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INSILICO DESIGN AND MOLECULAR DOCKING STUDIES OF NOVEL PYRIDINE DERIVATIVES AS XANTHINE OXIDASE INHIBITORS

Shiny George*, Mohammed Shafeek V, Lisana P.V, Anagha Baiju T, Babu G

Department of Pharmaceutical Chemistry, Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala, India-673634.

***Author for Correspondence: Shiny George**
Department of Pharmaceutical Chemistry, Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala, India-673634.

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ABSTRACT
Molecular docking is one of the best data-based screening methodology of virtual screening for ligand which minimized the works cost by filtering and also helps to predicted the toxicity study for designing the formulation or synthesis of New Chemical Entity in now a day of pharmaceutical research developments. The heterocyclic compounds are widely distributed in nature and they were found to posses various physiological activities. Pyridine and related fused heterocycles are of interest as potential bioactive molecules. The present work has focused on incorporation of pyridine and tetrazole and evaluate its xanthine oxidase inhibition. A new series of pyridyl tetrazole derivatives were designed as xanthine oxidase inhibitors based on docking studies and oral bioavailability scores based on Lipinski's rule evaluation. *Insilico* molecular docking was carried out using ArgusLab. To identify potential anti-gout agent lead compounds among compounds 5a1-5o2, docking calculations were performed into the 3D structure of the catalytic site of xanthine oxidase enzyme (pdb code: 1FIQ). Docking score of the novel compounds showed good fit against XO when compared with standard inhibitor salicylic acid.

KEY WORDS: Pyridine, tetrazole, docking, xanthine oxidase.

INTRODUCTION
The tetrazole group which is considered analogous to carboxylic group[1] as a pharmacore possesses wide range of biological activities. Several substituted tetrazoles have been shown to possess anticonvulsant [2], anti-inflammatory [3], CNS depressant[4], antimicrobial [5] anti-AIDS[6] and antifertility[7] agents. The first tetrazole was prepared in 1885 by the Swedish chemist, J. A. Bladin, at the University of Upsala during the course of an investigation of the reactions of dicyanophenylhydrazine, the condensation product of cyanogen and Phenylhydrazine[8]. Pyridine derivatives containing multi-functional groups such as streptonigrin, streptonigrone and lavendamycin are reported as anticancer drugs and cerivastatin is reported as the HMG-CoA enzyme inhibitor. Moreover, substituted pyridines are reported as leukotriene B-4 antagonists.[9]

Xanthine oxidase (XO) is a highly versatile enzyme that is widely distributed among different species from bacteria to man and within the various tissues of mammals. It is a member of group of enzymes known as molybdenum iron – sulphur flavin hydroxylase. It catalyses the oxidation of hypoxanthine to xanthine which further reduce to uric acid, the final reactions in the metabolism of purine bases. The accumulation of uric acid in the body is responsible for the formation of several diseases and thus it plays a vital role in producing hyperuricemia and gout. Inherited xanthine oxidase reductase (XOR) deficiency leads to xanthineuria and multiple organ failure syndrome caused by the accumulation of xanthine in different tissues [10].

The present study aimed to develop molecules with improved xanthine oxidase inibitory activity. The possible effective molecules were designed by incorporating the fusion of tetrazole and pyridine moiety. The objectives are to screen 1, 5 di-substituted tetrazole incorporated pyridine derivatives by using Lipinski rule of 5 for oral bioavailability and carry out docking simulation by using ArgusLab and find out the derivative with higher docking scores.

MATERIALS AND METHODS
All the compounds were constructed using Chem Draw Ultra software, Cambridge Soft Corporation, USA. Version-8.0 April 23, 2003. It is a Chem Tech tool used for the drawing of ligand molecules. The crystal structure of XO receptor used for docking was recovered from Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/home) (entry code: 1FIQ).

Docking study

Lead optimization

Lead optimization was done through insilico Lipinski filter. Molinspiration server was used for this purpose. The structure drawn in the JME editor was subjected to calculate the druglikeness score through calculate the properties module. The datas are given in the **Table 2**.

Input File Preparations for Energy Minimization of Protein

For each of the protein-ligand complexes chosen for the study, a "clean input file" was generated by removing water molecules, ions, ligands, and subunits not involved in ligand binding from the original structure file. Water molecules were removed because ArgusLab sometimes failed to dock the compounds having water molecules at their binding sites. All hydrogen atoms in the protein were allowed to optimize. The hydrogen locations are not specified by the X-ray structure but these are necessary to improve the hydrogen bond geometries, at the same time maintaining the protein conformation very close to that observed in the crystallographic model. The resulting receptor model was saved to a PDB file. Minimization was performed by geometry convergence function of ArgusLab software performed according to Hartree-Fock calculation method [11].

Ligand Input File Preparation and Optimization

Ligand input structure was drawn using Chem Draw software. The structure was cleaned in 3D format and energy was minimized. The resulting structure was then saved in "mdl mol" format for molecular docking studies.

Docking Methodology

After the preparation of the protein and ligand, molecular docking studies were performed by ArgusLab 4.0.1 to evaluate the interactions. The active site of protein was obtained from CASTp [12].

ArgusLab 4.0.1

ArgusLab is implemented with shapebased search algorithm. Docking has been done using "Argus Dock" exhaustive search docking function of ArgusLab with grid resolution of 0.40 A°. Docking precision was set to "Regular precision" and "Flexible" ligand docking mode was employed for each docking run. The stability of each docked pose was evaluated using ArgusLab energy calculations and the number of hydrogen bonds formed.

Molecular Docking Study

To perform docking one first needs to define atoms that make up the ligand and the binding sites of the protein where the ligand should bind. The prepared 3D structures of 1FIQ protein was downloaded into the ArgusLab program and binding sites were made by choosing "Make binding site for this protein" option. The ligand was then introduced and docking calculation was allowed to run using shape-based search algorithm and AScore scoring function. The scoring function is responsible for evaluating the energy between the ligand and the protein target. Flexible docking was allowed by constructing grids over the binding sites of the protein and energy-based rotation is set for that ligand's group of atoms that do not have rotatable bonds. For each rotation, torsions are created and poses (conformations) are generated during the docking proces. For each complex 10 independent runs were conducted and one pose was returned for each run. The best docking model was selected according to the lowest AScore calculated by ArgusLab and the most suitable binding conformation was selected on the basis of hydrogen bond interactions between the ligand and protein near the substrate binding site. The lowest energy poses indicate the highest binding affinity as high energy produces the unstable conformations [13].

RESULTS AND DISCUSSION

TABLE -1 LIST OF SUBSTITUENTS

CPD CODE	R`	R	n	CPD CODE	R`	R	n
5a1	H	H	0	5a2	CH_3	H	0
5b1	H	4-Cl	0	5b2	CH_3	4-Cl	0
5c1	H	4-OCH_3	0	5c2	CH_3	4-OCH_3	0
5d1	H	3-Cl	0	5d2	CH_3	3-Cl	0
5e1	H	H	1	5e2	CH_3	H	1
5f1	H	2-Cl	0	5f2	CH_3	2-Cl	0
5g1	H	3,5-Cl	0	5g2	CH_3	3,5-Cl	0
5h1	H	2-F	0	5h2	CH_3	2-F	0
5i1	H	3-F	0	5i2	CH_3	3-F	0
5j1	H	3,5-F	0	5j2	CH_3	3,5-F	0
5k1	H	3-F, 5- CH_3	0	5k2	CH_3	3-F, 5- CH_3	0
5l1	H	2-F, 4-CH_3	0	5l2	CH_3	2-F, 4-CH_3	0
5m1	H	3- CH_3,5-F	0	5m2	CH_3	3- CH_3,5-F	0
5n1	H	3- CH_3,4-F	0	5n2	CH_3	3- CH_3,4-F	0
5o1	H	4-CH_3	0	5o2	CH_3	4-CH_3	0

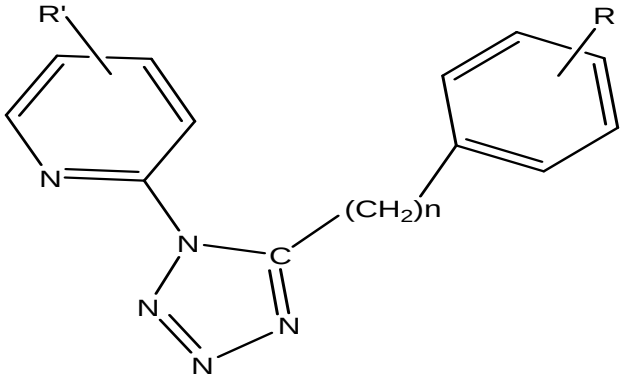

TABLE-2 LIPINSKI RULE ANALYSIS

Cpd Code	Log P	Molecular weight	Hydrogen donors	Hydrogen acceptor	Number of violation
5a1	2.232	223.239	5	0	0
5b1	2.91	257.684	5	0	0
5c1	2.289	253.265	6	0	0
5d1	2.886	257.684	5	0	0
5e1	2.182	237.266	5	0	0
5f1	2.862	257.684	5	0	0
5g1	3.516	292.12	5	0	0
5h1	2.348	241.229	5	0	0
5i1	2.372	241.229	5	0	0
5j1	2.487	259.219	5	0	0
5k1	2.772	255.256	5	0	0
5l1	2.772	255.256	5	0	0
5m1	2.772	255.256	5	0	0
5n1	2.772	255.256	5	0	0
5o1	2.68	237.266	5	0	0
5a2	2.685	237.266	5	0	0
5b2	3.363	271.711	5	0	0
5c2	2.742	267.292	6	0	0
5d2	3.339	271.711	5	0	0
5e2	2.635	251.293	5	0	0
5f2	3.315	271.711	5	0	0
5g2	3.969	306.156	5	0	0
5h2	2.825	255.256	5	0	0
5i2	2.801	255.256	5	0	0
5j2	2.94	273.246	5	0	0
5k2	3.225	269.283	5	0	0
5l2	3.225	269.283	5	0	0
5m2	3.225	269.283	5	0	0
5n2	3.225	269.283	5	0	0
5o2	3.134	251.293	5	0	0

TABLE-3 BINDING ENERGY OF DESIGNED ANALOGOUS

Compound code	Binding energy (kcal/mol)	Compound code	Binding energy (kcal/mol)
5a1	-8.3839	5h2	-8.2352
5a2	-9.4304	5i1	-8.6752
5b1	-8.29826	5i2	-8.337
5b2	-7.88293	5j1	-8.06682
5c1	-7.93772	5j2	-7.77989
5c2	-7.38748	5k1	-8.50669
5d1	-7.6543	5k2	-8.3481
5d2	-8.89316	5l1	-8.3605
5e1	-8.59205	5l2	-7.46441
5e2	-9.57796	5m1	-7.7562
5f1	-8.54583	5m2	-8.11456
5f2	-8.79077	5n1	-8.0181
5g1	-9.07831	5n2	-7.57482
5g2	-8.86784	5o1	-8.3011
5h1	-6.5437	5o2	-8.07091
Salicylic acid	-7.694	-	

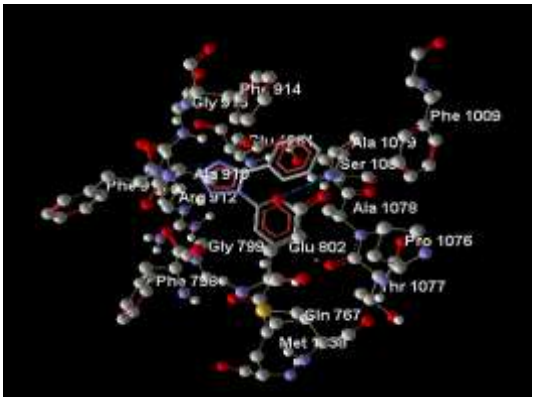

Fig 1: Hydrogen bonding interaction of 5a2 with Ala 1079 and bond length 2.79 A^o

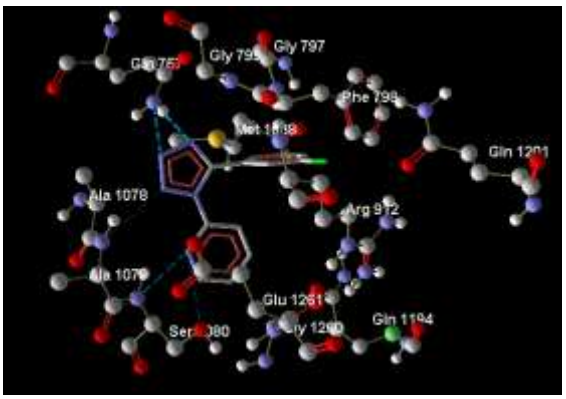

Fig 2: Hydrogen bonding interaction of 5g1 with Gln 767 and bond length 2.978 A^o

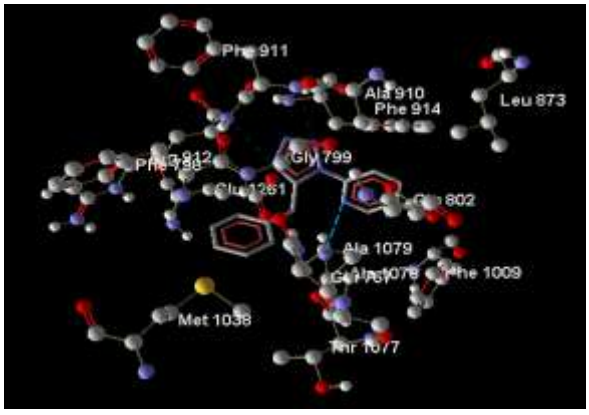

Fig 3: Hydrogen bonding interaction of 5e2 with Ala 1079 and bond length 2.912 A^o

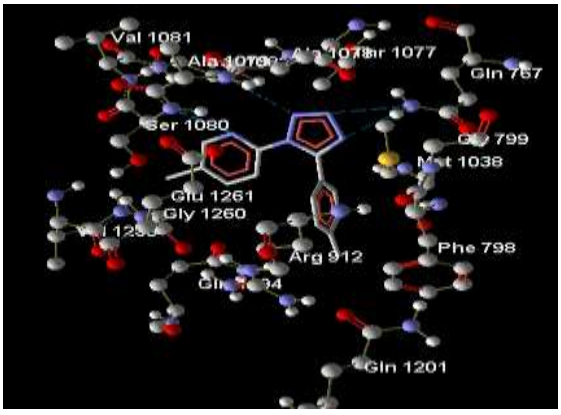

Fig 4: Hydrogen bonding interaction of 5o2 with Gln769 and bond length 3.288 A^o

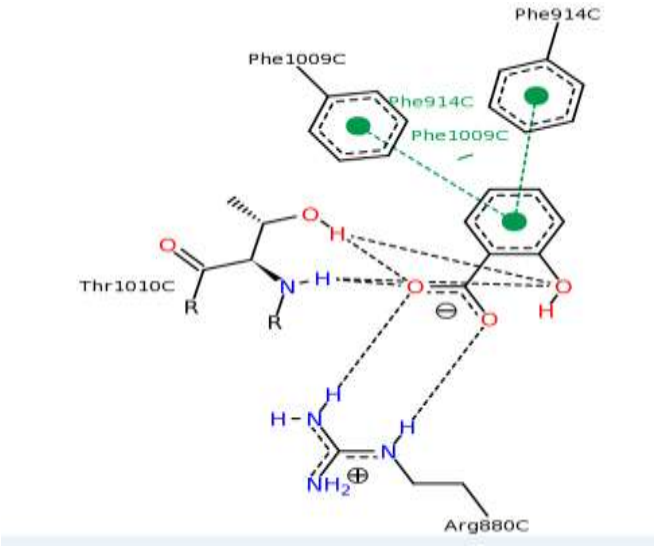

Fig 5: standard inhibitor salicylic acid shows hydrogen bonding with Thr 1010 and Arg 880.

CONCLUSION

Preliminary *in-silico* molecular modeling was carried out with the help of available softwares. All the proposed analogs obeyed Lipinski's Rule of Five. Docking studies were carried out on the proposed analogue to determine the affinity with the enzyme XO using Argus lab. The analogue 5e2 was found to have higher docking score and significant binding interaction. Molecular docking studies shows that hydrogen bond interaction and hydrophobic interaction plays a crucial role in the biological activity of novel compounds. From the present study it can be concluded that 1,5-disubstituted pyridyl tetrazole derivatives were found to possess good xanthine oxidase inhibition.

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