## **Research Article**



# Transmuting Methotrexate in to a Potential Binder of Dihydrofolate Reductase

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#### ABSTRACT

We report here design of some binders of DHFRase by modifying the structure of methotrexate on the basis of Rational Drug Design and predict their mode of attachment at the putative binding site by docking them with Autodock 4.2. The crystal structure of DHFRase was imported from Protein Data Bank, binding site was optimized and the best set of model was chosen for lead generation. Probably the ligand N-(1,3-di(1H-tetrazol-5-yl)propyl)-6-(((5,7-diaminoimidazo[1,2-c]pyrimidin-2-yl)methyl) (methyl)amino)nicotinamide will be a lead molecule having better binding affinity towards DHFRase than Methotrexate.

Keywords: DHFRase, Methotrexate, Docking, Bioisosteres.

### **INTRODUCTION**

ihydrofolate Reductase (DHFR) catalyzes the conversion of 7, 8-dihydrofolate to 5, 6, 7, 8-Tetrahydrofolate with NADPH. This enzyme is present in Vertebrates and in bacterial organism. Tetrahydrofolate plays a pivotal role in cell division by synthesizing purines, thymidylates and some other amino acids. The enzyme inhibitors play an important role in therapy for several infectious diseases like bacterial, fungal, protozoal and autoimmune disease, arthritis and in neoplasm. The antifolates trimethoprim and pyrimethamine are selective inhibitors of bacterial and protozoal DHFRase respectively. On the contrary Methotrexate (Mtx) fig.1 is a selective inhibitor of Human DHFRase and also acts as antiarthritic, and immunosuppressive agents.

Docking is a computational tool required to know the binding mode of potential ligands to the macromolecular structure. Structure based drug design is of immense importance as from the 3D-structure of macromolecules the chemistry of binding site or catalytic site can be adopted to design some new chemical entity as inhibitor. The starting point of design is to get a reliable homology model of the target with co-crystallized ligand which can highlight on the aspects of binding site analysis and features responsible for binding affinity and selectivity. These data are then used to develop a new ligand having better binding and activity. Molecular modeling with the knowledge of computational chemistry helps in hypothesis generation. They help to exploit information about the binding site geometry by constructing molecules de novo, by analyzing known molecules with respect to their affinity and binding geometry, or by searching compound libraries for potential hits to suggest new leads. Discovered hits that are commercially available or synthetically accessible are then experimentally tested and their binding properties examined by biochemical, crystallographic, and

spectroscopic methods. The 3D structure of new complexes together with the acquired activity data are subsequently used to start a new cycle of ligand design to improve the hypotheses stated in the previous round.

Bioisosteres are substituent or groups that have chemical or physical similarity, and which produce broadly similar biological property<sup>1</sup>. Bioisosterism is an important lead modification approach that has been shown to be useful to attenuate toxicity or to modify the activity of a lead, and may have a significant role in the alteration of pharmacokinetics of a lead. There are classical isosteres<sup>2, 3</sup> and non-classical isosteres<sup>4</sup>. Bioisosterism also can lead to changes in activity or potency.

The structure of methotrexate (FIG1.) shows three fragments i.e. it consists of substituted Pteridine ring, para amino benzoic acid (PABA) residue and Glutamic acid at the end (Fig.1). The 2, 4-Amino groups of pteridine nucleus are important for its binding with the receptor. From the crystal structure (PDB accession code: 4GH8) it is evident that 2-amino group forms hydrogen bond with oxygen atom of Asp 28 and structural water (water ID 305). The 4-amino group forms one hydrogen bond with oxygen atom of Ile 98 and another oxygen atom of Ile 5. The carbonyl group of PABA residue interacts with nitrogen atom of Asn 55 forming strong hydrogen bond. The oxygen atom of carboxyl group nearest to the -NH- of glutamic acid forms two hydrogen bonds with the nitrogen atom of Arg 61 at two different positions and another with structural water (water ID 395). The carboxyl group of glutamic acid which is furthest to -NH-group forms two hydrogen bonds with structural water (water ID 360). The carboxyl group nearest to -NH- has electrostatic interaction with the Arg 61 residue, where the protein surface nearest to this group is positive and augments binding. The electrostatic potential is the sum of the Coulomb potentials for each atom in the protein, with a distance-dependent dielectric constant. N-4 of pteridine ring and carbonyl group of



PABA have several non favorable steric interactions with Asp 28 and Asn 55 of amino acid residue respectively.

In this paper we have attempted to design a more potent binder than Methotrexate at the active site of dihydrofolate reductase keeping all the non favorable interactions low.



**Figure 1:** Structure of Methotrexate: **A** shows pteridine nucleus, **B** shows para amino benzoic acid (PABA) residue and **C** shows glutamic acid portion.

## **MATERIALS AND METHODS**

2D structure of the ligands was drawn with Chem Bio Draw Ultra 12.0. Ligands were imported to Chem Bio 3D-Ultra 12.0; energy was minimized using MM2 force field and was saved as .cdx format. All the designed ligands were converted to .pdb format after adding hydrogen using the programme Open Babel 2.3.2. Autodock 4.2<sup>5</sup> was used to dock the designed ligands with the target enzyme i.e. Dihydrofolate reductase (PDB accession code 4GH8). Molegro Molecular viewer<sup>6</sup> was used to read the different interactions and decoys generated of the protein ligand complex.

## Design

On the basis of some physicochemical property (Table 1.) such as logP, Molar Refractivity, Polar Surface Area, pi energy and number of hydrogen bond acceptors, the Pteridine nucleus of Methotrexate was substituted with imidazo[1,2-c]pyrimidine (Fig.2) and 1H-imidazo[2,1-i]purine (Fig.3)



Figure 2: imidazo [1, 2-c] pyrimidine



Figure 3: 1H-imidazo [2, 1-i] purine

The amino groups were added at 5, 7-position of imidazo[1,2-c]pyrimidine and at 5 position of 1H-imidazo[2,1-i]purine to complete the substituted ring form.

### Table 1: Physicochemical properties of the rings

Nucleus	logP	Molar refractivity	Polar Surface area	Pi energy	No. of hydrogen bond acceptors
Pteridine	-0.30	35.688	51.56	16.11	4
imidazo[1,2- c]pyrimidine	-0.44	34.132	30.19	15.00	2
1H-imidazo[2,1- i]purine	-0.79	42.996	58.87	21.75	3

The methylene group was kept constant at 2-position for both the rings. The benzene ring in Mtx was replaced by its non classical isosteres pyridine, Pyrimidine, thiophene, thiazole, furan, isoxazole and imidazole and optimized that pyridine was invariable at that position. Subsequently, the carbonyl group of amide linkage was replaced by its Bioisosteres like sulfonyl, sulfinyl, malononitrile, oximes etc. but no group was found to be vital for binding at the catalytic site. Hence, was left unchanged.

The more electron rich carboxyl group was also altered by its several bioisosteres. Tetrazole ring was found to be the best substitute as it enhances the binding energy of the designed ligand.

Finally two leads (Fig.4 and 5) were found to be the starting point for further result based design.







**Figure 5:** 6-(((5-amino-1H-imidazo[2,1-i]purin-2-yl)methyl) (methyl)amino)-N-(1,3-di(1H-tetrazol-5-yl)propyl) nicotinamide **[D-2]** 

## Docking

The protein ligand complex (PDB accession code: 4GH8) was downloaded from <u>www.rcsb.org</u> (Protein Data Bank). This is crystal structure of humanized E.coli dihydrofolate reductase complexed with methotrexate. The complex was imported for binding site analysis in Castp Server<sup>8</sup> where it shows 14 pockets of protein. Pocket no. 14 is the active site of the protein and Mtx is bound at this site.



The catalytic site is comprised of 15 amino acids most of them are non-polar in nature. There are 10 hydrogen bonds formed between the protein residue and ligand. Electrostatic force of attraction also plays an important role in binding. Favorable and non-favorable steric clashes were found. The bioactive conformation of Mtx was generated.

## **Protein preparation**

While docking is carried out, the protein should be free from cofactors', bound ligand and water molecules. This protein is comprised of two chains, A and B. The active site lays within chain A, so chain B is cropped before preparation. The crystal structure was checked for any structural deformation. We have found that the normal template of amino acid residue is not correct. The structure was rectified, all hydrogens were added and Gasteiger charge was calculated. The total charge over the protein surface was calculated and corrected. Nonpolar hydrogens were merged and the atoms were assigned as AD4 type.

### Ligand preparation

Designed ligands in pdb format were imported to the working space with simultaneous calculation of charge, TORSODOF, number of rotatable bonds. In torsion tab, number of rotatable bonds with their degree of freedom was specified. The ligand was saved in pdbqt format to make it ready for docking.

### **Grid preparation**

The macromolecule was imported along with ligand. The grid parameters were set. The coordinates for the center of grid box was set as X=6.13, Y=2.451, Z=7.217, keeping the space as 0.375  $A^{\circ}$ . Number of points in X, Y, Z direction was set to 60. The file was saved as gpf format.

Auto Grid was run where the algorithms writes map files for every atom.

### **Docking preparation**

The macromolecule was imported to the work space and the ligand was selected. The search parameter was chosen as Genetic Algorithm with short number of evaluations while other parameters like docking parameters were kept constant. The file was saved as dpf.

The docking programme was run and the output was read as dlg file.

## **RESULTS AND DISCUSSION**

The binding energy of Mtx at the active site of dihydrofolate reductase is -7.81 with  $k_i$  value 1.9  $\mu$ M and total dock energy -11.74. Different portions of Mtx was substituted with bioisosteres and was docked. Few of the designed ligands showed lower binding energy while the best ligand which came as a better binder than Mtx is shown in Fig 3. The comparative result is shown in the table 2.

**Table 2:** A Comparative result of binding energy of the reference ligand and the designed ligands.

Ligand	Binding Energy	k <sub>i</sub>	Total Dock energy (kcal/mol)
Methotrexate	-7.81	1.9 µM	-11.74
D-1	-9.05	233.05 nM	-13.56
D-2	-7.9	1.83µM	-11.83

D-1 forms 4 hydrogen bonds (FIG. 6) with the amino acid residues of protein surface. The N-7 atom of imidazo[1,2-c]pyrimidine ring forms hydrogen bond with Oxygen atom of Trp 22. The N-2 and N-3 atom of Tetrazole ring (nearest to the amide linkage) forms Hydrogen bond with Oxygen atom of Ile 98 and Thr 47 respectively. The N-2 of Tetrazole ring (furthest from the amide linkage) forms hydrogen bond with oxygen atom of Asp 28.

The imidazo[1,2-c]pyrimidine ring (FIG. 7)faces the positive surface of the protein residue while the Tetrazole rings face the negative surface of the protein. This electrostatic potential map suggests the better binding of this ligand at the catalytic site.

The two Tetrazole rings, pyridine ring and imidazo[1,2c]pyrimidine ring faces the grooves of the protein which are steric favored (FIG. 8). Replacing the two carboxyl groups with Tetrazole rings favors its binding to the putative site.



**Figure 6:** The Protein Ligand Complex (D-1) showing four hydrogen bonds (Yellow dotted lines)



**Figure 7:** The Protein Ligand Complex (D-1) showing electrostatic interaction. The blue dots show positive surface charges while the red dots show negative surface charges.





**Figure 8:** The Protein Ligand Complex (D-1) showing steric favorable interaction. The green dots forming the mesh near two Tetrazole rings, pyridine ring and imidazo[1,2-c]pyrimidine ring shows steric favorable interactions.

D-2 forms five hydrogen bonds (fig. 9) with the amino acid residue of protein. The N-3 and N-4 position of Tetrazole ring (nearest to the amide linkage) forms hydrogen bond with oxygen atom of Ser 50. The N-2 of Tetrazole (furthest from the amide linkage) forms hydrogen bond with oxygen atom of Ile 98, Ile 5 and Tyr 104.



**Figure 9:** The Protein Ligand Complex (D-2) showing five hydrogen bonds (Yellow dotted lines)



**Figure 10:** The Protein Ligand Complex (D-2) showing electrostatic interaction. The blue dots show positive surface charges while the red dots show negative surface charges.

The 1H-imidazo[2,1-i]purine ring (FIG. 10) faces the positive surface of the protein residue while the Tetrazole rings face the negative surface of the protein. This

electrostatic potential map suggests the better binding of this ligand at the catalytic site.

The two Tetrazole rings, pyridine ring and 1H-imidazo[2,1i]purine ring faces the grooves of the protein which are steric favored (FIG. 11). Replacing the two carboxyl groups with Tetrazole rings favors its binding to the putative site.



**Figure 11:** The Protein Ligand Complex (D-2) showing steric favorable interaction. The green dots forming the mesh near two Tetrazole rings, pyridine ring and 1H-imidazo[2,1-i]purine ring shows steric favorable interactions.

## CONCLUSION

We have proposed two designed ligands by transmuting some portions of Methotrexate with bioisosteres and found to be better binder at the active site of DHFRase *in silico*. To ascertain the probable biological activity of the compounds designed, the compounds should be synthesized and actual binding affinity will be evaluated through co-crystallization.

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