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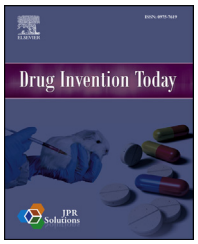

Original Article

In silico screening of chalcone derivatives as potential inhibitors of dihydrofolate reductase: Assessment using molecular docking, paired potential and molecular hydrophobic potential studies

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ABSTRACT

Objectives: Enzyme dihydro foliate reductase (DHFR) is involved in synthesis of DNA and consequently, has long been recognized to have utmost therapeutic significance, since its inactivation can be targeted in numerous infectious as well as noninfectious diseases. In the present studies molecular docking of chalcone derivatives with human as well as *Mycobacterial* DHFR, followed by paired potential and hydrophobic potential analysis were carried out to understand the novel chalcone–DHFR interactions.
Methods: Molecular docking was carried out using GOLD and AutoDock software, paired potential analysis was performed employing on-line program DSX-ONLINE and molecular hydrophobic potential (MHP) analysis was done using web-based program PLATINUM.
Results: Results obtained from docking study, drug score potential and MHP analysis coincide with experimental findings. Molecular property analysis indicates that given compounds follows Lipinski's rule of five. Compound number 1 exhibited best binding energy (−8.02 kcal/mol) in human DHFR while compound number 6 (−7.36 kcal/mol), 9 (−7.32 kcal/mol), 10 (−7.31 kcal/mol) and 11 (−8.25 kcal/mol) demonstrated favorable binding score in *Mycobacterial* DHFR.
Conclusions: Per atom score contribution of chalcone derivatives obtained by paired potential analysis indicate participation of conserved as well as few new residues are expected to be involved in inhibition of DHFR. MHP analysis of chalcone–DHFR complexes revealed important role of hydrophobic contact in inhibition; additionally, individual chemical scaffold on chalcone derivatives that contribute in lipophilicity has been identified. This data is expected to be further explored for the design and development of novel class of DHFR inhibitors using chalcone scaffold.

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1. Introduction

The enzyme dihydrofolate reductase (DHFR, EC 1.5.1.3) catalyzes the reduction of 7, 8-dihydrofolate (DHF) to 5, 6, 7, 8-tetrahydrofolate (THF) using NADPH as a cofactor.[1,2] THF provides the source of methyl groups in the synthesis of purines and pyrimidines, as well as some amino acids. The fact that, inhibition of DHFR activity leads to arrest DNA synthesis leading to cell death, made this enzyme an attractive target for the design and development of novel drugs against cancer,[3–7] bacterial,[8,9] protozoal[10–12] and fungal infections.[13,14] DHFR inhibitors are classified as either 'classical' or 'non-classical' antifolates; presence of p-aminobenzoylglutamic acid side-chain is the characteristic feature of 'classical' antifolates. Methotrexate (MTX) is distinguished and currently used drug among 'classical' antifolates. Non-classical inhibitors of DHFR such as trimetrexate (TMQ) and piritrexim (PTX) do not possess the *p*-aminobenzoylglutamic acid side-chain, but rather have a lipophilic side-chain. Other selective inhibitors of DHFR are trimethoprim (TMP) and pyrimethamine (PTM).[15,16] Chemical structures of some of these inhibitors are shown in Supplementary Fig. 1.

Plethora of literature has accumulated in the recent past describing the therapeutically important biological properties of chalcones and its derivatives and effectively gained momentum in pharmaceutical research owing to their role in prevention of various degenerative diseases and possessing promising anticancer activity. Chalcone moieties are known precursors of flavonoids and isoflavonoids abundantly found in natural sources. Structurally, chalcones consists of two aromatic rings joined by a three-carbon α, β-unsaturated carbonyl system. Chalcones are reputed for possessing several biological activities such as anticancer,[17–21] anti-inflammatory,[22–24] antimicrobial[25–27] and antioxidant.[28,29] By virtue of diverse pharmacological activities, these molecules have attracted medicinal chemists for the development of numerous strategies for synthesis of series of derivatives and testing their activity against several human ailments.

In the present work, molecular docking studies were performed using chalcone derivatives along with standard inhibitors like MTX and TMP onto the active site of human as well as *Mycobacterium tuberculosis* (MTB) DHFR, employing modern docking tools like GOLD[30,31] and AutoDock. Paired potential and molecular hydrophobic potential analysis has been carried out to understand the novel interactions that have potential to develop newer class of DHFR inhibitors.

2. Methods

2.1. Enzyme structure

X-ray crystal structure of human and tubercular DHFR, complexed with folate and trimethoprim (TMP) respectively, was obtained from the protein data bank (PDB ID 1DRF[32] and 1DG5[33] respectively). The obtained model of human DHFR consists of solvent molecules, folate, along with co-crystallized sulfate ions; while tubercular DHFR was found to be complex with TMP. Ligands (folate and TMP) were extracted from the target enzyme and were used to evaluate the validity of the docking system (using AutoDock 4.2). In order to use the target in GOLD software, it was appropriately protonated followed by its geometry optimization applying force field approach. The structure was subjected to energy minimization protocol in which the constraints on the enzyme were gradually removed and minimized until the RMS (root mean square) gradient was 0.1 kcal/mol (Å). The energy minimization was performed using discovery studio 2.5 and structure was used for docking studies in GOLD. On the other hand, target molecule was prepared in traditional pdbqt format after assigning charges to the target coordinate file by using 'Make macromolecule' command from AutoDock menu so that it can be used in AutoDock 4.2.

2.2. Compounds

A set of 11 synthetic chalcone derivatives (prepared *in house*) [Supplementary Fig. 2] were selected from the research project (scheme no-01(2023)/05/EMR-II) funded by Council of Scientific and Industrial Research (CSIR), New Delhi, Govt. of India. The details of the synthetic methodology and characterization are already reported (Final Technical Report 2010, submitted to CSIR, New Delhi, India). 2D structures of all compounds were drawn in ChemDraw® 8.0 (CambridgeSoft, Cambridge, MA, USA) and their SMILES were obtained. The 3D conformers of these compounds were then generated in SDF format using FROG2 Server.[34] AutoDock 4.2[35,36] implemented in Python Prescription 0.8 (PyRx) was used in Docking Analysis. The ligand molecules in sdf format were imported in PyRx environment via OpenBabel utility and were subject to energy minimization using UFF forcefield.[37–39] Conjugate gradient optimization algorithm was applied for over 200 steps while molecules were updated for every 1 step.

2.3. Docking using GOLD

Molecular docking studies on chalcone derivatives were initially performed using the CCDC GOLD 4.1.2 program, on windows operating system. GOLD is a genetic algorithm[31] for docking flexible ligands into protein binding sites. The energy minimized enzyme and ligands structures were imported as pdb and .sdf files respectively to GOLD program. Docking experiments were performed using the default GOLD fitness functions. The amino acids such as Ile7, Val8, Ala9, Ile16, Gly20, Asp21, Leu22, Trp24, Pro26, Leu27, Arg28, Glu30, Phe31, Arg32, Tyr33, Phe34, Gln35, Arg36, Met37, Thr38, Val50, Met52, Thr56, Ser59, Ile60, Pro61, Asn64, Arg65, Leu67, Arg70, Asn72, Val115, Gly116 and Tyr121 were selected as the binding site residues for all selected ligands. From these residues, the residues within a 5 Å radius from the solvent-accessible surface of the cavity were selected. The GOLD score was used to analyze the interactions between docked complexes. Discovery studio tool was used for visual inspections of the interactions between DHFR–chalcone derivatives complex.