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In-silico Design and Molecular Docking Studies of Novel Cinnoline Derivatives for Anti-tubercular Activity

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Abstract

Tuberculosis (TB) has become one of the most significant public health problems in recent years. Antibiotic therapy remains the mainstay of TB control strategies, but the increasing resistance of mycobacterial species has heightened alarm, requiring the development of novel drugs in order to improve treatment outcomes. Here, as an effort to identify novel and effective antitubercular agents,we designed a series of novel substituted cinnoline by various softwares such as ACD Lab ChemSketch, Molinspiration, PASS and AdmetSAR. The designed molecules having required physicochemical properties, drug likeness and obeying Lipinski rule of five were selected for docking studies. The docking studies were performed at M.tuberculosis enoyl-reductase InhA(PDB ID: 5JFO) by using the software, Biovia Discovery Studio. From the docking score, we identified some effective compounds may serve as a chemical probe of interest for further lead optimization studies with the general aim of developing novel and effective antitubercular agents.

Keywords: Cinnoline, Docking , In- silico design, Lipinski's rule of five, Mycobacterium tuberculosis,

INTRODUCTION

Tuberculosis (TB) is a common and deadly infectious disease caused by various strains of mycobacteria, usually Mycobacterium TB (MTB). In spite of availability of highly potent antitubercular agents, TB remains the primary reason for high mortality worldwide. According to recent reports by the World Health Organization (WHO), worldwide, 10.4 million new tuberculosis patients were identified in 2016, from which 1.67 (16.1%) million deaths were recorded. The majority of TB infected people can be cured with early diagnosis and proper treatment.Antibiotic therapy remains the mainstay of TB control strategies. In addition, the increasing resistance of Mycobacterium species to existing drugs has heightened alarm about TB in the international health community [8]. Hence, the development of novel drugs with anti-tubercular activity is an urgent need. The increasing global TB burden due to HIV, MDR-TB, and XDR-TB has led to the search for newer therapeutic agents to tackle the menace [1].

Target Enzyme

InhA

InhA, the enoyl-ACP reductase in MTB is an attractive target for the development of novel drugs against TB, a disease that kills more than two million people each year. InhA is the target of the current first-line drug isoniazid for the treatment of TB infections. Compounds that directly target InhA and do not require activation by the mycobacterial catalase-peroxidase KatG are promising candidates for treating infections caused by isoniazid-resistant strains [2].

The enzyme was downloaded from the Protein Data Bank (an information portal to biological macromolecular structures).

Heterocyclic rings, which have been reason for the activity of most of the drugs of natural origin leads to the discovery of many synthetic drugs possessing the heterocyclic rings and their fused analogs represent an important class of heterocyclic compounds exists in numerous natural products displaying a wide range of biological and pharmaceutical activities. On intensive research heterocyclic derivatives continue to yield new medicinal agents [3].

Cinnoline ring is a versatile lead molecule that has been investigated widely used in medicinal chemistry due to its important pharmacological activities. The nucleus gives out different derivatives with different biological activities such as anti-microbial, anti-tubercular, anti-malarial, anti-hypertensive, anti-convulsant, neurological disorders, anti-depressant, anti-pyretic, analgesic, anxiolytics, anti-diabetic, anaesthetic, anti-thrombolytic, cardiotonic, anti-tumor, herbicidal, agrochemical insecticidal, etc [4].

Our ongoing investigations have been directed toward the in-silico design and molecular docking studies of novel cinnoline derivatives for anti-tubercular effects.

MATERIALS AND METHODS:

In-silico molecular modification was the most important preliminary step in the rational drug designing of novel drugs. In the present study different proposed derivatives are screened for different physico-chemical properties by using different softwares. ACD Lab Chemsketch was used for 3-D drawing and calculating various molecular descriptors such as hydrophobicity, lipophilicity, steric and electronic parameters of the proposed molecules. The Molinspiration software was used for calculating log P values, Lipinski's rule of five and drug likeness.

The proposed molecules were screened for whether they obey the rule of five or not. The general biological activities of proposed molecules were predicted by using PASS (Prediction of activity spectra for substances) software and general pharmacokinetic properties of proposed molecules were predicted by using AdmetSAR programme. The designed molecules having required physicochemical properties, drug-likeness and obeying Lipinski rule of five were selected for docking studies.

Molecular docking studies

In cell biology, the function of proteins is a result of its interaction (i.e., docking) with other proteins as well as other molecular components. If we can understand how proteins interact (dock) with other molecules, the function

of the protein can be inferred. Thus, the results of the docking help us to find the molecules which are effective against the particular disease. Docking searches for a molecule's (ligands) favored orientation with receptors usually a protein for the best binding affinity [2].

The 3-D structure of the protein was obtained from the protein data bank (PDB) using their specific PDB ID (5JFO). Bio via Discovery studio 2018 was used for the molecular docking of proposed molecules. Before docking the target and ligands were preprocessed for optimizing and minimizing the structure and generating conformers respectively. Library docking is performed for identifying the binding affinity with the targets using CHARM as force field.

Ten derivatives of cinnoline were selected as ligands and their structures were drawn using ACD Lab Chemsketch and Converted To 3d Form For The Docking studies. The ligands are shown in table 1 and fig.1

TABLE 1: LIGAND MOLECULES AND THEIR IUPAC NAMES

Ligand code	Ligand IUPAC names
BN01	6-chloro-4-{[(4-nitrophenyl)methylidene]amino}cinnoline-3-carboxamide
BN02	6-chloro-4-{(Z)-[(3-nitrophenyl)methylidene]amino}cinnoline-3-carboxamide
BN03	6-chloro-4-{(Z)-[(4-methoxyphenyl)methylidene]amino}cinnoline-3-carboxamide
BN04	6-chloro-4-{[(2-chloro-4-hydroxyphenyl)methylidene]amino}cinnoline-3-carboxamide
BN05	6-chloro-4-{(Z)-[(4-chloro-2-iodophenyl)methylidene]amino}cinnoline-3-carboxamide
BN06	6-chloro-4-[(Z)-{[4-(dimethylamino)phenyl]methylidene}amino]cinnoline-3-carboxamide
BN07	6-chloro-4-{(Z)-[(4-hydroxy-3-methoxy-5-nitrophenyl)methylidene]amino}cinnoline-3-carboxamide
BN08	methyl 4-{(Z)-[(3-carbamoyl-6-chlorocinnolin-4-yl)imino]methyl}benzoate
BN09	6-chloro-4-{(Z)-[(2,4-dihydroxyphenyl)methylidene]amino}cinnoline-3-carboxamide
BN10	6-chloro-4-{(Z)-[(2-hydroxy-4-methoxyphenyl)methylidene]amino}cinnoline-3-carboxamide

TABLE 2: MOLECULAR DESCRIPTORS OF PROPOSED DERIVATIVES

Compound	Molar refractivity,cm^3	Molar volume,cm^3	Parachor,cm^3	Surface tension,dynes/cm	Polarizability,cm^3
BN-1	90.82±0.5	227.4±7.0	654.1±8.0	68.4±7.0	38.83±0.5 10^{-24}
BN-2	90.82±0.5	227.4±7.0	654.1±8.0	68.4±7.0	38.83±0.5 10^{-24}
BN-3	90.97±0.5	243.8±7.0	658.9±8.0	53.3±7.0	36.06±0.5 10^{-24}
BN-4	90.61±0.5	228.7±7.0	643.2±8.0	62.5±7.0	35.92±0.5 10^{-24}
BN-5	102.41±0.5	249.0±7.0	700.2±8.0	62.5±7.0	40.60±0.5 10^{-24}
BN-6	97.96±0.5	263.3±7.0	704.9±8.0	51.3±7.0	38.83±0.5 10^{-24}
BN-7	97.49±0.5	246.3±7.0	710.0±8.0	69.0±7.0	38.64±0.5 10^{-24}
BN-8	96.43±0.5	257.1±7.0	703.2±8.0	55.9±7.0	38.23±0.5 10^{-24}
BN-9	86.86±0.5	216.6±7.0	620.0±8.0	67.0±7.0	34.43±0.5 10^{-24}
BN-10	91.83±0.5	241.4±7.0	664.6±8.0	57.7±7.0	36.40±0.5 10^{-24}

TABLE 3: ANALYSIS OF LIPINSKI'S RULE OF FIVE

Compound	LogP	Mol. Wt	NHDon	nHAcc	Nrotb	Lipinski's rule alert index
BN-1	3.06	355.73	2	8	4	0
BN-2	3.04	355.73	2	8	4	0
BN-3	3.16	340.76	2	6	4	0
BN-4	3.08	361.18	2	7	4	0
BN-5	4.79	471.07	2	5	3	0
BN-6	3.21	353.80	2	6	4	0
BN-7	2.56	401.76	3	10	5	0
BN-8	3.27	368.77	2	7	5	0
BN-9	2.54	342.73	4	7	3	0
BN-10	3.08	356.76	3	7	4	0

TABLE 4: DRUG- LIKENESS ANALYSIS OF LIGANDS

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
BN-1	-0.44	-0.41	-0.11	-0.68	-0.40	-0.09
BN-2	-0.53	-0.42	-0.04	-0.68	-0.45	-0.21
BN-3	-0.43	-0.45	-0.07	-0.61	-0.39	-0.13
BN-4	-0.39	0.40	-0.12	-0.48	-0.34	-0.04
BN-5	-0.39	-0.30	-0.11	-0.59	-0.35	-0.16
BN-6	-0.37	-0.39	-0.14	-0.55	-0.33	-0.09
BN-7	-0.62	-0.59	-0.12	-0.67	-0.56	-0.19
BN-8	-0.47	-0.45	-0.01	-0.59	-0.39	-0.16
BN-9	-0.34	-0.43	-0.15	-0.43	-0.28	-0.04
BN-10	-0.39	-0.50	-0.09	-0.49	-0.32	-0.10
INH	-1.39	-1.45	-1.05	-2.33	-1.23	-0.66

TABLE 5: ADMET PROPERTY/DESCRIPTORS OF LIGANDS

Compounds	ADME prediction				Toxicity prediction	
	BBB	Caco2 cell permeability	HIA	Cytochrome p 450	Ames test	Carcinogenicity
INH	0.9895	0.6959	0.9892	inhibitor	Non-Ames toxic	Non-carcinogens
BN-1	0.8892	0.5326	0.9702	inhibitor	Non-AMES toxic	Non-carcinogens
BN-2	0.8892	0.5326	0.9702	inhibitor	Non-AMES toxic	Non-carcinogens
BN-3	0.9558	0.5178	1.0000	inhibitor	Non-AMES toxic	Non-carcinogens
BN-4	0.8693	0.5071	0.9930	inhibitor	Non-AMES toxic	Non-carcinogens
BN-5	0.9445	0.5205	0.9835	inhibitor	Non-AMES toxic	Non-carcinogens
BN-6	0.8615	0.5381	0.9939	inhibitor	Non-AMES toxic	Non-carcinogens
BN-7	0.6676	0.5810	0.7910	inhibitor	Non-AMES toxic	Non-carcinogens
BN-8	0.8876	0.5413	0.9859	inhibitor	Non-AMES toxic	Non-carcinogens
BN-9	0.7247	0.5717	0.9888	inhibitor	Non-AMES toxic	Non-carcinogens
BN-10	0.7203	0.5403	0.9918	inhibitor	Non-AMES toxic	Non-carcinogens

TABLE 6: SUMMARY OF PASS VALUES OF LIGANDS

Compounds	**Anti-tubercular activity**	
	P_a	**P_i**
BN01	0.665	0.004
BN02	0.665	0.004
BN03	0.669	0.007
BN04	0.508	0.012
BN05	0.554	0.008
BN06	0.408	0.029
BN07	0.557	0.008
BN08	0.453	0.019
BN09	0.417	0.027
BN10	0.527	0.10

TABLE 7: LIGAND INERACTING RESIDUES AND THE DOCKING SCORES

SL:No.	**Compound**	**Substituition (-R)**	**Docking score**	**Interacting Residue**
1	BN01	4-NO_2	92.9384	Met 98, Tyr 158.
2	BN02	3-NO_2	80.7197	Lys 165, Ala 191, Gly 192, Asp 148, Met 147, Phe 149.
3	BN03	4-OCH_3	74.3888	Lys 165
4	BN04	2-Cl,4-OH	68.7534	Ile 21
5	BN05	4-Cl,2-I	70.8758	Lys 165
6	BN06	4-$N(CH_3)_2$	65.748	Ser 94
7	BN07	4-OH,3-OCH_3,5-NO_2	78.2134	Lys 165
8	BN08	4-$COOCH_3$	66.9707	Ile 194, Lys 165
9	BN09	2,4-dihydroxy	72.6638	Lys 165
10	BN10	2-OH,4-OCH_3	74.9593	Ser 94, Lys 165.
11	INH	-	56.8785	Tyr 158

RESULTS AND DISCUSSION

In silico Design

Insilico molecular modifications of proposed derivatives were done by using different softwares. 3-D drawing, optimizing and calculating various descriptors of proposed derivatives were done by using ACD Lab Chemsketch 12.0 and the results are shown in Table 2.

Analysis of Lipinski's Rule of Five and Drug- likeness

Molinspiration software was used to study the logP values, violation of Lipinski's rule of five and drug likeness by comparing with the existing standard drug, Isoniazid. All the compounds obey Lipinski's rule of five and the results are shown in Table 3 and Table 4.

ADMET Property/Descriptors of ligands

The ADMET (absorption, distribution, metabolism and excretion and toxicity) profiles were predicted by AdmetSAR programme. The study showed all the ligands tested exhibited positive result to cross blood-brain barrier, human intestinal absorption, Caco-2 permeability, the possible pharmacological effects in the central nervous system need to be further studied. Similarly, all the compounds scored negative result for Ames toxicity and carcinogenic character. Hence all the compounds are found to be suitable for human consumption. The results are shown in Table 5 and Table 6.

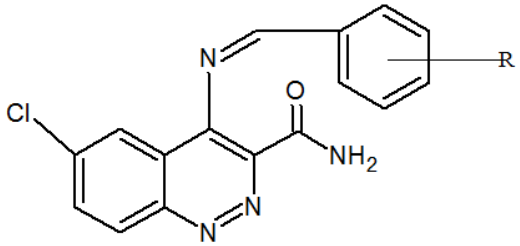

Fig. 1: Structure of novel cinnoline derivatives

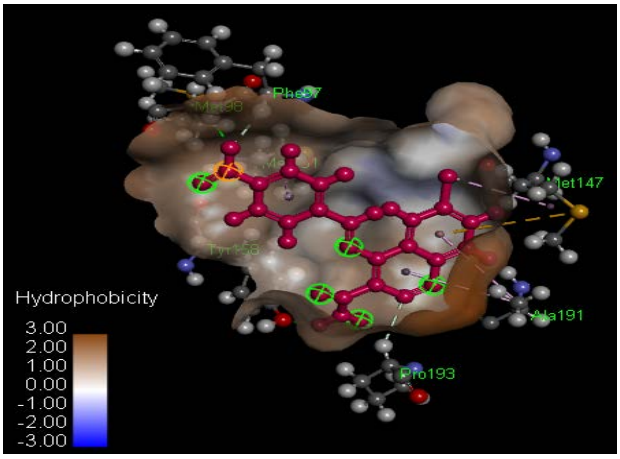

Fig. 2: Docking Image of BN01with 5JFO.

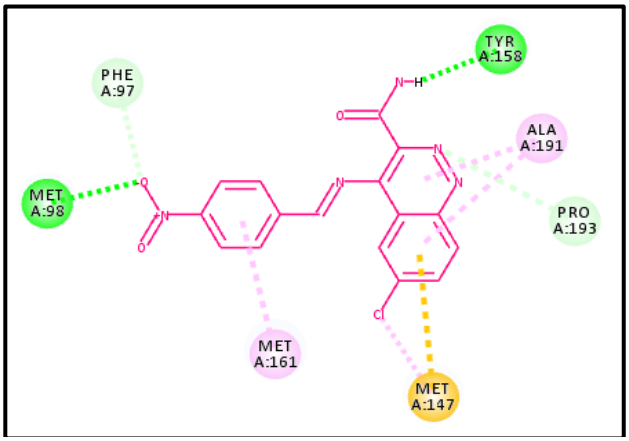

Fig. 3: Ligand Interactions of BN01 with 5JFO.

Molecular docking studies

The crystal structure of the M.tuberculosis enoyl-reductase InhA in complex with GSK625with PDB ID: 5JFO were retrieved from PDB with a resolution of 2.907A^0. The protein consists of polypeptide, chain with sequence length of 269 amino acids. The binding site of protein interaction with its inhibitor compound Gly 96, Met 98, Lys 165, Met 103 and Tyr 158. In preliminary docking analysis were conducted by selecting Gly 96, Met 98, Lys 165, Met 103 and Tyr 158 as binding site residues.

Docking scores of novel cinnoline derivatives with enzyme M. tuberculosis enoyl-reductase InhA (PDB ID: 5JFO) for anti-tubercular activity are given in Table 7.

All the ten compounds shows better docking score than the standard drug, isoniazid. Among the 10 compounds BN01 showed better interaction with the target active site amino acid by Hydrogen bond interaction with Met 98 and Tyr 158 with good docking score of 92.9384 compared to that of standard drug Isoniazid. So this molecule can be considered as ideal lead molecule in drug discovery after scientific validation.(fig.2, fig.3).

CONCLUSION

TB, the contagious disease caused by *M. tuberculosis*, has been threatening the mankind since ages. The present work was focused on the rational approach in designing and development of derivatives of well-known antitubercular drug INH not only as a mode to improve its antitubercular activity but also to minimize other problems associated with INH therapy.

A series of cinnoline derivatives were subjected to preliminary in silico designing. Docking studies of the designed derivatives were performed using Bio via Discovery studio 2018. Among the 10 derivatives docked at the active site of enoyl-reductase InhA (PDB ID: 5JFO) to study anti tubercular effect, the one containing nitro group (6-chloro-4-{[(4-nitrophenyl)methylidene]amino}cinnoline-3-carboxamide) shows better docking score when compared to that of standard drug, isoniazid. In addition to this, all the compounds shows better docking scores. So the in silico studies indicate the relevance of the work and further investigation can be done in future.

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