Abstract

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Project Title: Identification of effective antifolates against *Toxoplasma gondii* dihydrofolate reductase thymidylate synthase (TgDHFR-TS) and determination of crystal structure of inhibitor–TgDHFR-TS complex

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Abstract:

Pyrimethamine (Pyr), an inhibitor of the dihydrofolate reductase (DHFR) enzyme, has been used for decades to treat toxoplasmosis caused by *Toxoplasma gondii* (Tg) infection. However, Pyr treatment is effective only at high doses that causes antifolate associated toxicity to patients. Therefore, safer alternative treatments are required. In this study, we established a simple bacterial surrogate system, TgDKOTolC, and used it for screening BIOTEC antifolates against the bifunctional DHFR-thymidylate synthase target (TgDHFR-TS). We found that more than 50% of compounds tested are more effective against TgDHFR-TS than Pyr. Several of these compounds are also active by enzyme inhibition assay and *T. gondii* cell-based assay. To understand the binding mode of Pyr and selected inhibitors, high resolution X-ray structures of the full-length TgDHFR-TS-inhibitor complexes were explored. We found that there is conflict interaction of *p*-chlorophenyl of Pyr with Thr83 of TgDHFR which may deteriorate its binding affinity against TgDHFR. For those effective compounds, no such conflict interaction but additional electrostatic and hydrophobic interactions in the active site pocket was observed. A unique kinked crossover helix was identified in the TgDHFR-TS structure which plays an important role in DHFR-TS interdomain interactions. The identification of high-affinity inhibitors of TgDHFR-TS and their modes of binding together with the simple screening tools could aid development of new effective antifolates for toxoplasmosis treatment.

Keywords: *Toxoplasma gondii*, dihydrofolate reductase, antifolate, bacteria surrogate, crystal structure