexane, 3:7, developed twice). M.p. 141–143 °C. $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ = 8.06 (d, $J$ = 8.4 Hz, 1 H, Ar), 7.51 (s, 1 H, Ar), 7.35 (d, $J$ = 8.4 Hz, 2 H, Ar), 6.92 (d, $J$ = 8.4 Hz, 2 H, Ar), 6.80-6.68 (m, 3 H, Ar), 6.03 (s, 2 H, $OCH_2O$), 5.04 (s, 2 H, $OCH_2$), 4.90 (s, 2 H, $OCH_2OCH_3$), 4.80 (s, 2 H, $OCH_2OCH_3$), 3.82 (s, 3 H, $OCH_3$), 3.21 (s, 3 H, $OCH_3$), 3.17 (s, 3 H, $OCH_3$) ppm. $^{13}$C NMR (50 MHz, $CDCl_3$): $\delta$ = 191.4, 191.2, 164.8, 159.6, 159.4, 155.7, 153.7, 143.1, 131.9, 129.3 (2 C), 127.8, 117.2, 117.0, 114.0 (2 C), 108.6, 107.6, 102.2, 101.0, 97.1, 95.1, 94.2, 70.1, 56.3, 56.2, 55.3 ppm. IR (UATR): ν = 2907, 2836, 1655, 1596, 1477 cm$^{-1}$. EI-MS: *m/z* (%) = 510 (5) $[M]^+$, 301 (54), 209 (16), 121 (100). HRMS (microTOF): calcd. for $C_{27}H_{27}O_{10}$ $[M + H]^+$ 511.1599; found 511.1610.

*1-(4-Hydroxy-2-(methoxymethoxy)phenyl)-2-(6-(methoxymethoxy)-benzo[d][1,3]dioxol-5-yl)ethane-1,2-dione (**24**):* A solution of compound **23** (114 mg, 0.224 mmol) in $CH_2Cl_2$ (5 mL) was treated with DDQ (66.0

mg, 0.291 mmol) and reflux for 72 h. The resulting mixture was quenched with water and extracted with $CH_2Cl_2$ (3 × 5 mL), washed with water, brine, dried over anh. $Na_2SO_4$ and concentrated to give brown oil. The crude product was purified by PTLC using 40% ethyl acetate in hexane as developing solvent to compound **24** (63.5 mg, 73%) as a white solid and recovered of compound **23** (19.0 mg, 17%). **24**: $R_f$ = 0.26 (EtOAc/hexane, 4:6, developed twice). M.p. 183–184 °C. $^1$H NMR (200 MHz, $CDCl_3$ + $CD_3OD$ (2 drops)): $\delta$ = 7.96 (d, $J$ = 8.4 Hz, 1 H, Ar), 7.49 (s, 1 H, Ar), 6.71 (s, 1 H, Ar), 6.60 (dd, $J$ = 8.4, 2.2 Hz, 1 H, Ar), 6.53 (d, $J$ = 2.2 Hz, 1 H, Ar), 6.06 (s, 2 H, OC$H_2$O), 4.91 (s, 2 H, OC$H_2$OCH$_3$), 4.82 (s, 2 H, OC$H_2$OCH$_3$), 3.82 (br s, 1 H, O$H$), 3.21 (s, 3 H, OC$H_3$), 3.19 (s, 3 H, OC$H_3$) ppm. $^{13}$C NMR (50 MHz, $CDCl_3$ + $CD_3OD$ (2 drops)): $\delta$ = 191.8, 191.6, 164.4, 159.7, 155.8, 153.8, 143.0, 131.9, 116.7, 115.3, 109.9, 107.3, 102.2, 100.8, 96.8, 94.9, 93.8, 56.1 (2 C) ppm. IR (UATR): ν = 3318, 2908, 1647, 1582, 1477 cm$^{-1}$. EI-MS: *m/z* (%) = 390 (9) [M]$^+$, 209 (100), 181 (44), 149 (36). HRMS (microTOF): calcd. for $C_{19}H_{19}O_9$ [M + H]$^+$ 391.1024; found 391.1023.

*1-(2-(Methoxymethoxy)-4-(2-methylbut-3-yn-2-yloxy)phenyl)-2-(6-(methoxymethoxy)benzo[d][1,3]di- oxol-5-yl)ethane-1,2-dione* ***(26):*** A suspension of compound **24** ( 68.4mg, 0.175 mmol) and $K_2CO_3$ (48.3 mg, 0.350 mmol) in DMF (1.7 mL) was added with 3-chloro-3-methyl-1-butyne **25** (0.07 mL, 0. 614 mmol) and heated at 70 °C for 24 h. The resulting mixture was quenched with water and extracted with ethyl acetate (3 × 5mL), brine, dried over anh. $Na_2SO_4$ and concentrated to give yellow oil. The crude product was purified by PTLC using 30% ethyl acetate in hexane as developing solvent to afford compound **26** (52.5 mg, 66%) as a pale yellow oil and recovered of compound **24** (8.6 mg, 13%). **26**: $R_f$ = 0.42 (EtOAc/hexane, 3:7, developed twice). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 8.03 (d, $J$ = 8.7 Hz, 1 H, Ar), 7.52 (s, 1 H, Ar), 7.03 (dd, $J$ = 8.7, 2.1 Hz, 1 H, Ar), 6.97 (d, $J$ = 2.1 Hz, 1 H, Ar), 6.70 (s, 1 H, Ar), 6.04 (s, 2 H, OC$H_2$O), 4.91 (s, 2 H, OC$H_2$OCH$_3$), 4.81 (s, 2 H, OC$H_2$OCH$_3$), 3.22 (s, 3 H, OC$H_3$), 3.17 (s, 3 H, OC$H_3$), 2.65 (s, 1 H, C$H$≡C), 1.70 (s, 6 H, (C$H_3$)$_2$C) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 191.5, 191.4, 162.1, 158.9, 155.8, 153.7, 143.2, 131.2, 117.7, 117.3, 113.0, 107.7, 105.4, 102.2, 97.1, 95.2, 94.3, 85.0, 74.9, 72.6, 56.24, 56.2, 29.5 (2 C) ppm. IR (UATR): ν = 3281, 2908, 1655, 1595, 1477 cm$^{-1}$. EI-MS: *m/z* (%) = 456 (1) [M]$^+$, 247 (34), 209 (45), 181 (100), 151 (24). HRMS (microTOF): calcd. for $C_{24}H_{25}O_9$ [M + H]$^+$ 457.1493; found 457.1490.

*1-(2-(Methoxymethoxy)-4-(2-methylbut-3-en-2-yloxy)phenyl)-2-(6-(methoxymethoxy)-benzo[d][1,3] dioxol-5-yl)ethane-1,2-dione* ***(27):*** A solution of compound **26** (114 mg, 0.250 mmol) in $CH_2Cl_2$ (5 mL) was added with 10% Pd-$BaSO_4$ (27.0 mg, 0.025 mmol) and pyridine (0.17 mL, 2.11 mmol). The alkyne was hydrogenated at room temperature and atmospheric pressure of hydrogen was taken up about 80 min. The catalyst was filtered through celite and eluted with $CH_2Cl_2$, the solvent was concentrated to provide yellow oil. The residue was purified by short pad column chromatography using 40% ethyl acetate in hexane to afford compound **27** (94.0 mg, 82%) as a pale yellow oil. $R_f$ = 0.47 (EtOAc/hexane, 3:7, developed twice). $^1$H NMR (200 MHz, $CDCl_3$): $\delta$ = 7.96 (d, $J$ = 8.7 Hz, 1 H, Ar), 7.51 (s, 1 H, Ar), 6.75 (dd, $J$ = 8.7, 1.8 Hz, 1 H, Ar), 6.73 (s, 1 H, Ar), 6.69 (s, 1 H, Ar), 6.12 (dd, $J$ = 17.8, 10.4 Hz, 1 H, C$H$=CH$_2$), 6.03 (s, 2 H, OC$H_2$O), 5.28-5.16 (m, 2 H, C$H_2$=CH), 4.87 (s, 2 H, OC$H_2$OCH$_3$), 4.80 (s, 2 H, OC$H_2$OCH$_3$), 3.20 (s, 3 H, OC$H_3$), 3.16 (s, 3 H, OC$H_3$), 1.52 (s, 6 H, (C$H_3$)$_2$C) ppm. $^{13}$C NMR (50 MHz, $CDCl_3$): $\delta$ = 191.5, 191.4, 163.1, 158.9, 155.7, 153.7, 143.6, 143.1, 131.1, 117.3, 117.0, 114.0, 113.2, 107.7, 105.3, 102.2, 97.1, 95.1, 94.3, 80.7, 56.2, 56.1, 27.2 (2 C) ppm. IR (UATR): ν = 2924, 1656, 1617, 1595, 1477 cm$^{-1}$. EI-MS: *m/z* (%) = 458 (2) [M]$^+$, 293 (9), 249 (69), 217 (43), 209 (100), 181 (29), 149 (53). HRMS (microTOF): calcd. for $C_{24}H_{27}O_9$ [M + H]$^+$ 459.1650; found 459.1665.

*1-(2-(Methoxymethoxy)-4-(2-methylbut-3-en-2-yloxy)phenyl)-2-(6-(methoxymethoxy)benzo[d][1,3] dioxol-5-yl)ethane-1,2-dione* ***(28):*** In a 10 mL microwave vessel, compound **27** (108 mg, 0.240 mmol) was dissolved in DMF (1 mL). The solution was heated in microwave reactor (200 W, 50 psi) at 150 °C for 5 min. The solution was then diluted in ethyl acetate (15 mL) and washed with water (3 × 10 mL), brine (10 mL), dried over anh. $Na_2SO_4$, filtered and evaporated to give yellow oil. The crude product was purified by PTLC using 25% ethyl acetate in hexane to afford compound **28** (28.9 mg, 23%) as a pale yellow solid and a mixture of **29** (28.5 mg, 24%) as a pale yellow oil. **28**: $R_f$ = 0.16 (EtOAc/hexane, 3:7, developed twice). M.p. 110–112 °C. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.84 (s, 1 H, Ar), 7.50 (s, 1 H, Ar), 6.70 (s, 1 H, Ar), 6.55 (s, 1 H, Ar), 6.03 (s, 2 H, OC$H_2$O), 5.34-5.25 (m, 1 H, C=C$H$CH$_2$), 4.83 (s, 2 H, OC$H_2$OCH$_3$), 4.81 (s, 2 H, OC$H_2$OCH$_3$), 3.33 (d, $J$ = 7.2 Hz, 2 H, C=CHC$H_2$), 3.20 (s, 3 H, OC$H_3$), 3.19 (s, 3 H, OC$H_3$), 1.77 (s, 6 H, (C$H_3$)$_2$C) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 191.7, 191.6, 161.2, 158.2, 155.9, 153.8, 143.2, 135.4, 131.8, 121.4, 121.3, 117.2, 116.4, 107.7, 102.3, 101.8, 97.2, 95.2, 94.2, 56.22, 56.20, 29.0, 25.8, 17.9 ppm. IR (UATR): ν = 3299, 2910, 1648, 1587, 1504, 1477



$cm^{-1}$. EI-MS: *m/z* (%) = 458 (1) $[M]^+$, 249 (100), 209 (66). HRMS (microTOF): calcd. for $C_{24}H_{27}O_9$ $[M + H]^+$ 459.1650; found 459.1654.

**1. General Procedure for palladium(II)-catalyzed oxidative cyclization.**

**Method A.** The mixture of phenol (1.00 mmol), $Pd(TFA)_2$ (5 mol%), sparteine sulfate (40 mol%), $Na_2CO_3$ (2 equiv) and MS4Å (125 mg/mmol SM) in dry toluene (0.1 M) under $O_2$ was heated at 80 °C for 16 h. The resulting mixture was filtered over silica gel and eluted with ethyl acetate, concentrated to give crude product which was purified by preparative thin-layer chromatography (PTLC).

**Method B.** The mixture of phenol (1.00 mmol), $Pd(TFA)_2$ (5-20 mol%), pyridine (20-40 mol%), $Na_2CO_3$ (2 equiv) and MS4Å (125-500 mg/mmol SM) in dry toluene (0.1 M) under $O_2$ was heated at 80 °C for 16 h. The resulting mixture was filtered over silica gel and eluted with ethyl acetate, concentrated to give crude product which was purified by preparative thin-layer chromatography (PTLC).

*1-(6-(Methoxymethoxy)-2-(prop-1-en-2-yl)-2,3-dihydrobenzofuran-5-yl)-2-(6-(methoxymethoxy) benzo[d][1,3]dioxol-5-yl)ethane-1,2-dione* ***(30):*** Fllowing the general method A, using compound **28** (123 mg, 0.269 mmol), 5 mol% $Pd(TFA)_2$ (4.5 mg, 0.0135 mmol), 40 mol% sparteine sulfate (45 mg, 0.108 mmol), $Na_2CO_3$ (57 mg, 0.538 mmol) and MS4Å (34 mg) (125 mg/mmol SM) in dry toluene (0.1 M) (2.7 mL) and purification by PTLC using 40% ethyl acetate in hexane to furnish compound **30** (8.5 mg, 7%) as a yellow solid. Fllowing the general method B, using compound **28** (36 mg, 0.0786 mmol), 20 mol% $Pd(TFA)_2$ (5.2 mg, 0.0157 mmol), 20 mol% pyridine (1.2 µL, 0.0039 mmol), $Na_2CO_3$ (17 mg, 0.157 mmol) and MS4Å (9.8 mg) (125 mg/mmol SM) in dry toluene (0.1 M) (0.8 mL) and purification by PTLC using 40% ethyl acetate in hexane to furnish compound **30** (5.6 mg, 16%) as a pale yellow oil. $R_f$ = 0.38 (EtOAc/hexane, 4:6). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.90 (s, 1 H, Ar), 7.51 (s, 1 H, Ar), 6.71 (s, 1 H, Ar), 6.57 (s, 1 H, Ar), 6.03 (s, 2 H, $OCH_2O$), 5.28 (dd, *J* = 9.3, 8.1 Hz, 1 H, $CHCH_2$), 5.08 (s, 1 H, CHH=C), 4.94 (s, 1 H, CHH=C), 4.88 (s, 2 H, $OCH_2OCH_3$), 4.83 (s, 2 H, $OCH_2OCH_3$), 3.35 (dd, *J* = 15.3, 9.3 Hz, 1 H, CHH-CH), 3.21 (s, 6 H, 2 x $OCH_3$), 3.02 (dd, *J* = 15.3, 8.1 Hz, 1 H, CHH-CH), 1.76 (s, 3 H, $CH_3C$=) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 191.4 (2 C), 166.3, 160.0, 155.7, 153.7, 143.2, 143.1, 126.3, 121.1, 117.3, 116.8, 112.6, 107.8, 102.2, 97.2, 95.9, 95.2, 94.4, 87.8, 56.3, 56.2, 33.3, 17.1 ppm. IR (UATR): ν = 2917, 2851, 1651, 1611, 1476 $cm^{-1}$. EI-MS: *m/z* (%) = 456 (4) $[M]^+$, 247 (100), 217 (13), 209 (15). HRMS (microTOF): calcd. for $C_{24}H_{24}O_9Na$ $[M + Na]^+$ 479.1313; found 479.1309.

*2-(Prop-1-en-2-yl)-2,3-dihydrobenzofuran-5-carbaldehyde* ***(32):*** Fllowing the general method B, using 4-hydroxy-3-(3-methylbut-2-enyl)benzaldehyde (72.0 mg, 0.379 mmol), 5 mol% $Pd(TFA)_2$ (6.5 mg, 0.019 mmol), 20 mol% pyridine (6 µL, 0.0758 mmol), $Na_2CO_3$ (80 mg, 0.758 mmol) and MS4Å (47.4 mg) (125 mg/mmol SM) in dry toluene (0.1 M) (3.8 mL) and purification by PTLC using 10% ethyl acetate in hexane to furnish compound **32** (33.4 mg, 47%) as a pale yellow oil. $R_f$ = 0.24 (EtOAc/hexane, 1:9). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 9.82 (s, 1 H, -CHO), 7.71 (s, 1 H, Ar), 7.68 (d, *J* = 8.3 Hz, 1 H, Ar), 6.89 (d, *J* = 8.3 Hz, 1 H, Ar), 5.29 (t, *J* = 8.7 Hz, 1 H, $CH_2CH$), 5.10 (s, 1 H, CHH=C), 4.95 (s, 1 H, CHH=C), 3.40 (dd, *J* = 15.9, 9.6 Hz, 1 H, CHH-CH), 3.08 (dd, *J* = 15.9, 8.0 Hz, 1 H, CHH-CH), 1.77 (s, 3 H, $=CCH_3$) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 190.5, 165.1, 143.0, 133.0, 130.4, 128.1, 125.7, 112.7, 109.4, 87.1, 33.6, 17.0 ppm. IR (UATR): ν = 2920, 2820, 2733, 1683, 1604 $cm^{-1}$. EI-MS: *m/z* (%) = 188 (98) $[M]^+$, 173 (100), 159 (45), 144 (38), 141 (32), 131 (24), 115 (31), 91 (27), 77 (13). HRMS (microTOF): calcd. for $C_{12}H_{12}O_2Na$ $[M + Na]^+$ 211.0730; found 211.0735.

*Methyl 2-(prop-1-en-2-yl)-2,3-dihydrobenzofuran-5-carboxylate* ***(34):*** Fllowing the general method B, using methyl 4-hydroxy-3-(3-methylbut-2-enyl)benzoate (100 mg, 0.455 mmol), 5 mol% $Pd(TFA)_2$ (7.6 mg, 0.0228 mmol), 20 mol% pyridine (9 µL, 0.091 mmol), $Na_2CO_3$ (96 mg, 0.91 mmol) and MS4Å (57 mg) (125 mg/mmol SM) in dry toluene (0.1 M) (4.6 mL) and purification by PTLC using 10% ethyl acetate in hexane to furnish benzofuran **34 (**23.1 mg, 23%) as a pale yellow oil, chromene **35** (2.8 mg, 3%) as a pale yellow oil and recovered methyl 4-hydroxy-3-(3-methylbut-2-enyl)benzoate (7.0 mg, 7%). **34**: $R_f$ = 0.47 (EtOAc/hexane, 1:9, developed twice). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.87 (d, *J* = 9.0 Hz, 1 H, Ar), 7.85 (s, 1 H, Ar), 6.80 (d, *J* =

9.0 Hz, 1 H, Ar), 5.25 (t, *J* = 8.3 Hz, 1 H, $CH_2CH$), 5.09 (s, 1 H, C*H*H=C), 4.93 (s, 1 H, CH*H*=C), 3.87 (s, 3 H, $OCH_3$), 3.37 (dd, *J* = 15.8, 9.8 Hz, 1 H, C*H*H-CH), 3.05 (dd, *J* = 15.8, 8.3 Hz, 1 H, CH*H*-CH), 1.76 (s, 3 H, $=CCH_3$) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 166.9, 163.8, 143.4, 131.1, 127.0, 126.6, 122.7, 112.5, 108.9, 86.8, 51.8, 34.0, 17.1 ppm. IR (UATR): ν = 2951, 1712, 1611, 1489 cm$^{-1}$. EI-MS: *m/z* (%) = 219 (14), 218 (100) $[M]^+$, 203 (90), 187 (36), 171 (30), 159 (82), 144 (58). HRMS (microTOF): calcd. for $C_{13}H_{14}O_3Na$ $[M + Na]^+$ 241.0835; found 241.0832. **35**: $R_f$ = 0.51 (EtOAc/hexane, 1:9, developed twice). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.80 (d, *J* = 8.4 Hz, 1 H, Ar), 7.68 (s, 1 H, Ar), 6.77 (d, *J* = 8.4 Hz, 1 H, Ar), 6.34 (d, *J* = 9.9 Hz, 1 H, CH=C*H*Ar), 5.64 (d, *J* = 9.9 Hz, 1 H, C*H*=CHAr), 3.87 (s, 3 H, $OCH_3$), 1.45 (s, 6 H, $(CH_3)_2C$) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 166.8, 157.1, 131.1, 131.0, 128.1, 122.5, 121.7, 120.6, 116.1, 77.4, 51.8, 28.3 (2 C) ppm. IR (UATR): ν = 2976, 2927, 1714, 1641, 1609, 1576 cm$^{-1}$. EI-MS: *m/z* (%) = 218 (9) $[M]^+$, 203 (100), 144 (12), 115 (8). HRMS (microTOF): calcd. for $C_{13}H_{14}O_3Na$ $[M + Na]^+$ 241.0835; found 241.0832.

*Methyl 6-(methoxymethoxy)-2-(prop-1-en-2-yl)-2,3-dihydrobenzofuran-5-carboxylate* ***(36):*** Fllowing the general method B, using compound **44** (87.0 mg, 0.311 mmol), 10 mol% $Pd(TFA)_2$ (10.0 mg, 0.031 mmol), 40 mol% pyridine (11 µL, 0.124 mmol), $Na_2CO_3$ (66.0 mg, 0.622 mmol) and MS4Å (155 mg) (500 mg/mmol SM) in dry toluene (0.1 M) (3.1 mL) and purification by PTLC using 10% ethyl acetate in hexane to furnish compound **36** (42.8 mg, 49%) as a colorless oil and (6-(methoxycarbonyl)-7-(methoxymethoxy)-2-methyl-2H-chromen-2-yl)methylium **37** (9.1 mg, 11%) as a colorless oil. **36**: $R_f$ = 0.35 (EtOAc/hexane, 1.5:8.5, developed twice). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.67 (s, 1 H, Ar), 6.66 (s, 1 H, Ar), 5.24 (t, *J* = 8.7 Hz, 1 H, $CH_2CH$), 5.22 (s, 2 H, $OCH_2OCH_3$), 5.08 (s, 1 H, C*H*H=C), 4.92 (s, 1 H, CH*H*=C), 3.85 (s, 3 H, $OCH_3$), 3.52 (s, 3 H, $OCH_3$), 3.30 (dd, *J* = 15.5, 9.5 Hz, 1 H, C*H*H-CH), 2.98 (dd, *J* = 15.5, 8.0 Hz, 1 H, CH*H*-CH), 1.75 (s, 3 H, $=CCH_3$) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 166.2, 164.3, 159.0, 143.3, 127.8, 120.0, 112.8, 112.4, 98.3, 95.4, 87.4, 56.3, 51.6, 33.5, 17.0 ppm. IR (UATR): ν = 2950, 1723, 1622, 1592, 1487, 1439 cm$^{-1}$. EI-MS: *m/z* (%) = 279 (12), 278 (66) $[M]^+$, 263 (75), 233 (95), 202 (68), 187 (100). HRMS (microTOF): calcd. for $C_{15}H_{19}O_5$ $[M + H]^+$ 279.1227; found 279.1222. Minor product **37**: $R_f$ = 0.43 (EtOAc/hexane, 1.5:8.5, developed twice). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.53 (s, 1 H, Ar), 6.62 (s, 1 H, Ar), 6.28 (d, *J* = 9.9 Hz, 1 H, CH=C*H*), 5.54 (d, *J* = 9.9 Hz, 1 H, C*H*=CH), 5.22 (s, 2 H, $OCH_2OCH_3$), 3.85 (s, 3 H, $OCH_3$), 3.52 (s, 3 H, $OCH_3$), 1.43 (s, 6 H, $(CH_3)_2C$) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 165.9, 158.6, 157.6, 129.8, 128.9, 121.0, 114.8, 112.8, 104.2, 95.0, 77.4, 56.3, 51.6, 28.3 (2 C) ppm. IR (UATR): ν = 2975, 2947, 1724, 1615, 1565, 1493 cm$^{-1}$. EI-MS: *m/z* (%) = 279 (5), 278 (28) $[M]^+$, 263 (96), 247 (15), 233 (100), 187 (93), 160 (25), 77 (16). HRMS (microTOF): calcd. for $C_{15}H_{19}O_5$ $[M + H]^+$ 279.1227; found 279.1225.

*(6-(Methoxymethoxy)-2-(prop-1-en-2-yl)-2,3-dihydrobenzofuran-5-yl)methanol* ***(38):*** A solution of compound **36** (229 mg, 0.823 mmol) in dry THF (2 mL) was added into a suspension of LAH (78.0 mg, 2.06 mmol) in dry THF (3 mL) at 0 °C and allowed to warm to room temperature. After 2 h, the reaction mixture was cooled to 0 °C and quenched with a mixture of water:ethyl acetate (1:1) (10 mL) and filtered. The residue was extracted with ethyl acetate (3 × 10 mL), washed with water, brine, dried over anh. $Na_2SO_4$, and evaporated to give compound **38** (197 mg, 96%) as a colorless oil. $R_f$ = 0.29 (EtOAc/hexane, 3:7, developed twice). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 6.98 (s, 1 H, Ar), 6.54 (s, 1 H, Ar), 5.09 (s, 2 H, $OCH_2OCH_3$), 5.09 (apparent t, *J* = 8.9 Hz, 1 H, $CHCH_2$), 4.98 (s, 1 H, C*H*H=C), 4.81 (s, 1 H, CH*H*=C), 4.51 (s, 2 H, $CH_2OH$), 3.39 (s, 3 H, $OCH_3$), 3.18 (dd, *J* = 15.3, 9.6 Hz, 1 H, C*H*H-CH), 2.88 (dd, *J* = 15.3, 8.1 Hz, 1 H, CH*H*-CH), 2.37 (br s, 1 H, O*H*), 1.67 (s, 3 H, $CH_3C$=) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 160.5, 155.6, 143.8, 125.2, 122.0, 119.4, 112.0, 97.1, 95.0, 86.6, 61.6, 56.2, 34.0, 17.1 ppm. IR (UATR): ν = 3390, 2919, 1621, 1604, 1487 cm$^{-1}$. EI-MS: *m/z* (%) = 250 (29) $[M]^+$, 218 (17), 188 (100), 173 (53), 145 (38). HRMS (microTOF): calcd. for $C_{14}H_{18}O_4Na$ $[M + Na]^+$ 273.1097; found 273.1091.

*Methyl 6-((6-(methoxymethoxy)-2-(prop-1-en-2-yl)-2,3-dihydro-benzofuran-5-yl)methoxy)benzo[d][1,3]dioxole-5-carboxylate* ***(39):*** To a solution of compound **8** (180 mg, 0.918 mmol), alcohol **38** (153 mg, 0.612 mmol), and $PPh_3$ (241 mg, 0.918 mmol) in dry THF (6 mL) was added diisopropyl azodicarboxylate (0.17 mL, 0.918 mmol) dropwise at 0 °C under argon atmosphere. The solution was then refluxed for 24 h and concentrated *in vacuo* to give yellow oil. The crude product was purified by PTLC using 20% ethyl acetate in

hexane to furnish compound **39** (110 mg, 42%) as a pale yellow solid. $R_f$ = 0.26 (EtOAc/hexane, 2:8). M.p. 84–86 °C. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.36 (s, 1 H, Ar), 7.32 (s, 1 H, Ar), 6.67 (s, 1 H, Ar), 6.65 (s, 1 H, Ar), 5.97 (s, 2 H, OC$H_2$O), 5.177 (s, 2 H, OC$H_2$OCH$_3$), 5.176 (apparent t, $J$ = 8.7 Hz, 1 H, C$H$-CH$_2$), 5.08 (s, 3 H, OC$H_2$, C$H$H=C), 4.90 (s, 1 H, CH$H$=C), 3.85 (s, 3 H, OC$H_3$), 3.48 (s, 3 H, OC$H_3$), 3.29 (dd, $J$ = 15.0, 9.6 Hz, 1 H, C$H$H-CH), 2.99 (dd, $J$ = 15.0, 8.3 Hz, 1 H, CH$H$-CH), 1.76 (s, 3 H, C$H_3$C=) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 165.9, 160.5, 156.2, 154.8, 151.8, 143.9, 141.2, 124.8, 119.5, 117.4, 112.5, 111.9, 110.3, 101.8, 97.6, 96.5, 94.8, 86.6, 67.4, 56.0, 51.7, 34.2, 17.2 ppm. IR (UATR): ν = 2949, 2907, 1723, 1697, 1622, 1484 cm$^{-1}$. EI-MS: *m/z* (%) = 428 (0.2) [M]$^+$, 234 (16), 233 (100), 203 (18), 201 (21), 149 (34). HRMS (microTOF): calcd. for $C_{23}H_{25}O_8$ [M + H]$^+$ 429.1544; found 429.1533.

*Methyl 2-hydroxy-4-(2-methylbut-3-yn-2-yloxy)benzoate* ***(41):*** A suspension of compound **40** (500 mg, 2.98 mmol) and $K_2CO_3$ (617 mg, 4.47 mmol) in acetone (20 mL) under argon atmosphere was added 3-chloro-3-methyl-1-butyne **25** (1.23 mL, 10.4 mmol) and reflux for 16 h. The reaction mixture was quenched with water, extracted with ethyl acetate (3 × 20 mL), brine, dried over anh. $Na_2SO_4$ and concentrated to give yellow-brown oil. The crude product was purified by column chromatography using 5-10% ethyl acetate in hexane as developing solvent to afford compound **41** (427 mg, 61%) as a pale yellow solid. $R_f$ = 0.49 (EtOAc/hexane, 2:8). M.p. 89–90 °C. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 10.91 (s, 1 H, O$H$), 7.74 (d, $J$ = 9.0 Hz, 1 H, Ar), 6.92 (d, $J$ = 2.4 Hz, 1 H, Ar), 6.68 (dd, $J$ = 9.0, 2.4 Hz, 1 H, Ar), 3.93 (s, 3 H, OC$H_3$), 2.67 (s, 1 H, C$H$≡C), 1.72 (s, 6 H, (C$H_3$)$_2$C) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 170.2, 162.8, 161.9, 130.6, 111.3, 106.3, 106.1, 84.8, 74.8, 72.1, 51.8, 29.4 (2 C) ppm. IR (UATR): ν = 3291, 2992, 2955, 1669, 1620, 1578, 1497, 1439, 1347 cm$^{-1}$. EI-MS: *m/z* (%) = 234 (5) [M]$^+$, 219 (13), 175 (26), 168 (76), 136 (100). HRMS (microTOF): calcd. for $C_{13}H_{15}O_4$ [M + H]$^+$ 235.0965; found 235.0969.

*Methyl 2-(methoxymethoxy)-4-(2-methylbut-3-yn-2-yloxy)benzoate* ***(42):*** A suspension of compound **41** (639 mg, 2.73 mmol) and NaH (238 mg, 5.46 mmol) in dry DMF (6 mL) at 0 °C was added dropwise with MOMCl (0.37 mL, 4.10 mmol) under argon atmosphere. The ice-bath was removed and stirred at room temperature for 40 min. The reaction mixture was quenched with water, extracted with ethyl acetate (3 × 15 mL), washed with water, brine, dried over anh. $Na_2SO_4$ and concentrated to afford yellow oil. The crude product was purified by column chromatography using 20% ethyl acetate in hexane to furnish compound **42** (740 mg, 97%) as a colorless oil. $R_f$ = 0.26 (EtOAc/hexane, 2:8). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.80 (d, $J$ = 8.7 Hz, 1 H, Ar), 7.07 (d, $J$ = 1.7 Hz, 1 H, Ar), 6.94 (dd, $J$ = 8.7, 1.7 Hz, 1 H, Ar), 5.25 (s, 2 H, OC$H_2$OCH$_3$), 3.87 (s, 3 H, OC$H_3$), 3.53 (s, 3 H, OC$H_3$), 2.65 (s, 1 H, C$H$≡C), 1.70 (s, 6 H, (C$H_3$)$_2$C) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 166.0, 160.1, 158.2, 132.5, 114.4, 112.4, 107.9, 95.1, 85.1, 74.6, 72.3, 56.3, 51.7, 29.5 (2 C) ppm. IR (UATR): ν = 3284, 2991, 2951, 1723, 1603, 1575 cm$^{-1}$. EI-MS: *m/z* (%) = 278 (29) [M]$^+$, 247 (28), 233 (42), 219 (83), 212 (91), 197 (43), 181 (81), 165 (73), 151 (100), 136 (81). HRMS (microTOF): calcd. for $C_{15}H_{19}O_5$ [M + H]$^+$ 279.1227; found 279.1223.

*Methyl 2-(methoxymethoxy)-4-(2-methylbut-3-en-2-yloxy)benzoate* ***(43):*** A solution of compound **42** (761 mg, 2.74 mmol) in $CH_2Cl_2$ (50 mL) was added with 10% Pd-$BaSO_4$ (290 mg, 0.274 mmol) and pyridine (1.85 mL, 23.0 mmol). The alkyne **42** was hydrogenated at room temperature and atmospheric pressure of hydrogen was taken up about 3 h. The catalyst was filtered through celite and eluted with $CH_2Cl_2$, the solvent was concentrated to provide yellow oil. The residue was purified by column chromatography using 20-30% ethyl acetate in hexane to afford compound **43** (710 mg, 93%) as a colorless oil. $R_f$ = 0.31 (EtOAc/hexane, 2:8). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.72 (d, $J$ = 8.8 Hz, 1 H, Ar), 6.83 (d, $J$ = 2.2 Hz, 1 H, Ar), 6.65 (dd, $J$ = 8.8, 2.2 Hz, 1 H, Ar), 6.12 (dd, $J$ = 17.7, 11.1 Hz, 1 H, C$H$=CH$_2$), 5.26-5.15 (m, 4 H, OC$H_2$OCH$_3$, CH=C$H_2$), 3.85 (s, 3 H, OC$H_3$), 3.51 (s, 3 H, OC$H_3$), 1.51 (s, 6 H, (C$H_3$)$_2$C) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 166.0, 161.1, 158.2, 143.8, 132.4, 113.8, 113.6, 112.6, 107.9, 95.1, 80.3, 56.2, 51.6, 27.1 (2 C) ppm. IR (UATR): ν = 2983, 1725, 1603, 1572 cm$^{-1}$. EI-MS: *m/z* (%) = 280 (10) [M]$^+$, 212 (100), 197 (17), 181 (31), 165 (28), 151 (37), 136 (29), 69 (43). HRMS (microTOF): calcd. for $C_{15}H_{21}O_5$ [M + H]$^+$ 281.1384; found 281.1389.



*Methyl 4-hydroxy-2-(methoxymethoxy)-5-(3-methylbut-2-enyl)benzoate (**44**):* In a 10 mL microwave vessel, compound **43** (140 mg, 0.500 mmol) was dissolved in DMF (1 mL). The solution was heated in microwave reactor (200 W, 50 psi) at 150 °C for 5 min. The solution was then diluted in ethyl acetate (15 mL) and washed with water (3 × 10 mL), brine (10 mL), dried over anh. $Na_2SO_4$, filtered and evaporated to give pale yellow oil. The crude product was purified by PTLC using 20% ethyl acetate in hexane to afford compound **44** (63.2 mg, 45%) as a white solid and another isomer **45** (62.8 mg, 45%) as a white solid. **44**: $R_f$ = 0.29 (EtOAc/hexane, 3:7, developed twice). M.p. 106–107 °C. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.65 (s, 1 H, Ar), 6.71 (s, 1 H, Ar), 6.61 (br s, 1 H, O*H*), 5.35-5.25 (m, 1 H, =C*H*CH$_2$), 5.17 (s, 2 H, OC$H_2$OCH$_3$), 3.86 (s, 3 H, OC$H_3$), 3.47 (s, 3 H, OC$H_3$), 3.30 (d, $J$ = 7.2 Hz, 2 H, =CHC$H_2$), 1.76 (s, 6 H, (C$H_3$)$_2$C) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 166.7, 159.6, 157.5, 133.8, 133.2, 121.8, 121.0, 111.5, 103.6, 94.9, 56.1, 51.8, 28.2, 25.7, 17.8 ppm. IR (UATR): ν = 3267, 2925, 1678, 1607 cm$^{-1}$. EI-MS: *m/z* (%) = 280 (13) [M]$^+$, 279 (31), 256 (33), 178 (100), 167 (36), 149 (77). HRMS (microTOF): calcd. for $C_{15}H_{21}O_5$ [M + H]$^+$ 281.1384; found 281.1392. **45**: $R_f$ = 0.47 (EtOAc/hexane, 3:7, developed twice). M.p. 109–110 °C. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.69 (d, $J$ = 8.4 Hz, 1 H, Ar), 6.64 (d, $J$ = 8.4 Hz, 1 H, Ar), 6.52 (s, 1 H, O*H*), 5.27-5.19 (m, 1 H, =C*H*CH$_2$), 5.06 (s, 2 H, OC$H_2$OCH$_3$), 3.86 (s, 3 H, OC$H_3$), 3.58 (s, 3 H, OC$H_3$), 3.51 (d, $J$ = 6.9 Hz, 2 H, =CHC$H_2$), 1.81 (s, 3 H, =CC$H_3$), 1.73 (s, 3 H, =CC$H_3$) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 166.5, 159.7, 157.3, 134.6, 130.8, 122.1, 121.6, 116.0, 111.8, 101.4, 57.6, 51.9, 25.7, 23.3, 17.9 ppm. IR (UATR): ν = 3382, 2935, 2827, 1698, 1596, 1578, 1435 cm$^{-1}$. EI-MS: *m/z* (%) = 280 (0.2) [M]$^+$, 248 (59), 203 (100), 161 (47), 149 (56). HRMS (microTOF): calcd. for $C_{15}H_{21}O_5$ [M + H]$^+$ 281.1384; found 281.1381.

*Calophione A (**4**):* A solution of LTMP (4.5 equiv) was prepared by dropwise addition of *n*-BuLi (1.67 M in hexane) (0.50 mL, 0.837 mmol, 4.5 equiv) to tetramethylpiperidine (0.30 mL, 1.67 mmol) in dry THF (5 mL) under argon atmosphere at -78 °C then, warm to 0 °C and stirred at this temperature for 1 h. After 1 h, the reaction mixture was placed into -20 °C and added a solution of compound **39** (79.4 mg, 0.186 mmol) in dry THF (2 mL) and stirred at this temperature for 1 h. The pale yellow solution turned to brown solution and dimethyldioxirane (5 mL) was added dropwise at -78 °C and stirred at this temperature for 30 min. The reaction mixture was allowed to warm to room temperature for 1.5 h and quenched with saturated $NH_4Cl$, extracted with ethyl acetate (3 × 5 mL), washed with water, brine, dried over anh. $Na_2SO_4$ and concentrated to furnish yellow-brown oil. The residue was dissolved with toluene (2.4 mL) and treated with PTS-Si (230 mg, 0.186 mmol), MeOH (0.09 mL, 1.86 mmol) under heated at 80 °C for 1 h. The reaction mixture was filtered and concentrated to provide a yellow solid. The crude product was purified by PTLC using 20% ethyl acetate in hexane to furnish calophione A (**4**) (7.8 mg, 11%) as a pale yellow solid. $R_f$ = 0.34 (EtOAc/hexane, 2:8). M.p. 130–131°C. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 12.25 (s, 1 H, O*H*), 12.06 (s, 1 H, O*H*), 7.23 (s, 1 H, Ar), 6.83 (s, 1 H, Ar), 6.54 (s, 1 H, Ar), 6.45 (s, 1 H, Ar), 6.00 (s, 2 H, OC$H_2$O), 5.28 (dd, $J$ = 9.2, 7.5 Hz, 1 H, C*H*CH$_2$), 5.07 (s, 1 H, C*H*H=C), 4.95 (s, 1 H, CH*H*=C), 3.24 (dd, $J$ = 15.6, 9.2 Hz, 1 H, C*H*H-CH), 2.91 (dd, $J$ = 15.6, 7.5 Hz, 1 H, CH*H*-CH), 1.73 (s, 3 H, C$H_3$C=) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 194.0, 193.5, 168.5, 167.6, 164.3, 156.4, 142.7, 141.3, 128.0, 120.4, 113.2, 110.6, 109.5, 108.0, 102.4, 99.0, 98.5, 88.3, 32.6, 16.9 ppm. IR (UATR): ν = 2916, 1623, 1591, 1475, 1387 cm$^{-1}$. EI-MS: *m/z* (%) = 368 (7) [M]$^+$, 203 (100), 165 (32). HRMS (microTOF): calcd. for $C_{20}H_{17}O_7$ [M + H]$^+$ 369.0969; found 369.0966.

**3. Cytotoxicity Test :** All benzil derivatives **1-4** were solubilized in DMSO and tested for their cytotoxic activities against HuCCA-1, A-549, HepG2, and MOLT-3 cancer cell lines. The cells suspended in the corresponding culture medium were inoculated in 96-well microtiter plates (Corning Inc., NY, USA) at a density of 10000–20000 cells per well, and incubated at 37 °C in a humidified atmosphere with 95% air and 5% $CO_2$. After 24 h, an equal volume of additional medium containing either the serial dilutions of the test compounds, positive control (etoposide), or negative control (DMSO) was added to the desired final concentrations, and the microtiter plates were further incubated for an additional 48 h. The number of surviving cells in each well was determined using either MTT assay (for adherent cells) or XTT assay (for suspended cells) in order to determine the $IC_{50}$ which is defined as the concentration that inhibits cell growth by 50% (relative to negative control) after 48 h of continuous exposure to each test compound. Within each experiment, determinations were done in triplicate, and each compound was tested in at least two separate experiments. Any



experiments with a variation greater than 10% were excluded from the analysis. The results are expressed as the mean $IC_{50}$ value; standard deviations are omitted for visual clarity.

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## OUTPUT

ผลงานที่ได้ (Output) มีบทความระดับนานาชาติจำนวน 4 เรื่อง อยู่ระหว่างการดำเนิการ 2 เรื่อง และบทความที่มีความร่วมมือกับหน่วยงานทั้งภายในและภายนอกสถาบันวิจัยจุฬาภรณ์ และสถาบันบัณฑิตศึกษาจุฬาภรณ์ จำนวน 1 เรื่อง มีการนำเสนอผลงานวิจัยในการประชุมทั้งระดับชาติและนานาชาติ จำนวน 10 ครั้ง

### INTERNATIONAL PUBLICATIONS

1. Kanintronkul, Y.; Worayuthakarn, R.; **Thasana, N.**; Winayanuwattikun, P.; Pattanapanyasat, K.; Surarit, R.; Ruchirawat, S.; Svasti, J. Overcoming multidrug resistance in human lung cancer with novel benzo[*a*]quinolizin-4-one chemomodulators. *Anticancer Research* **2011**, *31*, 921-927. **(IF2008 = 1.725)**

2. Wittayalai, S.; Sathalalai, S.; Thorroad, S.; Worawittayanon, P.; Ruchirawat, S.; **Thasana, N.** Lycophlegmariols A-D: Cytotoxic serratene triterpenoids from the club moss *Lycopodium phlegmaria* L. *Phytochemistry* **2012**, *76*, 117-123, *doi: 10.1026/j.phytochem.2012.01.006.* **(IF2011 = 3.351)**

3. Worayuthakarn, R.; Nealmongkol, P.; Ruchirawat, S.; **Thasana, N.** Synthesis of benzoindoloquinolizines via Cu(I)-mediated C-N bond formation. *Tetrahedron* **2012**, *68*, 2864-2875, *doi: 10.1026/j.tet.2012.01.094*. **(IF2011 = 3.025)**

4. Boonya-udtayan, S.; Eno, M.; Ruchirawat, S.; Mahidol, C.; **Thasana, N.** Palladium-catalyzed intramolecular C-H activation: Synthesis and biological activities of indolobenzazocin-8-ones. *Tetrahedron* **2012**, *68*, 10293-10301, *doi: 10.1026/j.tet.2012.10.011*. **(IF2011 = 3.025)**

5. Nealmongkol, P.; Tangdenpaisal, K.; Ruchirawat, S.; **Thasana, N.** Cu(I)-mediated lactone formation in subcritical water: A benign synthesis of benzopyranones and urolithins A-C. *Tetrahedron* **2013**, *accepted.* **(IF2011 = 3.025)**

6. Worayuthakarn, R.; Boonya-udtayan, S.; Ruchirawat, S.; **Thasana, N.** Total synthesis of scandione and calophione A. **2013**, *manuscript in preparation.*

7. Nealmongkol, P.; Calmes, J.; Ruchirawat, S.; **Thasana, N.** Copper and palladium mediated C-N bond formation: Syntheis of phenanthridinones. **2013**, *manuscript in preparation*.

### PRESENTATIONS

1. Boonya-udtayan, S.; Yotapan, N.; Carson, B.; Woo, C.; Ruchirawat, S.; **Thasana, N.** Synthesis and biological activities of azalamellarins. *The 0th Junior International Conference on Cutting-Edge Organic Chemistry in Asia*, Hsinchu, ***Taiwan,*** **2010**, J-07, 7. (Presentation Award).

2. **Thasana, N.**; Worayuthakarn, R.; Boonya-udtayan, S.; Ruchirawat, S. Unsymmetrical bioactive benzils: Synthesis of licoagrodione, scandione, and calophione A. The Pre-Syposium of 5th International Conference on Cutting-Edge Organic Chemistry in Asia, National Sun Yat-sen University, Kaohsiung, ***Taiwan,*** **2010,** O-10, 12.



3. Boonya-udtayan, S.; Worayuthakarn, R.; Nealmongkol, P.; Tangdenpaisal, K.; Ruchirawat, S.; **Thasana, N.** Cu(I)/Pd(II)-mediated C-O and C-N bond formations: Synthesis of natural product-like alkaloids. *The 5th International Conference on Cutting-Edge Organic Chemistry in Asia/The 1st New Phase International Conference on Cutting-Edge Organic Chemistry in Asia (ICCEOCA-5/NICCEOCA-1)*, Ambassador Hotel, Hsinchu, ***Taiwan,* 2010**, IL-22, 22.

4. **Thasana, N.**; Worayuthakarn, R.; Boonya-udtayan, S.; Ruchirawat, S. Synthesis of unsymmetrical bioactive benzil derivatives. *The 9th NRCT-JSPS Joint Seminar: Natural Medicine Research for the Next Decade: New Challenges and Future Collaboration.* Chulalongkorn University, Bangkok, ***Thailand,* 2010**, OP-14, 38-41.

5. Worawittayanon, P.; Witttayalai, S.; Sathalalai, S.; Thorraoad, S.; Ruchirawat, S.; **Thasana, N.** Screening and isolation of biologically active compounds from lycopodium plants in Thailand. *Center of Excellence on Environmental Health, Toxicology and Management of Chemicals: Perspectives in Environmental Health Research.* CRI, Bangkok, ***Thailand,* 2011**, Abstract P-35, 43, Proceedings p162-166.

6. Nealmongkol, P.; Tangdenpaisal, K.; Ruchirawat, S.; **Thasana, N.** On-water chemistry: "Green synthesis" of benzopyranone derivatives with subcritical water. *The 1st Junior International Conference on Cutting-Edge Organic Chemistry in Asia*, Xiamen University, Xiamen, ***China,* 2011**, O-17, 24. (Impressive Presentation Award).

7. **Thasana, N.**; Worayuthakarn, R.; Boonya-udtayan, S.; Nealmongkol, P.; Ruchirawat, S. Copper(I)-mediated and palladium (II)-catalyzed indole formation: Synthesis of benzoindoloquinolizinone and indolobenzazipinone. *The 6th International Conference on Cutting-Edge Organic Chemistry in Asia/The 2nd New Phase International Conference on Cutting-Edge Organic Chemistry in Asia (ICCEOCA-6/NICCEOCA-2)*, The Chinese University of Hong Kong, ***Hong Kong,* 2011**, PA-21, 51.

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9. **Thasana, N.**; Boonya-udtayan, S.; Worayuthakarn, R.; Nealmongkol, P.; Tangdenpaisal, K.; Ruchirawat, S. Synthesis of natural product-like alkaloids using Cu(I)/Pd(II)-mediated C-O and C-N bond formations. *TRF Conference*. Cha-am, Petchabury, ***Thailand,* 2012**, O-PHY-B01, 13.

10. Nealmongkol, P.; Ruchirawat, S.; **Thasana, N.** A facile synthesis of nvobiocin analogs using copper-catalyzed and microwave-assisted 3-amidocoumarin formation. *The 7th International Conference on Cutting-Edge Organic Chemistry in Asia/The 3rd New Phase International Conference on Cutting-Edge Organic Chemistry in Asia (ICCEOCA-7/NICCEOCA-3)*, Nanyang Technological University, ***Singapore,* 2012**, PA-43, 102.



## AWARDS AND HONORS

**2011** Asian Core Program Lectureship Award (Visit to Korea and Taiwan): *The 6th International Conference on Cutting-Edge Organic Chemistry in Asia/The 2nd New Phase International Conference on Cutting-Edge Organic Chemistry in Asia (6th ICCEOCA/2nd NICCEOCA)*

## LECTURE

1. Thasana, N. *"Synthesis and biological activitie of natural product-like alkaloids"*, ACP Lectureship Award in Taiwan, National Taiwan Normal University (NTNU), Taipei, Taiwan, November 5, 2012.
2. Thasana, N. *"Synthesis and biological activitie of natural product-like alkaloids"*, ACP Lectureship Award in Taiwan, National Tsing Hua University (NTHU), Hsinchu, Taiwan, November 7, 2012.
3. Thasana, N. *"Synthesis and biological activitie of natural product-like alkaloids"*, ACP Lectureship Award in Taiwan, National Chung Hsing University (NCHU), Taichung, Taiwan, November 8, 2012.
4. Thasana, N. *"Synthesis and biological activitie of natural product-like alkaloids"*, ACP Lectureship Award in Taiwan, National Health Research Institute (NHRI), Miaoli, Taiwan, November 9, 2012.
5. Thasana, N. *"Microwave-assisted high speed chemistry, A technique in organic synthesis"*, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, November 12, 2012.
6. Thasana, N. *"Design and Synthesis of Bioactive Natural Product-like Alkaloids"*, ACP Lectureship Award in Korea, Ewha Womens University (EWU), Seoul, Korea, April 2, 2013.
7. Thasana, N. *"Design and Synthesis of Bioactive Natural Product-like Alkaloids"*, ACP Lectureship Award in Korea, Pohang University of Science and Technology (POSTECH), Pohang, Korea, April 3, 2013.
8. Thasana, N. *"Design and Synthesis of Bioactive Natural Product-like Alkaloids"*, ACP Lectureship Award in Korea, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea, April 5, 2013.
9. Thasana, N. *"Microwave chemistry: A high speed vehicle in organic synthesis and medicinal chemistry"*, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkla, Thailand, May 17, 2013.



# ภาคผนวก

# (Appendix)



ANTICANCER RESEARCH *31*: 921-928 (2011)

# Overcoming Multidrug Resistance in Human Lung Cancer with Novel Benzo[*a*]quinolizin-4-ones

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**Abstract.** *Aim: To investigate the ability of synthetic benzo[a]quinolizin-4-one derivatives to reverse multidrug resistance (MDR) in lung cancer cells. Materials and Methods: A cell line with MDR, A549RT-eto, was established by exposure to 1.5 μM etoposide. Cytotoxic activity was assayed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromine (MTT) method. The mechanism of drug resistance was studied by real-time PCR, Western blot analysis, and flow cytometry. Benzo[a]quinolizin-4-one derivatives were synthesized and tested for cytotoxic activity and ability to modulate MDR. Results: A549RT-eto cells had an $IC_{50}$ for etoposide of 176 μM, 28-fold higher than parental cells, due to increased levels of MDR1 gene and P-glycoprotein (P-gp), resulting in greater drug efflux. Three benzo[a]quinolizin-4-ones reduced etoposide $IC_{50}$ from 176 μM to 22.4 μM -24.7 μM. This resulted from increased drug accumulation without altering P-gp expression at the transcription or translation level. Conclusion: Non-toxic concentrations of benzo[a]quinolizin-4-one derivatives can reverse drug resistance of A549RT-eto by increasing the intracellular drug accumulation.*

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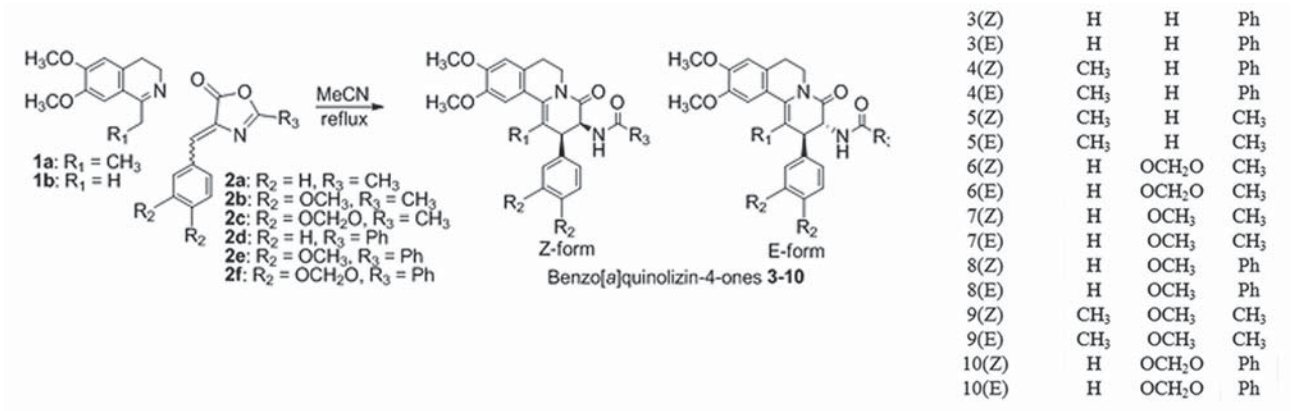
*Key Words:* Multidrug resistance, P-glycoprotein, benzo[*a*]quinolizin derivatives.

Multidrug resistance (MDR) in tumor cells is a major problem in the success of chemotherapy for many types of cancers. Of the various mechanisms proposed for drug resistance in cancer cells (1), a major mechanism involves the increase of drug efflux out of cells mediated by P-glycoprotein (P-gp). P-gp is a member of the ATP-binding cassette (ABC) superfamily of transporter proteins and utilizes the energy released from ATP hydrolysis to pump out cytotoxic drugs from cancer cells, leading to lower intracellular concentrations of chemotherapeutic drugs (1).

Drug resistance can be overcome by co-administering substances that inhibit transporters together with anticancer drugs to increase the efficiency of chemotherapy. Inhibition of P-gp as a way of reversing MDR has been extensively studied (2). Verapamil, cyclosporin, and taxoxifen have been shown to modulate the P-gp transporter (3, 4). Unfortunately, these reversing agents produced disappointing results *in vivo* because of their low binding affinities, so that reversal of MDR requires higher concentrations resulting in unacceptable toxicity. Thus, verapamil, a first-generation P-gp inhibitor, has effective concentrations in the range of 5 to 50 μM, which is cytotoxic to normal cells, resulting in clinical toxicity *e.g.* arterio-ventricular block, hypotension and cardiac toxicity at concentrations required to inhibit drug resistance (3). More recently, several natural products and synthetic compounds, such as valspodar (PSC833) (5), biricodar (VX-710) and elacridar (GF120918) (6), have been reported to overcome drug resistance through inhibition of P-gp activity *in vitro*. However, some compounds showed characteristics that limit their clinical usefulness, for example, significantly




| | | | |
|---|---|---|---|
| 3(Z) | H | H | Ph |
| 3(E) | H | H | Ph |
| 4(Z) | $CH_3$ | H | Ph |
| 4(E) | $CH_3$ | H | Ph |
| 5(Z) | $CH_3$ | H | $CH_3$ |
| 5(E) | $CH_3$ | H | $CH_3$ |
| 6(Z) | H | $OCH_2O$ | $CH_3$ |
| 6(E) | H | $OCH_2O$ | $CH_3$ |
| 7(Z) | H | $OCH_3$ | $CH_3$ |
| 7(E) | H | $OCH_3$ | $CH_3$ |
| 8(Z) | H | $OCH_3$ | Ph |
| 8(E) | H | $OCH_3$ | Ph |
| 9(Z) | $CH_3$ | $OCH_3$ | $CH_3$ |
| 9(E) | $CH_3$ | $OCH_3$ | $CH_3$ |
| 10(Z) | H | $OCH_2O$ | Ph |
| 10(E) | H | $OCH_2O$ | Ph |

Figure 1. *Schematic representation of synthesis of benzo[a]quinolizin-4-one and molecular structure of benzo[a]quinolizin-4-one derivatives 3-10.*

inhibiting metabolism *in vivo* or exhibiting severe side-effects (7-8). Therefore, new P-gp modulators with lower toxicity and greater potency are still needed.

The benzo[*a*]quinolizine ring system is an important basic structure found in many biologically active compounds including berberine, emetine and related ipecac alkaloids. These compounds have been reported to have interesting biological activities such as anti-neoplastic, antimicrobial and anti-depressant activities, as well as lowering cholesterol and blood sugar levels (9). Recently, our group has reported the synthesis of novel derivatives of benzo[*a*]quinolizin-4-ones (10). In the present study, some benzo[*a*]quinolizine derivatives were screened for cytotoxic activity and for ability to reverse MDR using a newly developed etoposide-resistant non-small cell lung carcinoma cell line (A549RT-eto) as an *in vitro* model. These derivatives showed moderate inhibition of P-gp-mediated efflux and reversed P-gp-dependent drug resistance at non-toxic concentrations.

## Materials and Methods

*Chemicals*. Etoposide, doxorubicin, cisplatin, colchicine, vinblastine, 5-fluorouracil (5-FU), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromine (MTT), calcein-acetoxymethylester (calcein-AM), verapamil, ProteoPrep Membrane Extraction Kit and P-glycoprotein antibody, C219, were purchased from Sigma, St. Louis, MO, USA. RNeasy kit, DNaseI and QuantiTect SYBR Green PCR master mix were purchased from Qiagen, (KJ Venlo, the Netherlands). Bradford reagent and taxol were purchased from Biorad Laboratories (Hercules, CA, USA) and Bristol Myers Squibb (New York, NY, USA), respectively. SuperscriptIII was obtained from Invitrogen (San Diego, CA, USA). All other chemicals were analytical grade. Non-small cell lung adenocarcinoma cell line, A549 was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Rabbit anti-mouse immunoglobulin G (IgG) was obtained from Dako Cytomation, (Glostrup, Denmark). KB-V1 was kindly provided by Professor Gottesman MM (Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, MD, USA)

*Development of drug-resistant lung cancer cells*. To develop etoposide resistant cells (A549RT-eto), parental A549 cells were continuously exposed to increasing concentrations of etoposide up to 1.5 μM over a period of 18 months. Cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco) and antibiotics at 37˚C in the presence of 5% $CO_2$. Resistant cells were maintained in 1.5 μM etoposide. Morphology of both etoposide-resistant and the parental lung cancer cell lines was studied using a Nikon TMS inverted microscope (Tokyo, Japan).

*Synthesis of chemomodulator benzo[a]quinolizin-4-one derivatives 3-10*. The tricyclic benzo[*a*]quinolizin-4-ones were prepared as described previously (10). The reaction of azlactones 2a-f (1 mmol) with various 1-substituted 3,4-dihydroisoquinolines 1a-b (1.5 mmol) containing methylene moiety by refluxing in acetonitrile (10 ml) for 2 h gave the corresponding benzo[*a*]quinolizine-4-ones 3-10 as a mixture of *cis*/*trans* (*Z*/*E*) form. The solvent was evaporated to dryness under vacuum. The crude product was purified by PLC on silica gel using 50% ethyl acetate in hexane as an eluent to give diastereoisomer of benzo[*a*]quinolizin-4-ones (*Z*/*E*) in moderate to good yield (Figure 1).

*Cytotoxicity assay*. The sensitivities of A549 and A549RT-eto cells to various chemotherapeutic drugs; etoposide, doxorubicin, cisplatin, taxol, colchicine, 5-FU, vinblastin and benzo[*a*]quinolizin-4-one derivatives were determined by MTT assay as previously described (11). Cells were cultured in drug-free medium for at least one week before the experiments were performed. Briefly, cells suspended in culture media were seeded at $5\times10^3$ cells per well into a 96-well plate, and incubated in humidified atmosphere, 5% $CO_2$ at 37˚C. After 24 hours, the cells were then cultured for 72 hours in the presence of chemotherapeutic drugs. Thereafter, the media were removed and the fresh media with MTT were added to each well. The plates were incubated at 37˚C for 2 hours. The dark blue formazan crystals formed by viable cells were dissolved in dimethyl sulfoxide (DMSO) solution. Absorbance of individual samples was determined at 550 nm using a Spectra Max Plus 384 microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). The concentrations required to inhibit growth by 50% ($IC_{50}$ values) were calculated. Each drug concentration was tested in at least three independent experiments and average values±S.D. were calculated. Relative resistance was calculated as the ratio of the $IC_{50}$ value of A549 cells to the $IC_{50}$ value of A549RT-eto cells.

*Chemosensitivity assay*. The effect of MDR-reversing agent of benzo[*a*]quinolizin-4-one derivatives on A549RT-eto cells to etoposide was also determined using MTT assay as described above. Non-cytotoxic benzo[*a*]quinolizin-4-one derivatives were selected and used to treat cells at final concentration of 10 μM in combination with several concentrations of etoposide. Reversing index was calculated as the ratio of the etoposide $IC_{50}$ value towards A549RT-eto in the absence and the presence of test compounds.

*Real-time PCR studies*. Quantitative analysis of mRNA expression for drug resistance related genes in A549 and A549RT-eto cells was performed by real-time PCR. Total cellular RNA was isolated from cells using RNeasy kit. QuantiTect SYBR Green PCR master mix was used with 2 μl of cDNA and 10 pmol of primers: *MDR1* F: 5'GTCTTTGGTGCCATGGCCGT3', R: 5'ATGTCCGGTCGGGTG GGATA3' *MRP1* F: 5'CTGACAAGCTAGACCATGAATGT3', R: 5'C CTTTGTCCAAGACGATCACCC3'*MRP2*F: 5'GCCAGATTGG CCCAGCAAA3', R: 5'AATCTGACCACCGGCAGCCT3', *MRP3*F: 5'GGGACCCTGCGCATGAACCTG3', R: 5'TAGGCAAGTCCAGC ATCTCTGG3', BCRPF: 5'TGGCTGTCATGGCTTCAGTA3', R: 5'GCCACGTGATTCTTCCACAA3', *LRP*F: 5'GAGCAGTTCACA GTGTTGTCC3', R: 5'AAAGCCAAAGACAGCAGTGCG3', *GSTF*: 5'TACGGGCAGCTCCCCAAGTT3', R: 5'TGCCCGCCTCATAGTT GGTG3', *TOPO2A*: 5'GGCTCGATTGTTATTTCCAC3', R: 5'ATGG TTGTAGAATTAAGAATAGC3', *TOPO2B*: 5'GCTGTGGATGAC AACCTC3', R: 5'GCTGTGGATGACAACCTC3', *actin*: 5'GACCT GACTGACTACCTCATGA3', R: 5'AGCATTTGCGGTGGACGA TGGAG3'. Real-time PCR was performed in capillary glass tubes on a LightCycler (Roche Applied Science, Indianapolis, IN, USA). During the amplification, SYBR green binds to double strand PCR products, so the fluorescence signal increases with increasing amounts of products. An initial activation step at 95˚C for 15 minutes was followed by 35 cycles comprising denaturation at 94˚C for 15 seconds, annealing at the respective temperature 55˚C for 30 seconds and extension at 72˚C for 30 seconds. Melting curve analyses and gel electrophoresis of products were used to validate the reactions. Reactions were photographed with Gene Genius Bio-Imaging system (Syngene, Cambridge, UK) and revealed single amplification products with the predicted sizes. The ratios of gene expression values were normalized using that of a housekeeping gene (β-actin as internal control) and calculated using the following formula:
Ratio of target gene expression=Fold change in target gene expression (A549RT-eto/A549)/Fold change in reference gene expression (A549RT-eto/A549)

*Western blot analysis*. For the investigation of the changes in protein expression, cells were treated as described above. Membrane proteins were extracted using ProteoPrep Membrane Extraction Kit. Protein concentrations were determined by Bradford assay. Solubilized membrane proteins (40 μg) were boiled in sodium dodecyl sulfate (SDS) sample buffer at 100˚C for 5 minutes and separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), then transferred to a polyvinylidene fluoride (PVDF) membrane. After blocking with 10% non-fat dried milk in TBS-T buffer (20 mM Tris buffer saline, pH 7.6 containing 0.1% Tween-20) overnight, membrane was probed with 1:1,000 diluted human P-gp monoclonal antibody C219 for 1 hour. Then, the membrane was incubated in 1:5,000 diluted secondary antibody linked with horseradish peroxidase for visualization by chemiluminescence (ECL Plus detection system with high-performance film; GE Healthcare, Waukesha, WI, USA).

*Calcein-AM efflux assay*. Evaluation of P-gp activity was performed by measuring the fluorescence due to intracellular accumulation of calcein produced by ester hydrolysis of the P-gp substrate calcein-AM. The transport capacity of P-gp is inversely proportional to the intracellular accumulation of fluorescent calcein (12). The ability of modulators to inhibit P-gp mediated efflux was investigated. Briefly, $1\times10^6$ cells cells were incubated with 0.5 μM of calcein-AM at 37˚C in the absence and presence of 10 μM benzo[*a*]quinolizin-4-one derivatives or P-gp inhibitor, verapamil for 1 hour. After incubation, cells were washed, resuspended and then calcein accumulation was measured using a FACSCalibur™ flow cytometer (Becton Dickinson Biosciences, San Jose, CA, USA) with excitation wavelength of 488 nm and emission of 530 nm.

*Statistical analysis*. Statistics were performed with the Unpaired *t*-test, one-way ANOVA and Tukey–Kramer multiple comparisons test using GraphPad Instat (GraphPad software, San Diego, CA, USA). Values significantly different from the control are shown by $*p<0.05$, $**p<0.01$ and $***p<0.001$.

## Results

*Development of drug-resistant lung cancer cells*. An etoposide resistant cell line (A549RT-eto) was established over a period of 18 months. There was no significant difference in growth curve and doubling time between the A549 parental cells and the A549RT-eto resistant cells (data not shown). However, the two cell lines showed differences in morphology. A549 cells showed an epithelioid-like shape and adhered to the dish, whereas morphology of A549RT-eto cells changed to a spindle-like shape, varying in size and having unclear cell borders (Figure 2).

*Cytotoxicity assay*. Cytotoxic effects of etoposide on the lung cancer cell lines were studied by measuring cell viability using the MTT assay. Dose–response curves were obtained after 72 hours of exposure of A549 and A549RT-eto cells to various concentrations of etoposide. The concentration of etoposide inhibiting growth of A549RT-eto cells by 50% ($IC_{50}$) was 176±9.5 μM, or 28-fold higher than that for A549 cells, which had an $IC_{50}$ of 6.33±1.46 μM (Table I). Moreover, the resistant cells exhibited cross-resistance to the structurally unrelated antitumor agents, doxorubicin, taxol, colchicine and vinblastin, but still remained sensitive to cisplatin and 5-FU, as shown in Table I.

*Mechanism of drug resistance*. In order to analyze changes in gene expression associated with drug resistance in A549RT-eto cells, real-time PCR was used to compare the expression of drug resistance related genes: P-glycoprotein (*MDR1*), multidrug resistance related protein (*MRP1, MRP2* and *MRP3*), breast cancer resistance protein (*BCRP*), lung resistance-related protein (*LRP*), glutathione-*S*-transferase π (*GSTP*) and topoisomeraseII (*topoIIa* and *topoIIb*) in resistant cells compared with parental cells. A549RT-eto cells

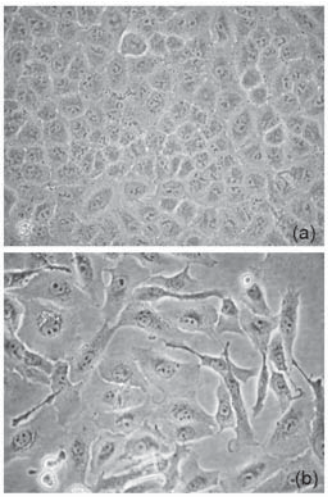



Figure 2. *Morphologies of parental (A549), and etoposide-resistant (A549RT-eto) cells shown under ×400 magnification by inverted microscopy.*

Table I. *$IC_{50}$ and resistance index of A549 cells and A549RT-eto cells to chemotherapeutic drugs. Each $IC_{50}$ value is an average value±S.D. from three independent experiments. Resistance index represents the ratio of $IC_{50}$ for A549RT-eto cells to the $IC_{50}$ for A549 cells. $IC_{50}$ values differing significantly between A549RT-eto cells and A549 cells by unpaired t-test are shown by *p<0.05, **p<0.01 and ***p<0.001.*

| Anticancer drug | $IC_{50}$ | | Resistance index |
|---|---|---|---|
| | A549 | A549RT-eto | |
| Etoposide (μM) | 6.33±1.46 | 176±9.5 | 27.7*** |
| Doxorubicin (μM) | 0.32±0.10 | 1.57±0.15 | 4.90** |
| Taxol (nM) | 3.50±1.00 | 12.2±1.0 | 3.48*** |
| Colchicine (nM) | 18.2±10 | 56.3±8.0 | 3.09* |
| Vinblastin (nM) | 0.20±0.15 | 0.80±0.10 | 4.00* |
| Cisplatin (μM) | 8.09±4.63 | 12.2±4.7 | 1.50 |
| 5FU (μM) | 30.7±2.98 | 34.0±4.5 | 1.11 |

Table II. *Ratios of target gene expression in etoposide-resistant A549RT-eto cells compared with that in parental A549 cells using real-time PCR.*

| Gene | Ratio of target gene expression |
|---|---|
| *MDR1* | 16.0 |
| *MRP1* | 1.82 |
| *MRP2* | 0.52 |
| *MRP3* | 0.65 |
| *BCRP* | 0.50 |
| *LRP* | 1.51 |
| *GSTP* | 1.13 |
| *TOPO2A* | 0.26 |
| *TOPO2B* | 0.31 |

expressed *MRP1, MRP2, MRP3, BCRP, LRP, GSTP, TOPO2A* and *TOPO2B* at comparable levels to A549 cells (Figure 3A). In contrast, the expression level of *MDR1* in resistant cells was dramatically increased by 16-fold compared to the parental cell line (Table II). The results suggest that the major mechanism of acquired etoposide resistance in the A549 cell line involves the up-regulation of *mdr1* gene, which encodes for a drug efflux transporter, P-gp. P-gp expression of the resistant cell line was confirmed by Western blot analysis using C219 antibody. P-gp (170 kDa) was highly expressed in A549RT-eto resistant cells, whereas no detectable P-gp could be observed in A549 parental cells (Figure 3B). This indicates that resistance to etoposide is related to the overexpression of P-gp in A549RT-eto cells. A functional study of P-gp was performed by calcein accumulation using flow cytometry. Calcein-AM becomes fluorescent after cleavage by cellular esterase producing a fluorescent derivative calcein. P-gp actively extrudes the calcein-AM, but not the fluorescent calcein. Therefore, calcein-AM efflux measured by flow cytometric analysis can be used to assess the function of the P-gp pump. Increased accumulation of calcein is detectable as a shift in the fluorescence peak to the right. As shown in Figure 3C, calcein was accumulated at higher levels in A549 cells than in A549RT-eto cells, suggesting that A549RT-eto cells showed higher activity of the efflux pump transporting calcein-AM out of the cells. Moreover, the addition of established P-gp inhibitor, verapamil, resulted in a substantial increase in the accumulation of calcein compared to untreated A549RT-eto cells. The data strongly confirm that the acquired resistance in A549RT-eto cells is associated with overexpression of P-gp which lowers the intracellular drug accumulation.

*Reversal of etoposide resistance by benzo[a]quinolizine-4-one derivatives 3-10.* Fifteen synthetic benzo[*a*]quinolizine-4-one derivatives, *Z*- and *E*-isomers of compounds 3-10, and *Z*-isomer only of compound 6 were investigated for their ability to inhibit proliferation of A549RT-eto cells using the MTT assay. The cytotoxicity of these derivatives on the multidrug resistant cells, A549RT-eto, is shown in Table III. Four of the synthetic compounds, 3(*Z*), 4(*Z*), 4(*E*) and 8(*E*), displayed cytotoxic activity towards the A549RT-eto cell line with an $IC_{50}$ in the range of 30-40 μM, while 11 of the synthetic compounds did not show cytotoxicity at the concentrations tested ($IC_{50}$ more than 100 μM).

The $IC_{50}$ of etoposide alone towards A549RT-eto cells was 176 μM. The ability of the eleven non-cytotoxic compounds to restore drug sensitivity of A549RT-eto cells towards etoposide was investigated by determining the $IC_{50}$ of etoposide towards A549RT-eto in the presence of non-cytotoxic concentrations (10 μM) of these compounds. As shown in Table III, only 3 compounds, 5(*Z*), 7(*Z*) and 9(*Z*)

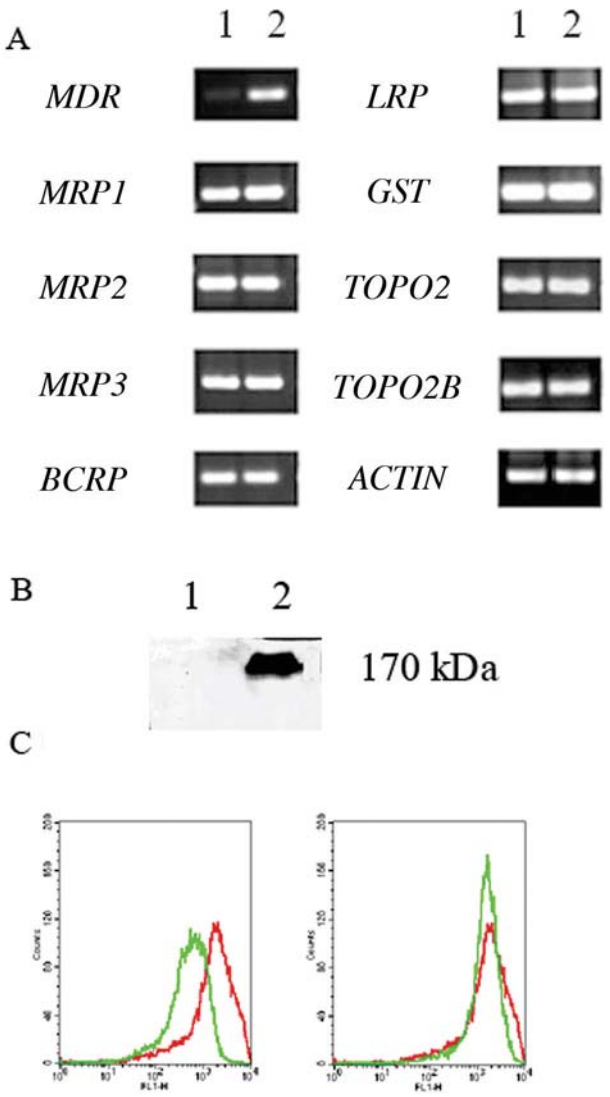



Figure 3. *Expression and activity of drug-resistance genes and proteins. A) RT-PCR characterization of drug resistance-related genes (MDR1, MRP1, MRP2, MRP3, BRCP, LRP, GST, TOPO2A, TOPO2B) and control (actin) in parental A549 (lane 1) and (lane 2) resistant A549RT-eto cells. B) P-gp expression in parental A549 cells (lane 1) and resistant A549RT-eto cells (lane 2) by immunoblotting. C) Accumulation of calcein-AM analyzed by flow cytometry. Left: Accumulation of calcein-AM in A549 cells (red line) compared to that with resistant A549RT-eto (green line) cells. Right: Accumulation of calcein-AM in A549 cells (red line) compared to that of resistant A549RT-eto cells in the presence of P-gp inhibitor, verapamil (green line).*

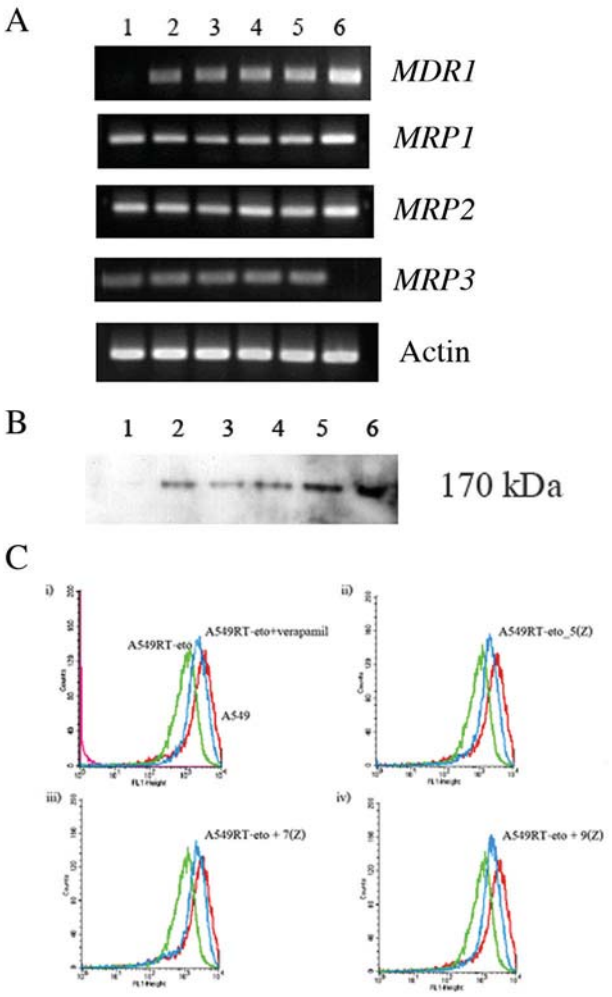



Figure 4. *Expression and activity of ABC transporter genes and proteins. A) Expression of genes (MDR1, MRP1, MRP2, MRP3) in lane 1: parental A549 cells; lane 2: resistant A549RT-eto cells; lanes 3-5; resistant A549RT-eto cells treated with 5(Z), 7(Z) and 9(Z) respectively; lane 6: positive control, KB-V1. B) protein expression of P-gp by immunoblotting in lane 1: parental A549 cells; lane 2: resistant A549RT-eto cells; lanes 3-5; resistant A549RT-eto cells treated with 5(Z), 7(Z) and 9(Z) respectively; lane 6: positive control, KB-V1. (C) Intracellular accumulation of calcein-AM by flow cytometry in A549 cells (red) compared to that of resistant A549RT-eto cells (green) and resistant A549RT-eto cells in the presence of the selected compounds (blue) namely i) verapamil or benzo[a]quinolizin-4-one derivatives (ii) 5(Z), (iii) 7(Z) and (iv) 9(Z).*

showed the ability to partially reverse drug resistance in A549RT-eto, by reducing the $IC_{50}$ of etoposide to 22.6, 24.7 and 22.4 μM respectively.

*Possible mechanism of action of potential reversing agents, 5(Z), 7(Z) and 9(Z) in sensitizing A549RT-eto cells*. First, the expression of drug resistance-related genes was studied by real-time PCR analysis in A549RT-eto cells treated with reversing agents 5(*Z*), 7(*Z*) and 9(*Z*) and in untreated A549RT-eto cells. Comparison of untreated and reversing agent-treated cells showed no significant difference in the expression levels of any of the drug resistance-related genes, namely the ABC transporters, *MDR1, MRP1, MRP2* and *MRP3* (Figure 4A). Similarly, immunoblotting of P-gp showed that the up-regulation of P-gp levels in A549RT-eto

Table III. *Effect of benzo[a]quinolizin-4-one derivatives on the sensitivity of A549RT-eto cells. Each $IC_{50}$ value is an average value±S.D. from three independent experiments.*

| Compound name | Cytotoxicity $IC_{50}$ (μM) of compound alone | $IC_{50}$ (μM) for etoposide in the presence of compound | Reversing index |
|---|---|---|---|
| Etoposide | 176 | | |
| Verapamil | >100 | 36.4±8.80 | 4.79 |
| 3(*Z*) | 40 | Nd | Nd |
| 3(*E*) | >100 | 153±5 | 1.14 |
| 4(*Z*) | 35 | Nd | Nd |
| 4(*E*) | 30 | Nd | Nd |
| 5(*Z*) | >100 | 22.6±7.0 | 7.74*** |
| 5(*E*) | >100 | 178.5±10.0 | 0.98 |
| 6(*Z*) | >100 | 118.9±5.7 | 1.47* |
| 6(*E*) | ND | Nd | Nd |
| 7(*Z*) | >100 | 24.7±6.4 | 7.10*** |
| 7(*E*) | >100 | 128±15 | 1.37 |
| 8(*Z*) | >100 | 68.0±10.6 | 2.57** |
| 8(*E*) | 38 | Nd | Nd |
| 9(*Z*) | >100 | 22.4±4.6 | 7.80*** |
| 9(*E*) | >100 | 153±10 | 1.14 |
| 10(*Z*) | >100 | 153±7 | 1.14 |
| 10(*E*) | >100 | 153±10 | 1.14 |

Cytotoxicity of each derivative was determined and derivatives showing an $IC_{50}$ greater than 100 μM were studied for their ability to affect the $IC_{50}$ of etoposide. Reversing index is defined as the ratio of $IC_{50}$ for etoposide in the absence and in the presence of added compound. Statistics were performed using one-way ANOVA and Tukey-Kramer Multiple Comparisons Test. Significantly different values are shown by *p<0.05, **p<0.01 and ***p<0.001. Nd, not determined since test compounds were toxic to cells.

cells was not diminished by treatment with benzo[*a*] quinolizine-4-one derivatives (Figure 4B). These results indicate that 5(*Z*), 7(*Z*) and 9(*Z*) do not inhibit expression of the drug transporter genes, including P-gp at either the gene expression level (Figure 4A) or the protein level (Figure 4B). The ability of the 5(*Z*), 7(*Z*) and 9(*Z*) to affect intracellular drug accumulation in A549RT-eto resistant cells was further investigated. Flow cytometric analysis of their action on reversal of drug resistance demonstrated that the addition of all modulating compounds caused a significant increase in accumulation of calcein in A549RT-eto (Figure 4C). In contrast, the tested substances did not cause any significant increase in the intracellular calcein levels of parental A549 cells (data not shown). Therefore, the calcein accumulation study was consistent with the reversal of drug resistance evaluated by the MTT assay.

## Discussion

In the present study, an etoposide-resistant A549RT-eto cell line was developed and used as an *in vitro* model of screening for inhibitors of P-gp. The resistant cells showed change in morphology from epitheloid shape to spindle-like shape. Morphological changes have also been reported in several studies of resistant cell lines (13, 14). Thus, cisplatin-resistant neuroblastoma cell lines demonstrated alterations in their morphology without changes in cell growth (13). Moreover, a correlation between the epithelial to mesenchymal transition (EMT) and drug resistance has been reported by Arumugam (15). Morphological changes in cells may also be caused by alterations of the actin cytoskeleton or by mutations of the *c-KIT* gene (16). In an adriamycin-resistant leukemia cell line, morphological changes have been reported to be associated with the tumor cell environment rather than the abundance of P-glycoprotein in the plasma membrane (14). However, the relationship between the changes in morphology and changes in gene expression of the A549RT-eto resistant cell line is still unclear.

The mechanisms of etoposide-resistance in A549 cells have been shown to vary. Long *et al*. demonstrated that the mechanism of acquired etoposide-resistance in A549 cell lines was likely to relate to decreased topoisomeraseII expression (17). On the other hand, Trussadi *et al*. reported that etoposide resistance in A549 cells paralleled the increased expression of the *MRP1* gene and decreased expression of the *LRP* gene (18). However, our studies by real-time PCR, Western blotting and drug efflux pump analysis clearly show that the etoposide resistance of A549RT-eto cells involves up-regulation of the *MDR1* gene, resulting in overexpression of P-gP.

To overcome multidrug resistance in human cancer cell, a number of natural or synthetic compounds have been discovered that exhibit MDR activity (19-21). For example, a benflumetrol derivative, LY980503, has been reported to inhibit P-gp activity in vincristine-resistant gastric cancer cells and increase drug sensitivity by about 6-fold (20).

Benzo[*a*]quinolizin-4-ones exhibit the common structure of most P-gp inhibitors in having a methoxy phenol group and a basic nitrogen atom, as reported by Pajeva (22). Therefore, a series of benzo[*a*]quinolizin-4-one derivatives was synthesized having substituents differing in molecular size, hydrophobicity and *E*- and *Z*- stereoisomer at C2-C3 position (10). These synthesized compounds were initially tested for their ability to inhibit cell proliferation. Certain derivatives showed cytotoxic activity. Thus only 11 compounds, which had $IC_{50}$ values higher than 100 μM, were further investigated for their ability to reverse MDR and potentiate etoposide cytotoxic activity in A549RT-eto resistant cells. The data demonstrated that three benzo[*a*]quinolizin-4-one derivatives of *Z*-isomer, 5(*Z*), 7(*Z*) and 9(*Z*), were able to

increase the sensitivity of A549RT-eto to etoposide. Interestingly, none of the E-isomers was effective, indicating the stereospecificity of the target site. The benzo[*a*]quinolizin-4-one derivatives appear to reverse MDR by enhancing drug accumulation in A549RT-eto cells without altering the levels of the transcription of the *MDR1* gene or the level of P-gp expression. Thus, the action of the active benzo[*a*]quinolizin-4-one derivatives may be due to direct binding to P-gp and competition with drugs, such as etoposide, thereby enhancing intracellular drug accumulation.

Thus, our studies show that derivatives of benzo[*a*] quinolizine-4-one can sensitize drug-resistant A549RT-eto cells apparently by decreasing the activity of P-gp at non-toxic concentrations (10 μM). Thus benzo[*a*]quinolizine-4-one derivatives may be promising lead compounds for developing new drugs which reverse MDR *in vivo*. Future studies are required in order to investigate the action of these compounds *in vivo*, as well as to develop more effective compounds by modifying their structures.

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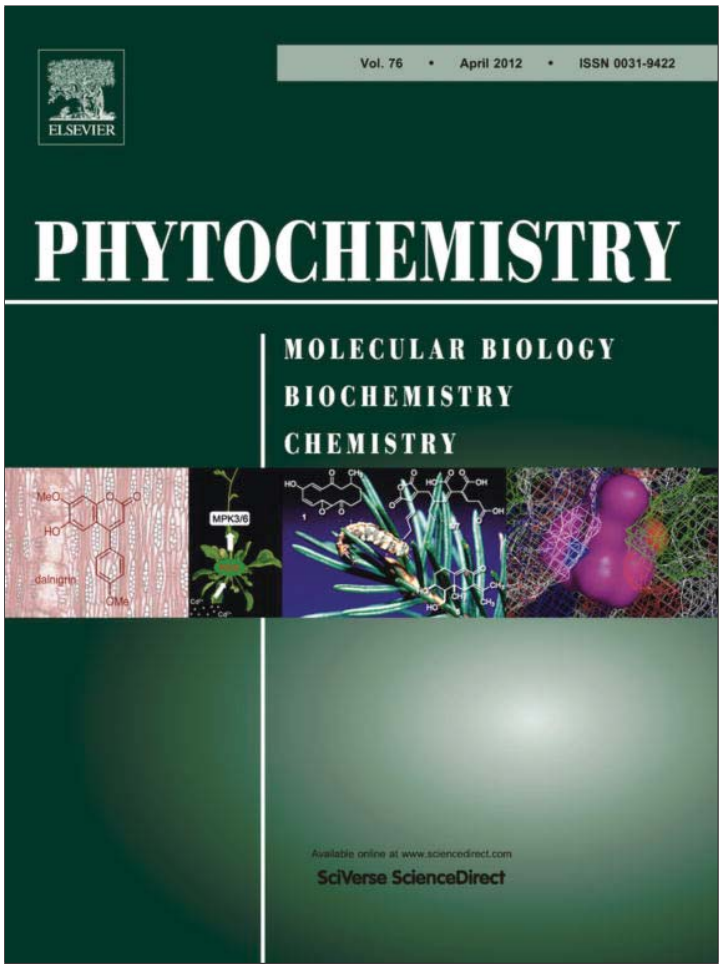

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# Lycophlegmariols A–D: Cytotoxic serratene triterpenoids from the club moss *Lycopodium phlegmaria* L.

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ABSTRACT

Lycopodium serratene triterpenoids, along with an abietane-type diterpene were isolated from the methanol extract of club moss *Lycopodium phlegmaria* L. The structures of these hitherto unknown lycopodium terpenoids were elucidated on the basis of spectroscopic analysis. Pentacyclic triterpenoids, 21β-hydroxy-serrat-14-en-3α-ol (**1**) and 21β-hydroxy-serrat-14-en-3α-yl acetate (**2**) were isolated together with four serratene triterpeneoids established as 21β,29-dihydroxyserrat-14-en-3α-yl dihydrocaffeate (lycophlegmariol A, **5**), 21β,24,29-trihydroxyserrat-14-en-3β-yl dihydrocaffeate (lycophlegmariol B, **6**), 21α,24-dihydroxyserrat-14-en-3β-yl 4-hydroxycinnamate (lycophlegmariol C, **7**), and 14β,21α,29-trihydroxyserratan-3β-yl dihydrocaffeate (lycophlegmariol D, **8**) as well as a known lycophlegmarin (**9**). An abietane-type diterpene, 8,11,13-abietatriene-3β,12-dihydroxy-7-one (margocilin, **10**), was isolated for the first time from a *Lycopodium* plant. Lycophlegmariol B (**6**), D (**8**) and compound **1** showed inhibitory effects against MOLT-3 acute lymphoblastic leukemia (T-lymphoblast) with $IC_{50}$ of 14.7, 3.0 and 2.9 μM, respectively.



## 1. Introduction

*Lycopodium phlegmaria* (Tagawa and Iwatsuki, 1979), also named *Huperzia phlegmaria*, is one of the club mosses (Lycopodiaceae) found in Thailand and used as a traditional herbal medicine (Jayaweera, 1981). The Lycopodiaceae is not only a famous source of lycopodium alkaloids (Hirasawa et al., 2009; Ma and Gang, 2004) having acetylcholine esterase (AChE) inhibition (Liu et al., 1986; Scarpini et al., 2003), particularly huperzine A (Ma et al., 2006, 2007; Ma and Gang, 2008), but they are also known as a source of serratene-type triterpenoids (Inubushi et al., 1971a,b; Orito et al., 1972; Shi et al., 2005; Tsuda et al., 1975; Zhang et al., 2002; Zhou et al., 2003). Serratene triterpenoids, possessing a seven membered ring C and seven tertiary methyl groups, are a common fused-pentacyclic triterpene with a double bond between C-14 and C-15 and oxymethines at both C-3 and C-21; they have been reported in fern alliances and conifers (Pinaceae) (Conner et al., 1981, 1984; Fang et al., 1991). Lycernuic acid C, isolated from *Lycopodium cernuum*, showed inhibitory effects against *Candida albicans* secreted aspartic protease (SAP) (Zhang et al., 2002). Previous work reported by Shi and co-worker that lycophlegmarin, isolated from *L. phlegmaria* L., showed modest growth-inhibitory activity against human hepatoma cells BEL 7402 (Shi et al., 2005). In this paper, the isolation and structure elucidation of three known serratene triterpenes (**1**, **2** and **9**) and four new serratene triterpenoids, lycophlegmariols A–D (**5**–**8**) are described (Fig. 1). A known 8,11,13-abietatriene-3β,12-dihydroxy-7-one (margocilin, **10**) was also isolated for the first time from *Lycopodium* plant, having been previously isolated from *Azadirachta indica* (Ara et al., 1990). Lycophlegmariol B (**6**), D (**8**) and compound **1** (Fang et al., 1991) showed inhibitory effects against MOLT-3 acute lymphoblastic leukemia (T-lymphoblast) with $IC_{50}$ of 14.7, 3.0 and 2.9 μM, respectively, while the other compounds were inactive.

## 2. Results and discussion

The whole plant of *L. phlegmaria* L. (948 g) was extracted with MeOH and then the greenish extract (204 g) was suspended in water and defatted with diethyl ether. Purification of the di-ethyl ether extract using a combination of silica gel column

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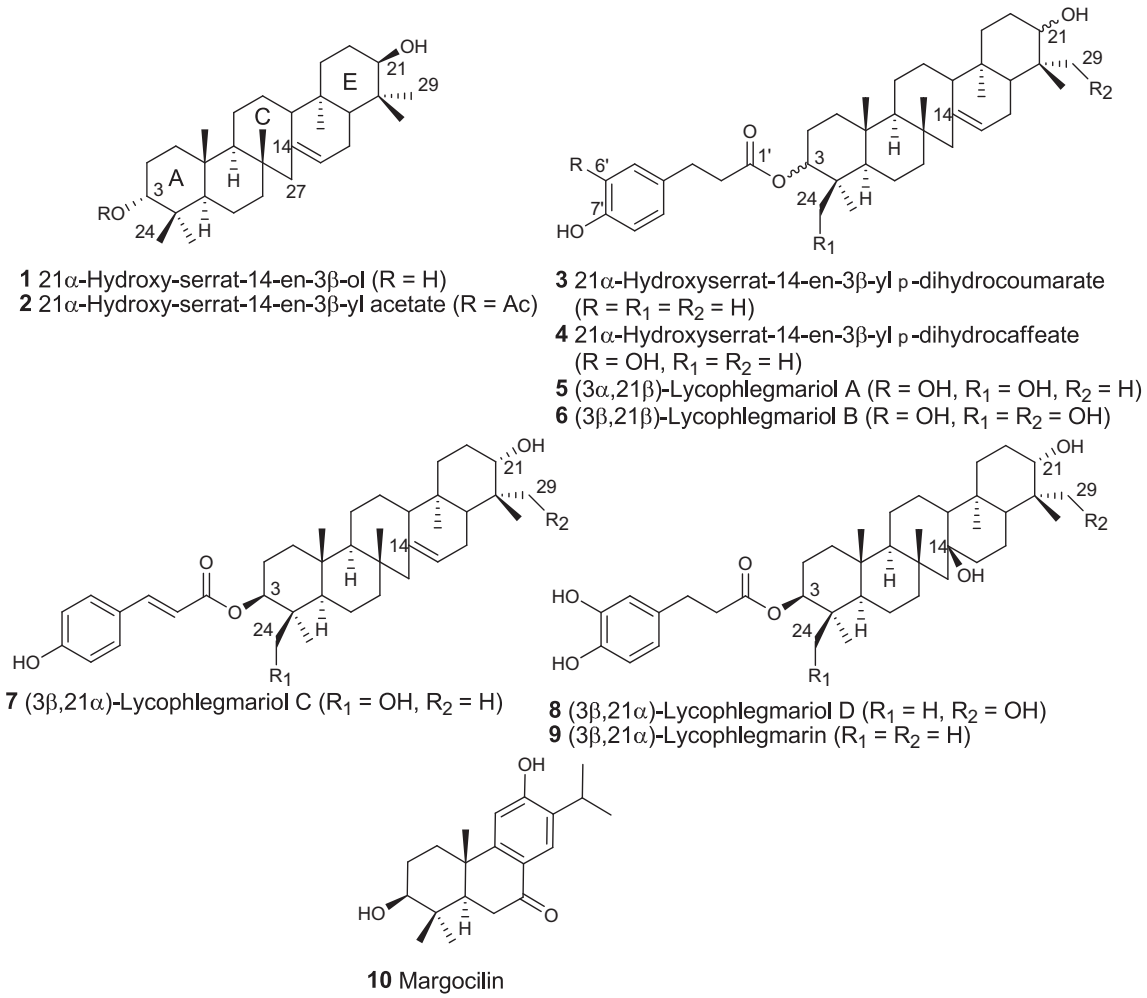


**Fig. 1.** Lycopodium serratene triterpenoids (**1**–**9**) and abietane diterpene (**10**).

chromatography and reversed phase C-18 high pressure liquid chromatography resulted in isolation of four new (**5–8**) and three known (**1**, **2**, **9**) (Fang et al., 1991; Shi et al., 2005) serratene triterpenoids, and an abietane diterpene margocilin (**10**) (Ara et al., 1990).

Compound **5**, obtained as a white powder, possessed the molecular formula $C_{39}H_{58}ClO_6$ on the basis of the positive HRAPCIMS (micro TOF) at 657.3907 $[M+Cl]^+$ requiring 11° of unsaturation. Analysis of the $^1H$ and $^{13}C$ NMR spectroscopic data indicated that compound **5** was related to **4** (Tables 1 and 2) (Zhou et al., 2003). In the $^{13}C$ NMR and DEPT spectra there was 30 carbons for six methyls, 10 methylenes, five methines, six quaternary carbons, two oxymethines ($\delta$ 69.8, C-3 and $\delta$ 75.3, C-21), and one oxymethylene ($\delta$ 68.2, C-24) group indicative of a serratene skeleton, together with signals of a dihydrocaffeoyl unit ($C_9H_9O_3$ with 5° of unsaturation) at C-1′ ($\delta$ 173.3, s), C-2′ ($\delta$ 36.8, t), C-3′ ($\delta$ 31.0, t), C-4′ ($\delta$ 132.6, s), C-5′ ($\delta$ 116.9, d), C-6′ ($\delta$ 147.3, s), C-7′ ($\delta$ 145.7, s), C-8′ ($\delta$ 116.6, d), and C-9′ ($\delta$ 119.7, d). Based on the NMR spectroscopic analysis of the core structure of compounds **5** and **4**, they differed only in ring A. Analysis of the $^{13}C$ NMR spectrum of **4**, indicated that the signal at $\delta$ 16.6 (q, C-24) was replaced in **5** by a resonance at $\delta$ 68.2 (t), indicating that an oxymethylene group was present at C-24. The NOESY experiment of an oxymethylene proton at C-24 of **5** also correlated to the C-25 methyl group, this being indicative of a β-configuration for an oxymethylene group. The orientations of the dihydrocaffeoyl group at C-3 and the hydroxyl group at C-21 were deduced by comparison of their carbon chemical shifts with compound **4** and its 2D NMR spectra (Fig. 2). Due to the difference of the stereochemistry at C-3 and C-21 of compounds **4** (3β, 21α) and **5** (3α, 21β), the $^{13}C$ NMR chemical shift of C-3 showed a 10.5 ppm difference ($\delta$ 80.3 for **4** and $\delta$ 69.8 for **5**) as well as a $^1H$ NMR chemical shift difference of H-3 for 0.65 ppm ($\delta$ 4.70 for **4** and $\delta$ 4.05 for **5**) as shown in Tables 1 and 2, respectively. Additionally the $^{13}C$ NMR chemical shift of C-21 of compounds **4** and **5** differed only 2.7 ppm ($\delta$ 78.0 for **4** and $\delta$ 75.3 for **5**) and showed a strong effect to the $^{13}C$ NMR chemical shift difference of C-29 for 7 ppm (Table 1). The relative configuration of compound **5** was also determined by optical rotation (see Section 4). Therefore, compound **5** was shown to be a 21β,24-dihydroxyserrat-14-en-3α-yl dihydrocaffeate as a new serratene triterpenoid named lycophlegmariol A.

Compound **6**, isolated as a yellowish solid, possessed the molecular formula $C_{39}H_{58}ClO_7$ on the basis of the positive HRAPCIMS (micro TOF) at 673.3877 $[M+Cl]^+$ requiring 11° of unsaturation and indicated that it had one more oxygen atom comparing with ompound **5**. Analysis of the $^1H$ and $^{13}C$ NMR spectra indicated that compound **6** was related to **5** (Tables 1 and 2). The $^{13}C$ NMR and DEPT spectra showed 30 carbons for five methyls, 10 methylenes, five methines, six quaternary carbons, two oxymethines ($\delta$ 74.3, C-3 and $\delta$ 75.7, C-21), and two oxymethylenes ($\delta$ 64.5, C-24 and $\delta$ 70.0, C-29) of a serratene skeleton, together with signals of a dihydrocaffeoyl unit. Based on the NMR spectroscopic analysis of core structure of compounds **6** and **5**, they differed only in the ring E. In the $^{13}C$ NMR spectrum of **5**, the resonance at $\delta$ 22.2 (q, C-29) was replaced in **6** by a signal at $\delta$ 70.0 (t), indicating that another

**Table 1**
$^{13}$C NMR spectroscopic data of lycopodium triterpenes **3**–**9**.

| Position | Lycopodium triterpenes | | | | | | |
|---|---|---|---|---|---|---|---|
| | **3**[a,c] | **4**[a,c] | **5**[a] | **6**[a] | **7**[a] | **8**[b] | **9**[a,d] |
| 1 | 38.4(t) | 38.1(t) | 33.8(t) | 34.3(t) | 37.7(t) | 38.0(t) | 38.2(t) |
| 2 | 28.7(t) | 28.4(t) | 26.6(t) | 26.8(t) | 28.7(t) | 26.3(t) | 26.3(t) |
| 3 | 80.7(d) | 80.3(d) | 69.8(d) | 74.3(d) | 74.2(d) | 80.7(d) | 80.9(d) |
| 4 | 38.3(s) | 37.9(s) | 42.4(s) | 43.3(s) | 43.6(s) | 38.4(s) | 38.3(s) |
| 5 | 55.8(d) | 55.5(d) | 50.0(d) | 51.7(d) | 52.0(d) | 55.7(s) | 55.7(d) |
| 6 | 19.1(t) | 18.8(t) | 19.5(t) | 19.4(t) | 19.5(t) | 19.2(t) | 19.3(t) |
| 7 | 45.3(t) | 45.0(t) | 45.7(t) | 45.7(t) | 45.8(t) | 44.6(t) | 44.7(t) |
| 8 | 37.4(s) | 37.1(s) | 38.4(s) | 38.4(s) | 38.5(s) | 38.1(s) | 38.2(s) |
| 9 | 62.6(d) | 62.3(d) | 62.9(d) | 63.0(d) | 63.2(d) | 59.5(d) | 59.6(d) |
| 10 | 38.2(s) | 37.9(s) | 37.6(s) | 37.6(s) | 37.6(s) | 38.6(s) | 38.6(s) |
| 11 | 25.5(t) | 25.2(t) | 25.4(t) | 25.4(t) | 25.8(t) | 25.6(t) | 25.8(t) |
| 12 | 27.5(t) | 27.3(t) | 27.6(t) | 27.5(t) | 27.6(t) | 24.1(t) | 24.2(t) |
| 13 | 57.6(d) | 57.3(d) | 57.4(d) | 57.6(d) | 57.7(d) | 60.3(d) | 60.5(d) |
| 14 | 138.6(s) | 138.4(s) | 139.1(s) | 139.1(s) | 138.7(s) | 74.8(s) | 75.0(s) |
| 15 | 122.9(d) | 122.6(d) | 122.8(d) | 122.6(d) | 122.9(d) | 46.0(t) | 46.0(t) |
| 16 | 24.7(t) | 24.4(t) | 24.6(t) | 24.2(t) | 24.7(t) | 19.5(t) | 19.5(t) |
| 17 | 50.1(d) | 49.8(d) | 43.8(d) | 38.2(d) | 50.2(d) | 56.8(d) | 56.2(d) |
| 18 | 36.5(s) | 36.2(s) | 36.4(s) | 36.1(s) | 36.6(s) | 38.7(s) | 39.1(s) |
| 19 | 37.6(t) | 37.3(t) | 31.9(t) | 31.7(t) | 34.6(t) | 38.2(t) | 39.0(t) |
| 20 | 24.3(t) | 24.0(t) | 26.5(t) | 23.5(t) | 23.7(t) | 28.0(t) | 28.5(t) |
| 21 | 78.3(d) | 78.0(d) | 75.3(d) | 75.7(d) | 78.4(d) | 80.4(d) | 78.5(d) |
| 22 | 39.5(s) | 39.3(s) | 38.0(s) | 40.8(s) | 39.6(s) | 43.5(s) | 39.9(s) |
| 23 | 28.2(q) | 27.9(q) | 23.4(q) | 23.0(q) | 23.1(q) | 28.7(q) | 28.1(q) |
| 24 | 16.9(q) | 16.6(q) | 68.2(t) | 64.5(t) | 64.7(t) | 17.0(q) | 16.9(q) |
| 25 | 16.0(q) | 15.7(q) | 16.3(q) | 16.3(q) | 16.4(q) | 16.7(q) | 16.7(q) |
| 26 | 20.1(q) | 19.8(q) | 20.0(q) | 20.2(q) | 20.2(q) | 23.1(q) | 23.4(q) |
| 27 | 56.4(t) | 56.1(t) | 56.7(t) | 56.7(t) | 56.6(t) | 62.0(t) | 62.2(t) |
| 28 | 13.8(q) | 13.6(q) | 13.8(q) | 14.2(q) | 13.8(q) | 16.6(q) | 16.5(q) |
| 29 | 15.5(q) | 15.2(q) | 22.2(q) | 70.0(t) | 15.5(q) | 64.6(t) | 16.7(q) |
| 30 | 28.3(q) | 28.0(q) | 28.7(q) | 17.6(q) | 28.3(q) | 23.9(q) | 29.2(q) |
| 1′ | 172.3(s) | 172.6(s) | 173.3(s) | 172.9(s) | 167.4(s) | 172.8(s) | 173.0(s) |
| 2′ | 36.9(t) | 36.7(t) | 36.8(t) | 37.0(t) | 116.1(d) | 37.0(t) | 37.2(t) |
| 3′ | 30.8(t) | 30.8(t) | 31.0(t) | 31.2(t) | 145.0(d) | 31.1(t) | 31.2(t) |
| 4′ | 131.6(s) | 132.3(s) | 132.6(s) | 132.7(s) | 126.9(s) | 132.7(s) | 132.8(s) |
| 5′ | 130.0(d) | 116.7(d) | 116.9(d) | 116.9(d) | 130.7(d) | 116.9(d) | 117.1(d) |
| 6′ | 116.4(d) | 147.1(s) | 147.3(s) | 147.3(s) | 116.2(d) | 147.3(s) | 147.4(s) |
| 7′ | 157.5(s) | 145.4(s) | 145.7(s) | 145.6(s) | 161.4(s) | 145.8(s) | 145.8(s) |
| 8′ | 116.4(d) | 116.3(d) | 116.6(d) | 116.6(d) | 116.2(d) | 116.6(d) | 116.8(d) |
| 9′ | 130.0(d) | 119.5(d) | 119.7(d) | 119.8(d) | 130.7(d) | 119.8(d) | 119.9(d) |

[a] 150 MHz in pyridine-$d_5$.
[b] 100 MHz in pyridine-$d_5$.
[c] Zhou et al. (2003).
[d] Shi et al. (2005).

oxymethylene group was present at C-29. The NOESY experiment (Figure in Supplementary data) of the C-29 oxymethylene proton of **6** correlated to the C-28 methyl group was indicative of α-configuration for the C-29 oxymethylene group. The orientations of the dihydrocaffeatyl group at C-3 and the hydroxyl group at C-21 were deduced by comparison of their carbon chemical shifts with compound **5** and its 2D NMR spectra (Figure in Supplementary data). The stereochemistry at C-3 of compound **6** (3β, 21β) was different from **5** (3α, 21β) and similar to **4** (3β, 21α). The difference in $^{13}$C NMR chemical shift of C-3 of **6** as compared to **4** was 6 and 4.5 ppm as compared with **6** ($\delta$ 80.3 for **4**, $\delta$ 69.8 for **5**, and $\delta$ 74.3 for **6**). Also the oxymethylene C-24 showed a significant effect to the $^1$H NMR chemical shift of H-3 of compounds **5** and **6** with an 1.7 ppm difference as shown in Table 2 (position 3, $\delta$ 4.05 for **5**, and $\delta$ 5.73 for **6**). The relative configuration of compound **6** was also determined by optical rotation (see Section 4). Therefore, compound **6** was shown to be 21β,24,29-trihydroxyserrat-14-en-3β-yl dihydrocaffeate as a new serratene triterpenoid named lycophlegmariol B.

Compound **7**, isolated as a white powder, possessed the molecular formula $C_{39}H_{57}O_5$ on the basis of the positive HRAPCIMS (micro TOF) at 605.419 $[M+H]^+$ requiring 12° of unsaturation. Analysis of the $^1$H and $^{13}$C NMR spectra indicated that compound **7** was related to **3** (Tables 1 and 2) (Zhou et al., 2003). The $^{13}$C NMR and DEPT spectra showed 30 carbons for six methyls, 10 methylenes, five methines, six quaternary carbons, and two oxymethines ($\delta$ 74.2, C-3 and $\delta$ 78.4, C-21) and one oxymethylene ($\delta$ 64.7, C-24) of serratene skeleton together with signals of a *para*-hydroxycinnamoyl unit ($C_9H_7O_2$ with 6° of unsaturation) at C-1′ ($\delta$ 167.4, s), C-2′ ($\delta$ 116.1, d), C-3′ ($\delta$ 145.0, d), C-4′ ($\delta$ 126.9, s), C-5′ ($\delta$ 130.7, d), C-6′ ($\delta$ 116.2, d), C-7′ ($\delta$ 161.4, s), C-8′ ($\delta$ 116.2, d), and C-9′ ($\delta$ 130.7, d). Based on the NMR spectroscopic analysis of core structure of compounds **7** and **3**, they differed in ring A. In the $^{13}$C NMR of **3**, the signal at $\delta$ 16.9 (q, C-24) was replaced in **7** by a resonance at $\delta$ 64.7 (t), indicating that an oxymethylene group was present at C-24. The orientations of the cinnamoyl group at C-3 and the hydroxyl group at C-21 were deduced by comparison of their carbon chemical shifts with compound **3** and its 2D NMR spectra (Figure in Supplementary data). The relative configuration of compound **7** was also determined by an optical rotation (see Section 4). Therefore, compound **7** was 21α,24-dihydroxyserrat-14-en-3β-yl 4-hydroxycinnamate as a new serratene triterpenoid named lycophlegmariol C.

Compound **8**, obtained as a colorless solid, possessed the molecular formula $C_{39}H_{60}NaO_7$ on the basis of the positive HRESIMS (micro TOF) at 663.4247 $[M+Na]^+$ requiring 10° of unsaturation

**Table 2**
$^1$H NMR spectroscopic data of lycopodium triterpenes **3–9**.

| Position | Lycopodium triterpenes | | | | | | |
|---|---|---|---|---|---|---|---|
| | **3**[a,c] | **4**[a,c] | **5**[a] | **6**[a] | **7**[a] | **8**[b] | **9**[a,d] |
| 1 | 1.68, 0.94(m) | 1.68, 0.94(m) | 1.75, 1.52(m) | 1.50, 1.30(m) | 1.85, 1.14(m) | 1.68, 1.04(m) | 1.70, 1.06(m) |
| 2 | 1.90, 1.64(m) | 1.90, 1.64(m) | 2.03, 1.85(m) | 1.96, 1.84(m) | 1.92, 1.89(m) | 2.00, 1.65(m) | 1.99, 1.73(m) |
| 3 | 4.70(dd, 11.5, 4.7) | 4.70 (dd 11.5, 4.7) | 4.05(br s) | 5.73(br s) | 5.90(m) | 4.58(dd, 11.0, 2.5) | 4.69(dd, 11.3, 4.6) |
| 5 | 0.80(m) | 0.80(m) | 1.83(m) | 1.59(m) | 1.75(m) | 0.93(m) | 0.94(m) |
| 6 | 1.40, 1.38(m) | 1.40, 1.38(m) | 1.54, 1.44(m) | 1.57, 1.47(m) | 1.59, 1.50(m) | 1.65, 1.50(m) | 1.64, 1.50(m) |
| 7 | 1.39, 1.16(m) | 1.39, 1.16(m) | 1.37, 1.20(m) | 1.34, 1.25(m) | 1.43, 1.32(m) | 1.85, 1.55(m) | 1.80, 1.49(m) |
| 9 | 0.74(m) | 0.74(m) | 0.95(m) | 0.97(d, 12.0) | 1.03(m) | 1.68(m) | 1.68(m) |
| 11 | 1.74, 1.13(m) | 1.74, 1.12(m) | 1.73, 1.02(m) | 1.63, 1.13(m) | 1.74, 1.09(m) | 1.85, 1.25(m) | 1.78, 1.26(m) |
| 12 | 1.90, 1.38(m) | 1.90, 1.38(m) | 1.99, 1.03(m) | 2.00, 1.08(m) | 2.40, 2.10(m) | 1.85, 1.68(m) | 1.80, 1.73(m) |
| 13 | 1.83(m) | 1.83(m) | 2.00(m) | 2.07(d, 8.8) | 1.84(m) | 1.15(m) | 1.18(m) |
| 15 | 5.47(br s) | 5.47(br s) | 5.43(br s) | 5.40(br s) | 5.47(br s) | 2.14, 1.98(m) | 2.03, 1.71(m) |
| 16 | 2.15, 2.01(m) | 2.15, 2.01(m) | 2.05, 1.95(m) | 2.20(d, 16.8), 1.96(m) | 2.16, 2.00(m) | 1.57, 1.35(m) | 1.63, 1.50(m) |
| 17 | 1.38(m) | 1.38(m) | 2.15(dd, 11.3, 5.3) | 2.6(dd, 12.4, 5.2) | 1.37(m) | 1.08(m) | 1.06(m) |
| 19 | 2.15, 2.01(m) | 2.15, 2.01(m) | 1.98, 1.61(m) | 1.95, 1.63(m) | 1.57, 1.32(m) | 2.14, 1.25(m) | 1.97, 1.21(m) |
| 20 | 1.75, 1.64(m) | 1.75, 1.64(m) | 1.93, 1.85(m) | 1.76, 1.63(m) | 1.91, 1.85(m) | 2.00, 1.56(m) | 2.03, 1.60(m) |
| 21 | 3.50(dd, 9.1, 6.4) | 3.52(dd, 9.3, 6.2) | 3.66(br s) | 4.04(br s) | 3.52(br s) | 3.73(m) | 3.63(dd, 11.2,4.6) |
| 23 | 0.88(s) | 0.86(s) | 1.35(s) | 1.31(s) | 1.43(s) | 0.77(s) | 0.89(s) |
| 24 | 0.92(s) | 0.89(s) | 4.47, 4.20(ABq,11.2) | 4.04, 3.73(ABq,10.8) | 4.14, 3.81(ABq,10.1) | 0.82(s) | 0.97(s) |
| 25 | 0.82(s) | 0.78(s) | 0.80(s) | 0.78(s) | 0.78(s) | 0.85(s) | 0.91(s) |
| 26 | 0.92(s) | 0.90(s) | 0.78(s) | 0.76(s) | 0.90(s) | 0.96(s) | 1.10(s) |
| 27 | 2.31, 1.85(ABq,14.3) | 2.31, 1.85(ABq,14.3) | 2.23, 1.76(ABq,14.8) | 2.30, 1.76(ABq,14.4) | 2.31, 1.85(ABq,14.3) | 1.85, 1.51(ABq, 15.1) | 1.80, 1.64(ABq, 14.9) |
| 28 | 0.78(s) | 0.78(s) | 0.78(s) | 0.82(s) | 0.78(s) | 1.56(s) | 1.44(s) |
| 29 | 1.08(s) | 1.10(s) | 0.93(s) | 3.85, 3.65(ABq, 10.8) | 1.10(s) | 4.63, 3.75(ABq, 11.1) | 1.22(s) |
| 30 | 1.19(s) | 1.19(s) | 1.15(s) | 0.84(s) | 1.19(s) | 1.28(s) | 1.37(s) |
| 2′ | 2.78(t, 7.5) | 2.80(t, 7.5) | 2.76(t, 7.5) | 2.87(t, 7.6) | 6.88(d, 15.5) | 2.75(t, 7.4) | 2.86(t, 7.5) |
| 3′ | 3.06(t, 7.5) | 3.08(t, 7.5) | 3.02(t, 7.5) | 3.08(t, 7.6) | 8.10(d, 15.5) | 3.03(t, 7.4) | 3.13(t, 7.5) |
| 5′ | 7.28(d, 8.4) | 7.28(s) | 7.25(d, 1.8) | 7.27(s) | 7.65(d, 8.6) | 7.25(d, 1.9) | 7.35(d, 1.5) |
| 6′ | 7.15(d, 8.4) | – | – | – | 7.15(d, 8.6) | – | – |
| 8′ | 7.15(d, 8.4) | 7.23(d, 8.1) | 7.22(d, 8.0) | 7.21(d, 8.0) | 7.15(d, 8.6) | 7.21(d, 8.0) | 7.31(d, 8.1) |
| 9′ | 7.28(d, 8.4) | 6.85(br d, 8.1) | 6.80(dd, 8.0, 1.8) | 6.83(d, 8.0) | 7.65(d, 8.6) | 6.82(dd, 8.0, 1.9) | 6.93(dd, 8.1, 1.5) |

[a] 600 MHz in pyridine-$d_5$.
[b] 400 MHz in pyridine-$d_5$.
[c] Zhou et al. (2003).
[d] Shi et al. (2005).

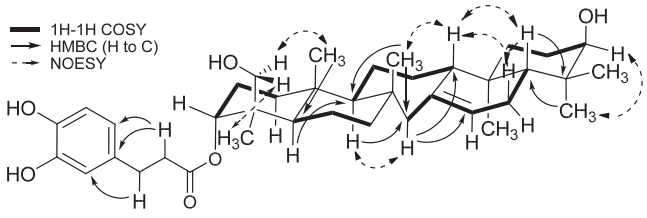


**Fig. 2.** Selected 2D NMR spectroscopic data and configurational analysis of lycophlegmariol A (**5**).

and indicated that it had two more hydrogen atoms as compared with compound **6**. Analysis of the $^{1}H$ and $^{13}C$ NMR spectra indicated that compound **8** was related to lycophlegmarin (**9**) (Tables 1 and 2) (Shi et al., 2005). The $^{13}C$ NMR and DEPT spectra showed 30 carbons for six methyls, 11 methylenes, four methines, five quaternary carbons, one oxyquaternary carbon ($\delta$ 74.8, C-14), two oxymethines ($\delta$ 80.7, C-3 and $\delta$ 80.4, C-21), and one oxymethylene ($\delta$ 64.6, C-29) of a serratene skeleton together with signals of a dihydrocaffeoyl unit. Based on the NMR analysis of the core structure of compound **8** and **9**, they differed only in ring E. In the $^{13}C$ NMR of **9**, the resonance at $\delta$ 29.2 (q, C-29) was replaced in **8** by a signal at $\delta$ 64.6 (t), indicating that an oxymethylene group was present at C-29. NOESY experiment of the C-29 oxymethylene proton of **8** correlated to the C-28 methyl group and a C-16 α-methylene proton was indicative of a α-configuration for the C-29 oxymethylene group. The orientations of the dihydrocaffeoyl group at C-3 and the hydroxyl group at C-21 were deduced by comparison of their carbon chemical shifts with compound **9** and 2D NMR (Figure in Supplementary data). The relative configuration of compound **8** was also determined by optical rotation (see Section 4). Therefore, compound **8** was shown to be 14β,21α,29-trihydroxyserrat-3β-yl dihydrocaffeate as a new serratane triterpenoid named lycophlegmariol D. Attempt to acetylate trihydroxyserratan **8** failed suggesting it was the 14β-tertiary alcohol of lycophlemariol D.

The effect of stereochemistry at C-3 and C-21 on the adjacent positions C-24 and C-29, was analysed and it was concluded that the difference in stereochemistry at C-3α and C-3β affected the $^{13}C$ and $^{1}H$ NMR chemical shifts of the oxymethylene C-24 as shown in lycophlegmariols A (**5**) and B (**6**); however no data supporting the effect to methyl C-24 was available. On the other hand, the oxymethylene C-24 also had an effect on the $^{13}C$ and $^{1}H$ NMR chemical shifts of C-3 as shown in lycophlegmariols B (**6**) and C (**7**). Also the difference in stereochemistry at C-21α and C-21β affected the $^{13}C$ NMR chemical shift of the methyl and oxymethylene C-29 moities. Only the oxymethylene C-29 showed a weak effect on the $^{13}C$ NMR chemical shift of C-21(α) as found in lycophlegmariol D (**8**). The variation in $^{13}C$ and $^{1}H$ NMR chemical shifts at C-3/C-24 and C-21/C-29 was the result of the effect of the difference of stereochemistry at C-3/C-21 and also the oxymethylene group at C-24/C-29 (Table 4 in Supplementary data).

Serratene triterpenes **1**, **6**, and **8** were evaluated for their cytotoxicities against the cancer cell lines: HuCCA-1 human cholangiocarcinoma, A549 human lung carcinoma, HepG2 human hepatocellular liver carcinoma, and MOLT-3 T-lymphoblast (acute lymphoblastic leukemia). MTT and XTT assays were used to estimate $IC_{50}$ values, that is, the drug concentration that causes 50% of cell growth inhibition after 72 h of continuous exposure to the test molecule. The results obtained are shown in Table 3. Interestingly, compound **1** showed cytotoxicity in a lower micromolar $IC_{50}$ range 2.62 and 2.42 μM against the HuCCA-1 and HepG2, respectively, but not against the A549 cell line. Lycophlegmariol B (**6**) was inactive against most cell lines tested, except the MOLT-3 cell line. Lycophlegmariol D (**8**) showed a weak activity with HuCCA-1 and A549 and inactive against HepG2. Moreover compounds **1** and **8** also showed cytotoxicity in a lower micromolar $IC_{50}$ range 2.9 and 3.0 μM against the MOLT-3 acute lymphoblastic leukemia, whereas compound **6** gave lower cytotoxicity in an order of magnitude (14.7 μM); therefore compounds **1** and **8** were ca five times more active than compound **6** (Table 3).

## 3. Conclusions

Investigation of the methanol extract obtained from club moss *L. phlegmaria* L. grown in Thailand resulted in the isolation of four new lycopodium serratene triterpenoids. Their structures were elucidated on the basis of extensive NMR spectroscopic analysis. Pentacyclic triterpenoids were established as 21β,29-dihydroxyserrat-14-en-3α-yl dihydrocaffeate (lycophlegmariol A, **5**), 21β,24,29-trihydroxyserrat-14-en-3β-yl dihydrocaffeate (lycophlegmariol B, **6**), 21α,24-dihydroxyserrat-14-en-3β-yl 4-hydroxycinnamate (lycophlegmariol C, **7**), and 14β,21α,29-trihydroxyserratan-3β-yl dihydrocaffeate (lycophlegmariol D, **8**). An abietane-type diterpene, margocilin (**10**), was isolated for the first time from a *Lycopodium* plant. The isolated lycopodium serratene triterpenoids, lycophlegmariol B (**6**), D (**8**) and compound **1**, showed inhibitory effects against MOLT-3 acute lymphoblastic leukemia (T-lymphoblast) with $IC_{50}$ of 14.7, 3.0 and 2.9 μM, respectively.

## 4. Experimental section

### 4.1. General experimental procedures

Optical rotations were determined on a JASCO DIP1020 polarimeter, whereas UV spectra were recorded on a UV 1700 Pharma Spec Shimadzu spectrophotometer. FTIR spectra were acquired using a Perkin Elmer Spectrum One using UATR. 1D and 2D NMR spectra were measured in pyridine-$d_5$ or $CD_3OD$ on a Bruker AVANCE-400 and 600 spectrometers with TMS as internal standard. High resolution mass spectra were obtained on a Bruker Daltonics MicroTOF instrument using atmospheric pressure chemical ionization (APCI) in either the positive or negative ion mode. Preparative HPLC was performed on a Thermo Separation Products (San Jose, CA) instruments with a UV 6000LP detector using a Cosmosil column 5C18-Ms-II, 20 × 250 mm, and Sunfire prep C18, 19 × 250 mm, 5 μm. Sephadex LH-20 were purchased from GE Healthcare. Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were carried out on Merck silica gel (70–230 mesh).

### 4.2. Plant material

*L. phlegmaria* L. (rhizome-stem-sporangia) was collected in 2010 from Satun province, Thailand. *L. phlegmaria* L. was identified by Dr. Tripeth Karnchanaphoom, and its voucher specimen (CRI-Lph-satun-Oct2010) has been deposited at Chulabhorn Research Institute, Bangkok Thailand.

### 4.3. Extraction and Isolation

Air-dried whole plants of *L. phlegmaria* (948 g) were soaked in MeOH (7 L × 3) at room temperature for 6 days, and the corresponding extracts were combined and evaporated to dryness. The crude extract (204 g) was partitioned with $H_2O$ (800 mL) and $Et_2O$ (400 mL × 3). The $Et_2O$ fraction (24.8 g) was applied to a silica gel column (980 g) and eluted with hexane and a gradient of EtOAc up to 100%, and followed by MeOH to 50% MeOH/EtOAc (each 2500 mL) to afford fractions A to N. Fraction G was purified by silica gel CC using n-hexane–EtOAc (1:4, v/v) as eluent to give

**Table 3**
Cytotoxic activities of serratane triterpenes **1**, **6**, and **8** in MTT and XTT assays.[a]

| Entry | Compound | Cytotoxic activity[c] ($IC_{50}$ [μM]); mean ± SD, $n$ = 3) | | | |
|---|---|---|---|---|---|
| | | HuCCA-1[d] | A549[d] | HepG2[d] | MOLT-3[e] |
| 1 | **1**[a] | 2.62 | Inactive | 2.42 | 2.94 |
| 2 | **6**[b] | Inactive | Inactive | Inactive | 14.7 |
| 3 | **8**[b] | 26.72 | 47.5 | Inactive | 3.0 |
| 4 | Doxorubicin | 0.97 | 0.28 | 0.33 | ND |
| 5 | Etoposide | ND | ND | 22.96 | 0.029 |

ND, not determined.
[a] Soluble in DMSO (0.263 mg.mL$^{-1}$).
[b] Soluble in DMSO (10 mg.mL$^{-1}$).
[c] Cytotoxicity was tested against the following cell lines: HuCCA-1 = human cholangiocarcinoma cell line; A549 = human lung carcinoma cell line; HepG2 = human hepatocellular liver carcinoma cell line; and MOLT-3 = T-lymphoblast (acute lymphoblastic leukemia) cell line.
[d] MTT assay.
[e] XTT assay.

21β-hydroxy-serrat-14-en-3α-yl acetate (**2**) (65.0 mg). 21β-Hydroxy-serrat-14-en-3α-ol (**1**) (126.6 mg) was obtained from fraction I by on silica gel chromatography eluted with n-hexane–EtOAc (3:7, v/v) as eluent. Fraction K (1.73 g) was reapplied to a Sephadex LH-20 column eluted with $CH_2Cl_2$/MeOH (1:1, v/v) to give $K_1$ to $K_3$. Fraction $K_3$ (0.999 g) was purified by HPLC (Sunfire prep C18) to give 10 fractions ($K_{3a}$–$K_{3j}$) and margocilin (**10**) (2.3 mg, MeOH/$H_2O$ (85:15, v/v) 8.5 mL/min; $t_R$ 19.6 min) from fraction $K_{3d}$. Fraction K3i was purified by HPLC (Cosmosil column 5C18-Ms-II) to give compounds **7** (2.0 mg, MeOH/$H_2O$ (85:15, v/v), 2.7 mL/min; $t_R$ 32.9 min) and **5** (4.3 mg, MeOH/$H_2O$ (85:15, v/v) 2.7 mL/min; $t_R$ 27.0 min), whereas fraction $K_{3j}$ was purified by HPLC (Cosmosil column 5C18-Ms-II) to give compound **9** (2.5 mg, MeOH/$H_2O$ (85:15, v/v) 8.5 mL/min; $t_R$ 36.5 min). Fraction M (1.30 g) was applied to Sephadex LH-20 eluted with $CH_2Cl_2$/MeOH (1:1, v/v) to give fractions $M_1$ to $M_3$. Fraction $M_3$ (0.464 g) was further purified by HPLC (Cosmosil column 5C18-Ms-II) to give compounds **6** (4.9 mg, 85% MeOH/$H_2O$, 8.0 mL/min; $t_R$ 22.3 min) and **8** (28 mg, MeOH/$H_2O$ (85:15, v/v), 8.0 mL/min; $t_R$ 34.8 min).

#### 4.3.1. 21β-Hydroxy-serrat-14-en-3α-ol (**1**)

White powder (126.6 mg); $[\alpha]^{27}_D$ − 21.5 (c 0.35, MeOH); $R_f$ 0.51 (5% MeOH/$CH_2Cl_2$); HRAPCIMS (microTOF) $m/z$ $[M+Cl]^+$ calcd for $C_{30}H_{50}ClO_2$: 477.3505, found 477.3497 and 479.3465 (isotopic peak) (Fang et al., 1991).

#### 4.3.2. 21β-Hydroxy-serrat-14-en-3α-yl acetate (**2**)

White powder (65.0 mg); $[\alpha]^{27}_D$ − 0.6 (c 1.1, MeOH); $R_f$ 0.68 (5% MeOH/$CH_2Cl_2$); HRAPCIMS (microTOF) $m/z$ $[M]^+$ calcd for $C_{32}H_{52}O_3$: 484.3911, found 484.3900 (Fang et al., 1991).

#### 4.3.3. Lycophlegmarinol A (**5**)

White powder (4.3 mg); $[\alpha]^{28}_D$ − 13.6 (c 0.45, MeOH); $R_f$ 0.34 (MeOH/$CH_2Cl_2$ 5:95, v/v); UV (MeOH): $\lambda_{max}$ (log ε 320 3.0, 284 3.35 nm; IR (UATR) $\upsilon_{max}$ 3400, 2927, 1716, 1603, 1452, 1386, 1264, 1187, 995, 737 cm$^{-1}$; for $^{13}$C and $^1$H NMR spectroscopic data are Tables 1 and 2; HRAPCIMS (microTOF) $m/z$ $[M+Cl]^+$ calcd for $C_{39}H_{58}ClO_6$: 657.3927, found 657.3907 and 659.3939 (isotopic peak).

#### 4.3.4. Lycophlegmarinol B (**6**)

Yellowish solid (4.9 mg); $R_f$ 0.29 (MeOH/$CH_2Cl_2$ 5:95, v/v); UV (MeOH) $\lambda_{max}$ (log ε 326 4.77, 289 4.75 nm; IR (UATR) $\upsilon_{max}$ 3355, 2924, 1733, 1517, 1454, 1379, 1266, 1164, 1029, 735 cm$^{-1}$; for $^{13}$C and $^1$H NMR spectroscopic data are Tables 1 and 2; HRAPCIMS (microTOF) $m/z$ $[M+Cl]^+$ calcd for $C_{39}H_{58}ClO_7$: 673.3877, found 673.3868 and 675.3834 (isotopic peak).

#### 4.3.5. Lycophlegmarinol C (**7**)

White powder (2.0 mg); $[\alpha]^{26}_D$ + 16.1 (c 0.20, MeOH); $R_f$ 0.35 (5% MeOH/$CH_2Cl_2$); UV (MeOH) $\lambda_{max}$ (log ε 311 4.72 nm; IR (UATR) $\upsilon_{max}$ 3355, 2929, 1704, 1604, 1515, 1452, 1385, 1265, 1167, 994, 736 cm$^{-1}$; for $^{13}$C and $^1$H NMR spectroscopic data are Tables 1 and 2; HRAPCIMS (microTOF) $m/z$ $[M+H]^+$ calcd for $C_{39}H_{57}O_5$: 605.4201, found 605.4190.

#### 4.3.6. Lycophlegmarinol D (**8**)

Colorless solid (28.0 mg); $[\alpha]^{27}_D$ + 6.9 (c 1.6, MeOH); $R_f$ 0.32 (5% MeOH/$CH_2Cl_2$); UV (MeOH) $\lambda_{max}$ (log ε 327 3.65, 283.4.16 nm; IR (UATR) $\upsilon_{max}$ 3353, 2926, 1713, 1452, 1367, 1264, 1190, 990, 736 cm$^{-1}$; for $^{13}$C and $^1$H NMR spectroscopic data are Tables 1 and 2; HRESIMS (microTOF) $m/z$ $[M+Na]^+$ calcd for $C_{39}H_{60}NaO_7$: 663.4231, found 663.4247.

#### 4.3.7. Lycophlegmarin (**9**)

White powder (2.5 mg); $R_f$ 0.39 (5% MeOH/$CH_2Cl_2$); UV (MeOH) $\lambda_{max}$ (log ε 323.5 4.29, 284 4.59 nm; IR (UATR) $\upsilon_{max}$ 3393, 2930, 1710, 1604, 1518, 1449, 1365, 1264, 1189, 991, 736 cm$^{-1}$; for $^{13}$C and $^1$H NMR spectroscopic data are Tables 1 and 2; HRAPCIMS (microTOF) $m/z$ $[M+Cl]^+$ calcd for $C_{39}H_{60}ClO_6$: 659.4084, found 659.4065 and 661.4053 (isotopic peak) (Shi et al., 2005).

#### 4.3.8. Margocilin (**10**)

Brown solid (2.3 mg); $[\alpha]^{26}_D$ + 3.2 (c 0.23, MeOH); $R_f$ 0.41 (5% MeOH/$CH_2Cl_2$); UV (MeOH) $\lambda_{max}$ (log ε 282 4.68, 232 4.79 nm; IR (UATR) $\upsilon_{max}$ 3305, 2926, 1652, 1595, 1460, 1375, 1303, 1268, 1177, 1081, 1023, 737 cm$^{-1}$; $^1$H NMR (600 MHz, $C_5D_5N$) ppm 0.96 (s, H18), 1.04 (s, H19), 1.23 (s, H20), 1.25 (d, $J$ = 6.9 Hz, H16), 1.28 (d, $J$ = 6.9 Hz, H17), 1.69 (m, H1α), 1.8–1.9 (m, H2, H5), 2.25 (d, $J$ = 12.7 Hz, H1β), 2.65 (dd, $J$ = 13.1, 18.0 Hz, H6α), 2.70 (dd, $J$ = 4.8, 18.0 Hz, H6β), 3.15 (h, $J$ = 6.7 Hz, H15), 3.33 (dd, $J$ = 4.3, 11.8 Hz, H3α), 5.50 (br s, OH), 6.64 (s, H11), 7.91 (s, H14); HRAPCIMS (microTOF) $m/z$ $[M+H]^+$ calcd for $C_{20}H_{29}O_3$: 317.2111, found 317.2113 (Ara et al., 1990).

### 4.4. Acetylation of Lycophlegmarinol D

A mixture of compound **8** (0.008 g, 0.01 mmol), dimethylaminopyridine (0.0023 g, 0.02 mmol) and $Et_3N$ (2.6 mL, 0.02 mmol) in dichloromethane (1 mL) was acetylated with $Ac_2O$ (0.01 mL, 0.09 mmol) at 0 °C. The reaction was allowed to room temperature for 3 h, poured into $H_2O$ (20 mL) and extracted with EtOAc (10 mL × 3). The organic layers were combined and then evaporated to dryness *in vacuo*. The residue was purified by PTLC using EtOAc/hexane (3:7, v/v) as eluent to afford lycophlegmariol D-21,29,6′,7′-tetraacetate (**11**, 0.01 g, 99%). $R_f$ 0.43 (5% MeOH/

$CH_2Cl_2$); IR (UATR) $\upsilon_{max}$ 3385, 2925, 1731, 1593, 1372, 1258, 1237, 1112, 1029, 802, 736 $cm^{-1}$; $^1H$ NMR (400 MHz, $C_5D_5N$) ppm 0.79, 0.85, 0.90, 1.02, 1.13, 1.28 (each 3H, s, 6 × methyls on triterpene nucleus), 0.86 (m, H5), 1.07 (m, H1a,17), 1.15 (m, H13), 1.25 (m, H11a,19a), 1.40 (m, H6a,16a), 1.56 (ABq, H27a), 1.60 (m, H7a,16b), 1.65 (m, H20a), 1.67 (m, H1b,2a), 1.68 (m, H6b,9,12a), 1.75 (ABq, H27b), 1.78 (m, H7b), 1.80 (m, H11b,12b), 1.89 (m, H20b), 1.93 (m, H19b), 1.98 (m, H15a), 2.02 (m, H2a), 2.03, 2.08, 2.25, 2.30 (each 3H, s, 4 × acetyls of acetylation), 2.12 (H, 15b), 2.70 (t, $J$ = 7.2 Hz, H2′), 2.99 (t, $J$ = 7.2 Hz, H3′), 4.32 (ABq, $J$ = 11.6 Hz, H29a), 4.57 (dd, $J$ = 10.8, 4.8 Hz, H21), 4.72 (ABq, $J$ = 11.6 Hz, H29b), 4.84 (dd, $J$ = 11.6, 4.6 Hz, H3), 7.17 (dd, $J$ = 8.3, 2.0 Hz, H9′), 7.29 (d, $J$ = 2.0 Hz, H5′), 7.32 (d, $J$ = 8.0 Hz, H8′); HRESIMS (microTOF) $m/z$ $[M+Na]^+$ calcd for $C_{47}H_{68}NaO_{11}$: 831.4654, found 831.4661.

### 4.5. Biological testing

The assay was conducted as previously reported (Boonya-udtayan et al., 2010). Serratene triterpenes **1**, **6**, and **8** were solubilized in DMSO and tested for their cytotoxic activities against HuCCA-1, A-549, HepG2, and MOLT-3 cancer cell lines. The cells suspended in the corresponding culture medium were inoculated in a 96-well microtiter plates (Corning Inc., NY, USA) at a density of 10,000–20,000 cells per well, and incubated at 37 °C in a humidified atmosphere with 95% air and 5% $CO_2$. After 24 h, an equal volume of additional medium containing either the serial dilutions of the test compounds, positive control (etoposide), or negative control (DMSO) was added to the desired final concentrations, and the microtiter plates were further incubated for an additional 48 h. The number of surviving cells in each well was determined using either MTT assay (for adherent cells) or XTT assay (for suspended cells) in order to determine the $IC_{50}$ which is defined as the concentration that inhibits cell growth by 50% (relative to negative control) after 48 h of continuous exposure to each test compound. Within each experiment, determinations were done in triplicate, and each compound was tested in at least two separate experiments. Two known anticancer drugs, doxorubicin and etoposide, were used as the reference drugs (Vaculova et al., 2010).

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2012.01.006.

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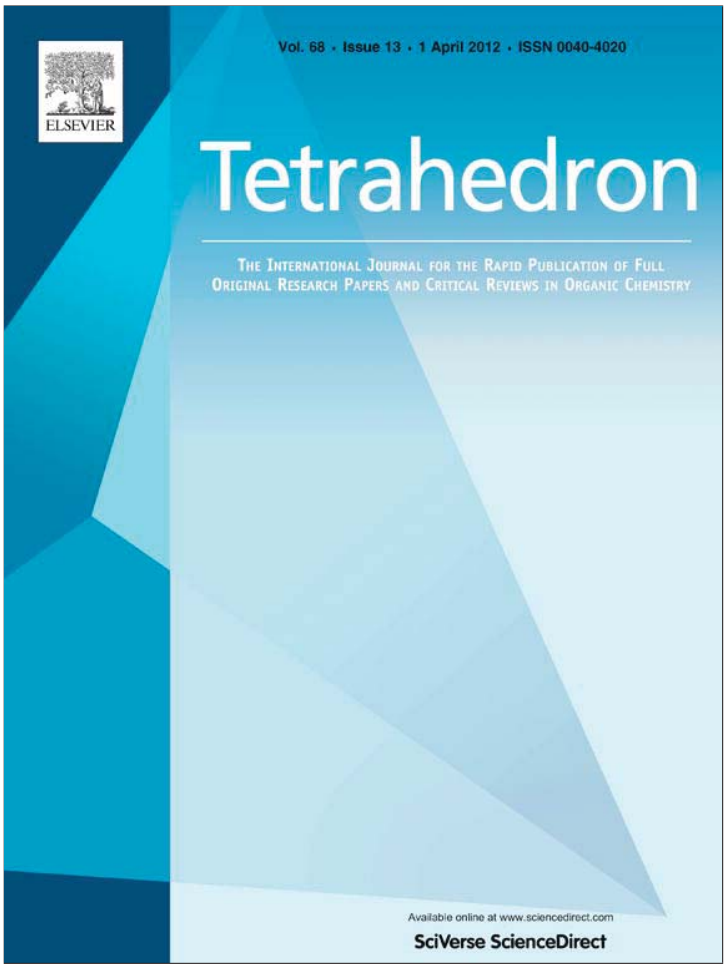

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# Synthesis of benzoindoloquinolizines via a Cu(I)-mediated C–N bond formation

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ABSTRACT

An effective synthesis of the multi ring-fused benzoindoloquinolizines has been accomplished by Cu(I)-mediated and MW-assisted C–$N_{amide}$ bond formation of benzo[*a*]quinolizin-4-ones. The deamination of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines was also studied and was found to give benzoquinolizines. The benzo[*a*]quinolizin-4-ones were prepared based on the annulations of C-1 substituted 3,4-dihydroisoquinolines and azlactones.



## 1. Introduction

The benzo[*a*]quinolizine ring system **1** is an important heterocyclic framework that can be found in numerous biologically active compounds[1] including alangiumkaloid A **2**, an oxoprotoberberine alkaloid, and isoalangioside **3**, isolated from a Thai folk medicinal plant, *Alangium salviifolium*.[2,3] Schulzeine A **4** and its analogues Schulzeines B–C, new α-glucosidase inhibitors, isolated from the marine sponge *Penares schulzei*, were the first three benzo[*a*]quinolizin-4-ones containing an amide moiety at the C-3 position.[4] The synthetic benzo[*a*]chinolizinone (Ro 41-3696) **5** was reported as an effective non-sedative hypnotic for the induction and maintenance of sleep.[5] 2-Amidobenzo[*a*]quinolizine **6** was synthesized as a novel dipeptidyl peptidase IV (DPP-IV) inhibitors (Fig. 1).[6]

The classical approaches for the synthesis of the benzo[*a*]quinolizine ring system involved the Dieckmann condensation of 1,2-dialkylesters of dihydroisoquinolines, the Bischler–Napieralski cyclization of arylethylpyridinones, and the reaction of 3,4-dihydroisoquinolines with α,β-unsaturated ketones.[7] Other new methods have been reported in the literature.[8] We have also reported a facile and direct synthetic entry to tricyclic imidazoloisoquinolinones and benzo[*a*]quinolizin-4-ones based on the annulations of 1-unsubstituted and 1-substituted dihydroisoquinolines **7** with azlactones **8** under neutral conditions.[9]

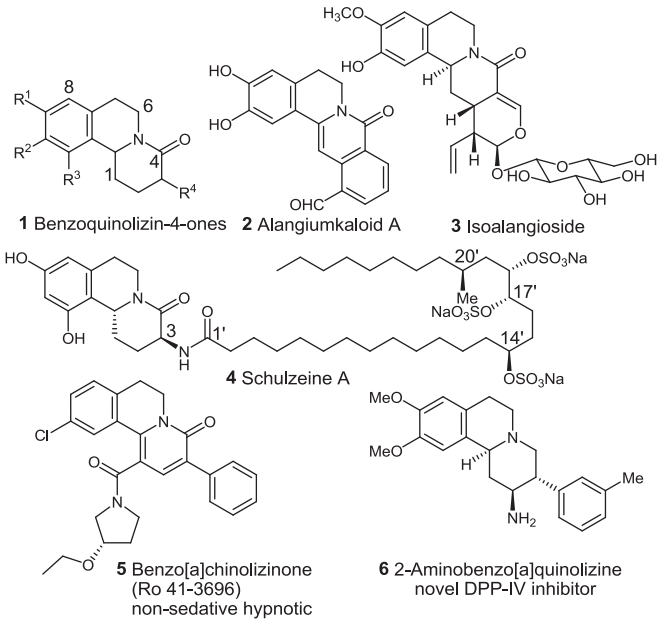


**Fig. 1.** Example of natural product alkaloids and biologically synthetic compounds containing benzoquinolizine system.

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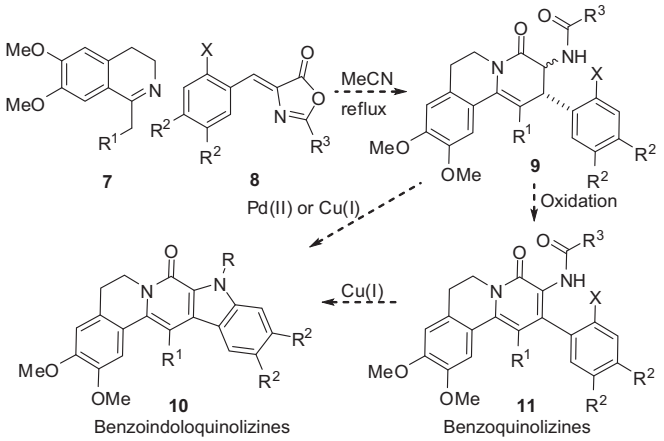
In continuation of our interests in the palladium- and copper-mediated formation of *O*- and *N*-aryl bonds[10,11] in general and particularly the C–N bond formation of indole alkaloids,[12] we have studied, in this work, the Pd(II)-catalyzed and Cu(I)-mediated $C–N_{amide}$ bond formations of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** and benzo[*a*]quinolizin-4-ones **11** in order to obtain the multi ring-fused benzoindoloquinolizines **10** as shown in Scheme 1.




**Scheme 1.** Synthesis of multi ring-fused benzoindoloquinolizinone.

## 2. Results and discussion

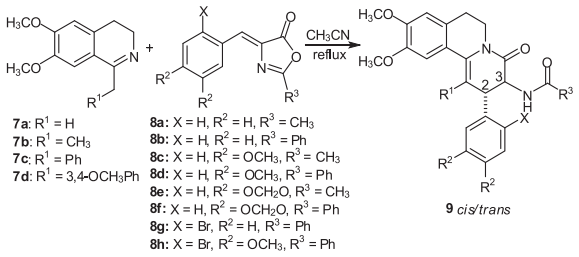
### 2.1. Synthesis of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines 9

The reaction of azlactones **8** with various 1-substituted 3,4-dihydroisoquinolines **7** in refluxing acetonitrile gave the corresponding 3,4-dihydropyridin-2(1*H*)-ones as a mixture of *cis*/*trans* tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** in moderate to good yields as shown in Table 1. It was found that when the substituent $R^3$ on the azlactones **8** was a phenyl group, the corresponding products **9** were obtained in higher yields than in the cases where $R^3$ was a methyl group. In the cases where the substituent $R^1$ on the 3,4-dihydroisoquinolines **7** was a proton ($R^1$=H) the *cis*-products predominated, however, increasing the steric bulk of the substituents where $R^1$=$CH_3$ and Ph derivatives, the trans isomers became the major products. The cyclocondensation of azlactones **8** ($R^3$=$CH_3$) with hindered 1-benzyl-3,4-dihydroisoquinolines **7** ($R^1$=Ph, 3,4-$(OCH_3)_2C_6H_3$) gave cyclocondensation products **9** in poor yield (entries 5–7 and 9–11). However, the yields could be improved with longer reaction times.

The structure of *cis*-tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9a** was assigned by interpretation of spectral data. The IR spectrum exhibited absorptions at 1683 and 1654 $cm^{-1}$ indicating the presence of two amide carbonyl groups, which corresponded to two peaks of carbonyl groups in the $^{13}C$ NMR spectrum at $\delta$ 165.3 and 170.0. In addition, the IR spectrum exhibited a secondary amide absorption at 3396 $cm^{-1}$. The $^1H$ NMR showed a coupling constant of 7.6 Hz for *H*-2 and *H*-3 inferring the cis-relationship. $^1H$ NMR of the *trans*-**9a** exhibited trans-relationship of *H*-2 and *H*-3 with larger coupling constant of 13.4 Hz.

The $^1H$ NMR chemical shift assignment for *cis*-**9a** was confirmed by a detailed observation of NOE effects (Fig. 2). In particular, irradiation at the frequency of the signal at $\delta$ 5.06 (*H*-3) enhanced the signal at $\delta$ 4.40 (*H*-2, 9%) and $\delta$ 6.84 (N*H*, 3%). In addition, irradiation at the frequency of the signal at $\delta$ 4.40 (*H*-2) enhanced the signal at $\delta$ 6.02 (*H*-1, 4%), thus confirming the chemical shifts and the stereochemical assignment between *H*-3 and *H*-2 as having a cis-relationship. Similarly, the $^1H$ NMR chemical shift assignment for

**Table 1**
Synthesis of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9**[a]




**7a**: $R^1$ = H
**7b**: $R^1$ = $CH_3$
**7c**: $R^1$ = Ph
**7d**: $R^1$ = 3,4-$OCH_3$Ph

**8a**: X = H, $R^2$ = H, $R^3$ = $CH_3$
**8b**: X = H, $R^2$ = H, $R^3$ = Ph
**8c**: X = H, $R^2$ = $OCH_3$, $R^3$ = $CH_3$
**8d**: X = H, $R^2$ = $OCH_3$, $R^3$ = Ph
**8e**: X = H, $R^2$ = $OCH_2O$, $R^3$ = $CH_3$
**8f**: X = H, $R^2$ = $OCH_2O$, $R^3$ = Ph
**8g**: X = Br, $R^2$ = H, $R^3$ = Ph
**8h**: X = Br, $R^2$ = $OCH_3$, $R^3$ = Ph

| Entry | X | $R^1$ | $R^2$ | $R^3$ | Yield % **9**[b] (*cis*/*trans*) |
|---|---|---|---|---|---|
| 1 | H | H | H | Ph | **a** 89 (74:26) |
| 2 | H | H | $OCH_3$ | Ph | **b** 79 (84:16) |
| 3 | H | $CH_3$ | $OCH_3$ | $CH_3$ | **c** 65 (40:60) |
| 4 | H | $CH_3$ | $OCH_3$ | Ph | **d** 86 (40:60) |
| 5 | H | Ph | H | $CH_3$ | **e** 42 (17:83) |
| 6 | H | Ph | $OCH_3$ | $CH_3$ | **f** 35[c] (0:100) |
| 7 | H | Ph | $OCH_2O$ | $CH_3$ | **g** 45[d] (20:80) |
| 8 | H | Ph | $OCH_3$ | Ph | **h** 80 (36:64) |
| 9 | H | 3,4-$(OMe)_2$Ph | H | $CH_3$ | **i** 34 (27:73) |
| 10 | H | 3,4-$(OMe)_2$Ph | $OCH_3$ | $CH_3$ | **j** 45[e] (16:84) |
| 11 | H | 3,4-$(OMe)_2$Ph | $OCH_2O$ | $CH_3$ | **k** 39[f] (15:85) |
| 12 | H | 3,4-$(OMe)_2$Ph | H | Ph | **l** 86 (35:65) |
| 13 | H | 3,4-$(OMe)_2$Ph | $OCH_3$ | Ph | **m** 85 (32:68) |
| 14 | H | 3,4-$(OMe)_2$Ph | $OCH_2O$ | Ph | **n** 84 (38:62) |
| 15 | Br | H | H | Ph | **o** 98 (78:22) |
| 16 | Br | H | $OCH_3$ | Ph | **p** 80 (76:24) |
| 17 | Br | $CH_3$ | H | Ph | **q** 70 (71:29) |
| 18 | Br | $CH_3$ | $OCH_3$ | Ph | **r** 77 (56:44) |

[a] All reaction times were 2 h.
[b] Isolated yields of pure product after PTLC on silica.
[c] Reaction time was 7 h, **9f**, 41% (17:83).
[d] Reaction time was 7 h, **9g**, 68% (18:82).
[e] Reaction time was 12 h, **9j**, 62% (21:79).
[f] Reaction time was 12 h, **9k**, 80% (19:81).

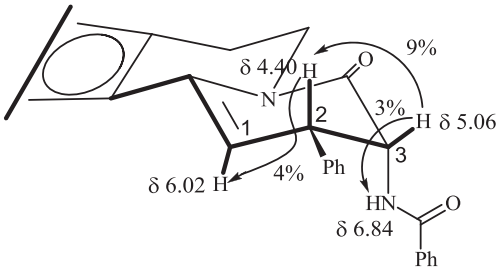



**Fig. 2.** NOEs effects observed for *cis*-**9a** ($CDCl_3$, 400 MHz).

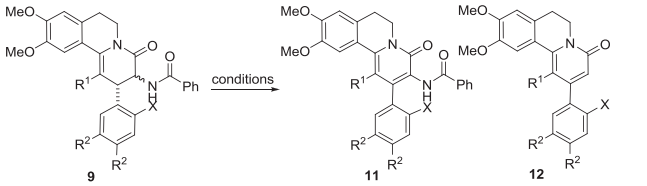
*trans*-**9a** was confirmed by a detailed observation of NOE effects. It was found that irradiation of the signal at $\delta$ 5.16 (*H*-3) did not enhance the signal at $\delta$ 4.04 (*H*-2), thus confirming the stereochemical assignment between *H*-2 and *H*-3 as having a trans-relationship.

### 2.2. Survey of C–N bond formation of tetrahydro-2*H*-pyrido [2,1-*a*]isoquinolines 9

Various approaches for the synthesis of indole ring systems have been reported in the literature.[12a,13] We sought to develop the synthesis of benzoindoloquinolizines **10** via the C–N bond formation of the corresponding tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9**. Initially, our approach to synthesize the indole ring was inspired by the recent findings of palladium-catalyzed C–H activation/C–N bond formation.[13,14] First, we examined the possibility of C–H activation of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9a** using $Pd(OCOCF_3)_2$ as the catalyst in the presence of $Cu(OAc)_2$ and $AgOCOCF_3$ as reoxidant at 80–85 °C under an argon atmosphere in

DMSO.[13c] The oxidation product benzoquinolizine **11a** was obtained in moderate yield (35%) instead of the expected indole product (Table 2, entry 1). Other palladium-catalyzed intramolecular amination conditions were also studied using $PdCl_2$ and $Pd(dba)_2$ hoping to construct the indole system from various tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9**. In all cases, deamidation led to the formation of benzoquinolizines **12**, which were obtained in moderate to good yields as shown in Table 2 (entries 2–8).

**Table 2**
Survey of C–N bond formation of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9**




| Entry | SM **9** | X | Condition | Time (h) | Yield % **11**[a] | Yield % **12**[a] |
|---|---|---|---|---|---|---|
| 1 | *cis*-**9a** | H | A | 23 | **a**, 35 | — |
| 2 | *cis*-**9a** | H | B | 20 | — | **a**, 68 |
| 3 | *cis*-**9o** | Br | B | 72 | — | **b**, 32[b] |
| 4 | *trans*-**9o** | Br | B | 72 | — | **b**, 50 |
| 5 | *cis*-**9p** | Br | B | 72 | — | **c**, 81 |
| 6 | *cis*-**9q** | Br | B | 72 | — | **d**, 72 |
| 7 | *cis*-**9r** | Br | B | 72 | — | **e**, 52[c] |
| 8 | *cis*-**9p** | Br | C | 24 | — | **c**, 37[d] |
| 9 | *cis*-**9o** | Br | D | 16 | — | **b**, 97 |
| 10 | *trans*-**9o** | Br | D | 16 | — | **b**, 91 |
| 11 | *cis*-**9q** | Br | D | 96 | — | **d**, 15[e] |
| 12 | *cis*-**9r** | Br | D | 96 | — | **e**, 12[f] |

Condition A: 30 mol % $Pd(OCOCF_3)_2$, 3 equiv $Cu(OAc)_2$, 6 equiv $AgOCOCF_3$, DMSO, Ar atmosphere, 80–85 °C.[13c] Condition B: 5 mol % $PdCl_2$, 10 mol % Xantphos, 2 equiv $Cs_2CO_3$, 1,4-dioxane, reflux. Condition C: 10 mol % $Pd(dba)_2$, 15 mol % Xantphos, 1.4 equiv $Cs_2CO_3$, 1,4-dioxane, 100 °C. Condition D: 2 equiv $Cs_2CO_3$, 1,4-dioxane, 100 °C.

[a] Isolated yields of pure product after PTLC on silica.
[b] Benzoquinolizine **13** was obtained in 21% as a side product.[15]
[c] Recovered starting materials 25%.
[d] Recovered starting materials in 45%.
[e] Recovered starting materials in 11%.
[f] Recovered starting materials in 63%.

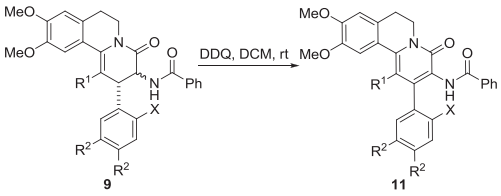
To study the deamidation reaction further, we then examined the reaction of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** under basic conditions using $Cs_2CO_3$. Both the *cis*/*trans* compounds **9o** underwent the deamidation reaction smoothly to give the benzoquinolizinone **12a** in excellent yield (Table 2, entries 9 and 10). The reaction gave poor yield when increasing of steric bulk of the substituent on tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** ($R^1$=$CH_3$) (Table 2, entries 11 and 12).

### 2.3. Synthesis of benzoindoloquinolizines 10

Since the synthesis of benzoindoloquinolizines **10** via the C–N bond formation from tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** failed due to the competing deamidation reaction, another approach was then attempted based on the C–N bond amination of the benzo[*a*]quinolizinones **11**. The benzo[*a*]quinolizinones **11** could be used to synthesize the benzoindoloquinolizines **10** using the palladium(II)-catalyzed and copper(I)-mediated C–N bond formation without the competing deamination reaction.

As shown in Table 3, a number of compounds **11** were obtained by the oxidation of selected *cis*-tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** with DDQ in dichloromethane at room temperature for 0.5–2 h. It was found that when the substituent $R^1$ on *cis*-**9** was a proton ($R^1$=H), the corresponding products **11** were obtained in higher yields than when was a methyl group ($R^1$=$CH_3$) (Table 3, entries 1, 2, 6, and 7). The reaction of *trans*-**9b** was also studied and

**Table 3**
Synthesis of benzo[*a*]quinolizin-4-ones **11**




| Entry | SM **9** | X | $R^1$ | $R^2$ | Time (h) | Yield % **11**[a] |
|---|---|---|---|---|---|---|
| 1 | *cis*-**9a** | H | H | H | 0.5 | **a**, 56 |
| 2 | *cis*-**9b** | H | H | $OCH_3$ | 0.5 | **b**, 67 |
| 3 | *trans*-**9b** | H | H | $OCH_3$ | 0.5 | **b**, 23 |
| 4 | *cis*-**9**[b] | H | $CH_3$ | H | 16 | **c**, 15 |
| 5 | *cis*-**9d** | H | $CH_3$ | $OCH_3$ | 16 | **d**, 8[c] |
| 6 | *cis*-**9o** | Br | H | H | 1 | **e**, 57[d] |
| 7 | *cis*-**9p** | Br | H | $OCH_3$ | 1.5 | **f**, 53 |
| 8 | *cis*-**9q** | Br | $CH_3$ | H | 16 | N/A[e] |
| 9 | *cis*-**9r** | Br | $CH_3$ | $OCH_3$ | 16 | N/A[e] |

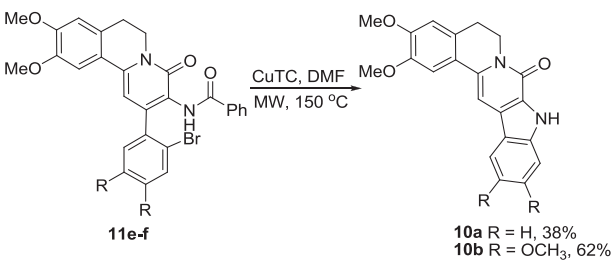
[a] Isolated yields of pure product after PTLC on silica.
[b] From previous work see Ref. 9.
[c] Recovered starting material 47%.
[d] Recovered starting material 41%.
[e] Recovered starting material >99%.

gave the oxidation product **11b** in lower yield (Table 3, entry 3). In the cases where the substituent X on *cis*-**9** was a proton (X=H) and $R^1$ was a methyl group ($R^1$=$CH_3$), the product yields were poor (Table 3, entries 4 and 5). Moreover, increasing the steric bulk of the substituents (X=Br and $R^1$=$CH_3$) resulted in no reaction (Table 3, entries 8 and 9).

We then re-examined the palladium-catalyzed intramolecular amination conditions to synthesize benzoindoloquinolizines **10**. Various Pd(II)-catalyzed C–H activation/amination[13] and C–N bond formation[14] conditions were studied. The cyclization of benzoquinolizinone **11b** (X=H) was studied using $Pd(OAc)_2$ as a catalyst in the C–H activation amidation reaction, and benzoquinolizinones **11e** and **11f** were examined using palladium(II)-catalyzed C–N bond formation using various conditions, as previously reported.[13,14] However all attempts failed to afford the indole system.

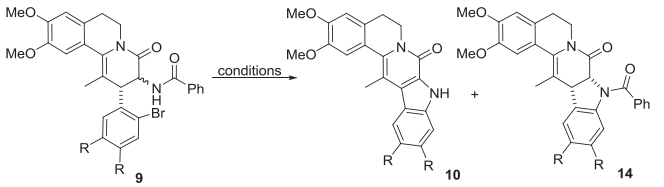
Recently, we reported the synthesis of azalamellarin derivatives using CuTC-mediated/MW-assisted C–N bond formation.[11] Based on our developed method, using CuTC (1 equiv) in DMF with microwave irradiation at 150 °C for 15 min, benzoindoloquinolizines **10a** and **10b** were obtained in moderate yields (38–62%) from the corresponding benzoquinolizin-4-ones **11e** and **11f**, respectively (Scheme 2).

We then applied these conditions to the tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9q** and the reaction gave a mixture of benzoindoloquinolizine **10c** (20%) and *N*-benzoyl benzodihydroindoloquinolizine **14a** (42%) (Table 4, entry 1). Increasing the reaction time to 30 min afforded a mixture of **10c** and **14a** in higher yield (85%) (Table 4, entry 2). We also studied the other Cu(I)-mediated C–N bond formation conditions using




**Scheme 2.** Synthesis of benzoindoloquinolizines **10a**,**b**.

**Table 4**
Synthesis of benzoindoloquinolizines **10** and *N*-benzoyl benzodihydroindoloquinolizines **14**




| Entry | SM **9** | R | Condition | Time (h) | Yield % **10**[a] | Yield % **14**[a] |
|---|---|---|---|---|---|---|
| 1 | *cis*-**9q** | H | A | 0.25 | **c**, 20 | **a**, 42 |
| 2 | *cis*-**9q** | H | A | 0.5 | **c**, 14 | **a**, 71 |
| 3 | *cis*-**9r** | $OCH_3$ | A | 0.5 | **d**, 16 | **b**, 57 |
| 4 | *cis*-**9q** | H | B | 46 | **c**, 11 | **a**, 42[b] |
| 5 | *trans*-**9q** | H | B | 48 | **c**, 11 | **a**, 19[b] |
| 6 | *cis*-**9r** | $OCH_3$ | C | 0.5 | **d**, 17 | **b**, 29[b] |

Condition A: CuTC, DMF, MW, 150 °C. Condition B: Cu(Phen)($PPh_3$)Br, $K_3PO_4$, toluene, Ar atmosphere, 115–120 °C, Condition C: CuBr, TMEDA, DMF, MW, 150 °C.
[a] Isolated yields of pure product after PTLC on silica.
[b] Recovered starting material in 31%, 22%, and 23% yields, respectively.

Cu(Phen)($PPh_3$)Br and CuBr as catalysts. However the reaction gave either lower yields or a complex mixture of products (Table 4, entries 3–6).

## 3. Conclusion

In summary, we have reported a facile pathway to synthesize multi ring-fused benzoindoloquinolizines **10** and benzoquinolizines **11**. The annulations of 1-substituted dihydroisoquinolines **7** with azlactones **8** under neutral conditions followed by Cu(I)-mediated/MW-assisted C–N bond formation of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** gave benzoindoloquinolizines **10** and *N*-benzoyl benzodihydroquinolizines **14**. Under basic conditions tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** were converted into benzoquinolizines **11** in moderate to good yields.

## 4. Experimental section

### 4.1. General methods

Microwave reactions were performed with CEM Discover (250 W, 100 psi). Melting points were determined on electrothermal melting point apparatus and reported without correction. $^1$H Nuclear magnetic resonance ($^1$H NMR) spectra were recorded on 200 MHz, 300 MHz, and 400 MHz instruments at 200, 300 and at 400 MHz, respectively. $^{13}$C Nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded on 200 MHz, 300 MHz, and 400 MHz instruments at 50, 75 and at 100 MHz, respectively. FTIR spectra were recorded using Universal Attenuated Total Reflectance (UATR). Low resolution mass spectra were obtained on an LC/MS instrument using Electron Impact Ionization (EI). High resolution mass spectra were obtained on a MicroTOF instrument using atmospheric pressure chemical ionization (APCI) in positive or negative mode. Column chromatography was carried out using aluminum oxide (100–125 mesh) or Merck silica gel (70–230 mesh). Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were carried out on silica. All reagents were purified and dried according to the standard procedures.

### 4.2. General procedure: tetrahydro-2*H*-pyrido[2,1-*a*] isoquinolines (9)

A solution of azlactones[9] **8** (1.00 mmol) and 6,7-dimethoxy-1-alkyl-3,4-dihydroisoquinolines **7** (1.50 mmol) in acetonitrile (10 mL) was heated at reflux for 2 h. The solvent was evaporated to dryness in vacuo. The crude product was purified by PTLC on silica using 50–90% ethyl acetate in hexane as an eluent to give a mixture of diastereoisomers of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** *cis*/*trans* in moderate to good yields.

*4.2.1. N-[2-Phenyl-9,10,-dimethoxy-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9a**).* The general procedure was used with azlactone **8b** (249 mg, 1.00 mmol) and 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline **7a** (308 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9a** *cis*/*trans* (74:26) (405 mg, 89%): **9a**-*cis* as a bright yellow solid. $R_f$ (50% EtOAc/hexane) 0.38; Mp: 156–158 °C; IR (cm$^{-1}$): 3396, 1683, 1654, 1604, 1581, 1509; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.63–7.59 (m, 2H), 7.36–7.30 (m, 3H), 7.19–7.14 (m, 3H), 7.08 (s, 1H), 7.05–7.00 (m, 2H), 6.84 (d, $J$=5.5 Hz, 1H), 6.61 (s, 1H), 6.02 (d, $J$=7.2 Hz, 1H), 5.06 (dd, $J$=7.6, 5.5 Hz, 1H), 4.40 (m, 2H), 3.85 (s, 3H), 3.83 (s, 3H), 3.49 (ddd, $J$=12.9, 10.2, 4.0 Hz, 1H), 2.92 (ddd, $J$=15.7, 10.2, 4.9 Hz, 1H), 2.79 (dt, $J$=15.7, 4.0 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 167.4, 166.9, 149.7, 148.3, 136.3, 135.2, 134.2, 131.6, 128.6 (2C), 128.5 (4C), 127.6, 127.1, 127.0 (2C), 121.2, 110.7, 106.7, 103.1, 56.0, 55.9, 54.3, 39.7, 39.5, 28.6; EIMS: $m/z$ 454 ($M^+$, 5), 349 (26), 334 (24), 333 (100), 332 (33), 105 (2), 77 (3); HRMS-FAB $m/z$ $[M+H]^+$ calcd for $C_{28}H_{27}N_2O_4$: 455.1971, found: 455.1970; Anal. Calcd for $C_{28}H_{26}N_2O_4$: C, 73.99; H, 5.77; N, 6.16. Found: C, 73.67; H, 5.92; N, 6.16: **9a**-*trans* as a white solid. $R_f$ (50% EtOAc/hexane) 0.19; Mp: 143–144 °C; IR (cm$^{-1}$): 3292, 1669, 1634, 1549, 1514; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.64–7.59 (m, 2H), 7.43–7.23 (m, 8H), 7.04 (s, 1H), 6.78–6.69 (m, 1H), 6.65 (s, 1H), 5.76 (d, $J$=2.8 Hz, 1H), 5.16 (dd, $J$=14.3, 8.8 Hz, 1H), 4.53 (ddd, $J$=12.7, 4.7, 4.2 Hz, 1H), 4.04 (dd, $J$=14.3, 2.8 Hz, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.45 (ddd, $J$=12.7, 10.4, 4.2 Hz, 1H), 2.94 (ddd, $J$=15.7, 10.4, 4.7 Hz, 1H), 2.79 (dt, $J$=15.7, 4.2 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 168.4, 167.8, 149.6, 148.2, 140.6, 134.6, 134.1, 131.3, 128.7 (2C), 128.3 (2C), 128.2 (2C), 127.4, 127.0 (3C), 121.1, 110.7, 106.5, 104.7, 56.0, 55.9, 54.7, 44.3, 39.6, 28.7; EIMS: $m/z$ 455 ($M^+$+1, 0.33), 349 (6), 334 (26), 333 (100), 332 (42), 105 (2), 77 (3); HRMS-FAB $m/z$ $[M+H]^+$ calcd for $C_{28}H_{27}N_2O_4$: 455.1971, found: 455.1978; Anal. Calcd for $C_{28}H_{26}N_2O_4$: C, 73.99; H, 5.77; N, 6.16. Found: C, 74.23; H, 5.94; N, 6.29.

*4.2.2. N-[2-(3′,4′-Dimethoxyphenyl)-9,10,-dimethoxy-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9b**).* The general procedure was used with azlactone **8d** (309 mg, 1.00 mmol) and 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline **7a** (308 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9b** *cis*/*trans* (84:16) (408 mg, 79%): **9b**-*cis* as a bright yellow solid. $R_f$ (70% EtOAc/hexane) 0.19; Mp: 160–161 °C; IR (cm$^{-1}$): 3302, 1678, 1635, 1513; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.75–7.71 (m, 2H), 7.54–7.48 (m, 1H), 7.45–7.40 (m, 2H), 7.17 (s, 1H), 6.98 (d, $J$=5.4 Hz, 1H), 6.76 (d, $J$=8.3 Hz, 1H), 6.70 (s, 1H), 6.67 (dd, $J$=8.3, 2.0 Hz, 1H), 6.61 (d, $J$=2.0 Hz, 1H), 6.10 (d, $J$=7.4 Hz, 1H), 5.09 (dd, $J$=7.4, 5.4 Hz, 1H), 4.50 (ddd, $J$=14.0, 4.9, 4.1 Hz, 1H), 4.45 (t, $J$=7.4 Hz, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 3.84 (s, 3H), 3.61 (s, 3H), 3.55 (ddd, $J$=14.0, 10.2, 4.1 Hz, 1H), 3.01 (ddd, $J$=15.7, 10.2, 4.9 Hz, 1H), 2.87 (dt, $J$=15.7, 4.1 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 167.3, 166.9, 149.7, 148.7, 148.3, 148.2, 135.1, 134.0, 131.7, 128.6 (2C), 128.4, 127.0, 126.9 (2C), 121.1, 120.0, 111.8, 111.2, 110.7, 106.7, 103.3, 56.0, 55.9, 55.8, 55.5, 54.5, 39.5, 39.1, 28.6; EIMS: $m/z$ 514 ($M^+$, 29), 409 (16), 394 (27), 393 (100), 378 (20); HRMS-FAB $m/z$ $[M+H]^+$ calcd for $C_{30}H_{31}N_2O_6$: 515.2182, found 515.2183; Anal. Calcd for $C_{30}H_{30}N_2O_6$: C, 70.02; H, 5.88; N, 5.44. Found: C, 69.89; H, 5.88; N, 5.58: **9b**-*trans* as a white solid. $R_f$ (70% EtOAc/hexane) 0.06; Mp: 165–166 °C; IR (cm$^{-1}$): 3322, 1702, 1635, 1606, 1513; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.66–7.61 (m, 2H), 7.44–7.39 (m, 1H), 7.35–7.29 (m, 2H), 7.03 (s, 1H), 6.95–6.91 (m, 2H), 6.83 (d, $J$=8.7 Hz, 1H), 6.71 (d, $J$=8.9 Hz, 1H), 6.65 (s, 1H), 5.73 (d, $J$=2.7 Hz, 1H), 5.16 (dd, $J$=14.4, 8.9 Hz, 1H), 4.55 (ddd, $J$=12.5, 4.6, 4.3 Hz, 1H), 4.00 (dd, $J$=14.4, 2.7 Hz, 1H), 3.91 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.46–3.38 (m, 1H), 2.93 (ddd, $J$=15.6, 10.6,

4.6 Hz, 1H), 2.78 (dt, *J*=15.6, 4.3 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 168.5, 167.8, 149.7, 148.9, 148.2 (2C), 134.4, 134.2, 133.0, 131.4, 128.3 (2C), 127.5, 127.0 (2C), 121.0, 120.2, 111.2, 111.1, 110.7, 106.5, 105.1, 56.1, 55.9 (2C), 55.8, 54.5, 43.7, 39.5, 28.8; EIMS: *m*/*z* 514 ($M^+$, 2), 409 (4), 394 (26), 393 (100), 378 (25), 77 (3); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{30}H_{31}N_2O_6$: 515.2182, found 515.2180; Anal. Calcd for $C_{30}H_{30}N_2O_6$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.16; H, 5.73; N, 5.59.

*4.2.3. N-[2-(3′,4′-Dimethoxyphenyl)-9,10-dimethoxy-1-methyl-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]acetamide (**9c**).* The general procedure was used with azlactone **8c** (247 mg, 1.00 mmol) and 6,7-dimethoxy-1-ethyl-3,4-dihydroisoquinoline **7b** (329 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9c** *cis*/*trans* (40:60) (303 mg, 65%): **9c**-*cis* as a white solid. $R_f$ (70% EtOAc/hexane) 0.18; Mp: 171–172 °C; IR ($cm^{-1}$): 3276, 1675, 1664, 1635, 1547, 1515; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.01 (s, 1H), 6.78 (d, *J*=8.1 Hz, 1H), 6.66 (dd, *J*=8.1, 1.9 Hz, 1H), 6.77 (s, 1H), 6.62 (d, *J*=1.9 Hz, 1H), 6.31 (d, *J*=5.5 Hz, 1H), 4.89 (br t, *J*=6.7 Hz, 1H), 4.57 (ddd, *J*=12.4, 4.5, 2.4 Hz, 1H), 4.04 (d, *J*=6.7 Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H), 3.11 (ddd, *J*=12.4, 12.3, 4.5 Hz, 1H), 2.98–2.85 (m, 2H), 2.04 (s, 3H), 2.01 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 170.1, 165.6, 148.9, 148.8, 148.4, 146.9, 129.8, 129.6, 127.4, 123.3, 120.6, 117.1, 112.2, 111.8, 111.1, 110.0, 56.1, 56.0, 55.8, 55.6, 53.6, 45.6, 40.6, 28.6, 23.2, 19.1; EIMS: *m*/*z* 466 ($M^+$, 8), 423 (4), 409 (7), 408 (27), 407 (100), 393 (10), 392 (36); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{26}H_{31}N_2O_6$: 467.2182, found 467.2189; Anal. Calcd for $C_{26}H_{30}N_2O_6$: C, 66.94; H, 6.48; N, 6.00. Found: C, 67.05; H, 6.36; N, 6.26: **9c**-*trans* as a white solid. $R_f$ (70% EtOAc/hexane) 0.06; Mp: 192–193 °C; IR ($cm^{-1}$): 3254, 1669, 1648, 1558, 1513; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.97 (s, 1H), 6.88 (d, *J*=1.7 Hz, 1H), 6.84 (d, *J*=8.2 Hz, 1H), 6.81 (dd, *J*=8.2, 1.7 Hz, 1H), 6.75 (s, 1H), 6.24 (d, *J*=6.2 Hz, 1H), 5.01 (br t, *J*=9.7 Hz, 1H), 3.95–3.86 (m, 13H), 3.75 (d, *J*=9.7 Hz, 1H), 3.57 (ddd, *J*=12.9, 6.5, 6.2 Hz, 1H), 2.78 (t, *J*=6.2 Hz, 2H), 1.93 (s, 3H), 1.81 (d, *J*=0.8 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 170.3, 167.2, 149.0, 148.9, 148.2, 146.8, 130.3, 130.2, 127.5, 123.3, 121.0, 115.3, 112.6, 111.8, 111.0, 110.1, 56.3, 56.0 (2C), 55.8, 54.0, 49.3, 41.0, 28.6, 23.2, 19.5; EIMS: *m*/*z* 467 ($(M+1)^+$, 1), 409 (6), 408 (33), 407 (100), 392 (63); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{26}H_{31}N_2O_6$: 467.2182, found 467.2183.

*4.2.4. N-[2-(3′,4′-Dimethoxyphenyl)-9,10-dimethoxy-1-methyl-4-oxo-3,4,6,7,-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9d**).* The general procedure was used with azlactone **8d** (309 mg, 1.00 mmol) and 6,7-dimethoxy-1-ethyl-3,4-dihydroisoquinoline **7b** (329 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9d** *cis*/*trans* (40:60) (457 mg, 86%): **9d**-*cis* as a white solid. $R_f$ (70% EtOAc/hexane) 0.34; Mp: 168–169 °C; IR ($cm^{-1}$): 3369, 1672, 1652, 1605, 1578, 1513; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.78–7.74 (m, 2H), 7.54–7.49 (m, 1H), 7.46–7.40 (m, 2H), 7.06 (s, 1H), 7.00 (d, *J*=5.4 Hz, 1H), 6.80 (s, 1H), 6.76 (d, *J*=8.3 Hz, 1H), 6.68 (dd, *J*=8.3, 1.9 Hz, 1H), 6.62 (d, *J*=1.9 Hz, 1H), 5.07 (dd, *J*=6.9, 5.4 Hz, 1H), 4.60 (ddd, *J*=12.7, 4.8, 2.1 Hz, 1H), 4.24 (d, *J*=6.9 Hz, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 3.84 (s, 3H), 3.62 (s, 3H), 3.15 (ddd, *J*=12.7, 12.7, 3.6 Hz, 1H), 2.98 (ddd, *J*=13.7, 12.7, 4.8 Hz, 1H), 2.94–2.87 (m, 1H), 2.06 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 167.3, 165.6, 149.0, 148.7, 148.4, 147.0, 134.0, 131.7, 130.1, 129.7, 128.6 (2C), 127.2, 127.0 (2C), 123.3, 120.3, 117.0, 112.3, 112.2, 111.1, 110.1, 56.2, 56.0, 55.7, 55.4, 54.0, 45.4, 40.7, 28.6, 19.2; EIMS: *m*/*z* 528 ($M^+$, 5), 423 (3), 408 (31), 407 (100), 392 (35), 77 (4); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{31}H_{33}N_2O_6$: 529.2339, found 529.2341; Anal. Calcd for $C_{31}H_{32}N_2O_6$: C, 70.44; H, 6.10; N, 5.30. Found: C, 70.78; H, 6.09; N, 5.60: **9d**-*trans* as a white solid. $R_f$ (70% EtOAc/hexane) 0.18; Mp: 204–205 °C; IR ($cm^{-1}$): 3284, 1685, 1674, 1636, 1509; $^1$H NMR (400 MHz, $CDCl_3$+$C_6D_6$ (8:1)) $\delta$ 7.61–7.57 (m, 2H), 7.34–7.29 (m, 1H), 7.24–7.18 (m, 2H), 6.97 (d, *J*=8.8 Hz, 1H), 6.94 (s, 1H), 6.93 (d, *J*=2.0 Hz, 1H), 6.85 (dd, *J*=8.2, 2.0 Hz, 1H), 6.73 (d, *J*=8.2 Hz, 1H), 6.63 (s, 1H), 5.19 (dd, *J*=10.0, 8.8 Hz, 1H), 3.94–3.86 (m, 2H), 3.83 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.72 (s, 3H), 3.56–3.47 (m, 1H), 2.65 (t, *J*=5.9 Hz, 2H), 1.79 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$+$C_6D_6$ (8:1)) $\delta$ 167.5, 167.4, 149.0, 148.9, 148.2, 146.9, 134.0, 131.3, 130.3, 130.2, 130.1, 128.2 (2C), 127.0 (2C), 123.4, 121.1, 115.5, 112.7, 111.9, 111.0, 110.1, 56.1, 55.9, 55.8, 55.6, 54.6, 49.3, 41.1, 28.6, 19.5; EIMS: *m*/*z* 528 ($M^+$, 0.31), 408 (27), 409 (100), 392 (44), 77 (4); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{31}H_{33}N_2O_6$: 529.2339, found 529.2339.

*4.2.5. N-(9,10-Dimethoxy-4-oxo-1,2-diphenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl)acetamide (**9e**).* The general procedure was used with azlactone **8a** (88.2 mg, 0.472 mmol) and 1-benzyl-6,7-dimethoxy-3,4-dihydroisoquinoline **7c** (154 mg, 0.707 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9e** *cis*/*trans* (17:83) (93.0 mg, 42%): **9e**-*cis* as a yellow-brown solid. $R_f$ (70% EtOAc/hexane) 0.17; Mp: 267–268 °C; IR ($cm^{-1}$): 3328, 2936, 2833, 1651, 1606, 1510, 1403; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.28–7.04 (m, 10H), 6.67 (s, 1H), 6.32 (s, 1H), 6.27 (d, *J*=6.3 Hz, 1H), 5.19 (t, *J*=6.3 Hz, 1H), 4.58 (d, *J*=6.3 Hz, 1H), 4.48 (dt, *J*=12.1, 4.4 Hz, 1H), 3.87 (s, 3H), 3.36 (td, *J*=12.1, 4.4 Hz, 1H), 3.19 (s, 3H), 3.16–2.98 (m, 2H), 2.05 (s, 3H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 170.2, 166.2, 148.9, 146.6, 139.6, 134.7, 131.5, 130.2 (2C), 129.4, 128.7 (2C), 128.5 (3C), 127.5, 127.1, 122.7, 121.1, 113.3 (2C), 109.6, 55.8, 55.2, 54.1, 46.9, 40.7, 28.6, 23.2; EIMS: *m*/*z* 468 ($M^+$, 4), 409 (100), 394 (25); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{29}H_{29}N_2O_4$: 469.2122, found 469.2126: **9e**-*trans* as yellow solid. $R_f$ (70% EtOAc/hexane) 0.08; Mp: 257–258 °C; IR ($cm^{-1}$): 3315, 2924, 2853, 1642, 1515, 1448, 1393; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.34–6.98 (m, 10H), 6.64 (s, 1H), 6.39 (d, *J*=8.1 Hz, 1H), 6.31 (s, 1H), 4.97 (dd, *J*=8.1, 3.3 Hz, 1H), 4.38–4.23 (m, 1H), 4.22 (d, *J*=3.3 Hz, 1H), 3.88 (s, 3H), 3.42–3.24 (m, 1H), 3.17 (s, 3H), 3.08–2.76 (m, 2H), 2.11 (s, 3H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 169.9, 165.6, 148.7, 146.3, 140.1, 136.4, 129.9 (3C), 128.7 (2C), 128.6 (2C), 127.9 (3C), 127.3, 126.9, 122.1, 117.6, 113.4, 109.6, 55.8, 55.5, 55.1, 50.8, 40.5, 28.7, 23.4; EIMS: *m*/*z* 468 ($M^+$, 1), 409 (100), 394 (37); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{29}H_{29}N_2O_4$: 469.2122, found 469.2126.

*4.2.6. N-[2-(3″,4″-Dimethoxyphenyl)-9,10-dimethoxy-4-oxo-1-phenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]acetamide (**9f**).* The general procedure was used with azlactone **8c** (247 mg, 1.00 mmol) and 1-benzyl-6,7-dimethoxy-3,4-dihydroisoquinoline **7c** (423 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9f** *cis*/*trans* (0:100) (203 mg, 35%): **9f**-*cis* as a white solid. $R_f$ (80% EtOAc/hexane) 0.15; Mp: 229 °C; IR ($cm^{-1}$): 3317, 1646, 1516; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.17–7.02 (m, 5H), 6.69–6.58 (m, 3H), 6.53 (br s, 1H), 6.28 (d, *J*=5.7 Hz, 1H), 6.25 (s, 1H), 5.07 (dd, *J*=6.8, 5.7 Hz, 1H), 4.47 (d, *J*=6.8 Hz, 1H), 4.45–4.35 (m, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 3.69 (s, 3H), 3.30 (ddd, *J*=12.2, 12.2, 4.0 Hz, 1H), 3.12 (s, 3H), 3.02–2.80 (m, 2H), 1.99 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 170.1, 166.2, 148.8, 148.7, 148.3, 146.6, 139.5, 131.1, 130.2 (2C), 129.2, 128.5 (2C), 127.1, 126.9, 122.7, 121.4, 120.5, 113.2, 111.9, 111.1, 109.5, 55.8, 55.7, 55.6, 55.1, 54.2, 46.3, 40.5, 28.5, 23.2; EIMS: *m*/*z* 528 ($M^+$, 8), 471 (6), 470 (31), 469 (100), 455 (10), 454 (36); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{31}H_{33}N_2O_6$: 529.2339, found 529.2339: **9f**-*trans* as a white solid. $R_f$ (80% EtOAc/hexane) 0.06; Mp: 170–171 °C; IR ($cm^{-1}$): 3356, 1670, 1637, 1611, 1513; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.24–7.10 (m, 3H), 7.08–7.00 (m, 2H), 6.82 (br d, *J*=7.9 Hz, 1H), 6.81 (br s, 1H), 6.72 (d, *J*=7.9 Hz, 1H), 6.62 (s, 1H), 6.59 (d, *J*=7.9 Hz, 1H), 6.30 (s, 1H), 4.97 (dd, *J*=7.9, 3.2 Hz, 1H), 4.30–4.20 (m, 1H), 4.17 (d, *J*=3.2 Hz, 1H), 3.86 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.35–3.22 (m, 1H), 3.17 (s, 3H), 2.98–2.75 (m, 2H), 2.12 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 170.2, 165.7, 149.0, 148.7, 148.1, 146.3, 140.1, 131.3, 129.9 (2C), 129.8, 128.7, 128.6 (2C), 127.0, 122.1, 119.7, 118.0, 113.4, 111.3, 111.2, 109.6, 55.8, 55.7, 55.6, 55.5, 55.1, 50.2, 40.5, 28.6, 23.2; EIMS: *m*/*z* 528 ($M^+$, 1), 513 (9), 471 (6), 470 (29),

469 (100), 454 (44); HRMS-FAB *m/z* $[M+H]^+$ calcd for $C_{31}H_{33}N_2O_6$: 529.2339, found 529.2338.

*4.2.7. N-[9,10-Dimethoxy-2-(3″,4″-methylenedioxyphenyl)-4-oxo-1-phenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]acetamide (**9g**).* The general procedure was used with azlactone **8e** (231 mg, 1.00 mmol) and 1-benzyl-6,7-dimethoxy-3,4-dihydroisoquinoline **7c** (423 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9g** *cis*/*trans* (20:80) (230 mg, 45%): **9g**-*cis* as a pale yellow solid. $R_f$ (80% EtOAc/hexane) 0.27; Mp: 229–230 °C; IR ($cm^{-1}$): 3325, 1645, 1614, 1516; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.23–7.12 (m, 5H), 6.67 (s, 1H), 6.66 (d, *J*=8.2 Hz, 1H), 6.61 (d, *J*=1.7 Hz, 1H), 6.56 (dd, *J*=8.2, 1.7 Hz, 1H), 6.32 (s, 1H), 6.31 (br d, *J*=4.2 Hz, 1H), 5.89 (d, *J*=7.5 Hz, 1H), 5.88 (d, *J*=7.5 Hz, 1H), 5.13 (br t, *J*=6.5 Hz, 1H), 4.53–4.46 (m, 2H), 3.88 (s, 3H), 3.37 (ddd, *J*=12.2, 11.9, 3.6 Hz, 1H), 3.20 (s, 3H), 3.06 (ddd, *J*=15.3, 11.9, 4.4 Hz, 1H), 2.92 (dt, *J*=15.3, 3.6 Hz, 1H), 2.07 (s, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 170.2, 166.2, 148.9, 147.9, 147.0, 146.6, 139.5, 131.4, 130.2 (2C), 129.5, 128.6 (2C), 128.2, 127.1, 122.6, 122.2, 121.2, 113.3, 109.6, 108.7, 108.3, 100.9, 55.8, 55.1, 54.2, 46.4, 40.6, 28.6, 23.3; EIMS: *m/z* 512 ($M^+$, 9), 470 (31), 469 (100), 455 (14), 454 (71), 453 (91), 438 (26); HRMS-FAB *m/z* $[M+H]^+$ calcd for $C_{30}H_{29}N_2O_6$: 513.2026, found 513.2026: **9g**-*trans* as a yellow amorphous solid. $R_f$ (80% EtOAc/hexane) 0.11; IR ($cm^{-1}$): 3347, 1669, 1608, 1512; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.24–7.18 (m, 2H), 7.17–7.12 (m, 1H), 7.04 (br d, *J*=7.0 Hz, 2H), 6.79 (d, *J*=1.7 Hz, 1H), 6.78 (dd, *J*=7.2, 1.7 Hz, 1H), 6.68 (d, *J*=7.2 Hz, 1H), 6.63 (s, 1H), 6.39 (d, *J*=8.0 Hz, 1H), 6.29 (s, 1H), 5.88 (d, *J*=3.4 Hz, 1H), 5.87 (d, *J*=3.4 Hz, 1H), 4.89 (dd, *J*=8.0, 3.0 Hz, 1H), 4.30 (ddd, *J*=12.4, 4.2, 4.0 Hz, 1H), 4.13 (d, *J*=3.0 Hz, 1H), 3.86 (s, 3H), 3.31 (ddd, *J*=12.4, 11.6, 4.0 Hz, 1H), 3.17 (s, 3H), 2.96 (ddd, *J*=15.2, 11.6, 4.2 Hz, 1H), 2.82 (dt, *J*=15.2, 4.0 Hz, 1H), 2.11 (s, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 170.0, 165.5, 148.8, 147.9, 146.8, 146.4, 140.1, 131.5, 130.1, 130.0, 129.9 (2C), 128.7 (2C), 127.1, 122.0, 121.3, 117.7, 113.4, 109.6, 108.6, 108.3, 100.9, 55.8, 55.7, 55.1, 50.5, 40.5, 28.7, 23.3; EIMS: *m/z* 513 ($M^+$+1, 4), 475 (6), 454 (37), 453 (100), 452 (9), 439 (20), 438 (59); HRMS-FAB *m/z* $[M+H]^+$ calcd for $C_{30}H_{29}N_2O_6$: 513.2026, found 513.2027.

*4.2.8. N-[2-(3″,4″-Dimethoxyphenyl)-9,10-dimethoxy-4-oxo-1-phenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9h**).* The general procedure was used with azlactone **8d** (309 mg, 1.00 mmol) and 1-benzyl-6,7-dimethoxy-3,4-dihydroisoquinoline **7c** (423 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9h** *cis*/*trans* (36:64) (470 mg, 80%): **9h**-*cis* as a white solid. $R_f$ (60% EtOAc/hexane) 0.27; Mp: 237 °C; IR ($cm^{-1}$): 3289, 1672, 1632, 1526, 1514; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.82–7.75 (m, 2H), 7.56–7.39 (m, 3H), 7.27–7.11 (m, 5H), 7.05 (d, *J*=5.2 Hz, 1H), 6.77–6.66 (m, 3H), 6.59 (br s, 1H), 6.37 (s, 1H), 5.32 (br t, *J*=6.6 Hz, 1H), 4.76 (d, *J*=6.6 Hz, 1H), 4.56–4.46 (m, 1H), 3.88 (s, 3H), 3.79 (s, 3H), 3.56 (s, 3H), 3.42 (ddd, *J*=12.1, 12.1, 3.8 Hz, 1H), 3.22 (s, 3H), 3.14–2.90 (m, 2H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 167.3, 166.2, 148.9, 148.6, 148.3, 146.6, 139.6, 133.9, 131.8, 131.4, 130.2 (2C), 129.3, 128.6 (2C), 128.5 (2C), 127.2, 127.0 (2C), 126.7, 122.7, 121.3, 120.2, 113.3, 112.3, 111.1, 109.6, 55.8, 55.7, 55.4, 55.1, 54.7, 46.1, 40.6, 28.6; EIMS: *m/z* 590 ($M^+$, 8), 470 (33), 469 (100), 454 (28); HRMS-FAB *m/z* $[M+H]^+$ calcd for $C_{36}H_{35}N_2O_6$: 591.2495, found 591.2502; Anal. Calcd for $C_{36}H_{34}N_2O_6$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.52; H, 5.80; N, 4.63: **9h**-*trans* as yellow amorphous solid. $R_f$ (60% EtOAc/hexane) 0.13; IR ($cm^{-1}$): 3336, 1668, 1604, 1579, 1513; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.85–7.77 (m, 2H), 7.56–7.48 (m, 1H), 7.48–7.39 (m, 2H), 7.21–7.08 (m, 3H), 7.07–7.00 (m, 2H), 6.94–6.83 (m, 3H), 6.75 (d, *J*=8.8 Hz, 1H), 6.67 (s, 1H), 6.33 (s, 1H), 5.15 (dd, *J*=7.6, 3.6 Hz, 1H), 4.37 (d, *J*=3.6 Hz, 1H), 4.34–4.25 (m, 1H), 3.88 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.49–3.37 (m, 1H), 3.15 (s, 3H), 3.04–2.82 (m, 2H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 167.6, 165.8, 149.1, 148.8, 148.2, 146.5, 140.0, 134.0, 131.8, 131.5, 129.9 (2C), 129.8, 128.6 (5C), 127.1 (2C), 127.0, 122.2, 119.8, 118.3, 113.3, 111.4, 111.2, 109.7, 56.0, 55.8, 55.7, 55.1 (2C), 50.0, 40.6, 28.7; EIMS: *m/z* 590 ($M^+$, 0.83), 470 (32), 469 (100), 454 (21); HRMS-FAB *m/z* $[M+H]^+$ calcd for $C_{36}H_{35}N_2O_6$: 591.2495, found 591.2502.

*4.2.9. N-(1-(3,4-Dimethoxyphenyl)-9,10-dimethoxy-4-oxo-2-phenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl)acetamide (**9i**).* The general procedure was used with azlactone **8a** (190 mg, 1.01 mmol) and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline 7d (494 mg, 1.52 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9i** *cis*/*trans* (27:73) (184 mg, 34%): **9i**-*cis* as a yellow solid. $R_f$ (90% EtOAc/hexane) 0.26; Mp: 203–204 °C; IR ($cm^{-1}$) 3329, 2935, 2835, 1654, 1606, 1508, 1405; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.28–7.02 (m, 5H), 6.68 (d, *J*=8.3 Hz, 1H), 6.64 (s, 1H), 6.57 (dd, *J*=8.3, 2 Hz, 1H), 6.46 (d, *J*=2 Hz, 1H), 6.41 (s, 1H), 6.37 (d, *J*=8.4 Hz, 1H), 5.02 (dd, *J*=8.4, 4.8 Hz, 1H), 4.22 (d, *J*=4.8 Hz, 1H), 4.18 (dt, *J*=11.0, 4.4 Hz, 1H), 3.87 (s, 3H), 3.78 (s, 3H), 3.62 (s, 3H), 3.54–3.34 (m, 1H), 3.26 (s, 3H), 3.04–2.74 (m, 2H), 2.08 (s, 3H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 170.2, 166.1, 148.9, 148.8, 148.3, 146.7, 134.8, 131.9, 131.1, 129.4 (2C), 128.6 (2C), 128.5 (3C), 127.4, 122.9, 120.9, 113.4, 113.1, 111.3, 109.6, 56.0, 55.8, 55.3, 54.1, 46.9, 40.7, 28.6, 23.2; EIMS: *m/z* 528 ($M^+$, 12), 469 (100), 455 (9); HRMS-FAB *m/z* $[M+H]^+$ calcd for $C_{31}H_{33}N_2O_6$: 529.2333, found 529.2335: **9i**-*trans* as a brown solid. $R_f$ (90% EtOAc/hexane) 0.08; Mp: 145–146 °C; IR ($cm^{-1}$) 3329, 2935, 2835, 1654, 1606, 1508, 1405; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.32–7.15 (m, 5H), 6.68 (d, *J*=8.3 Hz, 1H), 6.65 (s, 1H), 6.58 (dd, *J*=8.3, 1.8 Hz, 1H), 6.46 (s, 1H), 6.43 (s, 1H), 6.13 (d, *J*=8.2 Hz, 1H), 5.03 (dd, *J*=8.2, 4.6 Hz, 1H), 4.22 (d, *J*=4.6 Hz, 1H), 4.19 (m, 1H), 3.88 (s, 3H), 3.78 (s, 3H), 3.62 (s, 3H), 3.53–3.44 (m, 1H), 3.26 (s, 3H), 2.96 (ddd, *J*=15.0, 10.3, 4.5 Hz, 1H), 2.85 (dt, *J*=15.0, 4.4 Hz, 1H), 2.09 (s, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 170.0, 165.9, 148.9, 148.8, 148.2, 146.5, 136.6, 132.4, 131.3, 130.0, 128.7 (2C), 128.2 (2C), 127.3, 122.7, 122.4, 117.7, 113.5, 113.2, 111.3, 109.7, 56.0, 55.9 (2C), 55.3, 55.1, 50.6, 40.7, 28.8, 23.4; EIMS: *m/z* 528 ($M^+$, 2), 469 (100), 455 (9); HRMS-FAB *m/z* $[M+H]^+$ calcd for $C_{31}H_{33}N_2O_6$: 529.2333, found 529.2337.

*4.2.10. N-[1,2-Bis-(3′,4′-dimethoxyphenyl)-9,10-dimethoxy-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]acetamide (**9j**).* The general procedure was used with azlactone **8c** (247 mg, 1.00 mmol) and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline **7d** (512 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9j** *cis*/*trans* (16:84) (265 mg, 45%): **9j**-*cis* as a yellow oil. $R_f$ (70% EtOAc/hexane; developed twice) 0.11; IR ($cm^{-1}$): 3329, 1656, 1606, 1509; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 6.74 (d, *J*=8.2 Hz, 1H), 6.73 (s, 1H), 6.70–6.63 (m, 4H), 6.61 (d, *J*=1.6 Hz, 1H), 6.42 (s, 1H), 6.36 (d, *J*=5.6 Hz, 1H), 5.13 (br t, *J*=6.8 Hz, 1H), 4.53 (d, *J*=6.8 Hz, 1H), 4.47 (ddd, *J*=12.3, 4.3, 4.0 Hz, 1H), 3.88 (s, 3H), 3.81 (s, 3H), 3.78 (s, 6H), 3.72 (s, 3H), 3.38 (ddd, *J*=12.3, 12.0, 4.3 Hz, 1H), 3.29 (s, 3H), 3.09–2.88 (m, 2H), 2.07 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.2, 166.1, 148.9, 148.8, 148.7, 148.3, 148.3, 146.6, 131.8, 130.8, 129.1, 127.0, 123.0, 122.9, 121.2, 120.5, 113.3, 113.0, 111.9, 111.2, 111.1, 109.5, 56.0, 55.8, 55.7 (2C), 55.6, 55.2, 54.2, 46.3, 40.6, 28.5, 23.2; EIMS: *m/z* 588 ($M^+$, 10), 531 (7), 530 (33), 529 (100), 515 (16), 514 (20); HRMS-FAB *m/z* $[M+H]^+$ calcd for $C_{33}H_{37}N_2O_8$: 589.2550, found 589.2551: **9j**-*trans* as a yellow amorphous solid. $R_f$ (70% EtOAc/hexane; developed twice) 0.05; IR ($cm^{-1}$): 1668, 1606, 1513; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 6.78–6.50 (m, 6H), 6.45 (d, *J*=8.2 Hz, 1H), 6.40 (br s, 1H), 6.32 (s, 1H), 4.93 (dd, *J*=8.0, 4.7 Hz, 1H), 4.18–4.05 (m, 2H), 3.79 (s, 3H), 3.73 (s, 3H), 3.72 (s, 3H), 3.71 (s, 3H), 3.55 (s, 3H), 3.41–3.26 (m, 1H), 3.19 (s, 3H), 2.90–2.66 (m, 2H), 2.01 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.1, 166.1, 149.0, 148.9, 148.7, 148.2, 148.1, 146.4, 132.3, 131.0, 129.8, 128.9, 122.6, 122.4, 119.9, 118.2, 113.5, 113.2, 111.4, 111.3, 111.1, 109.6, 55.9, 55.8 (2C), 55.7 (2C), 55.3, 55.1, 50.0, 40.6, 28.7, 23.3; EIMS: *m/z* 588 ($M^+$, 3), 545 (4), 531

(9), 530 (42), 529 (100), 515 (11), 514 (30); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{33}H_{37}N_2O_8$: 589.2550, found 589.2552.

*4.2.11. N-[9,10-Dimethoxy-1-(3′,4′-dimethoxyphenyl)-2-(3″,4″-methylenedioxyphenyl)-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a] isoquinolin-3-yl]acetamide (**9k**).* The general procedure was used with azlactone **8e** (231 mg, 1.00 mmol) and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline **7d** (512 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9k** *cis*/*trans* (15:85) (225 mg, 39%): **9k**-*cis* as a pale yellow oil. $R_f$ (70% EtOAc/hexane; developed twice) 0.20; IR ($cm^{-1}$): 3326, 1660, 1608, 1580, 1509; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 6.73–6.63 (m, 5H), 6.59 (d, *J*=1.5 Hz, 1H), 6.55 (dd, *J*=8.0, 1.5 Hz, 1H), 6.41 (s, 1H), 6.35 (d, *J*=6.1 Hz, 1H), 5.89 (d, *J*=5.6 Hz, 1H), 5.88 (d, *J*=5.6 Hz, 1H), 5.11 (br t, *J*=6.1 Hz, 1H), 4.53–4.43 (m, 2H), 3.88 (s, 3H), 3.79 (s, 3H), 3.71 (s, 3H), 3.36 (ddd, *J*=12.4, 12.1, 3.9 Hz, 1H), 3.29 (s, 3H), 3.05 (ddd, *J*=13.4, 12.1, 4.7 Hz, 1H), 2.97–2.87 (m, 1H), 2.07 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.3, 166.1, 148.9, 148.8, 148.3, 147.8, 146.9, 146.6, 131.7, 131.0, 129.4, 128.3, 122.9 (2C), 122.2, 121.0, 113.3, 113.0, 111.1, 109.5, 108.6, 108.3, 100.9, 56.0, 55.8, 55.7, 55.2, 54.1, 46.4, 40.6, 28.5, 23.2; EIMS: *m*/*z* 572 ($M^+$, 8), 515 (6), 514 (34), 513 (100), 498 (39); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{32}H_{33}N_2O_8$: 573.2237, found 573.2242: **9k**-*trans* as a white solid. $R_f$ (70% EtOAc/hexane; developed twice) 0.09; Mp: 167–169 °C; IR ($cm^{-1}$): 3384, 1664, 1578, 1509; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 6.80–6.62 (m, 5H), 6.62–6.55 (m, 1H), 6.49 (br s, 1H), 6.40 (s, 1H), 6.21 (d, *J*=8.0 Hz, 1H), 5.88 (s, 2H), 4.92 (dd, *J*=8.0, 3.9 Hz, 1H), 4.28–4.18 (m, 1H), 4.14 (d, *J*=3.9 Hz, 1H), 3.87 (s, 3H), 3.79 (s, 3H), 3.65 (s, 3H), 3.46–3.36 (m, 1H), 3.27 (s, 3H), 3.03–2.89 (m, 1H), 2.89–2.78 (m, 1H), 2.09 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.0, 165.7, 149.0, 148.8, 148.3, 147.9, 146.8, 146.5, 132.3, 131.2, 130.3, 130.0, 122.6, 122.3, 121.4, 117.8, 113.4, 113.2, 111.4, 109.7, 108.5, 108.4, 100.9, 56.0, 55.9 (2C), 55.5, 55.3, 50.3, 40.6, 28.7, 23.4; EIMS: *m*/*z* 572 ($M^+$, 7), 529 (2), 515 (7), 514 (36), 513 (100), 498 (11); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{32}H_{33}N_2O_8$: 573.2237, found 573.2236.

*4.2.12. N-[1-(3′,4′-Dimethoxyphenyl)-9,10-dimethoxy-4-oxo-2-phenyl-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9l**).* The general procedure was used with azlactone **8b** (249 mg, 1.00 mmol) and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline **7d** (512 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9l** *cis*/*trans* (35:65) (513 mg, 86%): **9l**-*cis* as a white solid. $R_f$ (60% EtOAc/hexane) 0.26; Mp: 146–148 °C; IR ($cm^{-1}$): 3371, 1645, 1603, 1579, 1515; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.86–7.82 (m, 2H), 7.63–7.57 (m, 1H), 7.55–7.49 (m, 2H), 7.33–7.20 (m, 5H), 7.07 (d, *J*=5.6 Hz, 1H), 6.86 (s, 1H), 6.82 (d, *J*=8.4 Hz, 1H), 6.79 (s, 1H), 6.75 (d, *J*=8.4 Hz, 1H), 6.56 (s, 1H), 5.44 (dd, *J*=7.0, 5.6 Hz, 1H), 4.83 (d, *J*=7.0 Hz, 1H), 4.60 (ddd, *J*=12.4, 4.6, 3.5 Hz, 1H), 3.98 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 3.50 (ddd, *J*=12.4, 12.0, 3.5 Hz, 1H), 3.40 (s, 3H), 3.20 (ddd, *J*=15.2, 12.0, 4.6 Hz, 1H), 3.04 (dt, *J*=15.2, 3.5 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 167.6, 166.2, 149.0, 148.8, 148.3, 146.7, 134.6, 134.1, 131.9, 131.7, 131.3, 129.4, 128.8 (2C), 128.6 (4C), 127.5, 127.1 (2C), 122.9 (2C), 120.9, 113.3, 113.0, 111.2, 109.6, 56.1, 55.9, 55.8, 55.3, 54.4, 46.7, 40.7, 28.6; EIMS: *m*/*z* 590 ($M^+$, 17), 513 (8), 470 (33), 469 (100), 454 (26), 105 (1), 77 (1); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{36}H_{35}N_2O_6$: 591.2495, found 591.2496; Anal. Calcd for $C_{36}H_{34}N_2O_6$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.31; H, 5.80; N, 4.68: **9l**-*trans* as a white solid. $R_f$ (60% EtOAc/hexane) 0.20; Mp: 183 °C; IR ($cm^{-1}$): 3285, 1667, 1634, 1601, 1579, 1532, 1513; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.82–7.77 (m, 2H), 7.56–7.51 (m, 1H), 7.47–7.42 (m, 2H), 7.39–7.34 (m, 2H), 7.25–7.31 (m, 2H), 7.23–7.17 (m, 1H), 6.76 (d, *J*=7.8 Hz, 1H), 6.69 (s, 1H), 6.66 (d, *J*=8.3 Hz, 1H), 6.59 (dd, *J*=8.3, 1.8 Hz, 1H), 6.48 (d, *J*=1.8 Hz, 1H), 6.44 (s, 1H), 5.19 (dd, *J*=7.8, 4.5 Hz, 1H), 4.43 (d, *J*=4.5 Hz, 1H), 4.25 (ddd, *J*=12.8, 4.6, 4.5 Hz, 1H), 3.90 (s, 3H), 3.78 (s, 3H), 3.55 (s, 3H), 3.58–3.48 (m, 1H), 3.26 (s, 3H), 3.01 (ddd, *J*=15.2, 10.6, 4.6 Hz, 1H), 2.89 (dt, *J*=15.2, 4.5 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 167.6, 165.8, 149.0, 148.8, 148.2, 146.5, 136.5, 134.1, 132.3, 131.8, 131.4, 130.0, 128.8 (2C), 128.7 (2C), 128.2 (2C), 127.4, 127.0 (2C), 122.5 (2C), 118.0, 113.2, 113.0, 111.3, 109.7, 55.9 (2C), 55.8 (2C), 55.3, 50.4, 40.8, 28.8; EIMS: *m*/*z* 590 ($M^+$, 0.91), 470 (32), 469 (100), 454 (35); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{36}H_{35}N_2O_6$: 591.2495, found 591.2492; Anal. Calcd for $C_{36}H_{34}N_2O_6$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.04; H, 5.97; N, 4.45.

*4.2.13. N-[1,2-Bis-(3′,4′-dimethoxyphenyl)-9,10-dimethoxy-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]benzamide (**9m**).* The general procedure was used with azlactone **8d** (309 mg, 1.00 mmol) and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline **7d** (512 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9m** *cis*/*trans* (32:68) (553 mg, 85%): **9m**-*cis* as a pale brown oil. $R_f$ (70% EtOAc/hexane) 0.49; IR ($cm^{-1}$): 1652, 1604, 1509; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.82–7.74 (m, 2H), 7.56–7.48 (m, 1H), 7.48–7.39 (m, 2H), 7.05 (d, *J*=5.2 Hz, 1H), 6.82–6.65 (m, 6H), 6.60 (s, 1H), 6.46 (s, 1H), 5.30 (br t, *J*=6.5 Hz, 1H), 4.73 (d, *J*=6.5 Hz, 1H), 4.55–4.45 (m, 1H), 3.89 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.74 (s, 3H), 3.58 (s, 3H), 3.42 (ddd, *J*=12.1, 11.9, 4.0 Hz, 1H), 3.31 (s, 3H), 3.06 (ddd, *J*=13.4, 11.9, 4.8 Hz, 1H), 3.01–2.91 (m, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 167.4, 166.2, 149.1, 148.9, 148.7, 148.5, 148.4, 146.8, 134.0, 132.0, 131.8, 131.1, 129.2, 128.6 (2C), 127.0 (2C), 126.9, 123.1, 123.0, 121.2, 120.3, 113.6, 113.2, 112.3, 111.4, 111.3, 109.7, 56.1, 55.9 (2C), 55.7, 55.4, 55.3, 54.7, 46.3, 40.7, 28.6; EIMS: *m*/*z* 650 ($M^+$, 6), 531 (7), 530 (33), 529 (100), 515 (9), 514 (25), 470 (30), 469 (88), 455 (12), 454 (27); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{38}H_{39}N_2O_8$: 651.2706, found 651.2709: **9m**-*trans* as a pale brown oil. $R_f$ (70% EtOAc/hexane) 0.40; IR ($cm^{-1}$): 3336, 1668, 1604, 1579, 1513; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.81–7.74 (m, 2H), 7.55–7.46 (m, 1H), 7.46–7.36 (m, 2H), 7.02–6.83 (m, 3H), 6.74 (d, *J*=8.2 Hz, 1H), 6.66 (s, 1H), 6.65 (d, *J*=7.5 Hz, 1H), 6.56 (dd, *J*=8.2, 1.5 Hz, 1H), 6.48 (br s, 1H), 6.41 (s, 1H), 5.16 (dd, *J*=7.4, 4.7 Hz, 1H), 4.39 (d, *J*=4.7 Hz, 1H), 4.25–4.14 (m, 1H), 3.88 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.76 (s, 3H), 3.54 (s, 3H), 3.50–3.37 (m, 1H), 3.24 (s, 3H), 3.01–2.79 (m, 2H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 167.6, 166.1, 149.0, 148.9, 148.7, 148.1 (2C), 146.5, 134.0, 132.3, 131.7, 131.1, 129.7, 128.7, 128.5 (2C), 127.0 (2C), 122.5 (2C), 120.0, 118.5, 113.2, 113.0, 111.4, 111.1, 111.2, 109.7, 55.8 (2C), 55.7 (4C), 55.3, 49.7, 40.7, 28.7; EIMS: *m*/*z* 650 ($M^+$, 11), 531 (7), 530 (35), 529 (100), 514 (25), 105 (2), 77 (3); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{38}H_{39}N_2O_8$: 651.2706, found 651.2712.

*4.2.14. N-[9,10-Dimethoxy-1-(3′,4′-dimethoxyphenyl)-2-(3″,4″-methylenedioxyphenyl)-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a] isoquinolin-3-yl]-benzamide (**9n**).* The general procedure was used with azlactone **8f** (293 mg, 1.00 mmol) and 1-(3′,4′-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline **7d** (512 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9n** *cis*/*trans* (38:62) (532 mg, 84%): **9n**-*cis* as a white solid. $R_f$ (60% EtOAc/hexane) 0.23; Mp: 134–135 °C; IR ($cm^{-1}$): 3392, 1677, 1651, 1607, 1580, 1515; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.80–7.72 (m, 2H), 7.55–7.50 (m, 1H), 7.47–7.42 (m, 2H), 7.03 (d, *J*=5.3 Hz, 1H), 6.76 (br s, 1H), 6.73 (br d *J*=8.5 Hz, 1H), 6.69 (s, 1H), 6.68 (d, *J*=8.5 Hz, 1H), 6.65 (d, *J*=1.6 Hz, 1H), 6.63 (dd, *J*=8.0 Hz, 1H), 6.57 (dd, *J*=8.0, 1.6 Hz, 1H), 6.45 (s, 1H), 5.88 (d, *J*=4.2 Hz, 1H), 5.87 (d, *J*=4.2 Hz, 1H), 5.28 (br t, *J*=6.8 Hz, 1H), 4.68 (d, *J*=6.8 Hz, 1H), 4.52 (ddd, *J*=12.5, 4.7, 3.5 Hz, 1H), 3.89 (s, 3H), 3.80 (s, 3H), 3.74 (s, 3H), 3.40 (ddd, *J*=12.5, 12.3, 3.5 Hz, 1H), 3.31 (s, 3H), 3.14–3.04 (m, 1H), 2.94 (dt, *J*=15.0, 3.5 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 167.6, 166.2, 149.0, 148.9, 148.3, 147.9, 147.0, 146.7, 134.1, 131.8, 131.7, 131.2, 129.4, 128.6 (2C), 128.2, 127.1 (2C), 123.0, 122.9, 122.5, 121.0, 113.3, 113.0, 111.2, 109.6, 108.5, 108.3, 100.9, 56.1, 55.9, 55.8, 55.3, 54.6, 46.4, 40.7, 28.6; EIMS: *m*/*z* 634 ($M^+$, 20), 515 (8), 514 (28), 513 (100), 498 (17), 77 (6); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{37}H_{35}N_2O_8$: 635.2393, found 635.2405; Anal. Calcd for $C_{37}H_{34}N_2O_8$: C, 70.02; H, 5.40; N, 4.41.

Found: C, 69.72; H, 5.69; N, 4.32: **9n**-*trans* as a white solid. $R_f$ (60% EtOAc/hexane) 0.17; Mp: 221–222 °C; IR ($cm^{-1}$): 3279, 1669, 1634, 1601, 1578, 1509; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.82–7.74 (m, 2H), 7.55–7.50 (m, 1H), 7.46–7.41 (m, 2H), 6.88–6.84 (m, 2H), 6.81 (d, *J*=7.7 Hz, 1H), 6.71 (d, *J*=8.6 Hz, 1H), 6.67 (s, 1H), 6.66 (d, *J*=8.2 Hz, 1H), 6.58 (dd, *J*=8.2, 1.7 Hz, 1H), 6.49 (d, *J*=1.4 Hz, 1H), 6.40 (s, 1H), 5.90 (d, *J*=3.1 Hz, 1H), 5.89 (d, *J*=3.1 Hz, 1H), 5.08 (dd, *J*=7.7, 4.0 Hz, 1H), 4.35 (d, *J*=4.0 Hz, 1H), 4.27 (ddd, *J*=12.3, 4.4, 3.9 Hz, 1H), 3.88 (s, 3H), 3.77 (s, 3H), 3.56 (s, 3H), 3.45 (ddd, *J*=12.3, 11.4, 3.9 Hz, 1H), 3.24 (s, 3H), 2.99 (ddd, *J*=15.1, 11.4, 4.4 Hz, 1H), 2.86 (dt, *J*=15.1, 3.9 Hz, 1H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 167.5, 165.7, 149.0, 148.9, 148.2, 148.0, 146.8, 146.5, 134.0, 132.3, 131.9, 131.3, 130.1, 130.0, 128.7 (2C), 127.0 (2C), 122.5, 122.4, 121.5, 118.0, 113.1, 113.0, 111.3, 109.7, 108.6, 108.4, 101.0, 56.2, 55.9, 55.8 (2C), 55.3, 50.0, 40.7, 28.7; EIMS: *m*/*z* 634 ($M^+$, 22), 529 (3), 515 (7), 514 (33), 513 (100), 498 (18); HRMS-FAB *m*/*z* $[M+H]^+$ calcd for $C_{37}H_{35}N_2O_8$: 635.2393, found 635.2390; Anal. Calcd for $C_{37}H_{34}N_2O_8$: C, 70.02; H, 5.40; N, 4.41. Found: C, 70.12; H, 5.45; N, 4.45.

*4.2.15. N-2-(2″-Bromophenyl)-4-oxo-3,4,6,7-tetrahydro-2H-pyrido [2,1-a]isoquinolin-3-yl]-benzamide (**9o**).* The general procedure was used with azlactone **8g** (327 mg, 1.00 mmol) and 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline **7a** (308 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9o** *cis*/*trans* (78:22) (520 mg, 98%): **9o**-*cis* as a pale green solid. $R_f$ (60% EtOAc/hexane) 0.32; Mp: 179–180 °C; IR ($cm^{-1}$): 3410, 3058, 2938, 1656, 1609, 1511; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.75–7.65 (m, 2H), 7.60–7.35 (m, 4H), 7.30–7.05 (m, 4H), 6.74 (d, *J*=6.8 Hz, 1H), 6.67 (s, 1H), 6.08 (d, *J*=6.8 Hz, 1H), 5.37 (t, *J*=6.8 Hz, 1H), 4.90 (t, *J*=6.8 Hz, 1H), 4.47 (dt, *J*=12.7, 4.0 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.58 (ddd, *J*=12.7, 9.6, 4.0 Hz, 1H), 3.08–2.76 (m, 2H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 167.8, 167.1, 149.8, 148.2, 136.8, 135.4, 134.3, 133.3, 131.4, 128.9, 128.6, 128.3 (3C), 128.0, 127.1 (2C), 125.7, 121.0, 110.7, 106.7, 102.7, 56.0, 55.9, 53.1, 39.6, 39.3, 28.6; EIMS: *m*/*z* 534 ($M^+$+2, 5), 532 ($M^+$, 6), 429 (29), 427 (31), 413 (98), 411 (100), 332 (63), 105 (53), 77 (36); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{28}H_{26}BrN_2O_4$: 533.1071, 535.1054, found 533.1082, 535.1083: **9o**-*trans* as a pale yellow sticky gum. $R_f$ (60% EtOAc/hexane) 0.22; IR ($cm^{-1}$): 3325, 3053, 2938, 1645, 1513; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.72–7.65 (m, 2H), 7.62 (dd, *J*=7.8, 1.5 Hz, 1H), 7.53 (d, *J*=7.8 Hz, 1H), 7.50–7.30 (m, 4H), 7.16–7.06 (m, 1H), 7.03 (s, 1H), 6.68 (d, *J*=9.5 Hz, 1H), 6.66 (s, 1H), 5.67 (d, *J*=2.1 Hz, 1H), 5.28 (dd, *J*=14.7, 9.5 Hz, 1H), 4.66 (dd, *J*=14.7, 2.1 Hz, 1H), 4.55 (dt, *J*=12.6, 4.5 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 3.55–3.35 (m, 1H), 3.05–2.70 (m, 2H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 167.9, 167.7, 149.7, 148.1, 139.7, 135.1, 133.9, 132.6, 131.4, 129.6, 128.9, 128.3 (2C), 128.2, 127.5, 127.1 (2C), 124.9, 120.9, 110.7, 106.5, 103.8, 56.1, 55.9, 54.6, 42.9, 39.6, 28.8; EIMS: *m*/*z* 534 ($M^+$+2, 2), 532 ($M^+$, 10), 453 (30), 413 (23), 411 (24), 105 (100), 77 (23); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{28}H_{26}BrN_2O_4$: 533.1071, 535.1054, found 533.1054, 535.1062.

*4.2.16. N-2-(2″-Bromo-4″,5″-dimethoxyphenyl)-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]-benzamide (**9p**).* The general procedure was used with azlactone **8h** (388 mg, 1.00 mmol) and 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline **7a** (308 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9p** *cis*/*trans* (76:24) (479 mg, 80%): **9p**-*cis* as a pale yellow sticky gum. $R_f$ (55% EtOAc/hexane) 0.21; IR ($cm^{-1}$): 3413, 3053, 2937, 1657, 1508; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.77–7.68 (m, 2H), 7.55–7.36 (m, 3H), 7.13 (s, 1H), 6.99 (s, 1H), 6.74–6.64 (m, 3H), 6.09 (d, *J*=7.4 Hz, 1H), 5.33 (t, *J*=7.4 Hz, 1H), 4.77 (t, *J*=7.4 Hz, 1H), 4.50 (dt, *J*=12.6, 4.9 Hz, 1H), 3.92 (s, 6H), 3.85 (s, 3H), 3.75 (s, 3H), 3.62–3.46 (m, 1H), 3.12–2.76 (m, 2H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 167.7, 167.3, 149.8, 148.8, 148.6, 148.3, 135.3, 134.4, 131.4, 128.4 (2C), 128.3, 127.1 (2C), 127.0, 121.0, 115.7, 115.6, 110.9, 110.7, 106.8, 103.4, 56.1 (2C), 56.0, 55.8, 53.3, 39.6, 39.2, 28.6; EIMS: *m*/*z* 594 ($M^+$+2, 12), 592 ($M^+$, 13), 511 (35), 473 (98), 471 (100), 392 (57), 363 (37), 105 (21); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{30}H_{30}BrN_2O_6$: 593.1282, 595.1267, found 593.1272, 595.1266: **9p**-*trans* as a pale yellow sticky gum. $R_f$ (55% EtOAc/hexane) 0.16; IR ($cm^{-1}$): 3344, 3053, 2937, 1650, 1603, 1508; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.69 (d, *J*=7.0 Hz, 2H), 7.50–7.28 (m, 3H), 7.08 (s, 1H), 7.03 (s, 1H), 6.99 (s, 1H), 6.79 (br d, *J*=8.8 Hz, 1H), 6.66 (s, 1H), 5.61 (d, *J*=2.7 Hz, 1H), 5.36 (dd, *J*=14.9, 8.8 Hz, 1H), 4.60 (m, 1H), 4.54 (dd, *J*=14.9, 2.7 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 6H), 3.82 (s, 3H), 3.50–3.32 (m, 1H), 3.04–2.72 (m, 2H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 168.3, 167.8, 149.7, 148.8, 148.6, 148.1, 134.8, 133.9, 131.4, 131.1, 128.3 (2C), 127.5, 127.0 (2C), 120.9, 115.1, 114.5, 111.5, 110.7, 106.6, 104.1, 56.3, 56.0 (2C), 55.9, 53.7, 42.7, 39.6, 28.8; EIMS: *m*/*z* 594 ($M^+$+2, 6), 592 ($M^+$, 5), 473 (93), 471 (100), 392 (31), 282 (37), 207 (66), 178 (36), 105 (84), 77 (20); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{30}H_{30}BrN_2O_6$: 593.1282, 595.1267, found 593.1278, 595.1260.

*4.2.17. N-2-(2″-Bromophenyl)-1-methyl-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]-benzamide (**9q**).* The general procedure was used with azlactone **8g** (327 mg, 1.00 mmol) and 6,7-dimethoxy-1-ethyl-3,4-dihydroisoquinoline **7b** (329 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9q** *cis*/*trans* (71:29) (384 mg, 70%): **9q**-*cis* as a white solid. $R_f$ (60% EtOAc/hexane) 0.34; Mp: 135–136 °C; IR ($cm^{-1}$): 3407, 3324, 3053, 2935, 1652, 1509, 1405; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.77–7.65 (m, 2H), 7.60–7.35 (m, 4H), 7.30–7.05 (m, 3H), 7.04 (s, 1H), 6.78 (s, 1H), 6.66 (d, *J*=7.3 Hz, 1H), 5.38 (t, *J*=7.3 Hz, 1H), 4.70 (d, *J*=7.3 Hz, 1H), 4.65 (m, 1H), 3.94 (s, 6H), 3.20–2.80 (m, 3H), 1.99 (s, 3H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 167.9, 165.5, 149.0, 146.9, 135.7, 134.4, 133.1, 131.3, 130.6, 129.7, 129.1, 128.5, 128.3 (2C), 128.1, 127.1 (2C), 126.9, 123.0, 117.0, 112.3, 110.1, 56.1, 55.9, 52.9, 45.5, 40.5, 28.5, 19.0; EIMS: *m*/*z* 548 ($M^+$+2, 8), 546 ($M^+$, 6), 425 (100), 391 (43), 373 (36), 346 (64), 149 (84), 105 (60); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{29}H_{28}BrN_2O_4$: 547.1227, 549.1211 found 547.1222, 549.1217: **9q**-*trans* as a pale orange solid. $R_f$ (60% EtOAc/hexane) 0.19; Mp: 148–149 °C; IR ($cm^{-1}$): 3324, 3053, 2936, 1647, 1510, 1390; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.66–7.50 (m, 4H), 7.48–7.28 (m, 4H), 7.09 (td, *J*=7.5, 1.5 Hz, 1H), 6.94 (s, 1H), 6.74 (s, 1H), 6.55 (d, *J*=9.2 Hz, 1H), 5.37 (dd, *J*=13.8, 9.2 Hz, 1H), 4.70–4.45 (m, 2H), 3.93 (s, 3H), 3.90 (s, 3H), 3.19 (ddd, *J*=11.8, 11.8, 5.2 Hz, 1H), 3.00–2.70 (m, 2H), 1.74 (d, *J*=1.6 Hz, 3H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 167.6, 167.1, 148.9, 146.7, 137.8, 134.2, 132.7, 131.5, 131.3, 130.5, 128.8, 128.3 (3C), 127.8, 127.0 (2C), 126.0, 123.2, 113.9, 112.7, 110.1, 56.3, 56.0, 53.5, 48.7, 41.0, 28.7, 20.0; EIMS: *m*/*z* 548 ($M^+$+2, 1), 546 ($M^+$, 3), 427 (100), 425 (99), 410 (45), 105 (47); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{29}H_{28}BrN_2O_4$: 547.1227, 549.1211, found 547.1234, 549.1213.

*4.2.18. N-2-(2″-Bromo-4″,5″-dimethoxyphenyl)-1-methyl-4-oxo-3,4,6,7-tetrahydro-2H-pyrido[2,1-a]isoquinolin-3-yl]-benzamide (**9r**).* The general procedure was used with azlactone **8h** (388 mg, 1.00 mmol) and 6,7-dimethoxy-1-ethyl-3,4-dihydroisoquinoline **7b** (329 mg, 1.50 mmol) to give tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9r** *cis*/*trans* (56:44) (470 mg, 77%): **9r**-*cis* as a pale yellow sticky gum. $R_f$ (55% EtOAc/hexane) 0.17; IR ($cm^{-1}$): 3413, 3320, 2934, 1655, 1507; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 7.78–7.70 (m, 2H), 7.56–7.36 (m, 3H), 7.04 (s, 1H), 7.00 (s, 1H), 6.77 (s, 1H), 6.73 (s, 1H), 6.67 (d, *J*=6.9 Hz, 1H), 5.34 (dd, *J*=7.5, 6.9 Hz, 1H), 4.72–4.60 (m, 1H), 4.58 (d, *J*=7.5 Hz, 1H), 3.94 (s, 6H), 3.85 (s, 3H), 3.80 (s, 3H), 3.26–3.08 (m, 1H), 2.98–2.84 (m, 2H), 2.02 (s, 3H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 167.9, 165.9, 149.1, 148.9, 148.6, 147.1, 134.5, 131.4, 130.2, 129.4, 128.4 (2C), 127.4, 127.2 (2C), 123.2, 117.7, 116.9, 115.6, 112.4, 110.7, 110.1, 56.2, 56.0 (2C), 55.7, 53.0, 45.5, 40.5, 28.6, 19.1; EIMS: *m*/*z* 608 ($M^+$+2, 6), 606 ($M^+$, 6), 487 (100), 485 (99), 406 (65); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{31}H_{32}BrN_2O_6$: 607.1438, 609.1423, found 607.1447, 609.1339: **9r**-*trans* as a pale yellow sticky gum. $R_f$ (55% EtOAc/hexane) 0.11; IR ($cm^{-1}$) 3333,

3053, 2936, 1651, 1508; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.68–7.60 (m, 2H), 7.50–7.33 (m, 3H), 7.04 (s, 1H), 6.98 (s, 1H), 6.93 (s, 1H), 6.74 (s, 1H), 6.59 (d, $J$=9.5 Hz, 1H), 5.42 (dd, $J$=14.0, 9.5 Hz, 1H), 4.60–4.45 (m, 2H), 3.93 (s, 6H), 3.89 (s, 3H), 3.83 (s, 3H), 3.30–3.12 (m, 1H), 3.00–2.72 (m, 2H), 1.74 (d, $J$=1.4 Hz, 3H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 167.7 (2C), 148.9, 148.6 (2C), 146.7, 134.2, 131.4, 130.8, 130.4, 129.2, 128.3 (2C), 127.0 (2C), 123.3, 116.1, 115.1, 115.0, 112.7, 112.4, 110.1, 56.4, 56.3, 56.0 (2C), 52.6, 48.2, 41.1, 28.7, 19.2; EIMS: $m/z$ 608 ($M^+$+2, 5), 606 ($M^+$, 5), 487 (72), 485 (100), 472 (49), 420 (35), 105 (35); HRMS (microTOF) $m/z$ [M+H]$^+$ calcd for $C_{31}H_{32}BrN_2O_6$: 607.1438, 609.1423, found 607.1459, 609.1446.

### 4.3. General procedure: synthesis of benzoquinolizinones (12)

*Condition B*: A suspension of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** (0.20 mmol), 5 mol % $PdCl_2$ (1.70 mg, 0.010 mmol), 10 mol % Xantphos (11.6 mg, 0.020 mmol), 2 equiv $Cs_2CO_3$ (130 mg, 0.40 mmol), and 1.25 M 1,4-dioxane (1.6 mL) in vessel was stirred under reflux for 3 day. The suspension was filtered through Celite and washed with dichloromethane (3×5 mL). The solvent was evaporated to dryness in vacuo. The crude product was purified by PTLC on silica using 90:10:1 $CH_2Cl_2$/hexane/MeOH as an eluent to give benzoquinolizin-4-ones **12** in moderate to good yield.

*Condition C*: A suspension of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** (0.10 mmol), 10 mol % $Pd(dba)_2$ (5.80 mg, 0.010 mmol), 15 mol % Xantphos (8.70 mg, 0.015 mmol), 1.4 equiv $Cs_2CO_3$ (45.6 mg, 0.14 mmol), and 1.25 M 1,4-dioxane (0.8 mL) in vessel was stirred at 100 °C for 24–56 h. The suspension was filtered through Celite and washed with dichloromethane (3×5 mL). The solvent was evaporated to dryness in vacuo. The crude product was purified by PTLC on silica using 90:10:1 $CH_2Cl_2$/hexane/MeOH as an eluent to give benzoquinolizin-4-ones **12** in moderate yield.

*Condition D*: A suspension of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** (0.10 mmol), 2 equiv $Cs_2CO_3$ (65.0 mg, 0.20 mmol), and 1.25 M 1,4-dioxane (0.8 mL) in vessel was stirred at 100 °C for 16–96 h. The suspension was filtered through Celite and washed with dichloromethane (3×5 mL). The solvent was evaporated to dryness in vacuo. The crude product was purified by PTLC on silica using 90:10:1 $CH_2Cl_2$/hexane/MeOH as an eluent to give benzoquinolizine-4-ones **12** in moderate to good yield.

*4.3.1. Benzoindoloquinolizinones (**12a**).* Using the general procedure (Condition B) with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9a**) (90.8 mg, 0.20 mmol), 5 mol % $PdCl_2$ (1.70 mg, 0.010 mmol), 10 mol % Xantphos (11.6 mg, 0.020 mmol), 2 equiv $Cs_2CO_3$ (130 mg, 0.40 mmol), and 1.25 M 1,4-dioxane (1.6 mL) gave benzoquinolizine-4-ones **12a** (45.6 mg, 68%) as a pale yellow solid. $R_f$ (90:10:1 $CH_2Cl_2$/hexane/MeOH; developed twice) 0.31; Mp: 182–183 °C; IR (cm$^{-1}$): 2936, 2847, 1652, 1564, 1506; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.68–7.60 (m, 2H), 7.53–7.41 (m, 3H), 7.23 (s, 1H), 6.80 (d, $J$=1.8 Hz, 1H), 6.77 (s, 1H), 6.74 (d, $J$=1.8 Hz, 1H), 4.30 (t, $J$=6.4 Hz, 2H), 3.96 (s, 3H), 3.95 (s, 3H), 2.95 (t, $J$=6.4 Hz, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 162.7, 151.1, 151.0, 148.4, 143.2, 138.2, 129.2, 129.1, 128.9 (2C), 126.7 (2C), 121.5, 114.3, 110.4, 108.1, 101.6, 56.2, 56.0, 39.2, 27.5; EIMS: $m/z$ 333 ($M^+$, 100), 318 (54), 166 (30), 152 (24), 105 (60), 77 (32); HRMS (microTOF) $m/z$ [M+H]$^+$ calcd for $C_{21}H_{20}NO_3$: 334.1438, found 334.1434.

*4.3.2. Benzoindoloquinolizinones (**12b** and **13**).* Using the general procedure (Condition B) with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9o**) (106 mg, 0.20 mmol) gave a mixture of benzoquinolizine-4-ones **12b** (26.2 mg, 32%) and **13** (18.9 mg, 21%). Employing the general procedure (Condition B) with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines (*trans*-**9o**) (53.2 mg, 0.10 mmol), 5 mol % $PdCl_2$ (0.85 mg, 0.005 mmol), 10 mol % Xantphos (5.80 mg, 0.010 mmol), 2 equiv $Cs_2CO_3$ (65.0 mg, 0.20 mmol), and 1.25 M 1,4-dioxane (0.8 mL) gave benzoquinolizine-4-ones **12b** (20.7 mg, 50%) as a pale yellow solid. $R_f$ (90:10:1 $CH_2Cl_2$/hexane/MeOH; developed twice) 0.27; Mp: 193–194 °C; IR (cm$^{-1}$): 3427, 3053, 2933, 1647, 1588, 1505; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.69 (dd, $J$=7.6, 1.2 Hz, 1H), 7.46–7.22 (m, 3H), 7.16 (s, 1H), 6.76 (s, 1H), 6.64 (d, $J$=1.8 Hz, 1H), 6.54 (d, $J$=1.8 Hz, 1H), 4.32 (t, $J$=6.5 Hz, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 2.97 (t, $J$=6.5 Hz, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 162.4, 151.5, 151.0, 148.5, 142.5, 140.1, 133.4, 130.2, 129.9, 129.0, 127.6, 121.5 (2C), 117.3, 110.4, 108.1, 103.9, 56.2, 56.1, 39.3, 27.6; EIMS: $m/z$ 413 ($M^+$+2, 62), 411 ($M^+$, 64), 396 (43), 191 (33), 149 (63), 114 (72), 104 (75), 95 (88), 83 (75), 71 (86), 57 (100), 55 (91); HRMS (microTOF) $m/z$ [M+H]$^+$ calcd for $C_{21}H_{19}BrNO_3$: 412.0543, 414.0525, found 412.0541, 414.0536: **13** as a pale yellow solid. $R_f$ (90:10:1 $CH_2Cl_2$/hexane/MeOH; developed twice) 0.16; Mp: 261–263 °C; IR (cm$^{-1}$): 3236, 3053, 2938, 1646, 1567, 1506; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.48 (br s, 1H), 8.31 (d, $J$=8.1 Hz, 1H), 7.86–7.83 (m, 2H), 7.51–7.46 (m, 2H), 7.42–7.34 (m, 3H), 7.30–7.25 (m, 1H), 6.98 (s, 1H), 6.71 (s, 1H), 6.67 (d, $J$=1.4 Hz, 1H), 6.54 (d, $J$=1.4 Hz, 1H), 4.22 (t, $J$=6.4 Hz, 2H), 3.92 (s, 3H), 3.64 (s, 3H), 2.90 (t, $J$=6.4 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 165.6, 162.4, 151.2, 149.6, 148.5, 143.7, 134.4, 134.3, 132.0, 131.1, 129.7, 129.1, 128.9, 128.8 (2C), 127.1 (2C), 125.3, 123.6, 120.9, 116.6, 110.3, 107.7, 103.1, 56.0, 55.9, 39.2, 27.4; EIMS: $m/z$ 452 ($M^+$, 9), 347 (33), 105 (100), 77 (64); HRMS (microTOF) $m/z$ [M+H]$^+$ calcd for $C_{28}H_{25}N_2O_4$: 453.1809, found 453.1818.

*4.3.3. Benzoindoloquinolizinone (**12c**).* Using the general procedure (Condition B) with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9p**) (119 mg, 0.20 mmol) gave benzoquinolizine-4-ones **12c** (75.5 mg, 81%). Employing the general procedure (Condition C) with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9p**) (59.3 mg, 0.10 mmol) gave benzoquinolizine-4-ones **12c** (17.5 mg, 37%) as a pale yellow solid. $R_f$ (90:10:1 $CH_2Cl_2$/hexane/MeOH; developed twice) 0.21; Mp: 104–106 °C; IR (cm$^{-1}$): 2933, 2893, 1720, 1651, 1587, 1502; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.18 (s, 1H), 7.14 (s, 1H), 6.87 (s, 1H), 6.77 (s, 1H), 6.68 (d, $J$=1.7 Hz, 1H), 6.54 (d, $J$=1.7 Hz, 1H), 4.32 (t, $J$=6.4 Hz, 2H), 3.95 (s, 3H), 3.93 (s, 6H), 3.89 (s, 3H), 2.97 (t, $J$=6.4 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 162.4, 151.5, 151.0, 149.6, 148.5, 148.4, 142.4, 132.0, 129.0, 121.5, 117.3, 115.9, 112.8, 111.5, 110.4, 108.1, 104.1, 56.3, 56.2, 56.1, 56.0, 39.2, 27.5; EIMS: $m/z$ 473 ($M^+$+2, 98), 471 ($M^+$, 100), 458 (31), 456 (39); HRMS (microTOF) $m/z$ [M+H]$^+$ calcd for $C_{23}H_{23}BrNO_5$: 472.0754, 474.0737, found 472.0767, 474.0750.

*4.3.4. Benzoindoloquinolizinone (**12d**).* Using the general procedure (Condition B) with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9q**) (109 mg, 0.20 mmol) gave benzoquinolizine-4-ones **12d** (61.4 mg, 72%): **12d** as a pale yellow sticky gum. $R_f$ (90:10:1 $CH_2Cl_2$/hexane/MeOH; developed twice) 0.22; IR (cm$^{-1}$): 3047, 2932, 1650, 1583, 1494; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 7.66 (d, $J$=8.0 Hz, 1H), 7.46–7.20 (m, 3H), 7.17 (s, 1H), 6.82 (s, 1H), 6.43 (s, 1H), 4.55–4.40 (m, 1H), 4.00 (m, 1H), 3.96 (s, 3H), 3.89 (s, 3H), 2.89 (t, $J$=6.0 Hz, 2H), 2.11 (s, 3H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 161.2, 154.5, 149.9, 146.6, 141.0, 140.5, 132.6, 132.2, 129.6, 129.4, 127.4, 122.1, 122.0, 117.9, 113.3, 111.3, 110.0, 56.2, 55.9, 40.8, 28.6, 18.4; EIMS: $m/z$ 428 ($M^+$+2, 2), 427 ($M^+$, 9), 412 (10), 410 (11), 149 (33), 111 (34), 109(52), 97 (55), 95 (65), 83 (82), 69 (99), 55 (100); HRMS (microTOF) $m/z$ [M+H]$^+$ calcd for $C_{22}H_{21}BrNO_3$: 426.0699, 428.0682, found 426.0696, 428.0676.

*4.3.5. Benzoindoloquinolizinone (**12e**).* Using the general procedure (Condition B) with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines (*cis*-**9r**) (121 mg, 0.20 mmol) gave benzoquinolizine-4-ones **12e** (57.6 mg, 52%) and recovered starting material *cis*-**9r** (30.1 mg, 25%): **12e** as a pale yellow sticky gum. $R_f$ (90:10:1 $CH_2Cl_2$/hexane/MeOH; developed twice) 0.19; IR (cm$^{-1}$): 3053, 2936, 1647, 1583, 1495; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.18 (s, 1H), 7.11 (s, 1H), 6.81 (s, 1H), 6.73 (s, 1H), 6.44 (s, 1H), 4.60–4.44 (m, 1H), 4.00 (m, 1H), 3.96

(s, 3H), 3.93 (s, 3H), 3.89 (s, 3H), 3.87 (s, 3H), 2.88 (t, $J$=6.0 Hz, 2H), 2.14 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 161.4, 154.6, 149.9, 149.2, 148.4, 146.7, 141.1, 132.6, 132.2, 122.3, 118.3, 115.3, 113.4, 112.2, 112.1, 111.8, 110.0, 56.2 (2C), 56.1, 56.0, 40.8, 28.7, 18.4; EIMS: $m/z$ 487 ($M^+$+2, 97), 485 ($M^+$, 100), 472 (83), 188 (66); HRMS (microTOF) $m/z$ $[M+H]^+$ calcd for $C_{24}H_{25}BrNO_5$: 486.0911, 488.0894, found 486.0915, 488.0894.

### 4.4. General procedure: synthesis of benzoquinolizin-4-ones (11)

A solution of tetrahydro-2*H*-pyrido[2,1-*a*]isoquinolines **9** (1.00 mmol) and DDQ (1.00 mmol) in dichlorometane (20 mL) was stirred at room temperature for 0.5–16 h. The solvent was evaporated to dryness in vacuo. The crude product was purified by PTLC on silica using 4% MeOH in $CH_2Cl_2$ as an eluent to give benzoquinolizin-4-ones **11** in moderate yield.

*4.4.1. Benzoquinolizin-4-one (**11a**).* The general procedure was used with tetrahydro-2H-pyrido[2,1-a]isoquinoline **9a** (45.4 mg, 0.10 mmol) and DDQ (22.7 mg, 0.10 mmol) to give benzoquinolizinone **11a** (25.5 mg, 56%) as a pale yellow oil. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.53; IR ($cm^{-1}$): 3253, 3053, 2936, 1636, 1590, 1508, 1476; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 8.27 (s, 1H), 7.82–7.72 (m, 2H), 7.64–7.54 (m, 2H), 7.52–7.28 (m, 6H), 7.17 (s, 1H), 6.76 (s, 1H), 6.71 (s, 1H), 4.33 (t, $J$=6.3 Hz, 2H), 3.94 (s, 3H), 3.93 (s, 3H), 2.96 (t, $J$=6.3 Hz, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 165.6, 159.6, 150.8, 148.6, 143.6, 139.2, 139.0, 134.4, 131.6, 128.5 (2C), 128.4 (2C), 128.2 (2C), 127.4 (2C), 127.0 (2C), 121.7, 121.3, 110.4, 107.8, 104.3, 56.3, 56.0, 40.3, 27.5; EIMS: $m/z$ 453 ($M^+$+1, 29), 452 ($M^+$, 100), 347 (89), 331 (35); HRMS (microTOF) $m/z$ $[M+H]^+$ calcd for $C_{28}H_{25}N_2O_4$: 453.1809, found 453.1821.

*4.4.2. Benzoquinolizin-4-one (**11b**).* The general procedure was used with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9b** (207 mg, 0.40 mmol) and DDQ (110 mg, 0.44 mmol) to give benzoquinolizinone **11b** (137 mg, 67%): as a pale yellow sticky gum. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.49; IR ($cm^{-1}$): 3248, 3000, 2936, 2836, 1635, 1592, 1507; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.22 (s, 1H), 7.81–7.78 (m, 2H), 7.51–7.46 (m, 1H), 7.42–7.37 (m, 2H), 7.21–7.14 (m, 3H), 6.93 (d, $J$=8.3 Hz, 1H), 6.76 (s, 1H), 6.71 (s, 1H), 4.32 (t, $J$=6.4 Hz, 2H), 3.95 (s, 3H), 3.93 (s, 3H), 3.87 (s, 3H), 3.78 (s, 3H), 2.95 (t, $J$=6.4 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 165.9, 159.7, 150.9, 149.0, 148.7, 148.6, 143.5, 139.3, 134.4, 131.8, 131.4, 128.5 (2C), 128.4, 127.4 (2C), 121.4, 121.3, 119.8, 111.2, 110.5, 110.2, 107.8, 104.3, 56.3, 56.0, 55.8, 55.7, 40.2, 27.5; EIMS: $m/z$ 513 ($M^+$+1, 33), 512 ($M^+$, 100), 407 (82), 105 (55), 77 (36); HRMS (microTOF) $m/z$ $[M+H]^+$ calcd for $C_{30}H_{29}N_2O_6$: 513.2020, found 513.2017.

*4.4.3. Benzoquinolizin-4-one (**11c**).* The general procedure was used with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9**[9] (46.8 mg, 0.10 mmol) and DDQ (22.7 mg, 0.10 mmol) to give benzoquinolizinone **11c** (7.10 mg, 15%) as a pale yellow oil. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.43; IR ($cm^{-1}$): 2920, 1631, 1580, 1501; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.73 (s, 1H), 7.63–7.60 (m, 2H), 7.45–7.25 (m, 8H), 7.17 (s, 1H), 6.80 (s, 1H), 4.26 (br t, $J$=5.8 Hz, 2H), 3.96 (s, 3H), 3.89 (s, 3H), 2.90 (t, $J$=5.8 Hz, 2H), 2.15 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 166.1, 158.7, 149.9, 149.2, 146.9, 138.8, 137.4, 134.6, 131.7, 131.4, 128.4 (2C), 128.3 (2C), 128.1 (2C), 127.7, 127.3 (2C), 122.8, 122.3, 113.6, 111.8, 110.1, 56.2, 56.0, 41.4, 28.5, 20.0; EIMS: $m/z$ 467 ($M^+$+1, 27), 466 ($M^+$, 100), 361 (89), 346 (38), 345 (42), 105 (100), 77 (64); HRMS (microTOF) $m/z$ $[M+H]^+$ calcd for $C_{29}H_{27}N_2O_4$: 467.1965, found 467.1963.

*4.4.4. Benzoquinolizin-4-one (**11d**).* The general procedure was used with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9d** (128 mg, 0.242 mmol) and DDQ (55.0 mg, 0.242 mmol) to give benzoquinolizinone **11d** (10.0 mg, 8%) as a pale yellow oil. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.26; IR ($cm^{-1}$): 3233, 2919, 2840, 1630, 1584, 1501; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.68–7.64 (m, 3H), 7.47–7.42 (m, 1H), 7.38–7.32 (m, 2H), 7.18 (s, 1H), 6.96–6.89 (m, 3H), 6.80 (s, 1H), 4.42 (s, 1H), 4.17–4.05 (m, 1H), 3.96 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 2.90 (t, $J$=5.7 Hz, 2H), 2.18 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 166.4, 158.7, 149.9, 149.3, 148.6, 148.4, 146.8, 139.0, 134.6, 131.8, 131.5, 129.7, 128.4 (2C), 127.4 (2C), 123.0, 122.3, 121.1, 113.5, 112.1, 111.8, 110.9, 110.1, 56.2, 56.0 (2C), 55.7, 41.4, 28.6, 20.1; EIMS: $m/z$ 527 ($M^+$+1, 23), 526 ($M^+$, 92), 421 (63), 105 (100), 77 (51); HRMS (microTOF) $m/z$ $[M+H]^+$ calcd for $C_{31}H_{31}N_2O_6$: 527.2177, found 527.2172.

*4.4.5. Benzoquinolizin-4-one (**11e**).* The general procedure was used with tetrahydro-2*H*-pyrido[2,1-a]isoquinoline **9o** (48.7 mg, 0.092 mmol) and DDQ (21.0 mg, 0.092 mmol) to give benzoquinolizinone **11e** (27.8 mg, 57%) as a pale yellow sticky gum. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.56; IR ($cm^{-1}$): 3233, 3056, 1637, 1595, 1509, 1477; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.30 (s, 1H), 7.75–7.71 (m, 2H), 7.62 (dd, $J$=8.1, 1.0 Hz, 1H), 7.49 (dd, $J$=7.7, 1.6 Hz, 1H), 7.46–7.41 (m, 1H), 7.38–7.32 (m, 3H), 7.18–7.12 (m, 2H), 6.75 (s, 1H), 6.63 (s, 1H), 4.47–4.39 (m, 1H), 4.29–4.20 (m, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 2.97 (t, $J$=6.4 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 165.5, 159.3, 150.7, 148.4, 142.7, 139.4, 139.0, 134.3, 132.8, 131.5, 130.0, 129.3, 128.3 (2C), 128.2, 127.4 (3C), 122.7, 121.2, 121.1, 110.3, 107.6, 104.3, 56.1, 55.9, 40.1, 27.3; EIMS: $m/z$ 532 ($M^+$+2, 61), 530 ($M^+$, 61), 451 (100), 346 (46); HRMS (microTOF) $m/z$ $[M+H]^+$ calcd for $C_{28}H_{24}BrN_2O_4$: 531.0914, 533.0898, found 531.0926, 533.0905.

*4.4.6. Benzoquinolizin-4-one (**11f**).* The general procedure was used with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9p** (119 mg, 0.20 mmol) and DDQ (46.0 mg, 0.20 mmol) to give benzoquinolizinone **11f** (62.2 mg, 53%) as a pale yellow solid. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.52; Mp: 137–139 °C; IR ($cm^{-1}$): 3233, 3053, 2935, 1638, 1591, 1505; $^1$H NMR (200 MHz, $CDCl_3$) $\delta$ 8.18 (s, 1H), 7.82–7.72 (m, 2H), 7.52–7.32 (m, 3H), 7.17 (s, 1H), 7.10 (s, 1H), 6.98 (s, 1H), 6.76 (s, 1H), 6.66 (s, 1H), 4.56–4.36 (m, 1H), 4.32–4.12 (m, 1H), 3.94 (s, 3H), 3.93 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H), 2.97 (t, $J$=6.4 Hz, 2H); $^{13}$C NMR (50 MHz, $CDCl_3$) $\delta$ 165.7, 159.4, 150.8, 149.1, 148.5, 148.2, 143.1, 139.0, 134.4, 131.6, 131.2, 128.3 (2C), 128.2, 127.4 (2C), 122.7, 121.2, 115.4, 112.4, 111.5, 110.4, 107.8, 104.7, 56.2, 56.1, 56.0 (2C), 40.2, 27.4; EIMS: $m/z$ 592 ($M^+$+2, 5), 590 ($M^+$, 5), 511 (100), 406 (52); HRMS (microTOF) $m/z$ $[M+H]^+$ calcd for $C_{30}H_{28}BrN_2O_6$: 591.1125, 593.1110, found 591.1130, 593.1121.

### 4.5. General procedure: synthesis of benzoindoloquinolizinone derivatives (10 and 14)

A solution of benzoquinolizin-4-ones **9** or **11** (0.10 mmol) and CuTC (0.11 mmol) in DMF (1 mL) was irradiated in a microwave reactor (250 W, 100 psi) at 150 °C for 15–30 min. The crude product was purified by PTLC on silica using 4% MeOH in $CH_2Cl_2$ as an eluent to give a mixture of benzoindoloquinolizin-4-ones **10** and/or *N*-benzoyl benzoindoloquinolizin-4-ones **14** in moderate yield.

*4.5.1. Benzoindoloquinolizinone (**10a**).* The general procedure was used with benzoquinolizin-4-one **11e** (48.3 mg, 0.090 mmol) and CuTC (19.0 mg, 0.099 mmol) to give benzoindoloquinolizinone **10a** (11.9 mg, 38%): as a pale yellow solid. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.52; Mp: >300 °C; IR ($cm^{-1}$): 3159, 1635, 1557, 1510, 1264; $^1$H NMR (400 MHz, $CDCl_3$+$CD_3OD$(2 drops)) $\delta$ 8.08 (d, $J$=8.2 Hz, 1H), 7.60 (d, $J$=8.2 Hz, 1H), 7.53–7.47 (m, 1H), 7.46 (s, 1H), 7.37 (s, 1H), 7.30–7.25 (m, 1H), 6.81 (s, 1H), 4.46 (t, $J$=6.1 Hz, 2H), 4.04 (s, 3H), 3.96 (s, 3H), 3.00 (t, $J$=6.1 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$+$CD_3OD$(2 drops))

$\delta$ 155.0, 149.5, 148.3, 139.7, 134.1, 127.5, 127.0, 126.0, 124.9, 122.9, 122.0, 121.0, 119.8, 112.2, 110.3, 107.7, 97.7, 56.0, 55.7, 39.8, 27.8; EIMS: *m*/*z* 347 ($M^+$+1, 30), 346 ($M^+$, 100); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{21}H_{19}N_2O_3$: 347.1390, found 347.1388.

*4.5.2. Benzoindoloquinolizinone (**10b**).* The general procedure was used with benzoquinolizin-4-one **11f** (39.5 mg, 0.067 mmol) and CuTC (14.0 mg, 0.74 mmol) to give benzoindoloquinolizinone **10b** (16.9 mg, 62%) as a pale brown solid. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.20; Mp: >300 °C; IR ($cm^{-1}$): 3173, 2927, 1635, 1586, 1557, 1512; $^1H$ NMR (400 MHz, $CDCl_3$+$CD_3OD$(2 drops)) $\delta$ 7.43 (s, 1H), 7.36 (s, 1H), 7.34 (s, 1H), 7.07 (s, 1H), 6.79 (s, 1H), 4.44 (t, *J*=6.0 Hz, 2H), 4.04 (s, 3H), 4.02 (s, 3H), 4.00 (s, 3H), 3.95 (s, 3H), 2.98 (t, *J*=6.0 Hz, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$+$CD_3OD$(2 drops)) $\delta$ 154.8, 150.9, 149.6, 148.3, 145.2, 135.2, 133.9, 127.6, 125.3, 125.2, 123.2, 114.4, 110.4, 107.8, 101.9, 97.5, 94.6, 56.2, 56.18, 55.9 (2C), 39.8, 28.0; EIMS: *m*/*z* 406 ($M^+$, 35), 203 (13), 149 (35), 104 (39), 97 (59), 85 (71), 71 (82), 57 (100); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{23}H_{23}N_2O_5$: 407.1602, found 407.1597.

*4.5.3. Benzoindoloquinolizinone (**10c** and **14a**).* The general procedure was used with tetrahydro-2*H*-pyrido[2,1-*a*]isoquinoline **9q** (50.4 mg, 0.092 mmol) and CuTC (19.0 mg, 0.101 mmol) to give a mixture of benzoindoloquinolizinone **10c** (6.50 mg, 20%) and benzoindoloquinolizinone **14a** (17.9 mg, 42%): **10c** as a pale yellow solid. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.24; Mp: 279–280 °C; IR ($cm^{-1}$): 3138, 2951, 1635, 1578, 1543, 1510; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 10.88 (s, 1H), 8.19 (d, *J*=8.1 Hz, 1H), 7.65 (d, *J*=8.1 Hz, 1H), 7.46 (dd, *J*=8.1, 8.1 Hz, 1H), 7.27–7.22 (m, 1H), 7.17 (s, 1H), 6.83 (s, 1H), 4.45 (br s, 2H), 3.97 (s, 3H), 3.96 (s, 3H), 2.97 (s, 3H), 2.96–2.88 (m, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 154.4, 148.8, 146.7, 139.8, 131.4, 131.3 (2C), 126.4, 126.3, 125.4, 123.4, 123.2, 120.0, 113.6, 112.7, 110.6, 110.2, 56.3, 56.0, 41.5, 29.3, 18.6. EIMS: *m*/*z* 361 ($M^+$+1, 25), 360 ($M^+$, 100), 345 (59). HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{22}H_{21}N_2O_3$: 361.1547, found 361.1552. **14a**: as a white solid. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.48; Mp: 205–207 °C; IR ($cm^{-1}$): 3053, 2934, 1667, 1511, 1472, 1385; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 8.06 (br s, 1H), 7.84–7.72 (m, 2H), 7.48–7.18 (m, 5H), 7.12–7.02 (m, 1H), 6.96 (s, 1H), 6.68 (s, 1H), 4.97 (d, *J*=8.5 Hz, 1H), 4.31(dt, *J*=11.7, 4.5 Hz, 1H), 4.19 (d, *J*=8.5 Hz, 1H), 3.90 (s, 3H), 3.88 (s, 3H), 2.93 (ddd, *J*=11.7, 11.3, 4.5 Hz, 1H), 2.70–2.55 (m, 2H), 2.38 (s, 3H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 170.4, 164.9, 148.9, 146.6, 142.1, 137.0, 132.2, 131.1, 130.0, 129.6, 128.4, 128.3 (2C), 127.7 (3C) 124.6, 122.5, 118.2, 112.4, 110.1, 108.5, 62.8, 56.2, 55.9, 44.7, 41.1, 28.8, 21.4; EIMS: *m*/*z* 467 ($M^+$+1, 2), 466 ($M^+$, 7), 361 (28), 105 (100), 77 (58); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{29}H_{27}N_2O_4$: 467.1965, found 467.1970.

*4.5.4. Benzoindoloquinolizinone (**10d** and **14b**).* The general procedure was used with tetrahydro-2*H*-pyrido[2,1-a]isoquinoline **9r** (52.9 mg, 0.087 mmol) and CuTC (18.0 mg, 0.096 mmol) to give a mixture of benzoindoloquinolizinone **10d** (5.80 mg, 16%) and benzoindoloquinolizinone **14b** (26.3 mg, 57%): **10d** as a pale yellow solid. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.18; Mp: >300 °C; IR ($cm^{-1}$): 3173, 2931, 1632, 1582, 1509; $^1H$ NMR (200 MHz, $CDCl_3$) $\delta$ 11.32 (br s, 1H), 7.55 (s, 1H), 7.18 (s, 1H), 7.15 (s, 1H), 6.84 (s, 1H), 4.47 (br s, 2H), 4.00 (s, 3H), 3.97 (s, 6H), 3.96 (s, 3H), 2.93 (br s, 5H); $^{13}C$ NMR (50 MHz, $CDCl_3$) $\delta$ 154.0, 150.3, 148.7, 146.7, 145.0, 135.6, 131.1, 130.9, 125.7, 125.5, 123.3, 115.4, 113.5, 110.1 (2C), 104.3, 94.8, 56.5, 56.2, 56.0, 55.8, 41.3, 29.4, 18.6; EIMS: *m*/*z* 421 ($M^+$+1, 27), 420 ($M^+$, 100), 405 (57), 361 (17), 210 (32), 105 (46); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{24}H_{25}N_2O_5$: 421.1758, found 421.1755: **14b** as a pale yellow oil. $R_f$ (4% MeOH/$CH_2Cl_2$) 0.42; IR ($cm^{-1}$): 2935, 2833, 1662, 1495, 1444, 1395; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 8.01 (br s, 1H), 7.83–7.73 (m, 2H), 7.47–7.36 (m, 3H), 6.97 (s, 1H), 6.72 (s, 1H), 6.70 (s, 1H), 4.92 (br s, 1H), 4.41–4.31 (m, 1H), 4.16 (d, *J*=8.1 Hz, 1H), 3.90 (s, 9H), 3.82 (s, 3H), 2.99–2.84 (m, 1H), 2.72–2.69 (m, 2H), 2.37 (s, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 170.1, 165.1, 148.9, 148.8, 146.6, 146.4, 137.1, 136.2, 131.0, 129.9, 129.6, 128.2 (2C), 127.7 (2C), 122.8, 122.6, 112.3, 110.1, 108.3, 106.2, 103.0, 63.2, 56.4, 56.2, 56.0, 55.9, 44.5, 41.0, 28.7, 21.4; EIMS: *m*/*z* 527 ($M^+$+1, 7), 526 ($M^+$, 29), 421 (50), 282 (26), 105 (100), 77 (41); HRMS (microTOF) *m*/*z* $[M+H]^+$ calcd for $C_{31}H_{31}N_2O_6$: 527.2177, found 527.2176.

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## Supplementary data

Experimental procedures and spectral data for all new compounds. Supplementary data related to this article can be found online at doi:10.1016/j.tet.2012.01.094.

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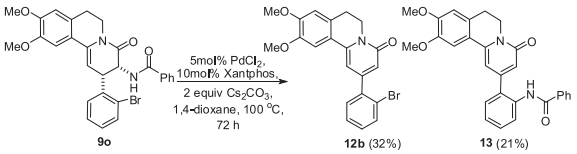
15. The reaction of *cis*-**9o** gave a mixture of **12b** (32% yield) and **13** (21% yield). For the details of this reaction see Supplementary data.




**9o** **12b** (32%) **13** (21%)

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# Palladium-catalyzed intramolecular C–H amidation: synthesis and biological activities of indolobenzazocin-8-ones

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ABSTRACT

The synthesis of multi ring-fused indolobenzazocinone derivatives, an antimitotic agent, has been carried out using palladium-catalyzed C–H activation/intramolecular amidation of benzo[*d*]azocinones, which were synthesized by the ring annulations of dihydroisoquinolines and azlactone in refluxing acetonitrile. The target compounds, indolobenzazocin-8-one derivatives, were evaluated for their cytotoxicity against the cancer cell lines HUCCA-1, A549, HepG2, and MOLT-3. The results showed that an unsubstituted indolobenzazocin-8-one **1e** exhibited very good activities in the nanomolar $IC_{50}$ value range (HepG2 and MOLT-3).



## 1. Introduction

Benzazocinone belongs to a class of alkaloid displaying a wide range of biological activities including hepatoprotective activity against chemical toxin and antiamnesia as well as acting as inhibitors of tubulin polymerization.[1] Recently our group reported a facile and convenient protocol based on ring annulations of dihydroisoquinoline with azlactone to synthesize benzo[*d*]azocin-4-ones **1**.[2] This protocol served as a useful template for the synthesis of eight-membered ring molecules. In our continuing interest concerning the application of this protocol, we focused our attention to the synthesis of indolobenzazocinone analogues from benzo[*d*]azocin-4-one derivatives. Indolobenzazocinone **2c** is closely related to some natural products and other biologically active compounds, such as indolobenzazepinones (with C5 substituted group) **3**, an antimitotic agent,[3] latonduine **4**, a cytotoxic agent,[4] and their regioisomers, paullones **5**, which act as cyclin-dependent kinase inhibitors (Fig. 1).[5]

In 2007, Joseph reported the synthesis of indolobenzazepinones **2b** using the intramolecular Heck reaction.[6] Similarly, Dodd revealed that the C5-alkylated indolobenzazepinones (**3a**) showed high cytotoxicity against various cancer cell lines and were categorized as antimitotic agents.[7] This methodology involved Suzuki coupling and lactamization steps. They also synthesized compounds with different substitution pattern **3b** at C5 position by using the application of an isocyanide-based multicomponent reactions (MCRs).[3]

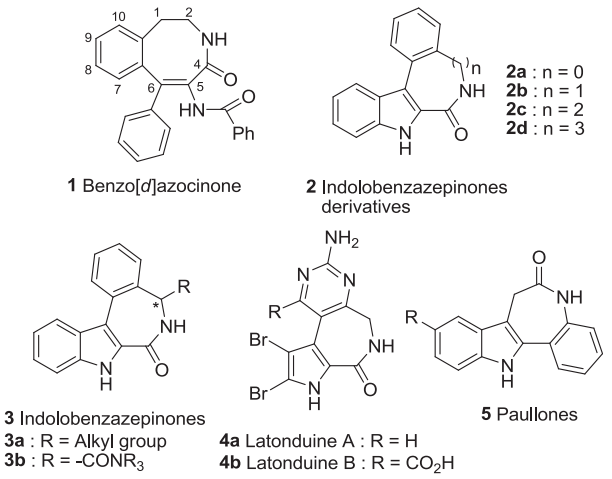


**Fig. 1.** Indolobenzazepinone derivatives and some related natural compounds.

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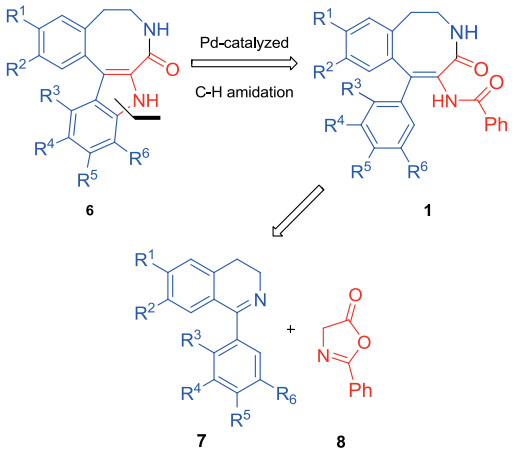
Recently, Joseph and colleagues reported the synthesis and SAR studies involving six-, seven-, eight- and nine-membered ring derivatives of indolobenzazepinones. The result showed that almost all of them exhibited good potency in both cell-based and target-based assays, especially, 5,6,7,9-tetrahydro-8*H*-indolo[2,3-*e*][3]benzazocin-8-one **2c**.[8] Moreover, the molecular modeling of indolobenzazepinones was also studied in comparison with tubulin polymerization inhibitor colchicine.[8] To accomplish these target indolobenzazocinones **6**, we have elaborated not only the condensation of various dihydroisoquinolines with azlactone but also investigated the C–N bond formation involving palladium-catalyzed intramolecular C–H amidation as depicted in Scheme 1.




**Scheme 1.** Retrosynthetic plan for the synthesis of indolobenzazocin-8-one derivatives.

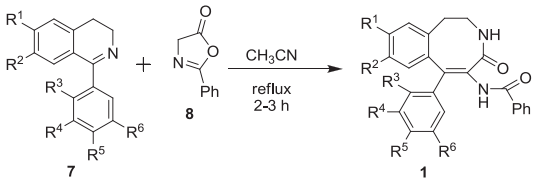
During the past decade, Pd-catalyzed C–H activation[9] has played a significant role in construction of the desired C–C, C–O, C–S, C–X bond formation and particularly the formation of C–N bond, as pioneered by Buchwald and co-workers.[10] The advantage of this approach is the support of green chemistry because reducing the number of steps as well as improving atom economy led to an increase in overall efficiency.[11] Accordingly, Pd-catalyzed C–N bond formation has attracted attention from many research groups.[9,10,12] Herein, this chemistry was used as the first time application to establish biologically active indolobenzazocinone derivatives.

## 2. Results and discussion

Ten analogues of dihydroisoquinolines **7a–j** were first investigated to expand our developed protocol.[2,13] Various dihydroisoquinolines **7** were prepared from different arylethylamines and various benzoic acids and nicotinic acid to generate amides followed by Bischler–Napieralski reaction.[2] Only dihydroisoquinoline **7e** was prepared using the Movassaghi's method.[14] Azlactone **8** was obtained from hippuric acid, as previously reported.[2,15] With both key starting materials in hand, we studied the ring annulations of dihydroisoquinolines **7a–j** with azlactone **8** in refluxing acetonitrile under dry conditions to afford compounds **1a–i** in moderate yields (30–74%) as shown in Table 1.

Notably, beta-lactam intermediate **9j** was isolated in 45% yield (Table 1, entry 10).[16] This supported our previously proposed mechanism that beta-lactams are intermediates in the formation of benzo[*d*]azocinones.[2] Furthermore, the isolation of beta-lactam **9j** shed some lights on the mechanism of ring expansion. Possible pathway might involve ring expansion via the carbocation **10** and the carbocation must be stabilized by both adjacent phenyl groups. In the case of beta-lactam **9j** ring expansion was not possible

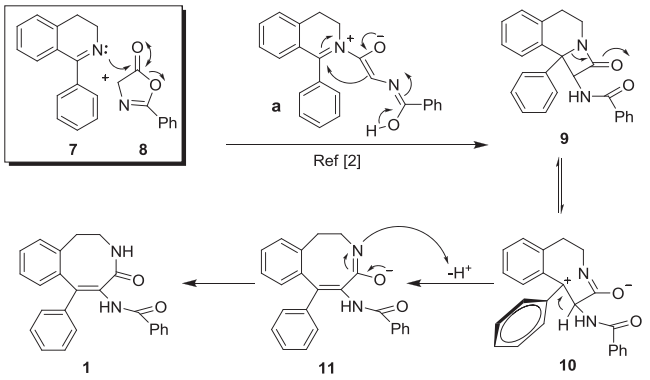
**Table 1**
Synthesis of 5-amido-8,9-dimethoxy-6-aryl-2,3-dihydrobenzo[*d*]azocin-4-ones **1**[a]




| Entry | Dihydroisoquinolines **7** | Products **1** | Yield [%] |
|---|---|---|---|
| 1 | **7a** | | **1a**, 74[a] |
| 2 | **7b** | | **1b**, 39[a] |
| 3 | **7c** | | **1c**, 40[a] |
| 4 | **7d** | | **1d**, 48[a] |
| 5 | **7e** | | **1e**, 37[a] |
| 6 | **7f** | | **1f**, 40[b] |
| 7 | **7g** | | **1g**, 37[b] |
| 8 | **7h** | | **1h**, 30[a] |
| 9 | **7i** | | **1i**, 58[a] |
| 10 | **7j** | | **9j**, 45[a] |

[a] Products were purified by crystallization.
[b] Products were purified by column chromatography.

because the ortho bromo substituent would interfere with the stability of carbocation via steric inhibition to resonance owing to the loss of co-planarity as shown in Scheme 2. Under refluxing toluene, a higher boiling point solvent, compound **9j** underwent retro-ring annulation reaction and imine **7j** was isolated.




**Scheme 2.** Proposed mechanism for the formation of compounds **1** and **9**.

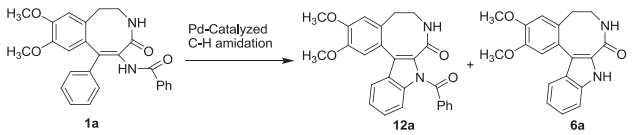
Moreover, the presence of electron-donating group $R^1$ of dihydroisoquinoline **7** may facilitate the ring expansion step but it is not an absolute requirement since we could prepare unsubstituted benzo[*d*]azocinone **1e** in moderate yield (Table 1, entry 5).

Benzo[*d*]azocinones **1a**–**i** thus obtained were further investigated as precursors for the indolobenzazocinones **6**. Palladium-catalyzed C–H activation chemistry and hydrolysis were studied with the aim to form the C–N bond followed by debenzoylation. We began to study the formation of the C–N bond by screening the catalytic system using benzo[*d*]azocinone **1a** as the substrate.

Hiroya[12b] and co-workers reported the synthesis of indazole derivatives using the palladium-catalyzed C–H activation and C–N bond formation. We initially attempted to modify Hiroya's conditions using 0.1 equiv of $Pd(OAc)_2$, 1.0 equiv of $Cu(OAc)_2$, and 2.0 equiv of $AgOCOCF_3$ in 0.05 M DMSO at 50 °C under argon atmosphere and obtained the desired indolobenzazocinone **12a** in 21% yield (Table 2, entry 1). Increasing the amount of catalyst and longer reaction time gave no significant increase in the yield of product **12a** (Table 2, entry 2). A mixture of indolobenzazocinone **12a** and hydrolyzed product **6a** was obtained when the reaction temperature was increased to 100 °C (Table 2, entry 3). When the palladium catalyst was changed to $Pd(OCOCF_3)_2$ more promising results were obtained (Table 2, entries 4–5). Increasing the reaction temperature to 80 °C gave a better yield in a shorter reaction time (Table 2, entry 6). We then optimized the reaction condition by screening other palladium catalysts and achieved the target product in the range of 60–71% yield (Table 2, entries 7–9). We also studied the re-oxidant and the use of atmospheric oxygen for oxidation, as reported by Buchwald.[10] Interestingly, using an oxygen atmosphere could eliminate the use of $AgOCOCF_3$ and gave the product in 68% yield (Table 2, entry 10) while using only $AgOCOCF_3$ without co-oxidant $Cu(OAc)_2$ under an $O_2$ atmosphere gave a very poor yield of **12a** (Table 2, entry 11). The catalytic amount of co-oxidant was also studied and found that 0.3 equiv of $Cu(OAc)_2$ was enough to mediate the reaction whereas 0.1 equiv of $Cu(OAc)_2$ was insufficient to complete the reaction (Table 2, entries 12–14).

The postulated reaction pathway of Pd-catalyzed C–H amidation for benzo[*d*]azocinones cores is depicted in Scheme 3.[12b] The amide nitrogen atom could complex to $Pd(OCOCF_3)_2$ and release trifluoroacetic acid (TFA) followed by the formation of palladacycle **15**. Subsequent reductive elimination could generate the product

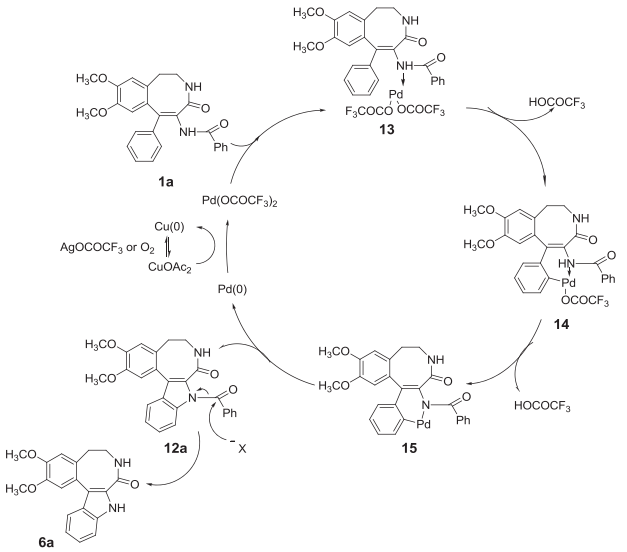
**Table 2**
Pd-catalyzed C–H activation[a]




| Entry | Pd(II) | Re-oxidant (equiv) | Temp (°C) | Time (h) | Yield [%] | | SM [%] |
|---|---|---|---|---|---|---|---|
| | | | | | 12a | 6a | |
| 1 | $Pd(OAc)_2$[b] | $Cu(OAc)_2$ (1)/ $AgOCOCF_3$ (2) | 50 | 24 | 21 | — | 67 |
| 2 | $Pd(OAc)_2$ | $Cu(OAc)_2$ (3)/ $AgOCOCF_3$ (6) | 50 | 144 | 37 | — | — |
| 3 | $Pd(OAc)_2$ | $Cu(OAc)_2$ (3)/ $AgOCOCF_3$ (6) | 100 | 24 | 13 | 33 | — |
| 4 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$ (3)/ $AgOCOCF_3$ (6) | 50 | 24 | 42 | — | 20 |
| 5 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$ (3)/ $AgOCOCF_3$ (6) | 50 | 44 | 54 | 26 | — |
| 6 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$ (3)/ $AgOCOCF_3$ (6) | 80 | 20 | 72 | 12 | — |
| 7 | $PdCl_2$ | $Cu(OAc)_2$ (3)/ $AgOCOCF_3$ (6) | 80 | 16 | 71 | 7 | 4 |
| 8 | $PdCl_2(PPh_3)_2$ | $Cu(OAc)_2$ (3)/ $AgOCOCF_3$ (6) | 80 | 16 | 60 | 8 | 19 |
| 9 | $Pd_2(dba)_3$ | $Cu(OAc)_2$ (3)/ $AgOCOCF_3$ (6) | 80 | 16 | 71 | 8 | 19 |
| 10 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$ (3) | 80 | 54 | 68 | 7 | — |
| 11 | $Pd(OCOCF_3)_2$ | $AgOCOCF_3$ (3) | 80 | 54 | 17 | — | 64 |
| 12 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$ (1) | 80 | 5 | 73 | 2 | — |
| 13 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$ (0.3) | 80 | 42 | 68 | 6 | — |
| 14 | $Pd(OCOCF_3)_2$ | $Cu(OAc)_2$ (0.1) | 80 | 89 | 21 | — | 72 |

[a] All reactions were performed with 0.3 equiv of palladium catalyst in 0.05 M DMSO under argon atmosphere except entries 10–14, the reactions were done under $O_2$ atmosphere.
[b] $Pd(OAc)_2$ (0.1 equiv) was required. SM=Starting material.

**12**. The debenzoylated product **6a**, which is sometimes, obtained in low yield, likely to be formed via simple benzoyl cleavage as depicted.

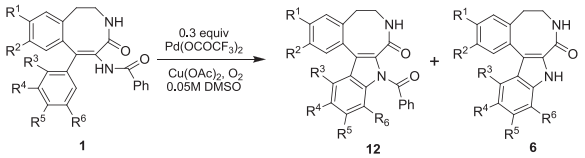


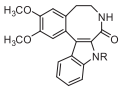
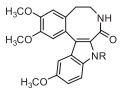
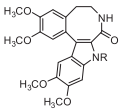
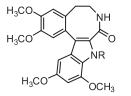
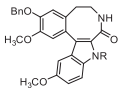
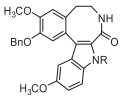

**Scheme 3.** Proposed mechanism for the formation of **12a** and **6a**.

With effective conditions for the synthesis of indolobenzazocinone **1a**, we then directed our attention to the synthesis of a small library of indolobenzazocinone analogues using $Pd(OCOCF_3)_2$ as the catalyst and $Cu(OAc)_2$ as the re-oxidant under the oxygen atmosphere. An increased amount of $Cu(OAc)_2$ was necessary for the

higher oxygenated analogues **1b**–**i** in the context of decreasing reaction time and obtaining optimal yield (Table 3). In addition, in some cases, increasing the amount of palladium catalyst to 0.5 equiv was required to obtain higher yield (Table 3, entry 7). We reasoned that the steric hindrance makes it difficult to incorporate the palladium species in the six-membered palladacycle complexes as shown in the postulated mechanism (Scheme 3).

**Table 3**
Pd-catalyzed C–H activation[a]



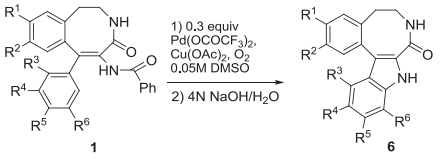

| Entry | SM | $Cu(OAc)_2$ (equiv) | Time (h) | Products | Yield [%] | |
|---|---|---|---|---|---|---|
| | | | | | **12** (R=Bz) | **6** (R=H) |
| 1 | **1a** | 0.3 | 42 |  | **a**, 68 | **a**, 6 |
| 2 | **1b**[d] | 0.3 | 50 |  | **b**, 25 | **b**, 5 |
| 3 | **1b** | 0.6 | 40 | | **b**, 59 | **b**, 6 |
| 4 | **1c**[d] | 0.3 | 50 |  | **c**, 31 | — |
| 5 | **1c** | 0.6 | 16 | | **c**, 88 | **c**, 7 |
| 6 | **1d**[d] | 0.6 | 40 |  | **d**, 14 | **d**, 5 |
| 7 | **1d**[b,d] | 1 | 47 | | **d**, 21 | **d**, 7 |
| 8 | **1e** | 0.6 | 18 |  | **e**, 91 | — |
| 9 | **1f** | 0.6 | 15 |  | **f**, 49 | **f**, 9 |
| 10 | **1g** | 0.6 | 23 |  | **g**, 50 | **g**, 3 |
| 11 | **1i** | 0.6 | 20 | — | NA[c] | — |
| 12 | **1i** | 1 | 20 | — | NA[c] | — |

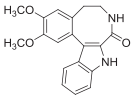
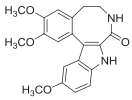
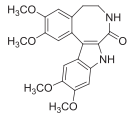
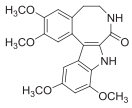
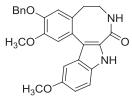
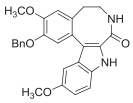
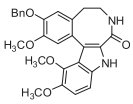
[a] All reactions were performed with 0.3 equiv $Pd(OCOCF_3)_2$ in 0.05 M DMSO at 80 °C under $O_2$ atmosphere and were monitored by TLC.
[b] $Pd(OCOCF_3)_2$ (0.5 equiv) was required.
[c] Complex mixture of products were detected.
[d] Starting materials were recovered as follows entry 2 (33%), entry 4 (39%), entry 6 (44%), and entry 7 (6%). SM=Starting material.

Moreover, we found that steric effect played an important role in the regioselectivity. Only the regioselective products were obtained from unsymmetrical materials **1b**,**c** and **1f**,**g**, which were confirmed by spectroscopic data. Unfortunately, the pyridine derivative **1i** gave no reaction even after increasing the amount of $Pd(OCOCF_3)_2$ or $Cu(OAc)_2$ (Table 3, entries 11–12). This could be due to the complexation of pyridine nitrogen with the transition metal rendering the catalyst inactive.[17]

With the success of Pd(II)-catalyzed C–H amidation of compounds **1**, we then turned our attention to find a way to effect the transformation of compounds **1** to indolobenzazocinones **6** in one pot. To effect such transformation, 4 N NaOH in $H_2O$ was added to the crude products to hydrolyze the amide group to give compounds **6** in moderate to good yields as shown in Table 4.

**Table 4**
Pd-catalyzed C–H activation and hydrolysis[a]

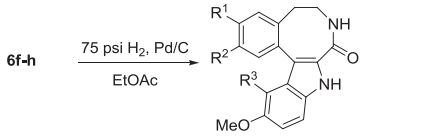



| Entry | SM | $Cu(OAc)_2$ (equiv) | Time (h) | Products | Yield [%] **6** |
|---|---|---|---|---|---|
| 1 | **1a** | 0.3 | 22 |  | **a**, 83 |
| 2 | **1b** | 0.6 | 20 |  | **b**, 60 |
| 3 | **1c** | 0.6 | 22 |  | **c**, 60 |
| 4 | **1d**[b] | 1 | 40 |  | **d**, 10 |
| 5 | **1e** | 0.6 | 42 |  | **e**, 66 |
| 6 | **1f** | 0.6 | 15 |  | **f**, 67 |
| 7 | **1g** | 0.6 | 20 |  | **g**, 54 |
| 8 | **1h** | 0.6 | 22 |  | **h**, 54 |

[a] All reactions were performed with 0.3 equiv $Pd(OCOCF_3)_2$ in 0.05 M DMSO at 80 °C under $O_2$ atmosphere and were monitored by TLC.
[b] $Pd(OCOCF_3)_2$ (0.5 equiv) was required.

Compounds **6f**–**h** required an additional step involving debenzylation in order to furnish indolobenzazocinone derivatives **16f**–**h**. The hydrogenolysis of compounds **6f**–**h** was conducted using palladium on activated charcoal in ethyl acetate at 75 psi in the Parr apparatus at room temperature to give the debenzylated products **16f**–**h** in moderate to good yield (54–84%, Scheme 4).

Interestingly, all $^1H$ NMR spectra of indolobenzazocinone derivatives **6** and **16** showed the absence of two sets of methylene protons at room temperature (Fig. 2, bottom). By decreasing the operating temperature to −20 °C, $^1H$ NMR spectra of four methylene protons appeared separately in the range of 2.6–4.0 ppm




**16f:** $R^1$= OH, $R^2$ = OMe, $R^3$ = H (82%)
**16g:** $R^1$ = OMe, $R^2$= OH, $R^3$ = H (84%)
**16h:** $R^1$ = OH, $R^2$-$R^3$ = OMe (54%)

**Scheme 4.** Debenzylation.

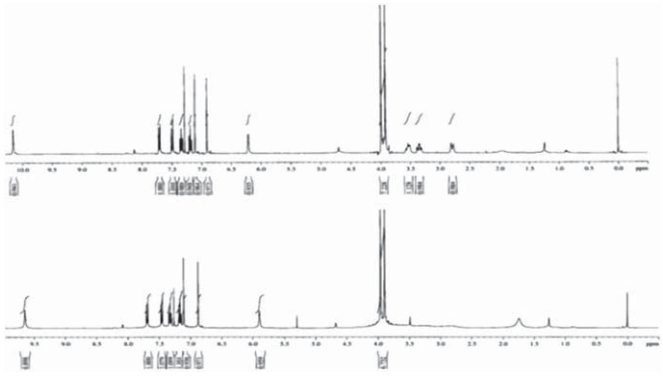


**Fig. 2.** Selected $^1$H NMR spectra of compound **6a** at −20 °C (top) and $^1$H NMR spectra of compound **6a** at room temperature (bottom).

(Fig. 2, top). This phenomenon possibly resulted from the restricted rotation of the secondary cyclic amide in this indolobenzazocin-8-one system.[18]

As reported by Joseph and co-workers, indolobenzazocin-8-one **6e** showed the most potent antiproliferative activity inhibiting cell growth in several cancer cell lines in the nanomolar $IC_{50}$ value range.[6] Our synthetic indolobenzazocinones **6a**–**d** and **16f**–**h** were evaluated for cytotoxicity as compared with unsubstituted compound **6e** as cytotoxic agents on a panel of four human tumor cell lines; cholangiocarcinoma HUCCA-1, lung carcinoma A549, hepatoblastoma HepG2, and T-lymphoblast (acute lymphoblastic leukemia) MOLT-3 using MTT and XTT assays depending on the cell-line types as shown in Table 5.

**Table 5**
Biological activities of synthetic indolobenzazocinone derivatives **6a**–**e** and **16f**–**h** in MTT and XTT assays[a]

| Entry | Compounds | IC50 (μM) | | | |
|---|---|---|---|---|---|
| | | HuCCA-1[b,f] | A549[c,f] | HepG2[d,f] | MOLT-3[e,g] |
| 1 | **6a** | Inactive | Inactive | 96.23 | 44.50 |
| 2 | **6b** | 83.8 | 71.0 | 39.8 | 17.12 |
| 3 | **6c** | Inactive | Inactive | 122.1 | 71.7 |
| 4 | **6d** | Inactive | Inactive | 11.04 | 3.98 |
| 5 | **6e** | 48.1 | 12.6 | 0.46 | 0.009 |
| 6 | **16f** | 102.8 | 66.4 | 69.80 | 26.6 |
| 7 | **16g** | Inactive | Inactive | 84.79 | 80.8 |
| 8 | **16h** | Inactive | 124.26 | 99.65 | 25.98 |
| 9 | Etoposide | ND | ND | 29.8 | 0.047 |
| 10 | Doxorubicin | 0.83 | 0.63 | 0.57 | ND |

ND=Not determined.
[a] DMSO solution (10 mg/mL).
[b] HuCCA-1: cholangiocarcinoma.
[c] A549: lung carcinoma.
[d] HepG2: hepatoblastoma.
[e] MOLT-3: T-lymphoblast (acute lymphoblastic leukemia).
[f] MTT assay.
[g] XTT assay.

The results showed that unsubstituted indolobenzazocin-8-one **6e** showed the highest $IC_{50}$ values for all cancer cell lines tested as compared to other substituted compounds. Compound **6e** exhibited hepatoblastoma HepG2 in the lower micromolar range ($IC_{50}$=0.46 μM) and T-lymphoblast (acute lymphoblastic leukemia) MOLT-3 in nanomolar range ($IC_{50}$=9 nM) (Table 5, entry 5). The presence of either methoxy or hydroxy groups at the C-4, C-5 or C-6 position of the indole ring and C-8 or C-9 of benzoazocinone ring led to decreasing cytotoxic activity or becoming inactive, whereas the presence of C-7 methoxy group of the indole ring in compound **6d** led to modest cytotoxicity in both MOLT-3 and HepG2 ($IC_{50}$=3.98–11.04 μM) (Table 5, entry 4). This study provided crucial information about the role of the oxygenated substitution on indolobenzazocin-8-one core skeleton in support of the previous reports by Dodd and Joseph groups.[8]

## 3. Conclusion

In summary, we have reported the synthesis and biological activities of indolobenzazocin-8-ones using ring annulations of various dihydroisoquinolines with azlactone and Pd-catalyzed C–H amidation. The method we have developed and reported here should be applicable to the synthesis of other biologically active indolobenzazocin-8-ones in just two steps and also capable of constructing a wide range of related compounds with different substitution patterns. Indolobenzazocin-8-one **6e** exhibited very good activities in the nanomolar $IC_{50}$ value range as compared to the oxygenated substitution on this core system. This study also delineated the structure–activity relationship studies involving substitution patterns of the indolobenzazocin-8-ones.

## 4. Experimental section

### 4.1. General methods

Melting points were measured with a Thermo Fisher Scientific IA920 digital melting point apparatus and reported without correction. $^1$H Nuclear magnetic resonance ($^1$H NMR) spectra were recorded on Varion Germini2000, Bruker AV-300, and Bruker AV-400 NMR instruments at 200, 300, and 400 MHz, respectively, using deuterochloroform as solvents with tetramethylsilane as an internal standard. $^{13}$C Nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded on Varion Germini2000, Bruker AV-300, and Bruker AV-400 NMR instruments at 50, 75, and 100 MHz, respectively, using deuterochloroform with tetramethylsilane as an internal standard and dimethylsulfoxide-$d_6$ for some compound. FTIR spectra were obtained on the Spectrum One FTIR spectrometer, Perkin Elmer System with the universal ATR (UATR) accessory. Mass spectra were performed with an AEI-MS-902. High-resolution mass spectra were performed with a MicroTOF$_{LC}$, Bruker Daltonics. Column chromatography was carried out using Fluka aluminum oxide (type 507 C neutral; 100–125 mesh) and Merck silica gel (70–230 mesh ASTM). Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were carried out on silica gel (E. Merck PF 254). All reagents were purified and dried according to the standard procedures. Solvents were removed by using Eyela Aspirator A-2S and Büchi Rotavapor R110. All products were evacuated by a Christ Freeze Dryer Unit Alpha l/6, to remove the last traces of solvents.

### 4.2. General procedure for the preparation of benzo[*d*]azocin-4-ones (1)

A solution of azlactone **8** (1 equiv) and 3,4-dihydroisoquinolines **7a**–**j** (1 equiv) in acetonitrile (6 mL/mmol of starting material) was heated at reflux for 2 h. The resulting reaction was allowed cool to

room temperature and was then concentrated under reduced pressure. The crude product was further purified by re-crystallization to furnish the desired product (37–74%).

*4.2.1. 5-Amido-6-(3′-methoxylphenyl)-8,9-dimethoxy-1,2-dihydrobenzo[d]azocin-4-one (**1b**).* Pale yellow solid (1.15 g, 39% yield). $R_f$ 0.33 (90% EtOAc/Hexane, two times). Mp: 191.2 °C. IR (UATR): $\nu_{max}$ 3231, 1655, 1653 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 2.88–2.96 (m, 1H), 3.36–3.48 (m, 2H), 3.86–4.07 (m, 1H), 3.72 (s, 3H), 3.76 (s, 3H), 3.87 (s, 3H), 4.06 (m, 1H), 5.99 (s, 1H), 6.63 (s, 1H), 6.68 (s, 1H), 6.83–6.96 (m, 3H). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$ 32.5, 41.7, 55.2, 55.9 (2C), 112.1, 113.0, 113.5, 114.0, 120.2, 127.2 (2C), 128.6 (2C), 129.2 (2C), 130.3 (2C), 131.1, 132.0, 132.8, 139.6, 147.6, 148.8, 160.2, 164.5, 169.2; EI-MS: *m*/*z* (%) 458 ($M^+$, 12), 298 (23), 105 (100); TOF-HRMS calcd for $C_{27}H_{27}N_2O_5$: 459.1915; found 459.1925.

*4.2.2. 5-Amido-6-(3′,4′-dimethoxylphenyl)-8,9-dimethoxy-1,2-dihydrobenzo[d]azocin-4-one (**1c**).* Pale yellow solid (1.84 g, 40% yield). $R_f$ 0.25 (90% EtOAc/Hexane, two times). Mp: 214 °C. IR (UATR): $\nu_{max}$ 3320, 3227, 1652, 1649 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 2.87–2.98 (m, 1H), 3.39–3.48 (m, 2H), 3.72 (s, 3H), 3.80 (s, 3H), 3.87 (s, 6H), 3.93–4.05 (m, 1H), 6.08 (br s, 1H), 6.63 (s, 1H), 6.69 (s, 1H), 6.84 (s, 1H), 6.89 (s, 2H) 7.35–7.72 (m, 5H), 8.54 (br s, 1H). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$ 32.4, 41.7, 55.8, 55.9 (2C), 56.0, 111.2, 111.6, 112.0, 113.0, 121.0, 127.1 (3C), 127.8, 128.5, 128.5, 128.9, 129.2, 130.6, 131.4, 132.0, 132.8, 147.5, 148.7, 149.3, 164.5, 169.5. EI-MS: *m*/*z* (%) 488 ($M^+$, 1), 105 (77), 77 (44), 69 (26), 57 (26). TOF-HRMS calcd for $C_{28}H_{29}N_2O_6$: 489.2020; found 489.1994.

*4.2.3. 5-Amido-6-(3′,5′-dimethoxylphenyl)-8,9-dimethoxy-1,2-dihydrobenzo[d]azocin-4-one (**1d**).* Pale yellow solid (0.64 g, 48% yield). $R_f$ 0.33 (90% EtOAc/Hexane, two times). Mp: 214 °C. IR (UATR): $\nu_{max}$ 3336, 3233, 1654, 1602 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 2.85–2.95 (m, 1H), 3.31–3.50 (m, 2H), 3.73 (s, 3H), 3.77 (s, 3H), 3.88 (s, 3H), 3.96–4.10 (m, 1H), 6.15 (br s, 1H), 6.41 (d, *J*=2.2 Hz, 1H), 6.48 (d, *J*=2.2 Hz, 2H), 6.61 (s, 1H), 6.70 (s, 1H), 7.37–7.51 (m, 3H), 7.71 (dd, *J*=8.6, 2.8 Hz, 2H). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$ 32.3, 41.5, 55.3 (2C), 55.8, 55.8, 99.9, 106.1 (2C), 112.0, 112.8, 127.1 (3C), 128.5 (2C), 129.1, 130.9, 132.0 (2C), 132.7, 140.1, 147.5, 148.8, 161.4 (2C), 164.6, 169.4. EI-MS: *m*/*z* (%) 489 ($M+H^+$, 1), 488 ($M^+$, 3) 328 (10), 105 (100), 77 (35). TOF-HRMS calcd for $C_{28}H_{29}N_2O_6$: 489.2020; found 489.2042.

*4.2.4. 5-Amido-6-phenyl-1,2-dihydrobenzo[d]azocin-4-one (**1e**).* Pale yellow solid (51.2 mg, 58% yield). $R_f$ 0.51 (90% EtOAc/Hexane, two times). Mp: 222 °C. IR (UATR): $\nu_{max}$ 3235, 1651 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 3.00 (m, 1H), 3.37–3.53 (m, 2H), 4.13 (m, 1H), 5.77 (br s, 1H), 7.12–7.69 (m, 14H), 8.30 (s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 32.8, 41.5, 126.8, 127.3 (2C), 128.2 (3C), 128.3, 128.7 (3C), 129.1, 129.3, 129.4 (2C), 130.1, 132.2, 132.7, 136.8, 138.2, 139.1, 164.7, 169.2. EI-MS: *m*/*z* (%) 369 ($M+H^+$, 4), 368 ($M^+$, 15), 219 (30), 208 (41), 105 (100), 77 (28); TOF-HRMS calcd for $C_{24}H_{21}N_2O_2$: 369.1598; found 369.1600.

*4.2.5. 5-Amido-6-(3′-methoxylphenyl)-9-benzyloxy-8-methoxy-1,2-dihydrobenzo[d]azocin-4-one (**1f**).* Yellow amorphous (0.92 g, 40% yield). $R_f$ 0.58 (90% EtOAc/Hexane, two times). Mp: 214 °C. IR (UATR): $\nu_{max}$ 3227, 1655, 1508, 1473 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.68–2.78 (m, 1H), 3.18–3.36 (m, 2H), 3.65 (s, 3H), 3.68 (s, 3H), 3.92–3.96 (m, 1H), 5.05 (s, 2H), 5.82 (br s, 1H), 6.57 (s, 1H), 6.62 (s, 1H), 6.75 (s, 1H), 6.77 (d, *J*=9.0 Hz, 2H), 6.88 (d, *J*=7.5 Hz, 1H), 7.20–7.43 (m, 9H), 7.58 (s, 1H), 7.59 (d, *J*=7.8 Hz, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 32.4, 41.7, 55.3, 56.1, 71.1, 113.5, 113.6, 114.0, 114.7, 120.3, 127.3 (4C), 127.9, 128.4, 128.6 (3C), 128.7 (2C), 129.2, 130.5, 131.7, 132.2, 132.8, 137.0, 139.6, 148.1, 148.3, 160.3, 164.6, 169.3. EI-MS: *m*/*z* (%) 535 ($M+H^+$, 3), 534 ($M^+$, 9), 388 (24), 105 (100), 91 (45), 77 (25). TOF-HRMS calcd for $C_{33}H_{31}N_2O_5$: 535.2228; found 535.2226.

*4.2.6. 5-Amido-6-(3′-methoxylphenyl)-8-benzyloxy-9-methoxy-1,2-dihydrobenzo[d]azocin-4-one (**1g**).* Yellow amorphous (0.51 g, 37% yield). $R_f$ 0.53 (90% EtOAc/Hexane, two times). Mp: 212 °C. IR (UATR): $\nu_{max}$ 3233, 1652, 1603, 1473 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.73–2.83 (m, 1H), 3.25–3.34 (m, 2H), 3.63 (s, 3H), 3.76 (s, 3H), 3.92–3.99 (m, 1H), 4.88 (q, *J*=12.0 Hz, 2H), 6.06 (s, 1H), 6.60 (s, 2H), 6.67 (s, 1H), 6.71–6.79 (m, 2H), 7.15–7.39 (m, 9H), 7.59 (d, *J*=7.8 Hz, 2H), 8.37 (s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 32.5, 41.5, 55.1, 55.9, 71.0, 112.6, 113.4, 113.8, 115.8, 120.2, 127.2 (5C), 127.6, 128.3 (3C), 128.5 (2C), 129.7, 130.2, 130.9, 132.0, 132.7, 136.8, 139.4, 146.6, 149.3, 160.0, 164.6, 169.4. EI-MS: *m*/*z* (%) 535 ($M+H^+$, 4), 534 ($M^+$, 13), 374 (12), 105 (100), 91 (56), 77 (26). TOF-HRMS calcd for $C_{33}H_{31}N_2O_5$: 535.2228; found 535.2211.

*4.2.7. 5-Amido-6-(2′,3′-dimethoxylphenyl)-9-benzyloxy-8-methoxy-1,2-dihydrobenzo[d]azocin-4-one (**1h**).* Yellow amorphous (0.33 g, 30% yield). $R_f$ 0.43 (100% EtOAc). IR (UATR): $\nu_{max}$ 3231, 1654, 1471 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.82–2.88 (m, 1H), 3.33–3.48 (m, 2H), 3.69 (s, 3H), 3.89 (s, 3H), 3.96 (s, 3H), 4.10–4.16 (m, 1H), 5.13 (s, 2H), 5.96 (br s, 1H), 6.49 (s, 1H), 6.75 (s, 1H), 6.86–6.99 (m, 2H), 7.29–7.47 (m, 9H), 7.81 (d, *J*=9.6 Hz, 2H), 9.34 (br s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 32.2, 41.3, 55.7, 55.9, 61.9, 70.9, 111.9, 114.0, 114.8, 122.5, 124.7, 127.2 (3C), 127.7 (2C), 128.3 (3C), 128.4 (2C), 129.6, 129.7, 131.8, 132.2, 132.3, 132.8, 136.8, 145.1, 147.9, 148.0, 153.1, 164.9, 169.5. EI-MS: *m*/*z* (%) 564 ($M^+$, 2), 105 (100), 91 (47), 77 (23). TOF-HRMS calcd for $C_{34}H_{33}N_2O_6$: 565.2333; found 565.2351.

*4.2.8. 5-Amido-6-(3′-pyridyl)-9-benzyloxy-8-methoxy-1,2-dihydrobenzo[d]azocin-4-on**e** (**1i**).* Pale yellow solid (0.29 g, 58% yield). $R_f$ 0.20 (90% EtOAc/Hexane, two times). Mp: 200 °C. IR (UATR): $\nu_{max}$ 3328, 3221, 1654 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.81–2.89 (m, 1H), 3.26–3.53 (m, 2H), 3.72 (s, 3H), 4.01–4.16 (m, 1H), 5.14 (s, 2H), 5.95 (br s, 1H, N*H*), 6.61 (s, 1H), 6.73 (s, 1H), 7.23–7.52 (m, 8H), 7.57–7.69 (m, 3H), 8.32 (s, 1H), 8.50 (d, *J*=3.9 Hz, 1H), 8.70 (s, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 32.2, 42.1, 56.1, 71.1, 113.5, 114.8, 123.9, 127.2, 127.3 (4C), 128.0 (2C) 128.6 (2C), 128.7 (2C), 129.3, 129.4, 130.9, 132.3, 132.7, 136.1, 136.9, 148.5, 148.6, 149.0, 149.2, 165.1, 169.3. EI-MS: *m*/*z* (%) 506 ($M^+$, 0), 105 (100), 91 (53), 77 (34). TOF-HRMS calcd for $C_{31}H_{28}N_3O_4$: 506.2074; found 506.2071.

*4.2.9. 1-Amido-9b-(2′-bromophenyl)-7,8-dimethoxy-1,4,5,9b-tetrahydro-2H-azeto[2,1-a]isoquinolin-2-one (**9j**).* White solid (1.22 g, 45% yield). $R_f$ 0.48 (90% EtOAc/Hexane). Mp: 157 °C. IR (UATR): $\nu_{max}$ 1684, 1247 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.52–2.79 (m, 2H), 3.59–3.65 (m, 2H), 3.92 (s, 1H), 4.08 (s, 1H), 5.85 (d, *J*=8.4 Hz, 1H), 6.74 (s, 1H), 7.04–7.22 (m, 4H), 7.33–7.53 (m, 5H), 7.62 (dd, *J*=7.8, 1.5 Hz, 1H), 7.93 (s, 1H, N*H*). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 26.5, 39.6, 56.0, 56.4, 66.6, 111.5, 111.6, 121.6, 126.7 (2C), 127.2 (2C), 127.7, 128.6 (2C), 129.8, 131.3, 131.9, 132.1, 133.4, 135.4 (2C), 147.8, 148.7, 166.3, 167.6. EI-MS: *m*/*z* (%) 506 ($M^+$, 0), 508 ($M+2H^+$, 0), 348 (7), 346 (27), 105 (100), 77 (63). TOF-HRMS calcd for $C_{26}H_{24}BrN_2O_4$: 507.0914; found 507.0928.

### 4.3. General procedure for the preparation of *N*-benzoylindolobenzazocinones (12a–g)

In a round bottle flask, mixture of benzo[*d*]azocinone **1**, Pd(O-$COCF_3)_2$ (0.3–0.5 equiv), $Cu(OAc)_2$ (0.3–1 equiv) was evacuated and refilled with Ar. Under argon atmosphere, DMSO (0.05 M) was added via syringe and flask was again evacuated and refilled with $O_2$. The reaction mixture was then stirred at 70–80 °C. The reaction was monitored via TLC until completion. After being cooled to room

temperature, the reaction mixture was filtered through Celite and then extracted with EtOAc. Combined organic layer was washed with water and dried over $Na_2SO_4$. The solvent was evaporated and the residue was purified by PTLC (80%EtOAc/Hexane) to give products **12**.

*4.3.1. 9-Benzoyl-(2,3-dimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3] benzazocin-8-one (**12a**).* White solid (26.1 mg, 68% yield). $R_f$ 0.43 (100% EtOAc). Mp: 255 °C. IR (UATR): $\nu_{max}$ 1687, 1647 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$): 2.80–2.90 (m, 1H), 3.24–3.51 (m, 2H), 3.70–3.80 (m, 1H), 3.90 (s, 3H), 3.96 (s, 3H), 5.78 (br s, 1H), 6.88 (s, 1H), 7.10 (s, 1H), 7.27–7.68 (m, 6H), 7.79–7.86 (m, 3H). $^{13}C$ NMR (50 MHz, $CDCl_3$): $\delta$ 32.6, 45.0, 56.0, 56.1, 112.4, 113.1, 114.7, 121.2, 123.7, 123.8, 124.3, 126.6, 127.7, 128.3 (2C), 129.3 (2C), 129.8, 131.2, 132.7, 135.8, 138.4, 147.8, 149.0, 165.1, 168.6. EI-MS: *m*/*z* (%) 427 ($M+H^+$, 15), 426 ($M^+$, 47), 304 (31), 293 (10), 105 (100), 77 (55). HRMS (microTOF) *m*/*z* calcd for $C_{26}H_{23}N_2O_4$ ($M+H^+$): 427.1652; found: 427.1651.

*4.3.2. 9-Benzoyl-(2,3,12-trimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one (**12b**).* White solid (43.7 mg, 59%). $R_f$ 0.45 (100% EtOAc). Mp: 262 °C. IR (UATR): $\nu_{max}$ 3333, 1683, 1646 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 2.81–2.87 (m, 1H), 3.25–3.34 (m, 1H), 3.39–3.47 (m, 1H), 3.74–3.80 (m, 1H), 3.81 (s, 3H), 3.89 (s, 3H), 3.95 (s, 3H), 5.63 (br s, 1H), 6.88 (s, 1H), 7.01 (dd, *J*=9.1, 2.6 Hz, 1H), 7.05 (d, *J*=2.4 Hz, 1H), 7.09 (s, 1H), 7.46–7.60 (m, 3H), 7.73 (d, *J*=9.1 Hz, 1H), 7.81 (d, *J*=7.0 Hz, 2H). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 32.6, 44.9, 55.7, 56.0, 56.1, 102.8, 112.5, 112.9, 115.8, 116.0, 123.7, 124.4, 128.3, 128.3, 128.5, 129.3 (2C), 129.8, 131.7, 132.6, 133.3, 136.0, 147.9, 149.1, 156.6, 165.0, 168.4. EI-MS: *m*/*z* (%) 457 ($M+H^+$, 311), 456 ($M^+$, 38), 352 (7), 105 (100), 77 (31). HRMS (microTOF) *m*/*z* calcd for $C_{27}H_{25}N_2O_5$ ($M+H)^+$: 457.1758; found: 457.1758.

*4.3.3. 9-Benzoyl-(2,3,11,12-tetramethoxyl)-5,6,7,9-tetrahydro indolo [2,3-e][3]benzazocin-8-one (**12c**).* White solid (64.1 mg, 88% yield). $R_f$ 0.38 (100% EtOAc). Mp: 258 °C. IR (UATR): $\nu_{max}$ 3328, 1681, 1644 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.81–2.87 (m, 1H), 3.20–3.33 (m, 1H), 3.45–3.56 (m, 1H), 3.70–3.75 (m, 1H), 3.89 (s, 6H), 3.92 (s, 3H), 3.96 (s, 3H), 5.41 (br s, 1H), 6.89 (s, 1H), 7.02 (s, 1H), 7.09 (s, 1H), 7.48–7.59 (m, 3H), 7.62 (s, 1H), 7.82 (d, *J*=6.9 Hz, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 32.5, 45.3, 56.0, 56.1 (3C), 97.8, 101.6, 112.2, 112.7, 120.0, 124.4, 124.6, 128.2 (2C), 129.1 (2C), 129.6, 130.0, 132.4, 133.6, 136.4, 147.5, 147.8, 149.0, 150.1, 165.0, 168.8. EI-MS: *m*/*z* (%) 488 ($M+2H^+$, 4), 487 ($M+H^+$, 19), 486 ($M^+$, 63), 364 (12), 105 (100), 77 (17). HRMS (microTOF) *m*/*z* calcd for $C_{28}H_{26}N_2O_6$ ($M+H)^+$: 487.1864; found: 487.1870.

*4.3.4. 9-Benzoyl-(2,3,10,12-tetramethoxyl)-5,6,7,9-tetrahydroindolo [2,3-e][3]benzazocin-8-one (**12d**).* White solid (18.6 mg, 21% yield). $R_f$ 0.48 (100% EtOAc). Mp: 236 °C; IR (UATR): $\nu_{max}$ 3333, 1703, 1648, 1516 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 2.72–2.78 (m, 1H), 3.21–3.33 (m, 2H), 3.34 (s, 3H), 3.72 (s, 3H), 3.84 (s, 3H), 3.86–3.90 (m, 1H), 3.88 (s, 3H), 5.59 (br s, 1H), 6.32 (d, *J*=2.1 Hz, 1H), 6.58 (d, *J*=2.1 Hz, 1H), 6.78 (s, 1H), 7.06 (s, 1H), 7.35 (d, *J*=7.4 Hz, 1H), 7.37 (d, *J*=7.8 Hz, 1H), 7.48 (dd, *J*=7.4, 7.4 Hz, 1H), 7.77 (d, *J*=7.1 Hz, 2H). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 33.0, 44.7, 55.1, 55.7, 56.0, 56.1, 93.7, 98.5, 109.5, 111.6, 112.9, 113.4, 121.4, 124.9, 128.1 (2C), 129.0, 129.6, 129.7 (2C), 132.2, 132.7, 136.0, 147.8, 156.9, 165.8, 169.4 (1C not observed). EI-MS: *m*/*z* (%) 487 ($M+H^+$, 30), 486 ($M^+$, 100), 381 (2), 105 (11), 77 (3). HRMS (microTOF) *m*/*z* calcd for $C_{28}H_{26}N_2O_6$ ($M+H)^+$: 487.1864; found: 487.1871.

*4.3.5. 9-Benzoyl-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one (**12e**).* White solid (52.9 mg, 91% yield). $R_f$ 0.43 (100% EtOAc). Mp: 223 °C; IR (UATR): $\nu_{max}$ 1690, 1651 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 2.85–2.90 (m, 1H), 3.20–3.30 (m, 1H), 3.37–3.45 (m, 1H) 3.69–3.74 (m, 1H), 5.91 (br s, 1H), 7.28–7.80 (m, 13H). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 32.8, 44.9, 114.7, 121.3, 123.6, 126.6, 126.9, 127.6, 128.3 (3C), 128.5, 129.3 (2C), 129.6, 130.2, 131.5, 132.2, 132.8, 135.8, 137.4, 138.4, 165.1, 168.6. EI-MS: *m*/*z* (%) 368 ($M+2H^+$, 6), 367 ($M+H^+$, 22), 366 ($M^+$, 79), 105 (100), 77 (27). HRMS (microTOF) *m*/*z* calcd for $C_{24}H_{19}N_2O_2$ ($M+H)^+$: 367.1441; found: 367.1435.

*4.3.6. 9-Benzoyl-(3-benzyloxyl-2,12-dimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one (**12f**).* Yellow amorphous (50 mg, 49% yield). $R_f$ 0.55 (100% EtOAc). IR (UATR): $\nu_{max}$ 3660, 1683, 1647, 1513 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.63–2.67 (m, 1H), 3.06–3.32 (m, 2H), 3.58–3.60 (m, 1H), 3.71 (s, 3H), 3.79 (s, 3H), 5.11 (s, 2H), 5.82 (br s, 1H), 6.81 (s, 1H), 6.91 (d, *J*=9.0 Hz, 1H), 6.96 (s, 1H), 7.01 (s, 1H), 7.20–7.46 (m, 8H), 7.63–7.69 (m, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 32.6, 44.9, 55.7, 56.3, 71.1, 102.9, 113.5, 115.1, 115.9, 116.0, 123.6, 124.9, 127.4 (2C), 128.0 (2C), 128.4 (2C), 128.6, 128.7 (2C), 129.3, 129.9, 131.8, 132.7, 133.3, 135.9, 136.9, 148.3, 148.5, 156.7, 165.2, 168.5. EI-MS: *m*/*z* (%) 532 ($M^+$, 0.3), 270 (100), 166 (96), 105 (11), 91 (61), 77 (21). HRMS (microTOF) *m*/*z* calcd for $C_{33}H_{29}N_2O_5$ ($M+H)^+$: 533.2071; found: 533.2077.

*4.3.7. 9-Benzoyl-(2-benzyloxyl-3,12-dimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one (**12g**).* Yellow amorphous (87.8 mg, 50% yield); $R_f$ 0.38 (100% EtOAc). IR (UATR): $\nu_{max}$ 3327, 1684, 1646, 1514 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 2.78–2.85 (m, 1H), 3.25–3.43 (m, 2H), 3.57–3.72 (m, 1H), 3.70 (s, 3H), 3.95 (s, 3H), 5.15 (q, *J*=12.6 Hz, 2H), 6.11 (br s, 1H), 6.79 (d, *J*=2.4 Hz, 1H), 6.90 (s, 1H), 6.96 (dd, *J*=9.0, 2.4 Hz, 1H), 7.11 (s, 1H), 7.25–7.56 (m, 8H), 7.69 (d, *J*=9.0 Hz, 1H), 7.78 (d, *J*=7.2 Hz, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 32.6, 45.0, 55.8, 56.1, 71.1, 102.9, 113.0, 115.2, 115.7, 116.0, 123.6, 124.3, 127.2 (3C), 128.0 (2C), 128.4, 128.7 (3C), 129.3, 130.5, 131.7, 132.7, 133.2, 136.0, 137.0, 147.1, 149.7, 156.7, 165.3, 168.5. EI-MS: *m*/*z* (%) 534 ($M+2H^+$, 2), 533 ($M+H^+$, 9), 532 ($M^+$, 30), 105 (100), 77 (33). HRMS (microTOF) *m*/*z* calcd for $C_{33}H_{29}N_2O_5$ ($M+H)^+$: 533.2071; found: 533.2071.

### 4.4. General procedure for the preparation of indolobenzazociones (6a–h)

In a round bottle flask, mixture of benzo[*d*]azocinone **1**, Pd(O-$COCF_3)_2$ (0.3–0.5 equiv), $CuOAc_2$ (0.3–1 equiv) was evacuated and refilled with Ar. Under argon atmosphere, DMSO (0.05 M) was added via syringe and the flask was again evacuated and refilled with $O_2$. The reaction mixture was then stirred at 70–80 °C. The reaction was monitored via TLC until completion. After being cooled to room temperature, the reaction mixture was filtered through Celite and washed with EtOAc. The resulting solution was evaporated to give a brown solution in DMSO, which was added with 4 M $NaOH/H_2O$. Reaction mixture was stirred at room temperature for 5 min and was then added with water and extracted with EtOAc three times. An organic layer was washed with water three times was then with brine. The organic layer was evaporated to give brown oil, which further purified by PTLC (80% EtOAc/Hexane) to give products **6** (10–83%).

*4.4.1. (2,3-Dimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3] benzazocin-8-one (**6a**).* White solid (25 mg, 83% yield). $R_f$ 0.35 (100% EtOAc). Mp: 256 °C. IR (UATR): $\nu_{max}$ 3273, 2953 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, at −20 °C): $\delta$ 2.77–2.81 (m, 1H), 3.31–3.39 (m, 1H), 3.51–3.57 (m, 1H), 3.91 (s, 3H), 3.92–3.97 (m, 1H), 3.99 (s, 3H), 6.21 (br s, 1H), 6.91 (s, 1H), 7.11 (s, 1H), 7.19 (ddd, *J*=8.0, 7.2, 0.6 Hz, 1H), 7.34 (ddd, *J*=8.0, 7.3, 0.8 Hz, 1H), 7.49 (d, *J*=8.3 Hz), 7.70 (d, *J*=8.1 Hz, 1H), 10.16 (s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 31.9, 48.6, 56.0, 56.1, 111.9, 112.0, 113.3, 118.0, 120.7, 121.2, 124.9, 125.8, 126.8, 128.3. 129.9, 136.8, 147.8, 148.5, 167.5. EI-MS: *m*/*z* (%) 323 ($M+H^+$),

322 (M+, 61), 293 (25), 278 (24), 250 (13), 69 (100). HRMS (microTOF): *m*/*z* calcd for $C_{19}H_{19}N_2O_3$: 323.1390; found: 323.1389.

*4.4.2. (2,3,12-Trimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one (**6b**).* White solid (39.8 mg, 60% yield). $R_f$ 0.33 (100% EtOAc). Mp: 206 °C. IR (UATR): $\nu_{max}$ 3330, 3329, 1652 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, at −20 °C): $\delta$ 2.75–2.78 (m, 1H), 3.27–3.35 (m, 1H), 3.47–3.53 (m, 1H), 3.79 (s, 3H), 3.90 (s, 3H), 3.93 (m, 1H), 3.96 (s, 3H), 6.82 (s, 1H), 6.86 (s, 1H), 6.94 (dd, *J*=8.8, 1.9 Hz, 1H), 7.04 (s, 1H), 7.09 (s, 1H), 7.35 (d, *J*=8.8 Hz, 1H), 10.86 (s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 32, 48.3, 55.6, 56.0, 56.1, 100.6, 111.5, 112.5, 113.4, 116.1, 117.5, 125.7, 126.6, 128.5, 129.6, 132.3, 147.3, 147.9, 154.4, 168.2. EI-MS: *m*/*z* (%) 353 ($M+H^+$, 21), 352 ($M^+$, 100), 323 (55), 308 (44). HRMS (microTOF) *m*/*z* calcd for $C_{20}H_{20}N_2O_4$ $(M+H)^+$: 353.1496 found: 353.1497.

*4.4.3. (2,3,11,12-Tetramethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one (**6c**).* Yellow amorphous (27.5 mg, 60% yield). $R_f$ 0.33 (100% EtOAc). IR (UATR): $\nu_{max}$ 3273, 1628, 1540, 1514 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, at −20 °C): $\delta$ 2.77 (d, *J*=14.1 Hz, 1H), 3.34 (td, *J*=12.9, 4.5 Hz, 1H), 3.54 (t, *J*=12.6 Hz, 1H), 3.78 (s, 3H), 3.85 (s, 3H), 3.91 (s, 3H), 3.91 (m, 1H), 4.00 (s, 3H), 6.57 (br s, 1H), 6.90 (s, 1H), 6.91 (s, 1H), 7.00 (s, 1H), 7.10 (s, 1H), 11.1 (br s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 32.0, 49.0, 56.0, 56.0, 56, 56.1, 93.9, 100.3, 111.3, 112.1, 118.4, 119.0, 126.0, 126.5, 129.8, 132.2, 145.6, 147.3, 147.9, 149.1, 168.4. EI-MS: *m*/*z* (%) 383 ($M+H^+$, 23), 382 ($M^+$, 100), 367 (30), 353 (35), 338 (23), 57 (55). HRMS (microTOF) *m*/*z* calcd for $C_{21}H_{23}N_2O_5$ $(M+H)^+$: 383.16015; found: 383.1591.

*4.4.4. (2,3,10,12-Tetramethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one (**6d**).* Pale yellow solid (14.1 mg, 10% yield). $R_f$ 0.35 (100% EtOAc). Mp: 243 °C. IR (UATR): $\nu_{max}$ 3284, 1628, 1517, 1455 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, at −20 °C): $\delta$ 2.71 (d, *J*=14.2 Hz, 1H), 3.25 (td, *J*=13.3, 4.6 Hz, 1H), 3.27 (t, *J*=12.4 Hz, 1H), 3.72 (s, 3H), 3.83 (s, 3H), 3.85 (m, 1H), 3.86 (s, 3H), 3.90 (s, 3H), 6.18 (s, 1H), 6.37 (d, *J*=1.2 Hz, 1H), 6.55 (d, *J*=0.8 Hz, 1H), 6.82 (s, 1H), 7.02 (s, 1H), 9.59 (s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 31.7, 48.3, 55.5, 55.6, 55.8, 55.9, 91.8, 96.5, 111.2, 112.1, 117.6, 123.1, 125.6, 126.7, 128.0, 129.6, 146.9, 147.1, 147.7, 155.2, 167.3. EI-MS: *m*/*z* (%) 383 ($M+H^+$, 18), 382 ($M^+$, 96), 353 (28), 83 (42), 57 (100), 55 (86). HRMS (microTOF) *m*/*z* calcd for $C_{21}H_{23}N_2O_5$ $(M+H)^+$: 383.1601 found: 383.1600.

*4.4.5. 5,6,7,9-Tetrahydro-8H-indolo[2,3-e][3]benzazocin-8-one (**6e**).* Pale yellow solid (7.5 mg, 66% yield). $R_f$ 0.48 (100% EtOAc). Mp: 269 °C. IR (UATR): $\nu_{max}$ 3173, 1620 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$+$CD_3OD$, at −20 °C): $\delta$ 2.83 (d, *J*=13.8 Hz, 1H), 3.35 (td, *J*=13.4, 4.9 Hz, 1H), 3.47 (td, *J*=12.7, 4.6 Hz, 1H), 3.93 (dd, *J*=11.8 Hz, 1H), 6.12 (s, 1H), 7.15 (dd, *J*=7.5, 7.5 Hz, 1H), 7.33 (dd, *J*=7.8, 7.4 Hz, 1H), 7.37–7.42 (m, 3H), 7.47 (d, *J*=8.2 Hz, 1H), 7.56 (d, *J*=7.1 Hz, 1H), 7.66 (d, *J*=8.1 Hz, 1H), 10.16 (s, 1H). $^{13}C$ NMR (100 MHz, $(CD_3)_2SO$): $\delta$ 33.3, 45.8, 112.6, 115.3, 120.5, 120.5, 123.9, 126.3, 126.7, 127.5, 130.4, 130.5, 134.1, 137.1, 138.0, 166.6, 1C not observed. EI-MS: *m*/*z* (%) 263 ($M+H^+$, 14), 262 ($M^+$, 97), 233 (57), 217 (21), 204 (100), 176 (24), 102 (14), 57 (42). HRMS (microTOF) *m*/*z* calcd for $C_{17}H_{15}N_2O$ $(M+H)^+$: 263.1179; found: 263.1139.

*4.4.6. (3-Benzyloxyl-2,12-dimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one (**6f**).* Yellow amorphous (185.1 mg, 53% yield). $R_f$ 0.45 (100% EtOAc). IR (UATR): $\nu_{max}$ 3282, 1652, 1515 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, at −20 °C): $\delta$ 2.58 (d, *J*=14.3 Hz, 1H), 3.16 (td, *J*=11.9, 4.5 Hz, 1H), 3.32 (t, *J*=12.1 Hz, 1H), 3.70 (s, 3H), 3.76–3.83 (m, 1H), 3.80 (s, 3H), 5.12 (s, 2H), 6.70 (br s, 1H), 6.77 (s, 1H), 6.85 (dd, *J*=8.9, 2.1 Hz, 1H), 6.96 (d, *J*=1.6 Hz, 1H), 7.01 (s, 1H), 7.25–7.41 (m, 6H), 10.7 (s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 33.1, 45.7, 55.7, 56.1, 70.6, 101.1, 113.5, 114.2, 114.9, 115.2, 115.7, 126.5, 126.6, 128.3 (3C), 128.8 (2C), 129.9, 130.5, 132.2, 137.7, 147.3, 147.9, 154.5, 166.6; EI-MS: *m*/*z* (%) 429 ($M+H^+$, 7), 428 ($M^+$, 29), 281 (14), 280 (30), 105 (11), 91 (100), 77 (6). HRMS (microTOF) *m*/*z* calcd for $C_{26}H_{25}N_2O_4$ $(M+H)^+$: 429.1809; found: 429.1815.

*4.4.7. (2-Benzyloxyl-3,12-dimethoxyl)-5,6,7,9-tetrahydroindolo[2,3-e][3]benzazocin-8-one (**6g**).* Yellow amorphous (185.1 mg, 53% yield). $R_f$ 0.35 (100% EtOAc). IR (UATR): $\nu_{max}$ 3297, 2925, 1706 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, at −20 °C): 2.64–2.68 (m, 1H), 3.16–3.24 (m, 1H), 3.37–3.43 (m, 1H), 3.51 (s, 3H), 3.79–3.83 (m, 1H), 3.89 (s, 3H), 5.09 (q, *J*=12.7 Hz, 2H), 6.61 (br s, 1H), 6.68 (d, *J*=1.9 Hz, 1H), 6.81 (s, 1H), 6.83 (dd, *J*=8.8, 2.4 Hz, 1H), 7.01 (s, 1H), 7.23–7.37 (m, 5H). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 32.1, 48.3, 55.8, 56.2, 71.1, 101.7, 112.7, 112.9, 115.6, 116.1, 117.4, 126.1, 126.9, 127.1 (2C), 127.8, 128.5 (2C), 128.8, 130.5, 132.3, 137.1, 147.1, 149.1, 154.9, 167.7. EI-MS: *m*/*z* (%) 429 ($M+H^+$, 18), 428 ($M^+$, 68), 337 (12), 281 (31), 250 (12), 105 (7), 91 (100), 65 (11). HRMS (microTOF) *m*/*z* calcd for $C_{26}H_{25}N_2O_4$ $(M+H)^+$: 429.1808; found: 429.1818.

*4.4.8. (3-Benzyloxyl-2,12,13-trimethoxyl)-5,6,7,9-tetrahydro indolo [2,3-e][3]benzazocin-8-one (**6h**).* Yellow amorphous (143.5 mg, 54% yield). $R_f$ 0.42 (100% EtOAc). IR (UATR): $\nu_{max}$ 3277, 1632, 1509, $cm^1$. $^1H$ NMR (400 MHz, $CDCl_3$, at −20 °C): 2.71–2.74 (m, 1H), 3.26 (s, 3H), 3.31–3.40 (m, 2H), 3.84–3.87 (m, 1H), 3.90 (s, 3H), 3.92 (s, 3H), 5.24 (s, 2H), 5.98 (br s, 1H), 6.84 (s, 1H), 7.10 (d, *J*=8.9 Hz, 1H), 7.19 (d, *J*=8.0 Hz, 1H), 7.20 (s, 1H), 7.36–7.53 (m, 5H), 9.82 (br s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$): $\delta$ 31.8, 48.2, 56.1, 58.1, 60.9, 71.4, 107.1, 113.6, 114.5, 116.2, 120.6, 122.6, 124.7, 126.8, 127.4 (2C), 127.8, 128.4 (2C), 128.5, 128.9, 129.5, 133.9, 145.9, 147.5, 147.9, 167.1. EI-MS: *m*/*z* (%) 459 ($M+H^+$, 8), 428 ($M^+$, 26), 368 (19), 367 (81), 105 (20), 91 (100), 77 (8). HRMS (microTOF) *m*/*z* calcd for $C_{27}H_{27}N_2O_5$ $(M+H)^+$: 459.1915; found: 459.1906.

### 4.5. General procedure for the synthesis of indolobenzazecinones 16f–h

A solution of **6f**–**h** (1 equiv) in EtOAc (60 mL/mmol) was placed in a high pressure Parr apparatus at room temperature. To this solution was added palladium on activated charcoal (ca. 100 mg). The resulting mixture was hydrogenated (75 psi) until all starting material was consumed (normally 16 h) as indicated by TLC. The mixture was then filtered through a plug of Celite and concentrated under reduced pressure to give a white solid. The crude material was further purified by recrystallization (PTLC) to give the desired product.

*4.5.1. (2,12-Dimethoxyl-3-hydroxyl)-5,6,7,9-tetrahydroindolo [2,3-e][3]benzazocin-8-one (**16f**).* White solid (113.5 mg, 82% yield). $R_f$ 0.40 (100% EtOAc). Mp: 238 °C. IR (UATR): $\nu_{max}$ 3280, 2929, 1652, 1513 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$+$CD_3OD$, at −20 °C): $\delta$ 2.65 (ddd, *J*=14.4, 4.3, 4.3 Hz, 1H), 3.17 (td, *J*=14.1, 4.9 Hz, 1H), 3.36 (td, *J*=12.4, 5.0 Hz, 1H), 3.73 (s, 3H), 3.80 (m, 1H), 3.80 (s, 1H), 6.47 (br s, 1H), 6.83 (s, 1H), 6.91 (dd, *J*=8.9, 2.3 Hz, 1H), 6.96 (s, 1H), 6.98 (d, *J*=2.1 Hz, 1H), 7.31 (d, *J*=8.9 Hz, 1H), 8.16 (br s, 1H). $^{13}C$ NMR (100 MHz, $CDCl_3$+$CD_3OD$): $\delta$ 31.4, 47.9, 55.4, 55.7, 100.5, 112.3, 113.0, 115.2, 115.8, 117.5, 124.6, 126.2, 127.9, 129.9, 131.9, 144.7, 145.7, 154.1, 167.7. EI-MS: *m*/*z* (%) 339 ($M+H^+$, 18), 309 (53), 294 (22), 266 (30), 97 (16), 83 (15). HRMS (microTOF) *m*/*z* calcd for $C_{19}H_{19}N_2O_4$: 339.1339; found: 339.1343.

*4.5.2. (3,12-Dimethoxyl-2-hydroxyl)-5,6,7,9-tetrahydroindolo [2,3-e][3]benzazocin-8-one (**16g**).* White solid (68.0 mg, 84% yield). $R_f$ 0.30 (100% EtOAc). Mp: 217 °C. IR (UATR): $\nu_{max}$ 3282, 2928, 1623, 1512 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$+$CD_3OD$, at −20 °C): 2.69 (dt, *J*=14.3, 3.6 Hz, 1H), 3.21 (td, *J*=12.7, 4.9 Hz, 1H), 3.40 (td, *J*=12.5, 4.9 Hz, 1H), 3.73 (s, 3H), 3.80–3.83 (m, 1H), 3.89 (s, 3H), 6.39 (br s,

1H), 6.81 (s, 1H), 6.90 (dd, *J*=8.9, 2.3 Hz, 1H), 6.98 (d, *J*=2.0 Hz, 1H), 7.02 (s, 1H), 7.29 (d, *J*=8.9 Hz, 1H), 8.01 (br s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$+$CD_3OD$): $\delta$ 31.6, 48.0, 55.5, 55.6, 100.6, 111.2, 112.8, 116.0, 116.0, 117.2, 125.8, 126.2, 127.9, 128.7, 131.9, 144.2, 146.3, 154.1, 168.5. EI-MS: *m*/*z* (%) 338 ($M^+$, 8), 178 (18), 149 (19), 97 (22), 85 (16), 69 (81), 57 (100). HRMS (microTOF) *m*/*z* calcd for $C_{19}H_{19}N_2O_4$: 339.1333; found: 339.1323.

*4.5.3. (3-Hydroxyl-2,12,13-trimethoxyl)-5,6,7,9-tetrahydroindolo [2,3-e][3]benzazocin-8-one (**16h**).* White solid (77.0 mg, 54% yield). $R_f$ 0.43 (100% EtOAc). Mp: 248 °C. IR (UATR): $\nu_{max}$ 3280, 2929, 1623, 1508, 1455 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$+$CD_3OD$, at −20 °C): 2.71–2.75 (m, 1H), 3.25 (s, 3H), 3.21–3.42 (m, 2H), 3.83–3.87 (m, 1H), 3.87 (s, 3H), 3.93 (s, 3H), 6.61 (br s, 1H), 6.83 (s, 1H), 7.10 (s, 1H), 7.11 (d, *J*=8.4 Hz, 1H), 7.22 (d, *J*=8.8 Hz, 1H), 8.10 (br s, 1H), 10.6 (br s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$+$CD_3OD$): $\delta$ 31.7, 47.6, 56.1, 58.1, 60.9, 107.4, 113.6, 114.3, 115.3, 116.5, 118.1, 120.3, 125.3, 129.6, 134.1, 143.3, 144.9, 145.0, 145.6, 167.6. EI-MS: *m*/*z* (%) 368 ($M^+$, 10), 57 (100), 55 (60). HRMS (microTOF) *m*/*z* calcd for $C_{20}H_{21}N_2O_5$: 369.1445; found: 369.1455.

### 4.6. Cytotoxicity test

All indolobenzazepinone derivatives **6** and **16** were solubilized in DMSO and tested for their cytotoxic activities against HuCCA-1, A549, HepG2, and MOLT-3 cancer cell lines. The cells suspended in the corresponding culture medium were inoculated in 96-well microtiter plates (Corning Inc., NY, USA) at a density of 10,000–20,000 cells per well, and incubated at 37 °C in a humidified atmosphere with 95% air and 5% $CO_2$. After 24 h, an equal volume of additional medium containing either the serial dilutions of the test compounds, positive control (etoposide), or negative control (DMSO) was added to the desired final concentrations, and the microtiter plates were further incubated for an additional 48 h. The number of surviving cells in each well was determined using either MTT assay (for adherent cells) or XTT assay (for suspended cells) in order to determine the $IC_{50}$, which is defined as the concentration that inhibits cell growth by 50% (relative to negative control) after 48 h of continuous exposure to each test compound. Within each experiment, determinations were done in triplicate, and each compound was tested in at least two separate experiments. Any experiments with a variation greater than 10% were excluded from the analysis. The results are expressed as the mean $IC_{50}$ value; standard deviations are omitted for visual clarity.

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## Supplementary data

Experimental procedures and spectral data for all new compounds. Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.tet.2012.10.011.

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Ms. Ref. No.: TET-D-13-00601
Title: Cu(I)-mediated lactone formation in subcritical water: A benign synthesis of benzopyranones and urolithins A-C
Tetrahedron

Dear Dr. Thasana

We have appreciated the opportunity to consider your paper for publication. It has been reviewed - see reviewer comments below. We shall be pleased to accept your paper subject to modifications as required by the referee and
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Yours sincerely,
Richard JK Taylor
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Tetrahedron

Reviewer's comments:
Overall Assessment: (where 5 is high and 1 is low)
1. Quality and originality 5__ 4__ 3_X_ 2__ 1__
2. General interest to Tetrahedron readers:5__ 4__ 3_X_ 2__ 1__
3. Presentation, layout and grammar: 5__ 4__ 3__ 2_X_ 1__
Compound Characterisation:
Do all new compounds have CHN analyses and/or HRMS? YES_X_ NO__
Is other characterisation [IR, NMR(inc. J values), mp, compound description (yellow oil, colourless needles, etc)] satisfactory? YES_X_ NO__
The authors report their studies on copper-mediated lactone formation in near-critical water. They have undertaken a systematic evaluation of various
ligands for this transformation, and having identified the optimal ligand, they have then applied the transformation to a variety of different substrates. They have demonstrated the utility of this methodology by carrying out the total synthesis of urolithins A-C.
An advantage of the methodology as described is the use of water as solvent, which carries various advantages that the authors describe. The "green" nature of the transformation is diminished somewhat by fact that "traditional" organic solvents arte still required for an extraction during
the workup, and for chromatography. Still, this is the case for most of the papers one sees in the literature on chemistry in

aqueous media.
A disadvantage of the method as described is the very high loadings of copper salt required to effect the transformation in good yield, i.e. 50
mol%. The authors have sensibly not tried to claim this as a catalytic method - although such a claim would be technically correct, a turnover number of 2 would not really impress anybody. Instead they simply describe it as "sub-stoichiometric" and focus instead on the advantages of the use of water in concert with microwave acceleration. After the sections describing the reaction optimisation and scoping, there then follows a description of the total syntheses, which require several steps to prepare highly functionalised substrates for the key transformation. The total syntheses are achieved concisely and good yield overall.
Overall, the paper contains a sufficient quantity of results or sufficient novelty to merit publication in tetrahedron. However, before I can recommend publication, extensive changes are required. The ESI show spectra to be of generally high purity, for which I compliment the authors. An exception is
30b which has appreciable amounts of grease in it, and iodinated anisole 27, which has a significant contaminant (the product of ortho-iodination?) Anyway, while the ESI is generally good,there are frequent errors in the experimental, which are listed below. Additionally some points to change in the manuscript are also included.
If these changes are made in a satisfactory manner, I would be willing to recommend publication.
* Page 1, column 1, line 43: "non-inflammability" should be "non-flammability"
* Page 2, column 1, line 29: "7a and b" - should be "7a and 7b", with
"7b" in BOLD
* Page 2, column 1, line 38: "CuTC" - this is not a standard abbreviation and it needs to be defined as it is not defined in the text or in the experimental. I am guessing it is copper thiocyanate, but this needs to be explicitly stated.
* Page 2, column 1, lines 50-53: The argument about the electronic character of the ligands is not clear and needs to be rewritten.
* Page 2, column 2, lines 50-53: "The presence of electron-

donating groups may play the important role in the C-O bond forming step and gave lower yield of the products." - It is not clear what is meant by this statement and it should either be clarified or removed.
* Table 2: THE SUBSTRATE FOR ENTRIES 7 & 8 IS MISSING!
* Page 3, column 1, line 59: The authors do not comment on the difference between the chloride and the bromide for formation of 23a/b and 24a/b. They should include a sentence or two commenting on this. It may be that the bromide generated from the Cu-mediated reaction is better able to
act as a nucleophile and attack the indole N-methyl group, giving MeBr and the N-demethylated products.
* Scheme 1: The label for 28c reads "R1, R2=OCH2O,71%" - needs an extra space
* Page 4, column 1, line 16. The standard deviation for the IC50 is missing (put in "13.6")
EXPERIMENTAL SECTION
* NEED TO INCLUDE DATA FOR 23a, 23b, 24a and 24b!!! Even though these are known compounds, the 1H data should still be reported.
*Page 4, column 1, line 58: "in three  portions" makes it sound like the solvent was added at three different times. Is this correct? More detail should be added here to make it clear exactly what is meant.
*Compound 12: There is a missing peak in the 1H NMR data: the ESI shows a peak at 8.51ppm, but it is not listed in the text!
* Compound 12: The 13C NMR peaks are not in the correct order: "52.4" should be the last number.
* Compound 18: 1H NMR: First two signals are in the wrong order!
* Compound 22: 1H NMR: for the resonance at 9.02ppm, a J value is given as "1.95Hz". This should be one decimal place only ("2.0 Hz")
*Compound 22: 13C NMR: extra space after "132.2" and no space at all after "117.3"
* Compound 30a: 13C NMR: MISSING PEAK - 123.1 is visible in the ESI but not reported in the text.
* Compound 30c: 1H NMR: "6.989-6.952" should be "6.99-6.95".
* Compound 30c: 13C NMR: "103.01" should be "103.0"
* Compound 30c: 13CNMR: MISSING PEAK 56.2 is visible in the ESI but not reported in the text.
* Page 5, column 1, line 51: "saturated Na2SO5" - working up an iodination with sodium peroxysulfate seems very strange to me.

Surely this should be "Na2S2O3"
* Page 5, column 1, line 55: "Rf 0.512" should be "Rf 0.51"
* Compound 28b: 1H NMR "J = 0.99, 1H" should be "J = 1.0 Hz, 1H"(missing "Hz")
* Compound 28b: 1H NMR "J = 4.62, 1H" should be "J = 4.6 Hz, 1H"
* Compound 28b: The 13C NMR peaks are not in the correct order: "55.6" should be the last number.
* Compound 29a: 1H NMR: "7.53-7.08" should be "7.23-7.08"
* Compound 29a: 13C NMR: MISSING PEAK. There is a peak visible in the ESI between the two peaks at 132.5 and 130.7, but it is not reported in the
text.
* Compound 2b: 1H NMR: "10.36 (s, 2H)" should be "10.36 (s, 1H)"
* Compound 2b: 13C NMR: MISSING PEAK - 127.7 is visible in the ESI but not reported in the text.
* Compound 2c: 13C NMR resonances are listed in ascending order, but for all other compounds they are listed in descending order.
* ESI: Page S21: This compound is labelled as "2b" but it is actually "2c"!!!!

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Manuscript Draft

Manuscript Number:

Title: Cu(I)-mediated lactone formation in subcritical water: A benign synthesis of benzopyranones and urolithins A-C

Article Type: Full Length Article

Corresponding Author: Dr nopporn thasana, PhD

Corresponding Author's Institution: Chulabhorn research institute

First Author: nopporn thasana, PhD

Order of Authors: nopporn thasana, PhD; Prattya Nealmongkol, BSc; Kassirin Tangdenpaisal, MSc; Somkid Sitthimonchai, PhD

Abstract: Benzopyranones were successfully synthesized using Cu(I)-mediated C-O bond formation in subcritical water. A number of benzopyranone derivatives including polymethoxy benzopyranones, benzopyranopyridones, chromenoindolones and furochromenones were synthesized in satisfactory yield. This methodology was further applied to synthesize the intestinal microbial metabolites, urolithins A, B, and C which were found to exhibit potent antioxidant activity

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He works on urolithins.

March 27, 2013

Dear Prof. Taylor,

Enclosed is a paper entitled "Cu(I)-mediated lactone formation in subcritical water: A benign synthesis of benzopyranones and urolithins A-C" by Prattya Nealmongkol, Kassrin Tangdenpaisal, Somkid Sittimonchai, Somsak Ruchirawat, and Nopporn Thasana for your consideration for publication in Tetrahedron as a full paper.

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The paper reports a benign synthesis of benzopyranone derivatives using copper-mediated C-O bond formation in subcritical water. Eight benzopyranone derivatives were synthesized. This methodology was further applied to synthesize the intestinal microbial metabolites, urolithins A, B, and C which were found to exhibit potent antioxidant activity.

The green method developed and reported here should be applicable to the synthesis of benzopyranone and other heterocyclic systems in "Green Chemistry" aspect and in the general synthesis of natural products and other biologically active compounds in particular. With the environmental consideration, the successful use of water, the ideal green solvent, in the key lactone formation further adds the merit of the protocol. The paper should draw interests of a significant portion of organic chemists. Further details of this paper were also submitted as Supporting Information.

Your kind consideration will be gratefully acknowledged.

Yours sincerely

Nopporn Thasana, Ph.D.

## Graphical Abstract

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**Cu(I)-mediated lactone formation in subcritical water: A benign synthesis of benzopyranones and urolithins A-C**

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Prattya Nealmongkol,[a] Kassrin Tangdenpaisal,[b] Somkid Sitthimonchai,[c] Somsak Ruchirawat[a,b,d] and Nopporn Thasana[a,b,d,*]

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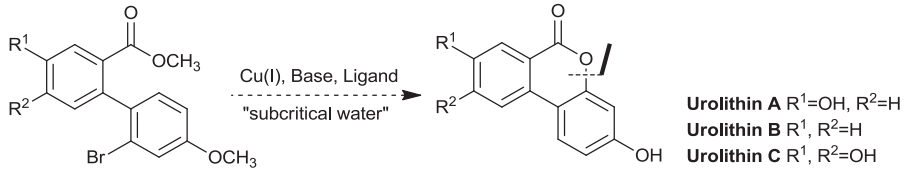

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# Cu(I)-mediated lactone formation in subcritical water: A benign synthesis of benzopyranones and urolithins A-C

Prattya Nealmongko[a] , Kassrin Tangdenpaisal[b], Somkid Sitthimonchai[c], Somsak Ruchirawat[a,b,d] and Nopporn Thasana[a,b,d,] *

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ABSTRACT

Benzopyranones were successfully synthesized using Cu(I)-mediated C-O bond formation in subcritical water. A number of benzopyranone derivatives including polymethoxy benzopyranones, benzopyranopyridones, chromenoindolones and furochromenones were synthesized in satisfactory yield. This methodology was further applied to synthesize the intestinal microbial metabolites, urolithins A, B, and C which were found to exhibit potent antioxidant activity.



## 1. Introduction

Recently, aqueous and microwave conditions have attracted both academic and industrial interests as an economical and environmentally friendly processes.[1-3] Water showed an important role as a reaction medium in organometallic reactions including Suzuki-Miyaura,[4,5] Negishi,[6] Stille,[7] and Sonogashira[8,9] cross-couplings. Because of its beneficial properties such as inexpensiveness, environmentally friendliness, non-inflammability, and safety, the role of water in research works has gained growing interest during the past decade.[10,11] Moreover, water has the dielectric constant ($\acute{\varepsilon}$) higher than other organic solvents at room temperature which can effectively absorb microwave energy and acts as pseudo-organic solvent at high temperature.[12] Apart from the superheated condition of water (>100°C), supercritical water (SCW, >374°C) has been widely studied in several fields[13-15] but some limitations retard its utilization due to its degenerative properties.[16] On the other hands, subcritical water (near-critical water, NCW), generated between 150 and 300°C, is reliable to use under a milder condition and is still able to maintain its pseudo-organic solvent properties.[17,18]

Benzopyranone **1** is the structural motif of various natural oxygen heterocycles which typically consist of dibenzo[*d*,*b*]pyran-6-one or 6*H*-benzo[*c*]chromen-6-one and these lactone containing natural products as shown in Figure 1 have been isolated from various sources. Urolithins A-C (**2a-c**), the intestinal microbial metabolites produced by *in vitro* fermentation of punicalagins, shows the antioxidant activity.[19,20] They also showed colon cancer chemopreventive activities by inhibiting TCDD-induced CYP1-mediated EROD activity.[21] Alternariol (**3**), a metabolite of toxin-producing *Alternaria* fungi, has been found to be the natural food contaminant in various grains, crops and decayed fruits.[22,23] TMC-264 (**4**), isolated from the fermentation broth of a fungus *Phoma* sp. TC 1674, displays potent inhibitory activity against tyrosine phosphorylation of STAT6.[24] Neo-tranchinlactone (**5**), isolated from *Salvia miltiorrhiza* and first synthesized by Lee,[25] shows potent and selective anti-breast cancer activity.[26] The complex benzopyranone lamellarin D (**6**), isolated from a marine organism,[27] displays potent cytotoxic activity against multidrug-resistant tumour cell lines and is highly cytotoxic to prostate-cancer cell lines.[28-30] Due to the potential uses as pharmacologically active compounds, benzopyranones have attracted much interest from various groups including ours.[31] Adhering to the economical aspect and "Green Chemistry" concept, in this study we report a short synthesis of benzopyranones using Cu(I)-mediated $C\text{-}O_{carboxylate}$ bond

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formation in subcritical water. Our protocol was also extended to synthesize the antioxidant benzopyranones, urolithins A (**2a**), B (**2b**), and C (**2c**).[1]

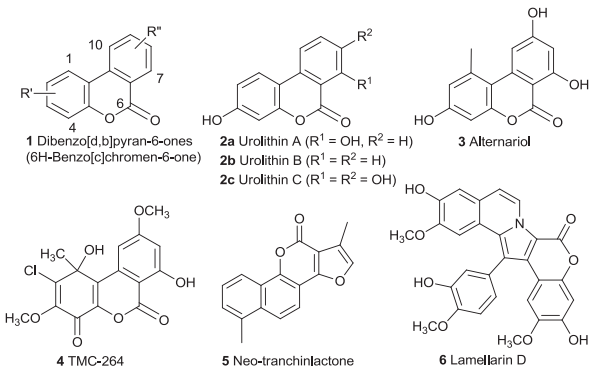



**Figure 1.** Various natural lactone containing heterocycles.

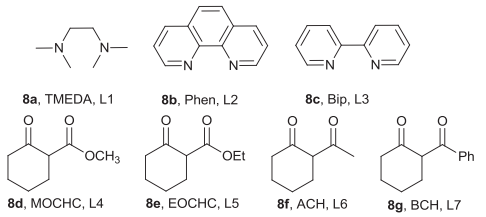


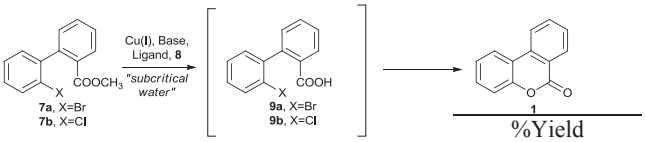

**Figure 2.** Ligands under screening.

## 2. Results and Discussion

To delve deeper and intensify our research in this direction, Cu(I)-catalyzed/microwave-assisted C-$O_{carboxylate}$ bond formation of 2-halobiarylcarboxylates **7a** and b was studied by varying the types of bidentate ligands **8** in subcritical water under the basic conditions. A set of bidentate ligands[32] (Figure 2), including N,N,N',N'-tetramethylethylenediamine (**8a**, TMEDA, L1), phenanthroline (**8b**, Phen, L2), bipyridine (**8c**, Bip, L3), methyl 2-oxocyclohexanecarboxylate (**8d**, MOCHC, L4), ethyl 2-oxocyclohexanecarboxylate (**8e**, EOCHC, L5), 2-acetyl-cyclohexanone (**8f**, ACH, L6), and 2-benzoylcyclohexanone (**8g**, BCH, L7), was examined together with sub-stoichiometric amount, 50 mol% of CuI or CuTC as catalysts and $Cs_2CO_3$ or $K_2CO_3$ as bases in subcritical water using microwave irradiation.

Interestingly, increasing the temperature to 300 $^oC$ for 10 min with $Cs_2CO_3$ and ligand L1, the lactone product (**1**) was obtained in excellent yield (Table 1, entry 9). Various bidentate ligands, L2-L7 (**8b-8g**) were then studied using these optimized conditions to give product **1** in poor to good yields (15 to 78%) (Table 1, entries 10 to 15). From these experiments, the effectiveness of various ligands for the lactone formation was found to be as followed: TMEDA, L1 > Bip, L3 > BCH, L7 > Phen, L2 > ACH, L6 > EOCHC, L5 > EOCHC, L4. The relative effectiveness of the ligands agrees well with the previous report by Buchwald.[33] The cu(I)-bidentate ligand L1 complex (LCu-X) provided the more electrophilicity than the co-ordination with β-diketone or β-keto ester ligands on the Cu(I) center, Which favored the more nucleophilic attraction to complex LCu-X. This mechanistic study also suggested that the bidentate ligand L1 was more suitable to furnish C-O bond than β-diketone or β-keto ester ligands.[33] This similar trend was also observed the comparison between bidentate and β-diketone or β-keto ester ligands (Table 1, entries 9 to 15). The C-$O_{carboxylate}$ bond formation was decreased when $K_2CO_3$ was used as a base instead of $Cs_2CO_3$ (Table 1, entries 16 to 18). The decreasing of CuTC to 25 mol% the lower yield of the lactone formation was obtained in 67% yield (Table 1, entry 19). This may support that the sub-stoichiometric amount was the suitable condition for our investigation. The lactone product was also furnished in low yield under refluxing condition (Table 1, entry 20).

**Table 1.** Synthesis of 5-amido-8,9-dimethoxy-6-aryl-2,3-dihydrobenzo[*d*]azocin-4-ones **1**.[a]

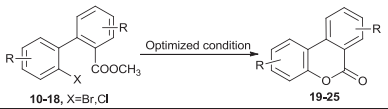


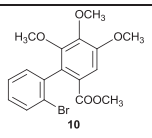
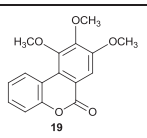
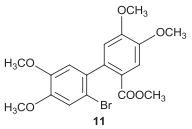
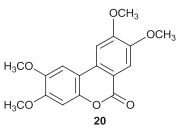
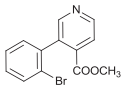
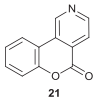
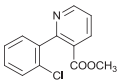
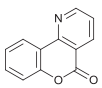
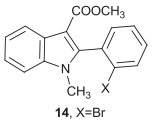
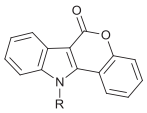
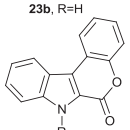
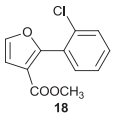
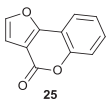

| Entry | X | Cu(I) | Condition[b] | Base/ Ligand, **8**[c] | **1** | **7** | **9** |
|---|---|---|---|---|---|---|---|
| 1 | Br | CuTC | A | - | 19 | 62 | - |
| 2 | Br | CuI | A | - | 8 | 56 | - |
| 3 | Br | CuTC[d] | B | - | 14 | 56 | - |
| 4 | Br | CuI[d] | B | - | 10 | 18 | - |
| 5 | Br | CuTC | B | $Cs_2CO_3$/**8a** | 60 | - | - |
| 6 | Br | CuI | B | $Cs_2CO_3$/**8a** | 57 | - | - |
| 7 | Cl | CuTC | B | $Cs_2CO_3$/**8a** | 16 | - | 53 |
| 8 | Cl | CuI | B | $Cs_2CO_3$/**8a** | 17 | - | 39 |
| **9** | **Br** | **CuTC** | **C** | **$Cs_2CO_3$/8a** | **99** | - | - |
| 10 | Br | CuTC | C | $Cs_2CO_3$/**8b** | 47 | - | - |
| 11 | Br | CuTC | C | $Cs_2CO_3$/**8c** | 78 | - | - |
| 12 | Br | CuTC | C | $Cs_2CO_3$/**8d** | 15 | - | 23 |
| 13 | Br | CuTC | C | $Cs_2CO_3$/**8e** | 30 | 19 | 24 |
| 14 | Br | CuTC | C | $Cs_2CO_3$/**8f** | 43 | 24 | 33 |
| 15 | Br | CuTC | C | $Cs_2CO_3$/**8g** | 60 | - | 40 |
| 16 | Br | CuTC | C | $K_2CO_3$/**8a** | 62 | - | - |
| 17 | Br | CuTC | C | $K_2CO_3$/**8b** | 36 | - | - |
| 18 | Br | CuTC | C | $K_2CO_3$/**8c** | 73 | - | - |
| 19 | Br | CuTC[e] | C | $Cs_2CO_3$/**8a** | 67 | 2 | 8 |
| 20 | Br | CuTC | D | $Cs_2CO_3$/**8a** | 29 | - | 11 |

[a] Unless otherwise noted, the reactions were performed in 10 mL microwave vessel, compound **7** (0.2 mmol), Cu(I) salts (0.1 mmol) in water (1 mL); [b] condition A: 200 Watt, 200 $^oC$, 100 psi, 20 min; condition B: 300 Watt, 250 $^oC$, 280 psi, 20 min; condition C: 300 Watt, 300 $^oC$, 250 psi, 10 min; condition D: refluxing condition up to 24 h; [c] Ligands under screening are shown in Figure 2; [d] Cu(I) salts (2 equiv) CuTC = copper(I) thiophene carboxylate; [d], [e] CuTC (0.25 equiv) was used.

In the above screening, we found that the best conditions to form the C-$O_{carboxylate}$ bond of benzopyranone was the use of TMEDA as ligand in the presence of $Cs_2CO_3$ in subcritical water at 300 $^oC$. Various biaryl carboxylates **10**-**18** were prepared using the Suzuki-Miyaura coupling reaction.[31a] With these starting materials in hands, we then studied the Cu(I)-mediated C-$O_{carboxylate}$ lactone formation of benzopyranones **19**-**25** as shown in Table 2. The polyoxygenated benzopyranones **19** and **20** were synthesized from tri- and tetra-methoxybiaryl carboxylates **10** and **11**, respectively, in moderate yields (Table 2, entries 1 and 2).[31a] In these reactions, the reaction time was increased to 20 min. The presence of electron-donating groups may play the important role in the C-O bond forming step and gave lower yield of the products.

**Table 2.** Synthesis of the benzopyranone derivatives using the optimized conditions.[a]

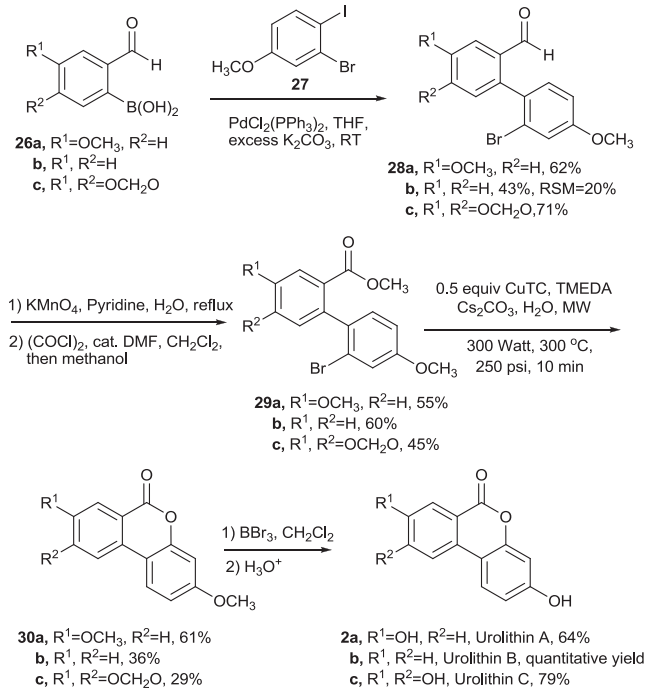



| Entry | Substrate | Product | Yield (%)[b] |
|---|---|---|---|
| 1 |  **10** |  **19** | 39[c] |
| 2 |  **11** |  **20** | 31[c] |
| 3 |  **12** |  **21** | 46 |
| 4 |  **13** |  **22** | 66 |
| 5 |  **14**, X=Br **15**, X=Cl |  **23a**, R=Me **23b**, R=H | 66, 10[d] |
| 6 | | | 73[e] |
| 7 | |  **24a**, R=Me **24b**, R=H | 45, 11[f] |
| 8 | | | 47, 4[g] |
| 9 |  **18** |  **25** | 30 |

[a] *Reaction conditions*: biaryl ester (0.2 mmol), CuTC (0.5 equiv), $Cs_2CO_3$ (0.5 equiv), TMEDA (1.0 equiv) in water (1 mL), MW (300 Watt), 300 °C, 250 psi, 10 min; [b] Isolated yields; [c] Reaction time 20 min.; [d] %yield of **23a** and **23b** from **14**; [e] % yield of **23a** from **15**; [f] % yield of **24a** and **24b** from **16**; [g] % yield of **24a** and **24b** from **17**.

In order to demonstrate the versatility of our strategy in the synthesis of benzopyranone derivatives, we then directed our attention to synthesize the chromenopyridinones **21** and **22**.[34] 3-Arylisonicotinate **12** and 2-arylnicotinate **13** were prepared from the corresponding pyridine derivatives using Suzuki-Miyaura cross-coupling reaction with 2-haloarylboronic acids as previously reported.[31a] The chromenopyridinones **21** and **22** were obtained in moderate yields (46-66%) (Table 2, entries 3 and 4). Fused heteroaromatic rings, tetracyclic chromenoindolones **23a** and **23b** were prepared from the corresponding 2-arylindole-3-carboxylates **14** (X = Br) or **15** (X = Cl).[31a,35] Compound **24a** and **24b** could also be obtained under the same conditions from 3-arylindole-2-carboxylates **16** (X = Br) or **17** (X = Cl).[31a,35] The reaction of 2-(2-bromophenyl)indole-3-carboxylate **14** gave a mixture of the lactone products, *N*-methyl chromenoindolone **23a** (66%) together with the demethylated chromenoindolone **23b** (10%) (Table 2, entry 5). In contrast, the reaction of 2-(2-chlorophenyl)indole-3-carboxylate **15** gave only *N*-methyl chromenoindolone **23a** in good yield (73%) (Table 2, entry 6). When 3- arylindole-2-carboxylates **16** and **17** were employed, both compounds gave a mixture of *N*-methyl chromenoindolone **24a** and demethylated chromenoindolone **24b** (Table 2, entries 7 and 8). The benzopyranone derivative, furochromenone **25**[36-39] was also successfully prepared from 2-arylfuran-3-carboxylate **18** in 30% yield (Table 2, entry 9).

The utility of this method was further demonstrated with the synthesis of natural benzopyranones urolithins A-C (**2a-c**).[19] Our synthetic route was performed using the same sequence of reactions requiring five steps from commercially available boronic acids **26a-c** as shown in Scheme 1. The reaction of boronic acids **26a-c** and 2-bromo-1-iodo-4-methoxybenzene **27** was performed using $PdCl_2(PPh_3)_2$ under basic conditions in THF at room temperature to afford the desired 2'-bromo-4'-methoxy-(1,1'-biphenyl)-2-carbaldehydes **28a-c** in moderate to good yields (43-71%). 2-Bromo-1-iodo-4-methoxybenzene **27** was prepared from the selective *para*-iodination of 3-bromoanisole.[40] The transformation of carbaldehydes **28a-c** to biaryl esters **29a-c** was conducted by oxidation followed by esterification. Treatment of biaryl esters **29a-c** with 0.5 equiv of CuTC in the presence of TMEDA and $Cs_2CO_3$ in subcritical water for 10 min furnished the methyl ether of urolithins A-C **(30a-c)** which were further demethylated with $BBr_3$ in dichlomethane at 0 °C. These conditions gave urolithins A-C (**2a-c**) in 15%, 9%, and 7% overall yield respectively over 4 steps.




**Scheme 1.** Synthesis of urolithins A-C.

The antioxidant radical-scavenging of urolithins A-C and cytotoxic activities are summarized in Table 3. Urolithin C (**2c**) scavenged 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical with $IC_{50}$ value of 12.6±0.9 μM which is better than the reference compound, ascorbic acid at 21.2 μM. Superoxide anion radical formation in the xanthine/xanthine oxidase (XXO) and xanthine oxidase (IXO) assays was not inhibited by urolithins A-C. Compounds **2a-c** inhibited aromatase activity (AIA) with $IC_{50}$ values of 13.2±1.4, 11.9±1.0, and 21.6±0.6 μM, respectively.

**Table 3.** Radical scavenging, antioxidant, and aromatase inhibitory activities of synthetic urolithins A-C[a]

| Urolithin | $IC_{50}$ [μM] | | | | unit |
|---|---|---|---|---|---|
| | DPPH | IXO | AIA | HL-60[b] | ORAC[c] |
| A | >250 | 157.6±14.5 | 13.2±1.4 | >100 | 3.0±0.5 |
| B | >250 | 334.3±1.8 | 11.9±1.0 | 86.8±13.6 | 3.0±0.7 |
| C | 12.6± 0.9 | 165.0±3.6 | 21.6±0.6 | >100 | 5.5±0.5 |

[a] Positive controls for each assay are as follows: DPPH: ascorbic acid ($IC_{50}$ = 21.2 μM); IXO: allopurinol ($IC_{50}$=3.0 μM); aromatase inhibition (AIA): ketoconazole ($IC_{50}$=2.4 μM). [b] HL-60: Human Promyelocytic Leukemia cells. [c] The results are expressed as ORAC units: 1 ORAC unit equals the net protection of β-phytoerythrin produced by 1 μM of Trolox.

They also exhibited the potent antioxidant activity in oxygen radical absorbance capacity (ORAC) assay with 3.0±0.5, 3.0±0.7 and 5.5±0.5 ORAC units respectively (Table 3).[41] The urolithin B can also inhibit the proliferation of leukemia cell with the $IC_{50}$ value of 86.8± μM.

## 3. Conclusion

In summary, we have reported the synthesis of various benzopyranones and urolithins A-C (**2a-c**) based on the green chemistry principle using the one-pot lactone formation from biaryl esters under microwave irradiation with the catalytic amount of CuTC. With the environmental consideration, the successful use of water, the ideal green solvent, in the key lactone formation further adds the merit of the protocol. The biological activity evaluation of urolithins A-C (**2a-c**) showed the potent antioxidant activity in ORAC assay while urolithin C (**2c**) exhibited the radical-scavenging activity.

## 4. Experimental Section

### 4.1. General Methods

Microwave reaction was performed in CEM Discover. Melting points were measured using a Thermo Fisher Scientific IA920 digital melting point instrument which was reported without correction. $^1$H-NMR spectra were recorded on Bruker AV-300 (300 MHz), Bruker AV-400 (400 MHz) and Varian Germini2000 (200 MHz) using the deuterochloroform as solvent with tetramethylsilane as an internal standard and dimethylsulfoxide-$d_6$ for some compounds. $^{13}$C-NMR spectra were recorded on Bruker AV-300 (75 MHz), Bruker AV-400 (100 MHz) and Varian Germini2000 (50 MHz) using the deuterochloroform as solvent with tetramethylsilane as an internal standard and dimethylsulfoxide-$d_6$ for some compounds. Infrared spectra (IR) were obtained on Perkin Elmer System 2000FT-IR and JASCO A-302 spectrometers. Mass Spectrometry was performed with an AEI-MS-902. High Resolution Mass Spectrometry was performed with a MicroTOFLC, Bruker Daltonics. Column chromatography was carried out using Fluka aluminum oxide (type 507 C neutral; 100-125 mesh) and Merck silica gel (70-230 mesh ASTM). Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were carried out on silica gel (E. Merck PF 254). All reagents were purified and dried according to the standard procedures. Solvents were removed using Eyela Aspirator A-2S and Büchi Rotavapor R110. All products were evacuated by a Christ Freeze Dryer Unit Alpha l/6, to remove the last traces of solvents.

### 4.2. General Procedure for the Preparation of Biarylcarboxylate Esters (7a, 7b, and 10–18)

A solution of 2-halophenylboronic acid (2.0 equiv), 2-halophenylcarboxylate (1.0 equiv) and 10 mol% $Pd(PPh_3)_4$ in three portion of toluene:EtOH:10%$Na_2CO_3$ (5:1:2) solution was refluxed overnight. After the complete reaction was observed by TLC, water was added to quench and partition with EtOAc to obtain the crude product. The crude product was then purified by flash column chromatography or the preparative thin layer chromatography (EtOAc/Hexane) to obtain the product. Compounds **7a, 7b, 10, 11,** and **14–17** were previously reported in the literature.[31a]

*4.2.1 Methyl 3-(2-bromophenyl)isonicotinate (**12**).* Yellow oil (143.0 mg, 68%). $R_f$ 0.43 (30% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2951, 1734, 1434, 1273, 1105, 756 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.59 (s, 1H), 7.89 (d, *J* = 4.5 Hz, 1H), 7.84 (d, *J* = 4.8 Hz, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.41 (t, *J* = 7.5 Hz, 1H), 7.31 – 7.25 (m, 2H), 3.72 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 165.6, 151.5, 149.5, 138.4, 137.1, 135.8, 132.2, 52.4, 130.2, 129.4, 127.1, 123.1, 122.7. EI-MS: *m/z* (%) 291 ($M+H^+$, 0) 212 (100), 197 (38), 126 (20). TOF-HRMS calcd for $C_{13}H_{11}BrNO_2$: 291.9967; found 291.9956, 293.9939.

*4.2.2 Methyl 2-(2-chlorophenyl)nicotinate (**13**).* Yellow oil (792.0 mg, quantitative yield). $R_f$ 0.14 (10% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2951, 1725, 1565, 1424, 1273, 754 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.81 (d, *J* = 3.9 Hz, 1H), 8.31 (d, *J* = 8.1 Hz, 1H), 7.43 – 7.34 (m, 5H), 3.70 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 166.2, 157.4, 151.8, 139.5, 138.0, 132.1, 130.0, 129.3, 128.8, 126.8, 126.6, 122.4, 52.3. EI-MS: *m/z* (%) 247 ($M^+$, 0), 212 (100), 197 (32). TOF-HRMS calcd for $C_{13}H_{11}ClNO_2$: 248.0472; found 248.0474, 250.0456.

*4.2.3 Methyl 2-(2-chlorophenyl)furan-3-carboxylate (**18**).* Yellow solid (92.0 mg, 80%). $R_f$ 0.34 (20% EtOAc Hexane). Mp: 61 °C. IR (UATR): $\nu_{max}$ 2952, 1720, 1619, 1470, 1439, 1299, 755 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.40 – 7.38 (m, 2H), 7.48 – 7.46 (m, 3H), 6.85 (s, 1H), 3.72 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 163.3, 155.0, 142.2, 134.1, 131.9, 130.6, 129.6, 129.3, 126.1, 116.2, 111.3, 51.4. EI-MS: *m/z* (%) 236 ($M^+$, 3), 201 (100), 186 (35), 170 (8). TOF-HRMS calcd for $C_{12}H_{10}ClO_3$: 237.0313; found 237.0315, 239.0284.

### 4.3 General Procedure for the Preparation of Benzopyranones (19–25, and 30a–c)

In a 10 mL microwave vessel, a mixture of methyl 2-halobiarylcarboxylate ester (0.2 mmol, 1.0 equiv), CuTC (0.1 mmol, 0.5 equiv), $Cs_2CO_3$ (0.1 mmol, 0.5 eqiuv) in deionized water (2 mL) was added with TMEDA (0.2 mmol, 1.0 eqiuv) via microsyinge. The mixture was allowed to stir at room temperature for 15 min and then placed into the microwave instrument. The reaction was then irradiated based on conditions appropriate for each reaction (see Table 1.) and reactions followed by TLC. After completion, the suspension was filtered through silica gel and washed with EtOAc (4 x 25 mL). The solvent was removed under reduced pressure to give a pale yellow solid which was then purified by PTLC (EtOAc/Hexane) to give the product. Compounds **1**, **19**, **20**, **23**, and **24** were previously reported in the literature.[34]

*4.3.1 5H-chromeno[4,3-c]pyridin-5-one (**21**).* White solid (15.3 mg, 46%). $R_f$ 0.34 (20% EtOAc/Hexane). Mp: 159 °C. IR (UATR): $\nu_{max}$ 1735, 1609, 1411, 1277, 1240, 1086, 757 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 9.57 (s, 1H), 8.87 (d, *J* = 5.1 Hz, 1H), 8.19 (d, *J* = 9.6 Hz, 1H), 8.17 (d, *J* = 7.2 Hz, 1H), 7.56 (t, *J* = 7.3 Hz, 1H), 7.44 – 7.39 (m, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 159.7, 151.6, 149.4, 145.4, 131.4, 128.5, 126.9, 125.2, 122.4, 122.0, 118.0, 115.7. EI-MS: *m/z* (%) 197 ($M^+$, 100), 169 (42), 142 (13), 114 (20). TOF-HRMS calcd for $C_{12}H_8NO_2$: 198.0549; found 198.0543.

*4.3.2 5H-chromeno[4,3-b]pyridin-5-one (**22**).* Yellow solid (49.3 mg, 62%). $R_f$ 0.40 (20% EtOAc/Hexane). Mp: 146 °C. IR (UATR): $\nu_{max}$ 1736, 1727, 1602, 1449, 764 cm$^{-1}$. $^1$H NMR (300 MHz,

$CDCl_3$): δ 9.02 (dd, *J* = 4.6, 1.95 Hz, 1H), 8.62 (dd, *J* = 8.1, 1.8 Hz, 1H), 8.58 (dd, *J* = 7.9, 1.5 1H), 7.59 (td, *J* = 8.2, 1.5 Hz, 1H), 7.52 (dd, *J* = 7.9, 4.5 Hz, 1H), 7.40 (td, *J* = 9.0, 1.2 Hz, 1H), 7.38 (d, *J* = 8.7 Hz, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 161.1, 155.6, 152.5, 151.8, 138.1, 132.2 , 124.9, 124.6, 123.7, 119.2, 117.3,117.1. EI-MS: *m/z* (%) 197 ($M^+$, 100), 169 (39), 140 (17), 114 (15), 70 (11). TOF-HRMS calcd for $C_{12}H_8NO_2$: 198.0549; found 198.0545.

*4.3.3 4H-furo[3,2-c]chromen-4-one (**25**).* Brown solid (20.1 mg, 30%): $R_f$ 0.14 (10% EtOAc/Hexane). Mp: 171 °C. IR (UATR): $\nu_{max}$ 1686, 1483, 1313, 1146, 753 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.50 (d, *J* = 1.5 Hz, 1H), 7.48 – 7.29 (m. 4H), 6.87 (d, *J* = 1.5 Hz, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 167.8, 156.3, 142.5, 134.3, 132.1, 130.9, 129.7, 129.2, 128.6, 128.1, 126.2, 111.6. EI-MS: *m/z* (%): 187 ($M+H^+$, 100), 170 (2), 149 (6), 131 (5), 115 (14). TOF-HRMS calcd for $C_{11}H_7O_3$: 187.0389; found 187.0385.

*4.3.4 3,8-Dimethoxy-6H-benzo[c]chromen-6-one (**30a**).* Yellow solid (37.5 mg, 61%). $R_f$ 0.43 (50% $CH_2Cl_2$/Hexane). Mp: 124 °C. IR (UATR): $\nu_{max}$ 2924, 2846, 1732, 1623, 1491, 1319, 1295 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.90 (d, *J* = 9.0 Hz, 1H), 7.85 (d, *J* = 8.7 Hz, 1H), 7.75 (d, *J* = 2.7 Hz, 1H), 7.36 (dd, *J* = 8.8, 3.0 Hz, 1H), 6.90 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.85 (d, *J* = 2.4 Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 161.5, 160.6, 159.0, 151.5, 128.5, 124.3, 122.7, 120.9, 112.3, 111.2, 110.9, 101.4, 55.6 (2C). EI-MS: *m/z* (%) 256 ($M^+$, 23), 241 (15), 178 (20), 149 (160). TOF-HRMS calcd for $C_{15}H_{13}O_4$: 257.0808; found 257.0811.

*4.3.5 3-Methoxy-6H-benzo[c]chromen-6-one (**30b**).* Yellow solid (3.3 mg, 36%): $R_f$ 0.46 (10% EtOAc/Hexane). Mp: 107 °C. IR(UATR): $\nu_{max}$ 2924, 2846, 1732, 1623, 1491, 1319, 1295 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 8.36 (d, *J* = 8.1 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.95 (d, *J* = 8.1 Hz, 1H), 7.79 (t, *J* = 7.8 Hz, 1H), 7.51 (t, *J* = 8.1 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 6.88 (s, 1H), 3.89 (s, 3H). $^{13}$C NMR (75 MHz, $CDCl_3$): δ 161.5, 135.1, 134.8, 130.5, 127.7, 124.4, 123.9, 123.7, 121.0, 119.1, 112.4, 111.1, 101.6, 55.6. EI-MS: *m/z* (%) 256 ($M^+$, 23), 241 (14), 178 (20), 149 (16). TOF-HRMS calcd for $C_{13}H_9O_3$: 213.0546; found 213.0554.

*4.3.6 3-Methoxy-6H-[1,3]dioxolo[4',5':4,5]benzo[1,2-c]chromen-6-one (**30c**).* Brown solid (2.3 mg, 29%): $R_f$ 0.43 (50% $CH_2Cl_2$/Hexane). Mp: 118 °C. IR (UATR): $\nu_{max}$ 2921, 2849, 2155, 1716, 1612, 1480, 1270, 1034, 935 cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): δ 8.19 (d, *J* = 8.4 Hz, 1H), 7.91 (s, 1H), 7.53 (s, 1H), 6.989 – 6.952 (m, 2H), 6.24 (s, 2H), 3.84 (s, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ 161.2, 154.6, 151.9, 148.3, 132.9, 130.0, 125.0, 113.4, 112.7, 111.9, 107.4, 103.01, 101.6, 101.5. EI-MS: *m/z* (%) 271 ($M+H^+$, 100), 256 (53), 241 (29), 128 (12). TOF-HRMS calcd for $C_{15}H_{11}O_5$: 271.0610; found 271.0603.

### 4.4 General Procedure for the Preparation of 2-Bromo-1-iodo-4-methoxybenzene (27)

A mixture of 3-bromophenol (0.53 mmol, 1.0 equiv), $I_2$ (0.53 mmol, 1.0 eqiuv) and $CF_3COOAg$ (0.80 mmol, 1.5 equiv) in $CHCl_3$ (5 mL) was stirred under Ar atmosphere for 3 h. The reaction was quenched with saturated $Na_2S_2O_5$ and extracted with $CH_2Cl_2$ (3 x 25 mL). The solvent was then removed by rotary evaporation to give the brown crude oil. The crude product was purified by flash column chromatography (Hexane 100%) to obtain product as pink oil (57.6 mg, 35%): $R_f$ 0.512 (20% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2975, 1578, 1460, 1283, 1223, 1032, 840 cm$^{-1}$. $^1$H NMR (300 MHz, $CDCl_3$): δ 7.69 (d, *J* = 8.7 Hz, 1H), 7.20 (d, *J* = 2.4 Hz, 1H), 6.60 (dd, *J* = 8.7, 2.1 Hz, 1H), 3.78 (s, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): δ 160.1, 140.1, 129.8, 118.3, 115.3, 89.4, 55.5. EI-MS: *m/z* (%) 312 ($M+H^+$, 87), 297 (17), 172 (30). TOF-HRMS calcd for $C_7H_6BrIO$: 311.8641; found 311.8645, 313.8622.

### 4.5 General Procedure for the Preparation of Biarylcarboxaldehyde (28a–c)

The mixture of 2-formylphenyl boronic acid **26a–c** (1.0 equiv), 2-bromo-1-iodo-4-methoxybenzene **27** (2.0 equiv) and 5 mol% $PdCl_2(PPh_3)_2$ in solution of THF was stirred under Ar atmosphere at room temperature. The solution of 2N $K_2CO_3$ was then transferred into the reaction via syringe until the reaction turned a brown-red solution. The reaction was allowed to stir at room temperature overnight. After checking by TLC, the reaction was quenched with water and partition with EtOAc to obtain the dark-brown crude oil. The crude product was purified by flash chromatography or preparative thin layer chromatography (EtOAc/Hexane) to obtain the product.

*4.5.1 2'-Bromo-4,4'-dimethoxy-[1,1'-biphenyl]-2-carbaldehyde (**28a**).* Yellow solid (32.0 mg, 62%): $R_f$ 0.40 (5% EtOAc/Hexane). Mp: 104 °C. IR (UATR): $\nu_{max}$ 2916, 2848, 2344, 1688, 1601, 1481 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$): δ 9.75 (s, 1H), 7.50 (d, *J* = 2.5, 1H), 7.19 – 7.25 (m, 4H), 6.94 (dd, *J* = 8.4, 2.5 Hz, 1H), 3.90 (s, 3H), 3.86 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 191.5, 159.9, 159.4, 137.3, 134.9, 132.4, 132.3, 130.5, 124.6, 121.0, 117.8, 113.4, 109.7, 55.6, 55.5. EI-MS: *m/z* (%) 230 ($M^+$, 1), 241 (100), 198 (28), 121 (14). TOF-HRMS calcd for $C_{15}H_{14}BrO_3$: 321.0120; found 321.0132, 323.0117.

*4.5.2 2'-Bromo-4'-methoxy-[1,1'-biphenyl]-2-carbaldehyde (**28b**).* Pink oil (20.1 mg, 43%): $R_f$ 0.26 (5% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2838, 2933, 1693, 1597, 1221, 1033, 776 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$): δ 9.81 (s, 1H), 8.02 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.64 (td, *J* = 7.5, 1.4 Hz, 1H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.32 (dd, *J* = 7.5, 0.6 Hz, 1H), 7.24 (d, *J* = 0.99, 1H), 7.22 (d, *J* = 4.62, 1H), 6.96 (dd, *J* = 8.4, 2.5 Hz, 1H), 3.86 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 191.6, 160.0, 55.6, 144.2, 133.9, 133.4, 132.0, 131.2, 130.8, 128.2, 126.9, 124.0, 117.8, 113.5. EI-MS: *m/z* (%) 290 ($M^+$, 0), 211 (100), 196 (11), 168 (25), 139 (30), 105 (6). TOF-HRMS calcd for $C_{14}H_{12}BrO_2$: 291.0015; found 291.0022, 293.0000.

*4.5.3 6-(2-Bromo-4-methoxyphenyl)benzo[d][1,3]dioxole-5-carbaldehyde (**28c**):* Brown oil (37.9 mg, 71%): $R_f$ 0.37 (5% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2909, 2849, 1680, 1602, 1476, 1235, 1034 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$): δ 9.55 (s, 1H), 7.44, (s, 1H), 7.21 (d, *J* = 2.4 Hz, 1H), 7.19 (d, *J* = 8.8 Hz, 1H), 6.92 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.20 (s, 2H), 3.85 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 189.9, 160.0, 151.9, 141.6, 132.2, 130.4, 124.3, 117.9, 114.4, 113.4, 110.7, 110.6, 105.9, 102.0, 55.6. EI-MS: *m/z* (%) 334 ($M+H^+$, 1), 255 (100), 212 (23), 197 (10), 154 (10). TOF-HRMS calcd for $C_{15}H_{12}BrO_4$: 334.9913; found 334.9911, 336.9891.

### 4.6 Genaeral Procedure for the Preparation of Biaryl Esters (29a–c)

To a solution of 2'-bromo-4'-methoxy-(1,1'-biphenyl)-2-carbaldehydes **28a–c** (1.0 equiv), pyridine (5 mL) in water (15 mL) was added $KMnO_4$ (2.0 equiv) at ambient atmosphere. The reaction was then refluxed and monitored by TLC until the starting material was completely oxidized. The reaction was cooled to room temperature and acidified with 2 N HCl to afford white solid precipitation. The white solid was recrystallized with EtOAc/hexane (1:4) to obtain the biaryl carboxylic acid. The obtained product was then placed into the round bottom flask and dissolved with $CH_2Cl_2$. To a precooled (0 °C) solution of carboxylic acid and DMF (3 drops) was added dropwise oxalyl chloride $(COCl)_2$ slowly. The reaction was stirred at the same temperature for 2 h, and then concentrated under reduced pressure to afford a yellow residue. The residue was then esterified with methanol (25 mL) at room temperature. The reaction was quenched with water and extracted with EtOAc (3 x 25 mL). The combined organic layer was washed with brine, dried with

anhydrous $Na_2SO_4$, and concentrated under reduced pressure to obtain the residue which was purified by flash column chromatography (5 – 95% EtOAc/Hexane) to give the biaryl esters.

*4.6.1 Methyl 2'-bromo-4,4'-dimethoxy-[1,1'-biphenyl]-2-carboxylate (**29a**).* Colorless oil (113.3 mg, 55%): $R_f$ 0.29 (20% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2949, 2837, 1727, 1601, 1479 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 7.53 (d, *J* = 2.4 Hz, 1H), 7.53 – 7.08 (m, 4H), 6.90 (dd, *J* = 8.4, 2.4 Hz, 1H), 3.90 (s, 3H), 3.84 (s, 3H), 3.70 (s, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 167.2, 158.9, 158.7, 134.5, 134.3, 132.5, 130.7, 123.6, 117.8, 117.3, 114.6, 113.0, 55.4 (2C), 52.1. EI-MS: *m/z* (%) 350 ($M^+$, 2), 271 (100), 256 (60), 241 (39), 225 (10), 187 (8). TOF-HRMS calcd for $C_{16}H_{16}BrO_4$: 351.0226; found 351.0230, 353.0212.

*4.6.2 Methyl 2'-bromo-4'-methoxy-[1,1'-biphenyl]-2-carboxylate (**29b**)* Yellow oil (18.6 mg, 60%): $R_f$ 0.34 (5% EtOAc/Hexane). IR (UATR): $\nu_{max}$ 2946, 2927, 1725, 1602, 1475, 1435, 1256, 1033 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 7.99 (dd. *J* = 9.0, 0.9 Hz, 1H), 7.53 (td, *J* = 7.5, 1.5 Hz, 1H), 7.43 (td, *J* = 7.6, 1.2 Hz, 1H), 7.23 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.17 (d, *J* = 2.7 Hz. 1H), 7.15 (d, *J* = 6.9 Hz, 1H), 7.11 (s, 1H), 6.89 (dd, *J* = 8.4, 2.7 Hz, 1H), 3.81 (s, 3H), 3.68 (s, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 167.2, 159.0, 141.8, 134.7, 131.6, 131.4, 130.4, 129.9, 127.6, 123.0, 117.3, 113.4, 112.9, 55.3, 51.9. EI-MS: *m/z* (%) 320 ($M^+$, 2), 241 (100), 226 (53), 139 (27). TOF-HRMS calcd for $C_{15}H_{14}BrO_3$: 321.0121; found 321.0119, 323.0111.

*4.6.3 Methyl 6-(2-bromo-4-methoxyphenyl)benzo[d][1,3]dioxole-5-carboxylate (**29c**).* Brown solid (18.2 mg, 45%): $R_f$ 0.31 (20% EtOAc/Hexane). Mp: 85°C. IR (UATR): $\nu_{max}$ 2949, 2901, 1724, 1601, 1478, 1240, 1032, 852 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): δ 7.48 (s, 1H), 7.17 (d, *J* = 2.7 Hz, 1H), 7.11 (d, *J* = 8.7 Hz, 1H), 6.89 (dd, *J* = 8.4, 2.7 Hz, 1H), 6.67 (s, 1H), 6.09 (s, 2H), 3.84 (s, 3H), 3.67 (s, 3H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 159.0, 150.2, 147.0, 138.1, 134.7, 130.7, 130.4, 123.7, 123.3, 117.3, 113.0, 111.4, 109.9, 102.0, 55.4, 51.9. EI-MS: *m/z* (%) 364 ($M^+$, 1), 285 (100), 270 (70), 255 (29), 143 (21). TOF-HRMS calcd for $C_{16}H_{11}BrO_5$: 365.0019; found 365.0021, 367.0007.

### 4.7 General Procedure for the Preparation of Urolithins a-c (2a–c).

A solution of methyl ether of urolithins A-C **30a–c** (1.0 equiv) was cooled at 0 °C under Ar atmosphere and added with $BBr_3$ slowly. Until the stating material was completely consumed by checked with TLC, the solution of 2N HCl was added to acidify and partitioned with EtOAc to give the crude product. The crude product was purified by size exclusion chromatography to obtain the product.

*4.7.1 3,8-Dihydroxy-6H-benzo[c]chromen-6-one (**2a**).* Brown solid (7.0 mg, 64%): $R_f$ 0.06 (20% EtOAc/Hexane). Mp: 331°C. IR (UATR): $\nu_{max}$ 3332, 3140, 1703, 1614, 1458, 1273 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): δ 10.20 (bs, 2H), 8.10 (d, *J* = 9.0 Hz, 1H), 8.01 (d, *J* = 8.7 Hz, 1H), 7.50 (d, *J* = 2.7 Hz, 1H), 7.31 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.79 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.71 (d, *J* = 2.1, 1H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ 160.6, 159.8, 152.1, 135.3, 135.1, 129.7, 127.7, 124.8, 121.6, 118.9, 113.2, 109.4, 102.9. EI-MS: *m/z* (%) 228 ($M^+$, 100), 200 (13), 115 (20). TOF-HRMS calcd for $C_{13}H_9O_4$: 229.0495; found 229.0493.

*4.7.2 3-Hydroxy-6H-benzo[c]chromen-6-one (**2b**).* Brown solid (10.0 mg, quantitative yield): $R_f$ 0.14 (20% EtOAc/Hexane). Mp 207 °C. IR (UATR): $\nu_{max}$ 3254, 2919, 1691, 1625, 1608, 1315, 1276 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): δ 10.36 (s, 2H), 8.25 (d, *J* = 8.1 Hz, 1H), 8.18 (dd, *J* = 7.3, 1.2 Hz, 1H), 8.15 (d, *J* = 8.7 Hz, 1H), 7.88 (td, *J* = 7.0, 1.5 Hz, 1H), 7.57 (td, *J* = 7.6, 0.9 Hz, 1H), 6.85 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.75 (d, *J* = 2.4 Hz, 1H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): δ 160.6, 159.8, 152.1, 135.3, 135.1, 129.7, 124.8, 121.6, 118.9, 113.2, 109.4, 102.9. EI-MS: *m/z* (%) 212 ($M^+$, 100), 184 (21), 128 (17), 127 (15). TOF-HRMS calcd for $C_{13}H_9O_3$: 213.0546; found 213.0548.

*4.7.3 3,8,9-Trihydroxy-6H-benzo[c]chromen-6-one (**2c**).* Olive-green solid (4.0 mg, 79%): $R_f$ 0.028 (10% EtOAc/Hexane). Mp: >333 °C. IR (UATR): $\nu_{max}$ 3230, 1690, 1614, 1461, 1278 $cm^{-1}$. $^1H$ NMR (400 MHz, DMSO-$d_6$): δ 10.12 (bs, 3H), 7.85 (d, *J* = 8.7 Hz, 1H), 7.47 (s, 1H), 7.41 (s, 1H), 6.77 (dd, *J* = 8.2, *J* = 2.4 Hz, 1H), 6.67 (d, *J* = 2.4 Hz, 1H). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): δ 103.4, 107.2, 110.2, 111.3, 113.2, 114.6, 124.1, 129.6, 146.5, 151.8, 153.8, 159.0, 160.7. EI-MS: *m/z* (%) 224 ($M^+$, 100), 216 (11), 129 (17), 97 (34), 83 (40), 69 (55). TOF-HRMS calcd for $C_{13}H_9O_5$: 245.0444; found 245.0445.

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## Supplementary Material

Experimental procedures and spectral data for all new compounds. Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.tet.2013.xx.xxxx.

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# Synthesis and Biological Activities of Azalamellarins

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Azalamellarins, lactam analogues of biologically active lamellarins, were synthesized for the purpose of improving their inhibitory potencies.[1,2] Accomplishing the synthesis of phenanthridinone derivatives, which used copper(I)-mediated/microwave-assisted[3] $C_{aryl}$-$N_{amide}$ bond formation, provided an easy way to prepare azalamellarin derivatives. All synthetic azalamellarins that consist of azalamellarin D, *N*-allylazalamellarins and *N*-propylazalamellarin χ-D, L-N and J-dehydro J, were synthesized and evaluated for their cytotoxicities against a panel of four human tumor cell lines: HuCCA-1, A-549, HepG2, and MOLT-3. The results showed that certain azalamellarins exhibited promising activities in micromolar $IC_{50}$ value range comparable to their parent lamellarin analogues.[4]


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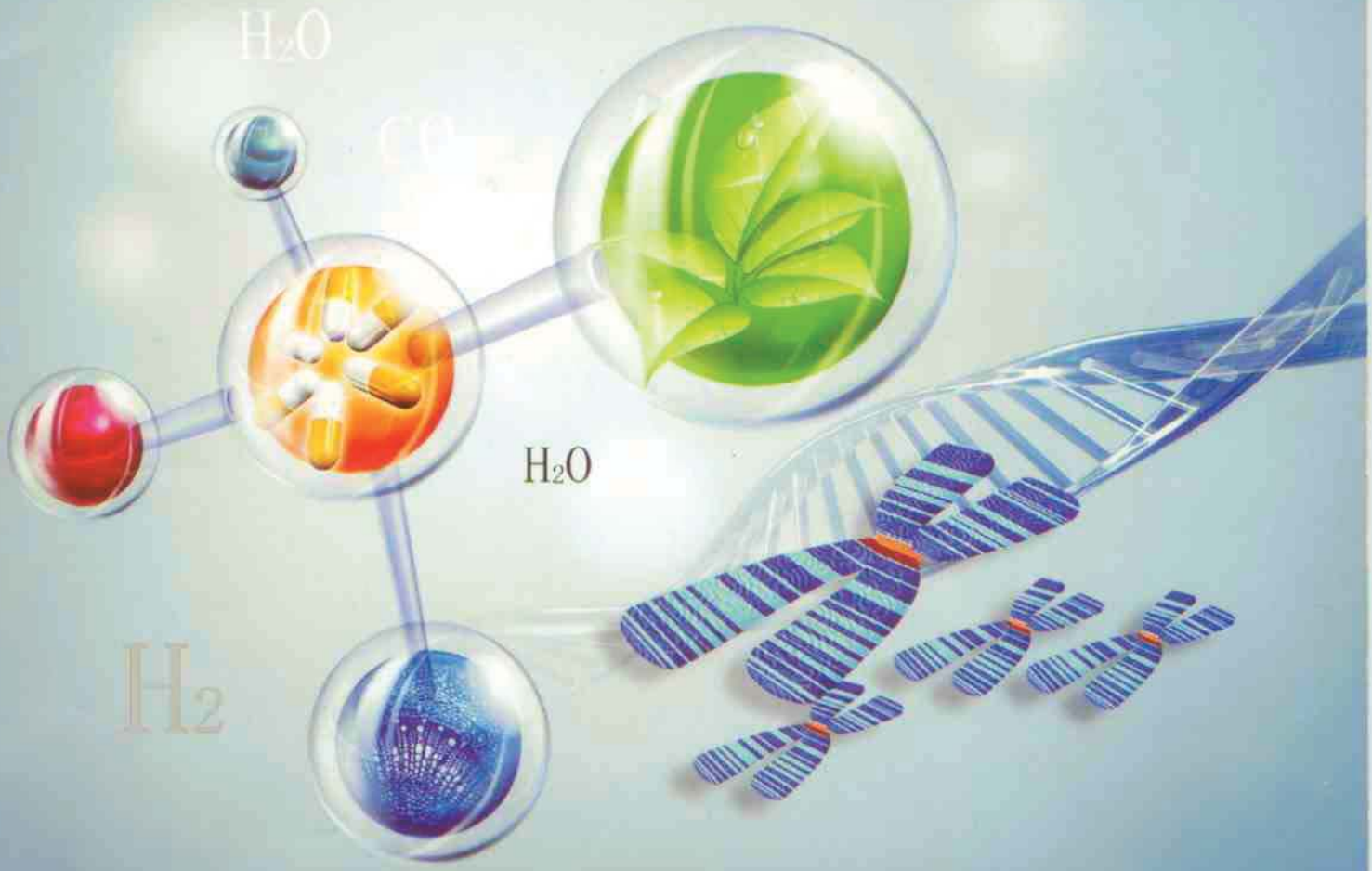


2010 11.5 - 7

理學院國際會議廳

International Conference Room, College of Science ,
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# Unsymmetrical Bioactive Benzils: Synthesis of Licoagrodione, Scandione, and Calophione A

Nopporn Thasana,* Rattana Worayuthakarn, Sasiwadee Boonya-udtayan, Somsak Ruchirawat

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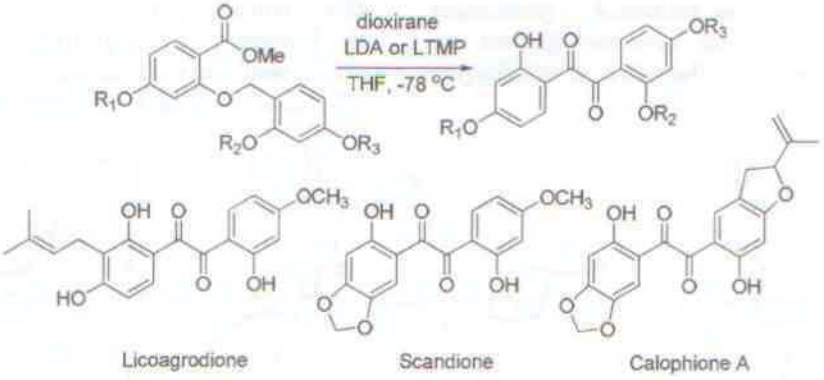
Licoagrodione, isolated from the hairy root cultures of Chinese herb, *Glycyrrhiza glabra* (licorice), was found to exhibit antimicrobial activity.[1] Scandione was isolated from the stem of Thai medicinal plant, *Derris scandens*, and showed antibacterial, hypertensive, and radical scavenging activities.[2] Calophione A, isolated from the roots of *Tephrosia calophylla*, was tested with mouse macrophage cells (RAW) and colon cancer cells HT-29 and showed significant cytotoxicity with $IC_{50}$ of 5.00 (RAW) and 2.9 μM (HT-29), respectively.[3] A concise method for the synthesis of unsymmetrical benzils was developed by the deprotonation of aryl benzyl ethers using lithiated bases (LDA and LTMP) in anhydrous media followed by reacting the intermediate with molecular oxygen or with dioxirane to afford unsymmetrical benzils.[4] Synthesis of licoagrodione, scandione, and calophione A from unsymmetrical benzil core was studied using alkynylation, Lindlar's reduction, Claisen rearrangement, and cyclization, respectively.[4,5]

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# Abstract Book

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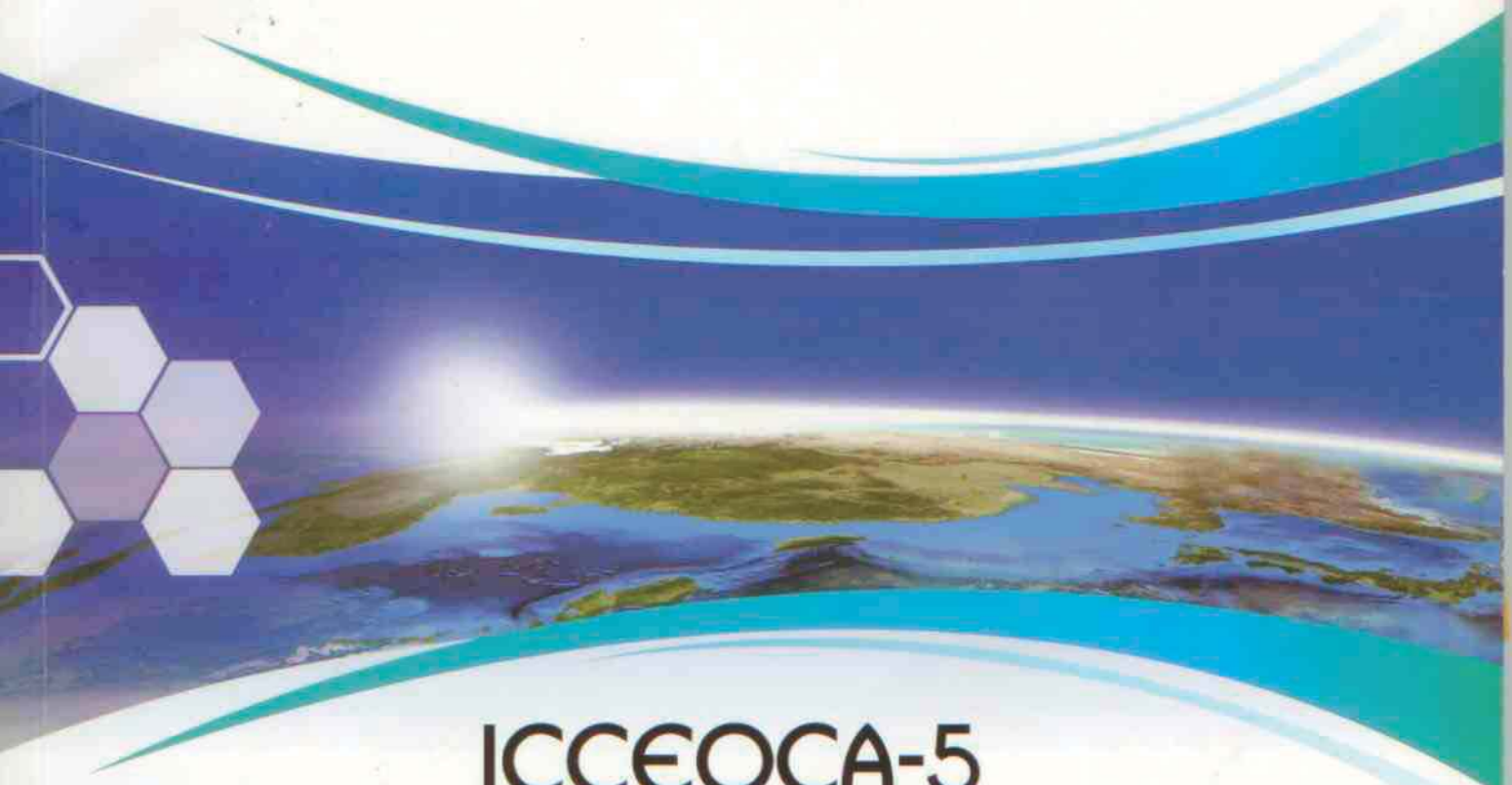

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# Cu(I)/Pd(II)-mediated C-O and C-N Bond Formations: Synthesis of Natural Product-like Alkaloids

Sasiwadee Boonya-udtayan, Rattana Worayuthakarn, Prattya Nealmongkol, Kassrin Tangdenpaisal, Somsak Ruchirawat, Nopporn Thasana*

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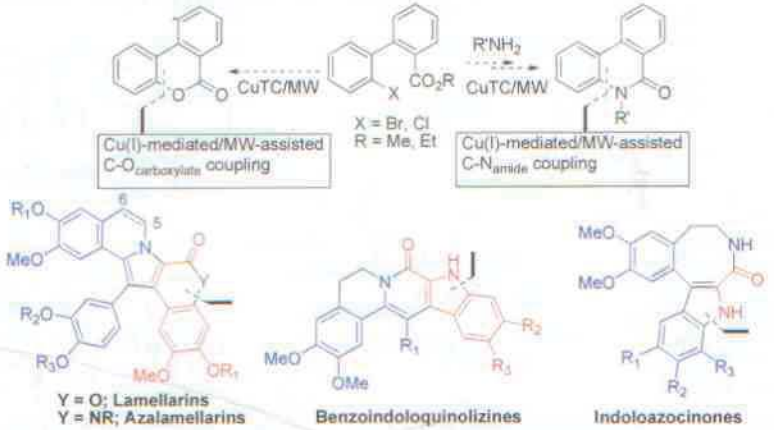
The synthesis of isolamellarins[1] and azalamellarins[2] was achieved using Cu(I)-mediated/MW-assisted C-$O_{carboxylic}$ and C-$N_{amide}$ bond formations, respectively. Seventeen azalamellarins, including *N*-allylazalamellarins and *N*-propyl-azalamellarins χ-D, L-N, and J-dehydro J, were synthesized and evaluated for their cytotoxicities against the cancer cell lines: HuCCA-1, A-549, HepG2, and MOLT-3.[2] The results showed that certain azalamellarins exhibited good activities in the micromolar $IC_{50}$ value range comparable to their parent lamellarins analogue.[2] A facile and direct synthetic entry to benzoquinolizin-4-ones and benzoazocinones was reported based on the ring annulation of 1-unsubstituted and 1-substituted dihydroisoquinolines with azlactones in a one-step procedure and under neutral condition.[3,4] The C-N indole bond formation of benzoindolo-quinolizinones and indoloazocinones is also studied using Cu(I) and Pd(II)-mediated coupling reaction.

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**Nopporn Thasana**, b 1970 in Pattani, Thailand. Ramkamhaeng University (BS 1992), Mahidol University (PhD 2003, Prof. Somsak Ruchirawat), Visiting fellow, Bristol U., UK (2001, Prof. T. Gallagher), and Nagoya U., Japan (2003, Prof. M. Isobe and 2009, Prof. T. Nishikawa), Researcher CRI (1997-present), Lecturer CGI (2007-present). Research field: total synthesis of bioactive natural and natural-like products, synthetic methodology (metal-mediated and microwave-assisted C-X (X = C, N, O) bond formation), natural product chemistry, and chemical biology.

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# PROCEEDINGS

OP 14

# YNTHESIS OF UNSYMMETRICAL BIOACTIVE BENZIL DERIVATIVES

...Nopporn Thasana, [1]Rattana Worayuthakarn, [2]Sasiwadee Boonya-udtayan, [1),2),3)]Somsak Ruchirawat

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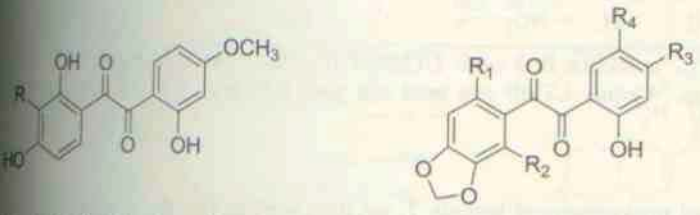
...Chemical Biology Program, Center on Environmental Health, Toxicology and Management of Chemicals, Chulabhorn Graduate Institute, ...Bangkok 10210, Thailand.
...Chulabhorn Research Centre, Institute of Molecular Biosciences, Mahidol University, Bangkok 10400, Thailand.

YWORDS: unsymmetrical benzil, licoagrodione, scandione, calophione A, anticancer, antioxidant, ...microbial

## RODUCTION

The unsymmetrical benzils were often isolated concurrently with other flavanoids as common constituents ...nts in the Fabaceae (Leguminosae) family such as Derris, Glycyrrhiza, Pterrocarpus, Tephrosia, Zollernia, etc. ...first natural benzil, 2,4,2'-trihydroxy-4'-methoxybenzil **1**, was isolated from the wood of *Zollernia paraensis*.[1] ...enylated derivative, licoagrodione **2**, was isolated from the hairy root cultures of Chinese herb, *Glycyrrhiza* ...a (licorice), which was found to exhibit antimicrobial activity.[2] Scandione **3** was isolated from the stem of Thai ...nal plant, *Derris scandens*, and showed antibacterial, hypertensive, and radical scavenging activities.[3] ...rly, tenuifodione **4** was isolated from the whole plant of *Iris tenuifolia* Pall (Iridaceae), the first plant which is ...congener with those from the previous works.[4] Calophione A **5**, isolated from the roots of *Tephrosia ...rella*, was tested with mouse macrophage cells (RAW) and colon cancer cells HT-29 and showed significant ...ucity with $IC_{50}$ of 5.00 (RAW) and 2.9 μM (HT-29), respectively.[5]



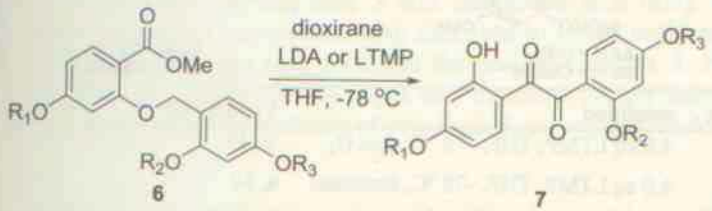

R = H, 2,4,2'-Trihydroxy-4'-methoxybenzil
R = $CH_2CH=CMe_2$, Licoagrodione

**3** $R_1$ = OH, $R_2$ = $R_4$ = H, $R_3$ = OMe, Scandione
**4** $R_1$ = OMe, $R_2$ = OH, $R_3$ = $R_4$ = H, Tenuifodione
**5** $R_1$ = OH, $R_2$ = H, $R_3$-$R_4$ = $OCH(CMe=CH_2)CH_2$, Calophione A

...1. Unsymmetrical bioactive benzil derivatives **1-5**

## RIALS AND METHODS

A concise method for the synthesis of unsymmetrical benzils was developed by the deprotonation of aryl ...ethers **6** using lithiated bases (LDA and LTMP) in anhydrous media followed by reacting the intermediate ...lecular oxygen or with dioxirane to afford unsymmetrical benzils **7**.[6]




**6** **7**

...2. Synthesis of unsymmetrical benzils **7**

...Synthesis of licoagrodione **2**,[6] scandione **3**,[7] and calophione A **5**[7] from unsymmetrical core skeleton **7** was ...d via alkynylation, Lindlar's reduction, Claisen rearrangement, and cyclization, respectively.

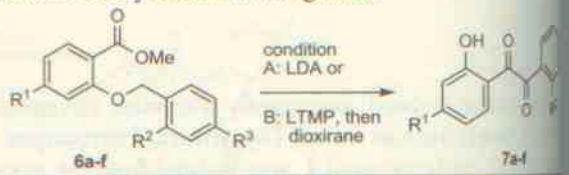
Biological potencies of licoagrodione **2**, scandione **3**, calophione A **5**, and
were evaluated as the $IC_{50}$ in comparison with etoposide and doxorubicin in Hep
cancer cell lines. The antioxidant and antimicrobial activities of unsymmetrical ben

## RESULTS AND DISCUSSION

### Method development: Synthesis of unsymmetrical benzils 7

We developed a concise method for the synthesis of unsymmetrical benzils b
ether using lithiated bases (LDA, and LTMP) in anhydrous media followed by reacti
oxygen or with dioxirane to afford benzils **7**. Methyl-*O*-benzylsalicylate derivatives
study. Our results are summarized in Table 1.

**Table 1.** Optimization of the anionic benzylic ester rearrangement.




6a-f 7a-f

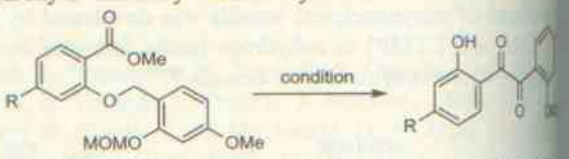
| Entry | 7 | $R^1$ | $R^2$ | $R^3$ | Yield (%) A[a] | Yield (% |
|---|---|---|---|---|---|---|
| 1 | **a** | H | H | H | 43[c] | 58 |
| 2 | **b** | H | H | OMe | 31 | 53 |
| 3 | **c** | OMe | H | H | 16 | 33 |
| 4 | **d** | OMe | H | OMe | 16 | 39 |
| 5 | **e** | H | $NO_2$ | H | - | NA[d] |
| 6 | **f** | H | H | $NO_2$ | - | NA[e] |

[a]condition A: 2 equiv LDA, THF, -78°C. [b]condition B: 3 equiv LTMP, THF, -78°C
was obtained after addition of oxygen gas. [d]4 equiv LTMP was used and gave 27%
recovery of benzyl ester **6f**.

### Synthesis of licoagrodione 2

Having successfully synthesized unsymmetrical benzils **7**, we then appli
B) for the synthesis of licoagrodione **2**. 2-Hydroxy-4-alkoxy-2'-methoxymet
modeled as the licoagrodione backbone and were synthesized from
(methoxymethoxy)benzyloxy benzoates **8**. With the optimized condition, increas
of LTMP gave benzils **9** in 16-54% yields as shown in Table 2.

**Table 2.** Preparation of 2-hydroxy-2'-methoxy-4'-methoxybenzils **9**.



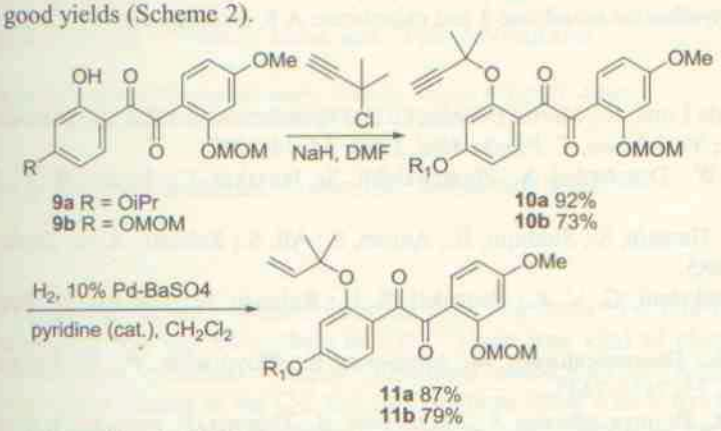

**8a** R = O*i*Pr
**8b** R = OMOM

**9a** R = OP
**9b** R = OM

| Entry | condition | Yield **9** (%) |
|---|---|---|
| 1 | 4.0 eq LTMP, THF, -78 °C, then $O_2$ | **a**, 47 |
| 2 | 4.0 eq LTMP, THF, -78 °C, dioxirane | **a**, 50 |
| 3 | 4.0 eq LTMP, THF, -78 °C, then $O_2$ | **b**, 16 |
| 4 | 4.0 eq LTMP, THF, -40 °C, dioxirane | **b**, 48[a] |
| 5 | 4.5 eq LTMP, THF, -40 °C, dioxirane | **b**, 54 |

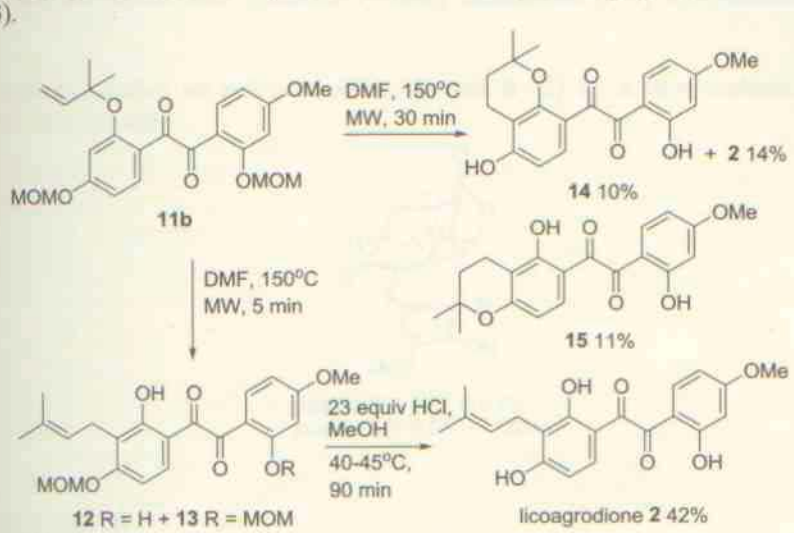
[a]15% recovery of benzyl ester **8b**.

"C-5" Dimethylallyl group equivalent was introduced into the molecule as the corresponding alkynyl ether of the phenolic group by reacting **9** with 3-chloro-3-methylbut-1-yne and NaH in DMF to give the corresponding alkynes **10** in good yields. Hydrogenaton of alkynes **10** using 10% Pd-$BaSO_4$ in $CH_2Cl_2$ yielded the 2-methylbut-3-en-2-yloxy ethers **11** in good yields (Scheme 2).




**Scheme 2.** Introduction of "C-5" dimethylallyl group

With the use of microwave irradiation, licoagrodione **2** was prepared via Claisen rearrangement of the corresponding allyl phenyl ether 1,2-diketone **11b** readily available from the Lindlar's reduction of the corresponding alkyne derivative **10b**. Subsequent removal of the protecting groups then furnished the desired product **2** (Scheme 3).




**Scheme 3.** Synthesis of licoagrodione **2**

**Synthesis of scandione 3 and calophione A 5**

We also succeeded in synthesizing scandione **3** and calophione A **5** using lithiated base (LTMP) in anhydrous media followed by reacting the intermediate with dioxirane to afford unsymmetrical benzil derivatives. Subsequent removal of the protecting groups then furnished the desired products **3**. Protection of the resulting hydroxy group and selective removal of the protecting group then furnished the key intermediate benzil which was further used to synthesize calophione A **5**. The synthesis of scandione **3** and calophione A **5** will be presented.

**Biological activity studies**

Licoagrodione **2** showed weak anticancer activity to HepG2 and antioxidant to HL-60 assay whereas other synthetic benzil derivatives showed no activities. The antioxidant and antimicrobial activities of unsymmetrical benzil derivatives will be reported.

## CONCLUSION

In summary, we have developed a facile route for the unsymmetrica
conversed to various natural benzils. Benzil **9b** was employed in the synthesi
**19a** and **19b** were used to synthesize scandione **3** and calophione A **5**, respectiv

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# Perspectives in Environmental Health Research

## (Abstracts)

August 21st, 2011

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P-35

# SCREENING AND ISOLATION OF BIOLOGICALLY ACTIVE COMPOUNDS FROM LYCOPODIUM PLANTS IN THAILAND

**Prateep Worawittayanon[1], Sawangjitt Wittayalai[2], Sakornrat Thorroad[2], Somsak Ruchirawat[1,3] and Nopporn Thasana[1,3]***

*[1]Chemical Biology Program, Center of Excellence on Environmental Health, Toxicology and Management of Chemicals, Chulabhorn Graduate Institute, Laksi, Bangkok 10210, Thailand, [2]Laboratory of Natural Product, Chulabhorn Research Institute, Laksi, Bangkok 10210, Thailand, [3]Laboratory of Medicinal Chemistry, Chulabhorn Research Institute, Laksi, Bangkok 10210, Thailand,*

*E-mail: nopporn@cri.or.th*

Eight lycopodium plants or club mosses, belonging to Lycopodiaceae and Huperziaceae families, have been screened and isolated for the biologically active compounds and acetylcholinesterase inhibitor. These lycopodium methanol extracts were analyzed by high resolution of mass spectrometry after the acid-base extraction. The chromatographic techniques were then applied to isolate the lycopodium alkaloids, particularly huperzine A, and serratene triterpene derivatives. The isolated compounds have also evaluated the biological activities.

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O-17

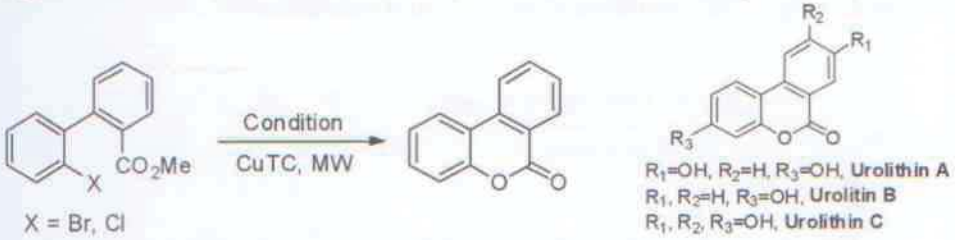
# On-Water Chemistry: "Green" Synthesis of Benzopyranone Derivatives with Subcritical Water[§]

Prattya Nealmongkol, Kassarin Tangdenpaisal, Somsak Ruchirawat and Nopporn Thasana*

*Laboratory of Medicinal Chemistry, Chulabhorn Research Institute, and Program in Chemical Biology, Center for Toxicology, Environmental Health and Management of Toxic Chemicals, Chulabhorn Graduate Institute, Kam Phaeng Phet 6 Road, Bangkok 10210 Thailand.*

E-mail: nopporn@cri.or.th

Under the "Green Chemistry" aspects[1], the synthesis of benzopyranone derivatives was studied using the Cu(I)-mediated C-O bond formation with subcritical water under the microwave irradiation. Methyl 2-halobiarylcarboxylates were used as a model study to optimize the methodology. The lactonization was accomplished using CuTC in catalytic amount incorporated with TMEDA as ligand and $Cs_2CO_3$ as base.[2,3] To extend the scope of methodology, the number of benzopyranone derivatives were synthesized from various 2-halobiarylcarboxylates in satisfied yield. Urolithins A-C,[4] antioxidant metabolite of the bacterial intestine in mice, was synthesized using the development method in modern to excellent yield. The developed methodology was applied to synthesize urolithins A-C in moderate to excellent yield (64-99%). Their biological activities were also evaluated for antioxidant activity.




X = Br, Cl

$R_1$=OH, $R_2$=H, $R_3$=OH, **Urolithin A**
$R_1$, $R_2$=H, $R_3$=OH, **Urolitin B**
$R_1$, $R_2$, $R_3$=OH, **Urolithin C**

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**Research field:** Copper/Palladium-mediated C-O bond formation

[§]*This work was supported by TRF Research Scholar (RMU5380021) and Center on Environmental Health, Toxicology and Management of Chemicals, ETM.*

# THE 6TH INTERNATIONAL CONFERENCE ON CUTTING-EDGE ORGANIC CHEMISTRY IN ASIA AND THE 2ND NEW PHASE INTERNATIONAL CONFERENCE ON CUTTING-EDGE ORGANIC CHEMISTRY IN ASIA

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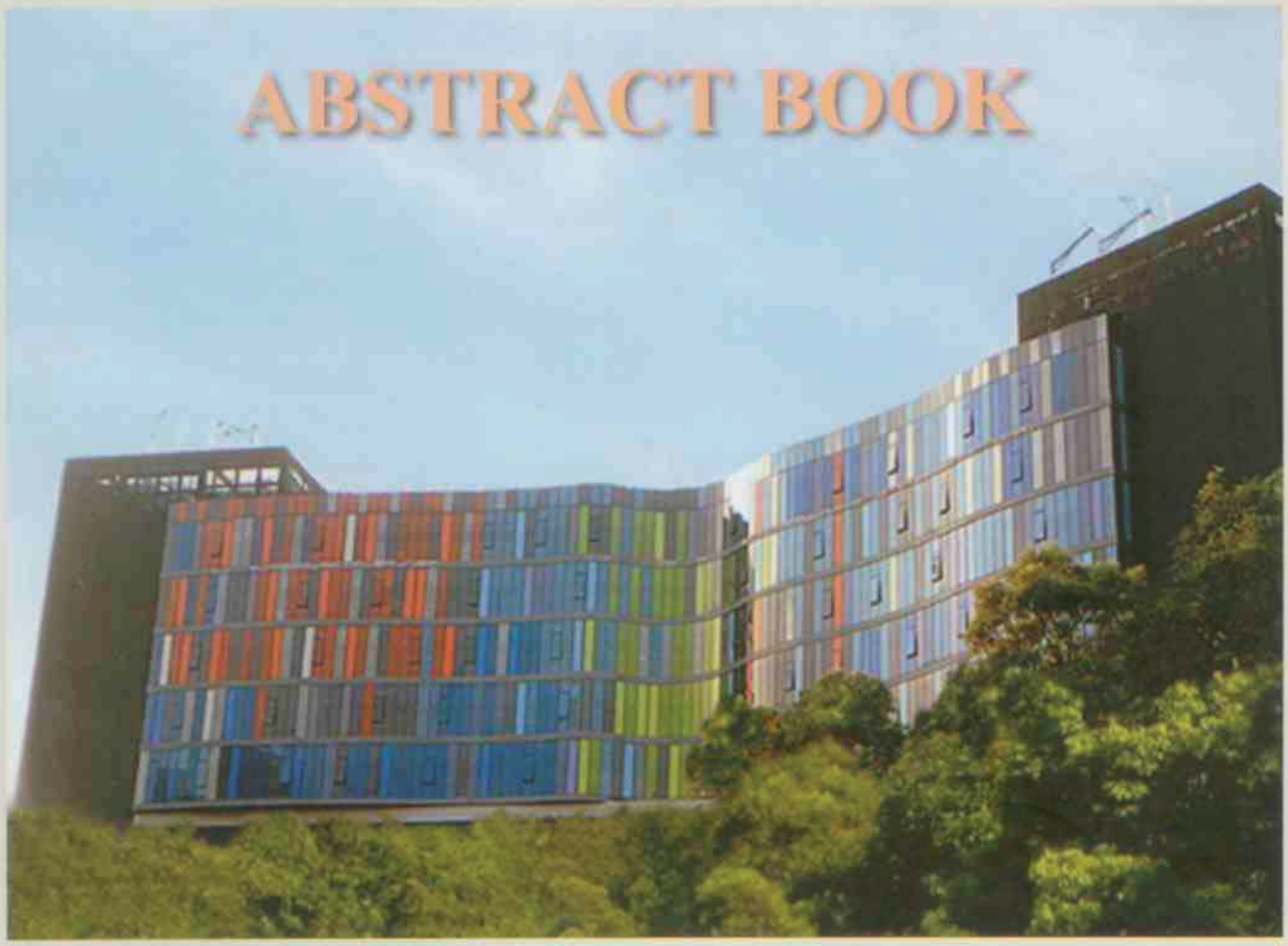

## Copper(I)-mediated and Palladium (II)-catalyzed Indole Formation: Synthesis of Benzoindoloquinolizinone and Indolobenzazipinone

PA-21

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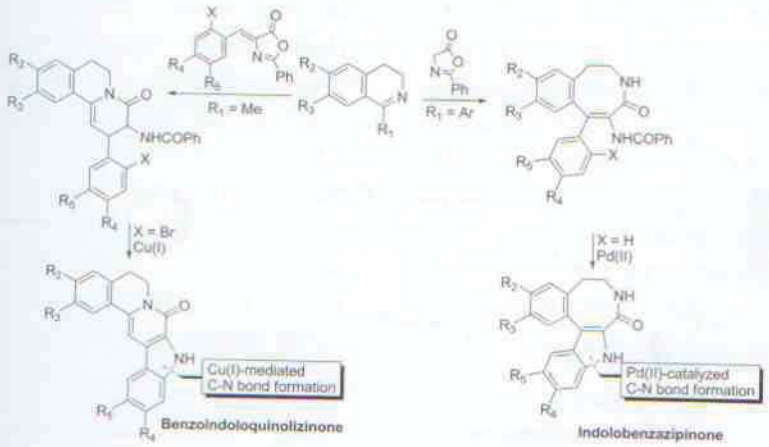
A facile and direct synthetic entry to tricyclic benzoquinolizinones and bicyclicbenzoazocinones is reported based on the ring annulations of 1-substituted dihydroisoquinolines with azlactones under neutral conditions.[1]

The copper(I)-mediated and palladium(II)-catalyzed C-N bond formation[2] of benzoindoloquinolizin ones and indolobenzazipinones will be presented with the focus on reaction mechanism.[3,4]

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3. Worayuthakarn, R.; Nealmongkol, P.; Ruchirawat, S. Thasana, N. *Manuscript in preparation.*
4. Boonya-udtayan, S.; Meredith Eno; Ruchirawat, S.; Thasana, N. *Manuscript in preparation.*

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# Synthesis of Natural Product-like Alkaloids Using Cu(I)/Pd(II)-mediated C-O and C-N Bond Formations

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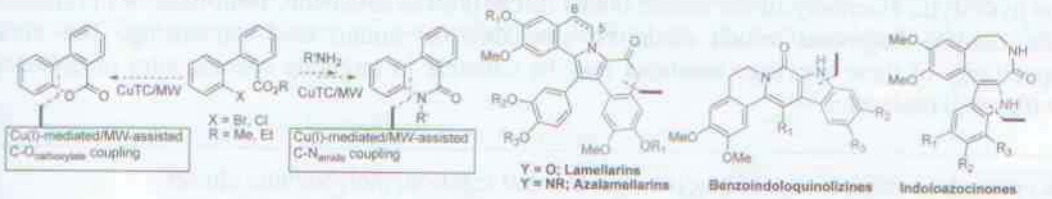
## Abstract

The synthesis of isolamellarins[1] and azalamellarins[2] was achieved using Cu(I)-mediated/MW-assisted C-$O_{carboxylic}$ and C-$N_{amide}$ bond formations, respectively. The results showed that certain azalamellarins exhibited good activities in the micromolar $IC_{50}$ value range comparable to their parent lamellarins analogue.[2] A facile and direct synthetic entry to benzoquinolizin-4-ones and benzoazocinones was reported based on the ring annulation of 1-unsubstituted and 1-substituted dihydroisoquinolines with azlactones in a one-step procedure and under neutral condition.[3,4] The C-N indole bond formation of benzoindoloquinolizinones[5] and indoloazocinones[6] is also studied using Cu(I) and Pd(II)-mediated coupling reaction.




**Keywords:** alkaloids, copper, cytotoxicity, green chemistry, indole, microwave, palladium

## Outputs

1. Boonya-udtayan S, Yotapan N, Woo C, Bruns C J, Ruchirawat S, Thasana N. *Synthesis and biological activities of azalamellarins*. Chemistry An Asian Journal **2010**; 5: 2113-2123.
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3. Worayuthakarn R, Nealmongkol P, Ruchirawat S, Thasana N. *Synthesis of benzoindoloquinolizines via Cu(I)-mediated C-N bond formation.* Tetrahedron **2012**; 68: 2864-2875.

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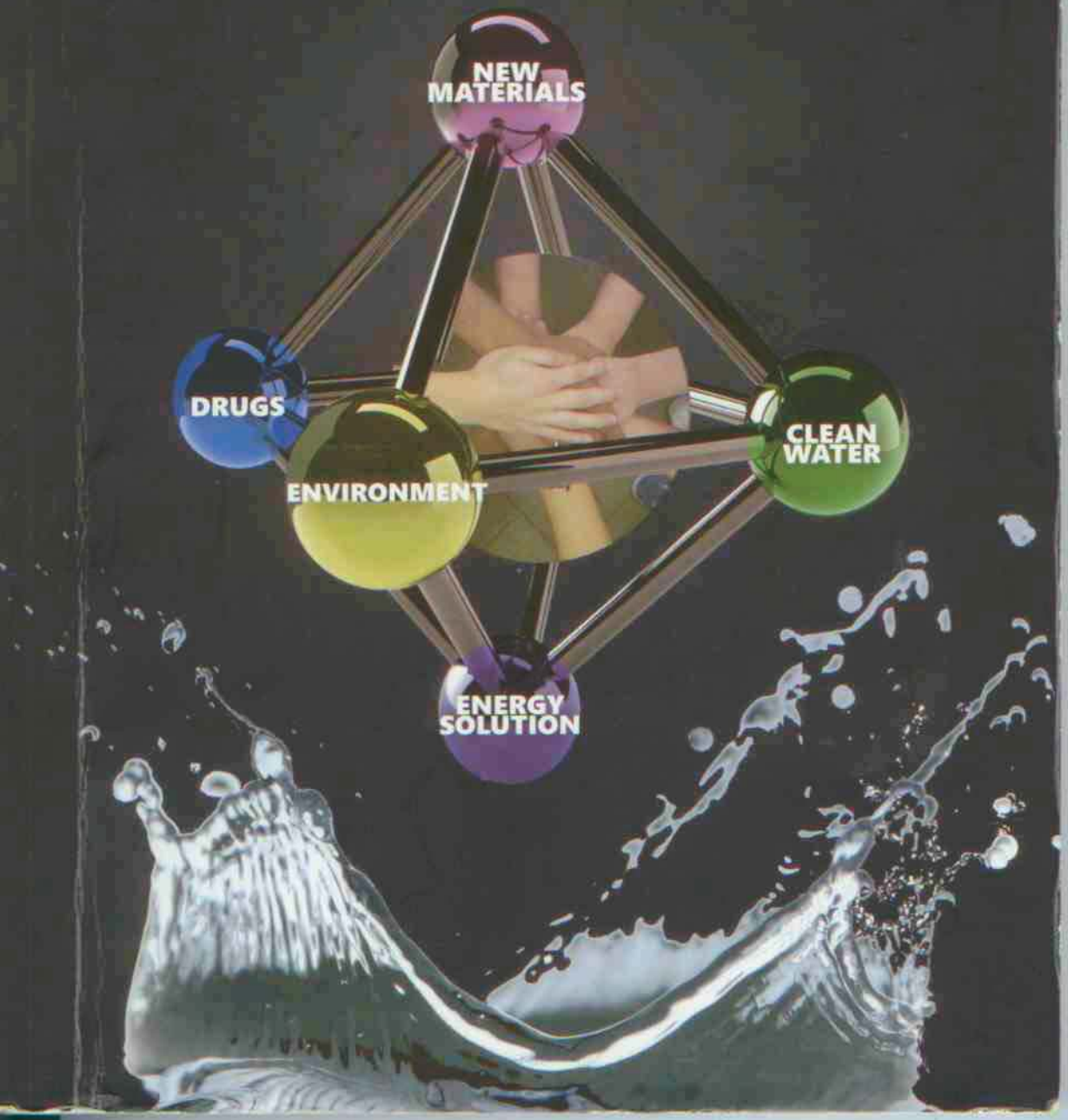

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## A Facile Synthesis of Novobiocin Analogs using Copper-catalyzed and Microwave-assisted 3-Amidocoumarin Formation

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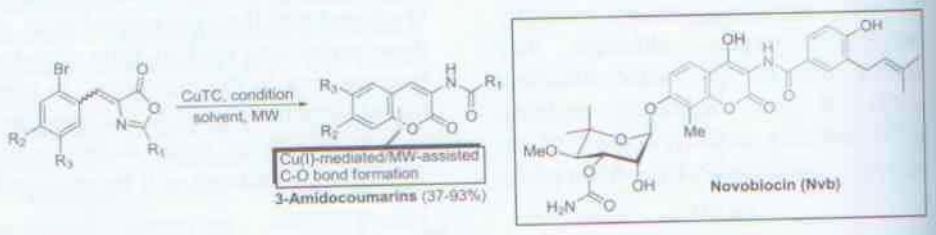
Novobiocin (Nvb) is a coumarin-derived antibiotic used as a competitive inhibitor of the bacterial ATP binding gyrase B subunit, blocking the negative supercoiling of relaxed DNA. Recent works showed that Nvb binds to heat shock protein 90 (hsp90) at the C-terminal nucleotide-binding region.[1] The structure-activity relationship, as revealed by Blagg, suggested that 4-hydroxy moiety of the coumarin ring and the 3-carbamate of the noviose appendage were detrimental to hsp90 inhibitory activity.[2] In this work, a facile and direct synthetic entry to 3-amidocoumarins is reported based on the Cu(I)-catalyzed and microwave-assisted C-O bond formation of coumarin under benign conditions in a one-pot procedure from azlactones.[3,4]

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4. (a) Boonya-udtayan, S.; Yotapan, N.; Woo, C.; Bruns, C. J.; Ruchirawat, S.; Thasana, N., *Chem. Asian. J.* **2010**, *5*, 2113–2123. (b) Worayuthakarn, R.; Nealmongkol, P.; Ruchirawat, S.; Thasana, N., *Tetrahedron* **2012**, *68*, 2864–2875.

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PA44

## Total Synthesis

Atsushi

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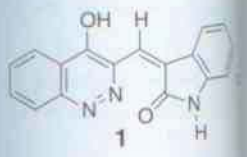
### Scheme 1



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Kawai, K-i., *Mycop*
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1 Nevira