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In Silico Screening of Pyridine Derivatives as Potential DHFR Inhibitors for Anticancer Activity

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In this paper, *in silico* screening prioritization of some pyridinyl Schiff bases (Schiff bases of isoniazid (INH)) as inhibitors of mammalian dihydrofolate reductase enzyme (DHFR) for anticancer activity, before actual synthesis of the molecules and anticancer evaluation are reported.

Key Words: *In silico* screening, Isoniazid, Schiff's bases, DHFR, ADME, Docking.

INTRODUCTION

Docking methods have a great advantage as compared to 2D similarity and 3D pharmacophore search methods as it utilizes use of the 3D receptor structure in a quantitative way[1]. Docking calculations alone or combined with the virtual screening has been carried out to develop the DHFR and tyrosine kinase inhibitors[2], agonists and antagonists of A3 adenosine receptors[3], acetylcholine esterase inhibitors[4], glycogen phosphorylase inhibitors[5], thymidyalte synthase inhibitors[6], glutathione and trypanothione reductase inhibitors[7], COX-1 inibitors[8], *etc*.

Pyridine nucleus: Pyridine nucleus has a potential to inhibit many receptor enzymes. Literature reveals that pyridine moiety has shown antitumour[9], antiproliferative[10], 11-β-steroid dehydrogenase inhibition[11], IRAK-4 inhibition[12], cGMP-dependent protein kinase inhibition (PKG)[13], PKC θ inhibition[14], either as fused or unfused ring systems. Further, there are reports that isoniazid (INH), a pyridine carbohydrazide to be potent inhibitor of *M. tuberculli* DHFR[15]. This motivated us to conduct *in silico* screening for alone INH as well as Schiff bases of INH against mammalian DHFR for anticancer activity. The comparision was made against the potent and clinically used inhibitors of DHFR enzyme like methotrexate (MTX).

Dihydrofolate reductase (E.C.1.5.1.3) is the most studied enzyme for the drug designing of anticancer agents. DHFR functions a the catalyst for reduction of the dihydrofolate to tetrahydrofolate that generates reduced folate carriers of one carbon fragments and is an important co-factor in the biosynthesis of nucleic acids and amino acids. The inhibition DHFR leads to the partial depletion of intracellular reduced folates with the subsequent limitation of cell growth[16]. Thus inhibitors of this enzyme are potential anticancer agents as DHFR plays important role in the

S-Phase of cell cycle. Recognition of methotrexate, chemically a pterin analogue, as an inhibitor of the DHFR attracted the attention towards the development of folate antagonists as anticancer agents[17]. MTX is a potent inhibitor of dihydrofolate reductase (DHFR) as a consequence of DHFR inhibition, intracellular levels of tetrahydrofolate coenzymes are decreased, resulting in inhibition of thymidylate synthase and consequently DNA and purine biosynthesis[18]. Herein, *in silico* prioritization of these INH-Schiff bases before actual synthesis and anticancer evaluation for *in silico* DHFR inhibition are reported.

EXPERIMENTAL

Molecular modeling: The computation was carried out in Schrodinger molecular modeling software. Molecular docking was performed for INH Schiff base analogues using the GLIDE® integrated Maestro® 7.5 interface on the Linux operation system. The ChemOffice 6.0 software was used to draw the 3-D structures and for the conversion of the structures to mol files.

Selection of the protein file[19]: For the docking purpose the PDB file 1BOZ was selected after evaluating several files from the protein database bank www.rcsb.org.

1. The file contains the 3-D crystalline structure of DHFR from human origin.
2. Further the enzyme file was subjected to structure validation procedures.

Structure validation of the enzyme: The errata report and the Ramachandran plot was obtained from the NIH MBI sever for evaluation of protein structures and are given in Figs. 1 and 2.

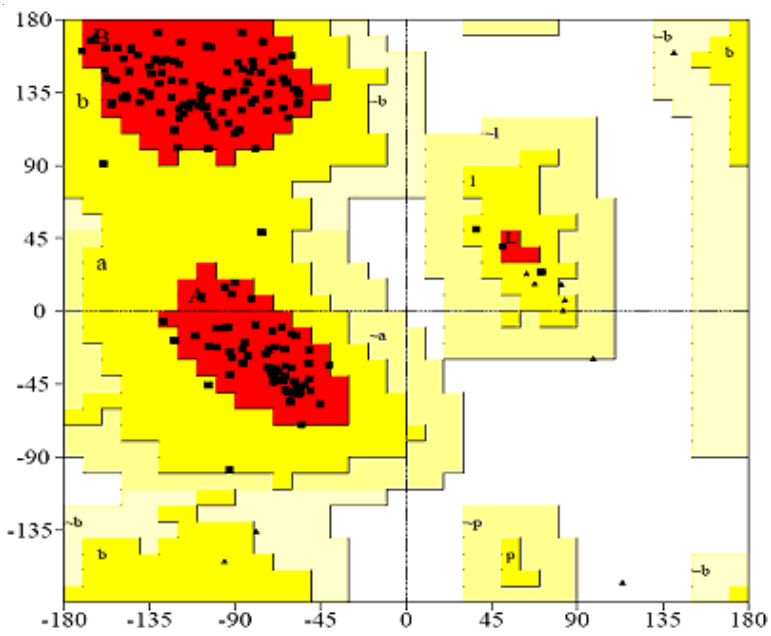
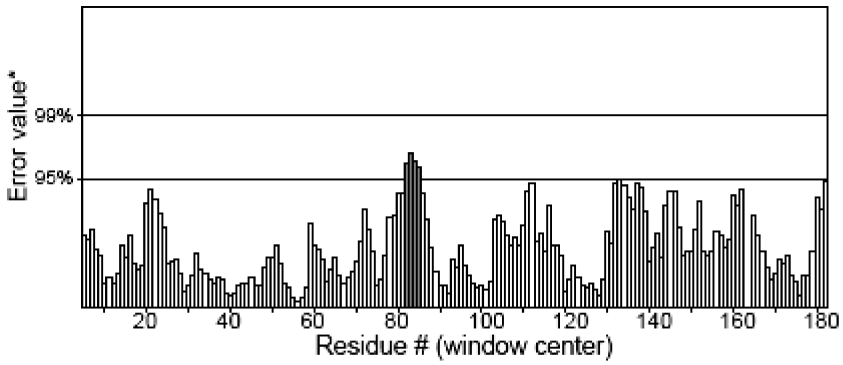


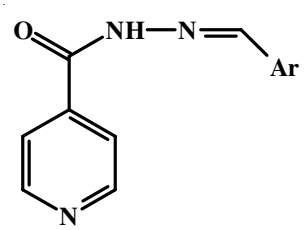
Fig. 1. Ramachandran plot of the PDB ID 1BOZ



Overall Quality Factor: 97.27%

Fig. 2. Errata report of the PDB ID1BOZ

Designing of the molecules: A set of 31 ligands from the aryl Schiff bases of INH shown in Fig. 3 were designed based upon their feasibility of synthesis and possible positional substitutions.



R = 3-Chloro-phenyl, 2-Chloro-phenyl, 4-Chloro-phenyl, 4-Bromo-phenyl, 3-Bromo-phenyl, 2-Bromo-phenyl, 2-Methoxy-phenyl, 3-Methoxy-phenyl, 4-Methoxy-phenyl, 2,6-Dichloro-phenyl, 2,5-Dihydroxy-phenyl, 3,4-Dihydroxy-phenyl, 3-hydroxy-phenyl, 4-hydroxy-phenyl, Phenyl, 2,3-Dimethoxy-phenyl, 2,4-Dimethoxy-phenyl, 2,5-Dimethoxy-phenyl, 3,4-Dimethoxy-phenyl, 4-Fluoro-phenyl, 2-Fufuryl, 2-Hydroxy-3-Methoxy-phenyl, 2-Hydroxy-4-Methoxy-phenyl, 3-Hydroxy-4-Methoxy-phenyl, 4-Isopropyl-phenyl, 2-Thiophenyl, 2-Pyrrolinyl, 2-Nitro-phenyl, 3-Nitro-phenyl, 4-Nitro-phenyl, 2-Napthalenyl

Fig. 3. Structures of the ligand molecules (ISB_{1-31})

Docking of the molecules: The ligands were prepared by LigPrep[20] module which produces a single low-energy 3D structure with correct chiralities, ionization states, tautomers, stereochemistry, ring conformations for each successfully processed input structure based upon the OPLS-2005 Molecular mechanics force field.

Energy minimization of the protein: The crystal structure of the human DHFR enzyme (1BOZ) was obtained from the protein data bank (RCSB PDB) and contains the chain A of the enzyme complexed with the known inhibitor. This chain A was selected for the docking studies in which, the energy minimization was carried out at the default cut off RMSD value of 0.30 Å.

Receptor grid preparation: The coordinates of the human DHFR chain A were obtained from the crystal structure of the protein file 1BOZ (processed file from the protein preparation wizard). The van der Waals radii were scaled up by the default value of 1.00 Å for the atoms with the partial charges of less than 0.25. The receptor grid was generated around the centroid of the ligand contained by PDB file and the ligands with cut off size of 10 Å were allowed to dock.

Docking of the ligands: Docking was carried out using the Glide module of software which uses the suite of hierarchical filters to remove unlikely solutions starting from low level approximation (distance matches) to high level calculations (force field based MCSA minimization) with free energy scoring[21]. The ligands were docked flexibly to write up to 10000 poses per ligand in the extra precision mode. This produced result of docking of the ligands having the G-Scores and $\Delta G_{binding}$ energy.

Validation of the docking protocol: Validation of the docking protocol was carried out by pose regeneration of the ligand as seen in the crystallized PDB and overlapping the best dock pose of the same ligand with the same downloaded PDB. The validation of the docking protocol was also carried out as reported by Vijjulatha *et al.*[22] with correlation coefficient r^2 of 0.9303.

RESULTS AND DISCUSSION

Docking protocol: Docking analysis was conducted for the selection of the potent inhibitors of the human DHFR enzyme prior to synthesis. The ligand preparation generated overall 76 different low energy conformations and tautomers in ionized form at a pH range 7.00 ± 2.00 for the set of 31 isoniazid aryl Schiff bases designed based upon their feasibility of synthesis. The receptor grid includes an area of 10 Å in a cuboid around the centroid of the ligand molecule. The docking analysis is carried out by default setting of low cut off RMSD value of 0.50 Å. The van der Waals radii are scaled at the default cut off distance of 1.00 Å which is an indication that only those ligands are allowed to dock and scored that have a receptor interaction with residues (amino acids) of the DHFR enzyme of 1.00 Å and interacting ligands below this distance shall not be considered suitable for the metal bondings for atoms with partial charge below 0.25. The H-bonding interactions were set to a cut off distance of 2.50 Å, donor bond angles at 120º and acceptor bond angles at 90º. Methotrexate was included in the ligand set for docking to optimize the docking protocol. The docking protocol was validated with correlation coefficient r^2 0.9303.

Selected molecules: From Table-1 it is evident that the G-Score of methotrexate being -10.10 and hence the ligand molecule scoring below -7.00 were discarded. Methotrexate has the 10 inter-hydrogen bondings with the receptor namely Thr-56 interacting with O-22 of the ligand atom, Ser-118 (OH) with O-22, Ser-118 (NH) with O-22, Gly-117 with O-31, Lys-55 with O-58, Thr-56 with O-58 Ser-119 (OH) with O-59, Ser-119 (NH) with O-59, Val-115 with H-40 and Ser-59 with H-60.

Thus it can be concluded that these amino acid residues contribute towards the active binding sites of the DHFR enzyme for interactions with methotrexate in the chain A of the enzyme and the interactions with these residues are taken standard for predicting the interactions with other ligands The thermodynamic $\Delta G_{binding}$ score for methotrexate is -163.3 kcal/mol and no ligand showed higher value than this value, proving the hypothesis of methotrexate being the potent inhibitor of the DHFR. The molecules were prioritized for synthesis and pharmacological evaluation.

TABLE-1
DOCKING SCORES OF THE BEST FIT 5 ANALOGUES FROM ISB SERIES

ISB	Ar	G-Score	$\Delta G_{binding}$ (Kcal/mol)	H-Bonds
3	Furfural	-8.35	-104.0	3
5	3-Hydroxy-phenyl	-8.22	-102.6	3
9	N,N-Dimethylamino-phenyl	-8.18	-102.0	2
4	3-Nitro-phenyl	-8.12	-98.7	3
6	*p*-Chloro-phenyl	-8.10	-100.0	3
MTX	–	-10.10	-163.3	7

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REFERENCES

1. A.K. Chakraborty and R. Thilagavathi, *Int. Electron. J. Mol. Des.*, **3**, 704 (2004).
2. A. Gangjee, Y. Zeng, M. Ihnat, L.A. Warnke, D.W. Green, R.L. Kisliuk and F.T. Lin, *Bio. Org. Med. Chem.*, **13**, 5475 (2005).
3. S.K. Kim, Z.G. Gao, L.S. Jeong and K.A. Jacobson, *J. Mol. Graph. Model.*, **25**, 562 (2006).
4. C.H.T.P. da Silva, V.L. Campo, I. Carvalho and C.A. Taft, *J. Mol. Graph. Model.*, **25**, 169 (2006).
5. Q. Deng, Z. Lu, J. Bohn, K.P. Ellsworth, R.W. Myers, W.M. Geissler, G. Harris, C.A. Willoughby, K. Chapman, B. Mckeever and R. Mosley, *J. Mol. Graph. Model.*, **23**, 457 (2005).
6. D.A. Gschwend, W. Sirawaraporn, D.V. Santi and I.D. Kuntz, *Science*, **259**, 1445 (1998).
7. M.V. Teijido, I. Caracelli and J. Zukerman-Schpector, *J. Mol. Graph. Model.*, **24**, 349 (2006).
8. M. Martic, I. Tatic, S. Markovic, N. Kujundzic and S. Kostrun, *Eur. J. Med. Chem.*, **39**, 141 (2004).
9. V. Stockmann and A. Fiksdahl, *Tetrahedron*, **64**, 7626 (2008).
10. F. Linger, F. Popowycz, T. Benson, L. Picot, C.M. Galmarini and B. Joseph, *Eur. J. Med. Chem.*, **15**, 5615 (2007).
11. H. Wang, Z. Ruan, J.J. Li, L.M. Simpkins, R.A. Smirk, S.C. Wu, R.D. Hutchins, D.S. Nirschl, K. Van Kirk, C.B. Cooper, J.C. Sutton, Z. Ma, R. Golla, R. Seethala, M.E.K. Salyan, A. Nayeem, S.R. Krystek Jr., S. Sheriff, D.M. Camac, P.E. Morin, B. Carpenter, J.A. Robl, R. Zahler, D.A. Gordon and L.G. Hamann, *Bioorg. Med. Chem. Lett.*, **18**, 3168 (2008).
12. G.M. Buckley, T.A. Ceska, J.L. Fraser, L. Gowers, C.R. Groom, A.P. Higueruelo, K. Jenkins, S.R. Mack, T. Morgan, D.M. Parry, W.R. Pitt, O. Rausch, M.D. Richard and V. Sabin, *Bioorg. Med. Chem. Lett.*, **18**, 3291 (2008).
13. A. Scribner, S. Meitz, M. Fisher, M. Wyvratt, P. Leavitt, P. Liberator, A. Gurnett, C. Brown, J. Mathew, D. Thompson, D. Schmatz and T. Biftu, *Bioorg. Med. Chem. Lett*., **18**, 5263 (2008).

14. L. Tumney, D.H. Boschelli, J. Lee and D. Chaudhary, *Bioorg. Med. Chem. Lett.*, **18**, 4420 (2008).
15. A. Argyrou, M.W. Vetting, B. Aladegbami and J.S. Blanchar, *Nat. Struct. Mol. Bio.*, **13**, 408 (2006).
16. J. Jolivet, K.H. Cowan, G.A. Curt, N.J. Clendeninn and B.A. Chabner, *New Eng. J. Med.*, **309**, 1094 (1983).
17. J.R. Bertino, E. Goker, R. Gorlick, W.W. Li and D. Banerjee, *Stem Cells*, **14**, 5 (1996).
18. J. Davoll and A.M. Jhonson, *J. Chem. Soc.*, 997 (1970).
19. www.rcsb.org.
20. Glide 4.0 User Manual, Schrodinger Press, Schrodinger Co. Ltd., USA.
21. H.D. Holtje, W. Sippl, D. Rognan and G. Folker, Molecular Modeling-Basic Principles and Applications, Wiley-VCH Gmbh & Co., Germany, edn. 2, p. 155 (2003).
22. L. Yamini and M. Vijjulatha, *E-J. Chem.*, **5**, 263 (2008).

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