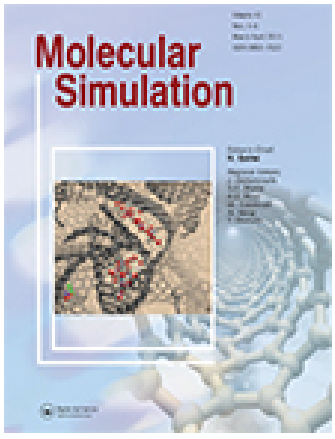

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Binding mode analysis of dual inhibitors of human thymidylate synthase and dihydrofolate reductase as antitumour agents via molecular docking and DFT studies

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Due to the diligence of inherent redundancy and robustness in many biological networks and pathways, multitarget inhibitors present a new prospect in the pharmaceutical industry for treatment of complex diseases. Nevertheless, to design multitarget inhibitors is concurrently a great challenge for medicinal chemists. Human thymidylate synthase (hTS) and human dihydrofolate reductase (hDHFR) are the key enzymes in folate metabolic pathway that is necessary for the biosynthesis of RNA, DNA and protein. Their inhibition has found clinical utility as antitumour, antimicrobial and antiprotozoal agents. The aim of this study is to elucidate the factors which are responsible for the potent inhibition of hTS and hDHFR, respectively, through the detailed analysis of the binding modes of dual TS–DHFR inhibitors at both active sites using molecular docking study. Moreover, this study is also accompanied by the exploration of electronic features of dual inhibitors via the density functional theory approach. This study demonstrates that appropriate substitution at the sixth position of thieno[2,3-d]pyrimidines moiety in non-classical dual inhibitors of hTS and hDHFR plays a key role in the inhibition of hTS and hDHFR enzymes. In general, the outcomes of this research exertion will significantly be helpful in drug design for cancer chemotherapy.

Keywords: TS; DHFR; dual inhibitors; molecular docking; DFT

Thymidylate synthase (TS) and dihydrofolate reductase (DHFR) are key enzymes in folate metabolic pathway that is necessary for the biosynthesis of RNA, DNA and protein.[1] Several TS and DHFR inhibitors, as separate entities, have found clinical utility as antitumour agents.[2–5] Gangjee et al. reported synthesis of a series of non-classical antifolates as dual TS–DHFR inhibitors.[6] In this series, compound **1** (Figure 1) represented most potent dual inhibitory activities against human TS and human DHFR than other compounds of the series. Gangjee et al. also reported the synthesis of another series of antifolate inhibitors and compound **2** (Figure 1) was the most potent compound in this series.[7] The aim of this study is to elucidate the factors which are responsible for the potent inhibition of compounds **1** and **2** against TS and DHFR, respectively, through the detailed analysis of their binding mode at both active sites. For molecular docking (GOLD 5.0.1 program), crystal structures of hDHFR (1U72) and hTS (1HVY) were used.[8–10] Best bioactive poses of each ligand generated with docking were used as input for density functional theory (DFT) calculations.

Analysis of docking results revealed that compound **1** exhibited a network of interactions with important residues such as Arg50 and Trp109 at the TS binding site (Figure 2). The thieno[2,3-*d*]pyrimidines moiety of compound **1** showed various π···π interactions with the indole of Trp109 (5.103 Å, 4.604 Å, 4.152 Å and 3.442 Å). A previous study has also indicated that close contacts with Trp109 enhance the potent inhibitory activity against hTS.[6] Benzene ring from the 4-nitrobenzene group also made important π···cation contact with the key residue Arg50 (6.685 Å). In addition, oxygen atom of the 4-nitrobenzene group from compound **1** also formed hydrogen bond with the amide of Arg50 (2.101 Å). For comparison purpose, the binding conformation of compound **2** was overlaid upon compound **1** in the binding pocket of TS. The binding conformations of both compounds were almost similar. Compound **2** also formed imperative interactions with key residues such as Arg50 and Trp109 at the TS binding site like compound **1**. However, the meticulous analysis of binding conformation of compound **2** at the active site of TS showed divergence in terms of orientation of thieno[2,3-*d*]pyrimidines moiety which was tilting away from the 6-ethyl of compound **2**. This divergence of binding conformation weakened the crucial interactions between key residue Trp109 and thieno[2,3-*d*]pyrimidines moiety of compound **2**. As a result, the π···π interactions between the thieno[2,3-*d*]pyrimidines moiety of compound **2** and indole of Trp109 were 5.964, 5.517, 5.771 and 5.322 Å. The range of this π···π interactions is considerably longer than those of compound **1**. Moreover, π···cationic and hydrogen bond

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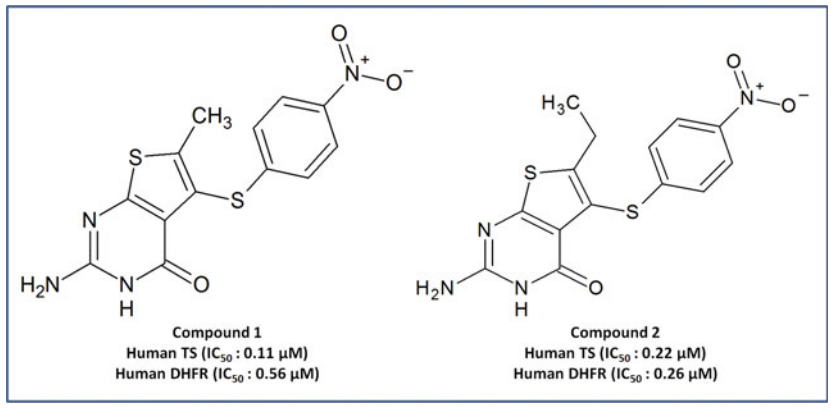

Figure 1. (Colour online) 2D chemical structures of compounds used in the study.

contacts of compound **2** were also relatively weaker than those of compound **1**. Thus, substitution of larger 6-ethyl moiety in compound **2** instigated its binding conformation away from the key residue Trp109 which leads to lessen the imperative interactions between thieno[2,3-*d*]pyrimidines moiety of compound **2** and indole of Trp109. This analysis not only indicates the importance of appropriate substitution at the sixth position of the thieno[2,3-*d*] pyrimidines moiety in non-classical dual inhibitors but also manifests the significance of interactions with Trp109 for potent TS inhibition.

The binding mode of compound **1** was elucidated at DHFR active site as well. The scrupulous analysis of binding conformation showed various contacts between compound **1** and important residues such as Phe34 and Ile60 in the binding pocket of DHFR. Key π···π interactions between the thieno[2,3-*d*]pyrimidines moiety and phenyl ring of Phe34 were observed. The binding

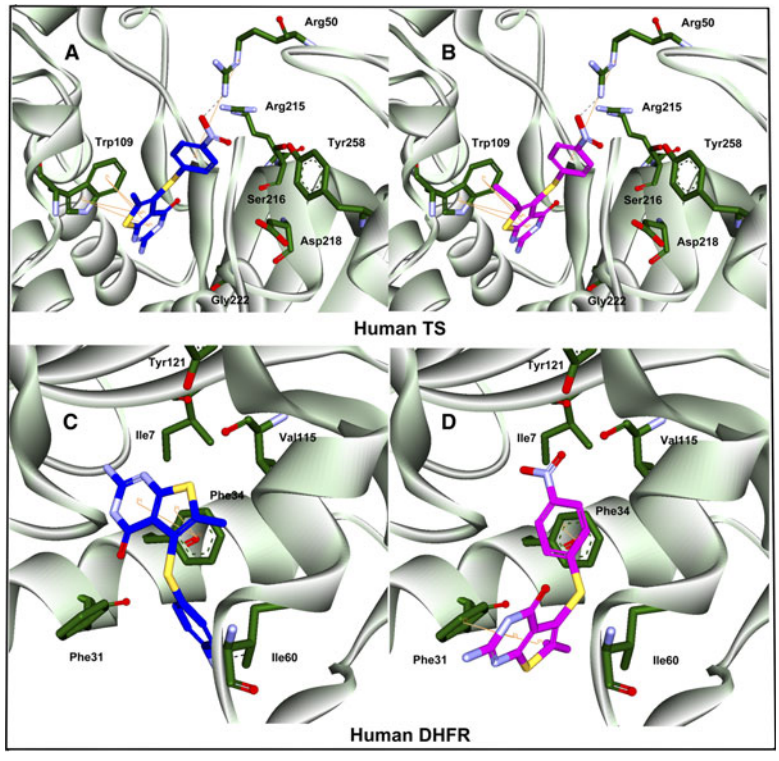

Figure 2. (Colour online) Binding modes of compounds **1** (A) and 2 (B) at the active site of hTS. Binding modes of compounds **1** (C) and **2** (D) at the active site of hDHFR. Key protein residues and ligands are represented by thick sticks. Hydrogen atoms have been removed for clarity.

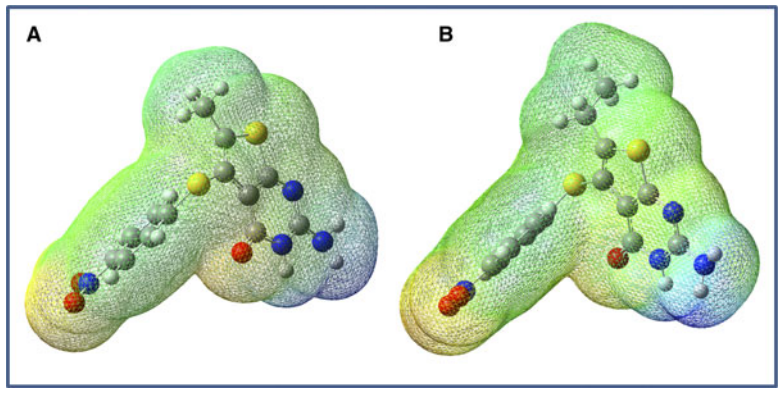

Figure 3. (Colour online) MEP maps of compounds **1** (A) and **2** (B). The red and the blue represent the electronegative and electropositive potentials, respectively, whereas the green represents a potential halfway between the two extremes.

conformation of compound **2** was also investigated at the DHFR active site. Although compound **2** occupied almost the same place in the binding pocket, however, its binding conformation was quite different from that of compound **1**. The substitution of 6-ethyl in compound **2** played a significant role in driving this compound to adopt an appropriate bioactive conformation oriented in the active site of the DHFR enzyme. Consequently, compound **2** exhibited more key interactions in the active site region than compound **1**. For instance, compound **2** not only formed π···π interactions with the Phe34 residue through the 4-nitrobenzene group, but also established close π···π interactions with the important Phe31residue via the thieno[2,3-*d*]pyrimidines moiety.

In the next phase of this research exertion, electrostatic feature analysis of compounds **1** and **2** was performed via DFT. The analysis of 3D isosurface maps of molecular electrostatic potential (MEP) for both compounds **1** and **2** revealed intriguing results (Figure 3). The thieno[2,3-*d*] pyrimidines moiety substituted with the methyl group at the sixth position in compound **1** showed a different conformation than that of compound **2**. In compound **1**, the pyrimidine of the thieno[2,3-*d*]pyrimidines moiety is oriented more towards the methyl group while replacement of methyl with ethyl in compound **2** at the sixth position leads to modify the orientation of pyrimidine away from the ethyl moiety as depicted in Figure 3. The more prominent localised positive charged region was seen over the amine group while electronegative potential was spread over the oxygen atoms of the 4-nitro group in both compounds. Docking studies have also shown the different orientation of thieno[2,3-*d*]pyrimidines in both compounds, which consequently affected their binding modes at both active sites.

On the whole, this study demonstrates that appropriate substitution at the sixth position of the thieno[2,3-*d*] pyrimidines moiety in non-classical dual inhibitors of TS and DHFR plays a key role in the inhibition of these enzymes. For potent inhibition of TS, the substituent at the sixth position of thieno[2,3-*d*]pyrimidines should not be bulky or a larger chemical group. While a relatively larger substituent plays a significant role in driving the ligand to adopt an appropriate bioactive conformation oriented in the active site of the enzyme, thus enhancing its potency against DHFR. The upshots obtained from this study should aid in efficient design and development of non-classical series of antifolates as potent dual inhibitors of TS and DHFR.

Acknowledgements

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