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INHIBITORS OF DIHYDROFOLATE REDUCTASE: MOLECULAR DOCKING AND ADME STUDIES

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ABSTRACT

Background: Dihydrofolate reductase (DHFR) is one of the indispensable targets and has important role in the survival of microorganisms. In traditional medicine, *A. majus* L. which is native to Egypt is an important plant due to its diverse pharmacology. It has been extensively studied to identify its phytoconstituents, biological activity, pharmacokinetic, and toxicity profile. Studies focusing on evaluation of *A. majus* against cancer, skin diseases, diuretic, etc. have been reported. **Methods:** In the present study we aimed to perform molecular docking studies along with ADME assessment for some of the important phytoconstituents of *A. majus* as antimicrobial agents. **Results:** Significant binding interactions were reported. **Conclusion:** The study results will be helpful to understand the pharmacodynamic profile and establish its mechanism of action as potential inhibitors of DHFR.

KEYWORDS : *A. majus*, DHFR inhibitor, Antimicrobial agents, Molecular docking, ADME

INTRODUCTION

Worldwide reports highlighting the alarming situation with increasing cases of antimicrobial resistance demands efforts to be focused on discovery and development of novel antimicrobial agents [1]. One of the promising approaches in this process is to target the enzymes essential for the survival of disease causing microorganisms. In *Escheria coli* (*E. coli*), dihydrofolate reductase (DHFR) is an important enzyme with crucial role. DHFR has been reported as an important molecular target for dental caries, bacterial infections, fungal infections in addition to cancer, influenza, malaria, etc. [2]. Some of the well reported inhibitors of DHFR include trimethoprim, iclaprim, aminopterine, methotrexate, pralatrexate, pemetrexed, etc. [3,4]. Potential of various reported phytoconstituents as antimicrobial agents has emphasized to investigate them as inhibitors of DHFR. *Ammi majus* L. (*A. majus*) is a well reported Egyptian plant has been studied for its diverse pharmacology [5-7]. It has a promising role in the treatment of skin diseases and has been emphasized in Unani medicine [8]. Based on its reported promising activity profile it is warranted to explore its antimicrobial activity at molecular level using novel approaches.

In preclinical phase of drug discovery, rational approaches have demonstrated promising role as compared to the traditional methods. With the availability of structural details of target enzymes, computational methods like molecular docking can be efficiently implemented as a structure-based method [8]. It contributes not only to identify the compounds having therapeutic potential but also to understand their interactions with the receptors [9].

In the present study, molecular docking studies have been carried out with an aim to the pharmacodynamic profile and establish its mechanism of action as potential inhibitors of DHFR and further as antimicrobial agents for the treatment of infections caused by *E. coli* or Gram negative bacteria. *In silico* ADME assessment has been simultaneously reported.

MATERIAL AND METHODS

Selection of Ligands

Various reported phytoconstituents of *A. majus* were selected for the *in silico* evaluation. Their names and structures are reported in Fig. 1.

Molecular Docking

Ligands were drawn and the SMILES notation was generated using ACD/ChemSketch 2019.1.3 (www.acdlabs.com). Molecular docking studies were performed using a computer system (Specifications: HP DESKTOP-LGE6608; Intel(R) Core(TM) i5-8265UC CPU @ 1.60 GHz 1.80 GHz, 16.0 GB RAM). Software used for this study was Pyrex virtual screening, PyMOL, and Chimera.

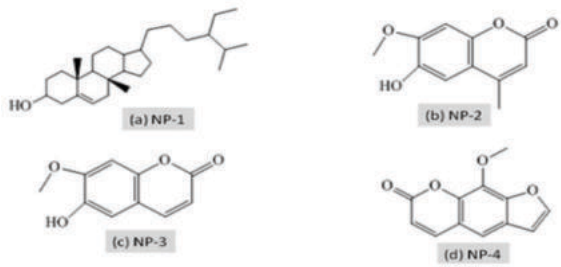


Figure 1: Chemical structures and names of *A. majus* phytoconstituents (a) NP-1, β-sitosterol; (b) NP-2, 6-Hydroxy-7-methoxy-4-methyl-2*H*-1-benzopyran-2-one; (c) NP-3, 6-Hydroxy-7-methoxy--2*H*-1-benzopyran-2-one; and (d) NP-4, 9-Methoxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one

The 3D crystal structure of the receptor i.e. *E. coli* of dihydrofolate reductase was accessed from the Protein Data Bank (www.rcsb.org). Receptor file was downloaded and was prepared using Discovery Studio Visualizer (PDB ID: 2ANO) [10]. During preparation addition of H and elimination of H_2O molecule, heteroatoms and co-ligands from the receptor structure and saved for further analysis. The ligands were optimized and saved in PDB format using UCSF Chimera Tool [11,12]. Docking studies was performed using Pyrex software which utilizes Autodock Vina [13]. Docking results were investigated with the help of Chimera, Discovery Studio Visualizer and PyMOL.

In Silico ADME Studies

For predicting the adsorption, distribution, metabolism, and excretion (ADME) properties or compliance for Lipinski rule, Swiss ADME online tool was used [14]. Also the skin permeation (log K_p) was reported.

RESULTS AND DISCUSSION

In *E. coli*, DHFR induces catalytic reduction of 7,8-dihydrofolate to get the 5,6,7,8-tetrahydrofolate which is required for purine, thymidylate and amino acid biosynthesis. It has been recently investigated as a potential target for antibiotics against the resistance cases. The binding interactions of some important phytoconstituents present in *A. majus* were investigated by molecular docking. Results are summarized in Table 1 showing significant binding energy score and suggesting potential inhibitors of the selected

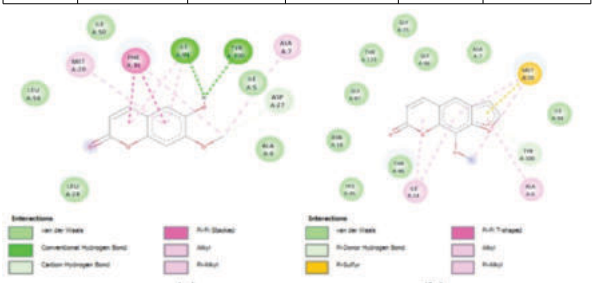
bacterial enzyme. The binding conformation is depicted in Fig. 2. The active site comprising of hydrophobic amino acid residues like Val726, Met766, Met790, Leu718, and Leu844 have shown strong hydrophobic interactions. The binding score was compared with the reported DHFR inhibitor Trimethoprim (- 10.7 kcal/mol) and all the selected compounds (NP-1 to -4) have exhibited comparable inhibition. The residues ALA A:7, ILE A:5, TRP A:30 with OCH_3 and ASP A:27 with OH showed interaction for NP-1. Pi-pi stacking interactions were observed with PHE A:31 for benzopyranone moiety. In addition to docking studies, ADME properties were investigated and NP-2 to -4 have shown zero violation for Lipinski rule while the skin permeation score was at higher side.

CONCLUSIONS

In this work, molecular docking studies have been performed to explore the mechanism of binding towards DHFR enzyme and obtained significant results. The most favorable docking conformations and will be helpful to understand the mechanism of inhibition and to design potent inhibitors to overcome resistant strains.

Table 1-Results of docking studies and Swiss ADME predictions

Ligand ID	Binding Energy	Amino Acid Residues	H-Bond (H-Bond length Å)	Lipinski Rule	Skin permeation (Log Kp)
NP-1	-10.4 Kcal/mol	LEU718, LEU844, MET766, VAL726, ALA743, LYS745, MET790	MET793 (2.49, 2.05), THR854 (3.77)	Yes; 1 violation; MLogP >4.15	-2.30 cm/s
NP-2	-10.3 Kcal/mol	LEU718, LEU844, MET766, VAL726, ALA743, LYS74, MET790	MET793 (2.61, 2.16), LYS745 (2.88)	Yes; 0 violation	-6.54 cm/s
NP-3	-10.2 Kcal/mol	LEU718, LEU844, MET766, VAL726, ALA743, LYS745, MET790	MET793 (2.54, 2.13), LYS745 (2.82), ASP800 (3.78)	Yes; 0 violation	-6.49 cm/s
NP-4 10	-10.1 Kcal/mol	ALA743, LYS745, MET790, ARG841, VAL726, LEU844	ARG841 (2.75, 3.04), LYS745 (2.48), LEU788 (3.58) , PHE723 (3.50)	Yes; 0 violation	-6.20 cm/s



(a) (b)

Figure 2: Binding interactions for (a) NP-3; (b) NP-4

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