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Docking analysis of potent inhibitors of topoisomerase IV as potential antimicrobial agents

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ABSTRACT

According to data of the World Health Organization, Tuberculosis (TB) caused by *Mycobacterium tuberculosis*, is considered to be the most chronic communicable disease in the world especially in Asia and Africa. This situation was made worse by the emergence of multi drug resistant TB (MDR-TB) and the increasing number of HIV-positive TB cases. Worldwide, TB accounts for approximately one-fourth of HIV-related deaths and is the leading cause of death in HIV-infected adults in developing countries, thus an urgent need exists for the development of new antimycobacterial agents with a unique mechanism of action. *Mycobacterium tuberculosis 3LPS*, an essential enzyme to pass one double strand of DNA to another there by changing the linking number of DNA is an attractive target for novel anti-tubercolosis agents. A series of Isatin, Thiazole with various acetophenones were computationally designed and energy minimized. The molecular properties were calculated from suitable computational tools. These ligands were investigated for drug like properties by calculating Lipinski's rule of five using mol inspiration. All of the derivatives showed a zero violations of the rule of 5 which indicates good bioavailability. The positive bioactivity score of the derivative were also in agreement with their probability of drug likeness. These compounds were docked using Topoisomerase IV using Argus lab docking software which showed good binding energy for the enzyme, when compared with the binding energies of standard drug isoniazid -5.83kcal/mol.) Among all the designed ligands, the ligand S_1A and S_5N showed more binding energy values (-8.05 and -8.37Kcal/mol). In future we planned to synthesize these ligand and to screen for their anti TB activity.

INTRODUCTION

Tuberculosis is one of the major reasons of death all across the world. The responsible microbe for this dreaded disease is none but a bacterium, *Mycobacterium tuberculosis*, which has an unusual cell wall composition for its survival. The cell wall component has mycolic acid which is synthesized due to the Fatty Acid Synthase-II enzyme (FAS-II). This prevents binding of broad range of drug molecule due to presence of a precursor of mycolic acid, the Meromycolic acid. This facilitates the bacterium with pathogenicity, survival and multi drug resistant functionality. Every year huge population is being chomped by *TB* at a rate of about 2-3 million annually. Thus an urgent need exists for the development of new antimycobacterial agents with a unique mechanism of action [1-3].

Enzyme-Topoisomerase IV

Topoisomerase IV is one of two type-II topoisomerases in bacteria, the other being DNA gyrase. Like gyrase, topoisomerase IV is able to pass one double-strand of DNA through another double-strand of DNA, there by changing the linking number of DNA by two in each enzymatic step [4].

Topoisomerase IV has two main functions in the cell. First, it is responsible for unlinking, or deactivating, DNA following DNA replication. The double-helical nature of DNA and its semi conservative mode of replication cause the two newly replicated DNA strands to be interlinked. These links must be removed in order for the chromosome (and plasmids) to segregate into

daughter cells so that cell division can complete. The second function in the cell is to relax positive supercoils. It shares this role with DNA gyrase, which is also able to relax positive supercoils [5-7]. Together, gyrase and topoisomerase IV remove the positive supercoils that accumulate ahead of a translocating DNA polymerase, allowing DNA replication to continue unhindered by topological strain. DNA gyrases are analogous enzymes in other organisms [8].

Isatin and Thiazole linked with various acetophenone derivatives are known to possess antitubercular, antifungal, anti-neoplastic activities. Construction of our compounds containing both the Isatin, Thiazole linked with various acetophenones towards the development of novel antimycobacterial agents. Based on that we planned to link Isatin, Thiazole with various acetophenones derivative systems to produce better anti tubercular agents and to evaluate the interactions with the target (Topoisomerase IV) by using ARGUS LAB docking software [8-10].

MATERIALS AND METHODS

Calculation of Molecular Physicochemical Properties [10,11]

The physiochemical properties involve determination of drug-like property of the designed compounds. It is based on Lipinski's rule of five and can be determined by using molinspiration cheminformatics software. All the designed compounds showed zero violation of Lipinski's rule of five, which indicates good bioactivity and bioavailability.

Lipinski's Rule of Five states that in general, an orally active drug has not more than one violation of the following criteria. It should have, Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms), Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms), A molecular weight under 500 g/ mol., A partition coefficient log P less than 5 and Not more than 15 rotatable bonds.

Molecular Docking [12,13]

Preparation of protein molecule

The experimental structure of Topoisomerase IV (PDB CODE: 3LPS) as shown in Figure 2 was retrieved from the RCSB protein data bank as a PDB file. The protein molecules were prepared mainly by using the software Swiss PDB viewer. Active site residues within a range of 4.0 A0 were selected and saved in PDB format.

Preparation of ligand

The ligands were drawn using ACD/ Chemsketch (12.0) (Alex, 2009) and saved in mol 2 format. The saved ligand compounds were later imported and minimized in Argus Lab after adding hydrogen bonds. The molecules thus obtained were saved in PDB format. Physiochemical properties and biological activities of the compounds are presented in table 1 and 2.

Docking of designed ligands to Topoisomerase IV (3LPS)

Docking of designed ligands (S_1A-S_5N) with Topoisomerase IV was performed using ARGUS LAB 4.0. The algorithm exhaustively searches the entire rotational and translational space of the ligand with respect to the receptors. The various solutions evaluated by a score, which is equivalent to the absolute value of the total energy of the ligand in the protein environment. The best docking solutions ARGUS LAB score for each compound was considered. It was noted that ARGUS LAB scores of compound S_1A and S_5N were 8.05 and -8.37Kcal/mol respectively, which is greater than isoniazid drug score value -5.83. as shown in Table 3, Figures 3, 4 and 5. The drug like activity of the ligand molecules are characterized using ADME properties. Isoniazid and designed compounds satisfy Lipinski rule of 5 and ADME properties results are shown in Table 1& 2.

RESULTS

Physiochemical Properties

Analysis of physiochemical properties and determination of drug-like property of the designed compounds were carried out based on Lipinski's rule of five by using molinspiration cheminformatics software. All the designed compounds showed zero violation of Lipinski's rule of five. As per Lipinski's Rule of Five, an orally active drug has not more than one violation, it should have not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms), not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms), a molecular weight under 500 g/ mol., and a partition coefficient log P less than 5 and Not more than 15 rotatable bonds.

The log P values of the compounds are ranges from 3.486 to 4.644 and topological polar surface area are ranges from 58.118 to 103.942 angstroms. Molecular weight of the compounds are within thin limit of Lipinski rule and are ranges from 305.362 to 350.359 Daltons. Most of the compounds had 4 or 5 hydrogen bond acceptor while compound S_5N was observed with 7 hydrogen bond acceptor. Majority of the compound were shown

Table 1. : Physicochemical Properties

COMP	Log P	TPSA	MW	No. of hydrogen bond acceptor	No. of hydrogen bond donor	Violations	No. of rotatable bond	Molar volume
S_1A	3.966	58.118	305.362	4	1	0	2	257.223
S_2H	3.486	78.346	321.361	5	2	0	2	265.238
S_3M	4.022	67.352	335.388	5	1	0	3	282.766
S_4C	4.644	58.118	339.807	4	2	0	2	270.756
S_5N	3.925	103.942	350.359	7	1	0	3	280.555

Table 2. : Biological Activities

COMP	GPCR	ION CHANNEL	KINASE INHIBITOR	NUCLEAR RECEPTOR LIGAND	PROTEASE INHIBITOR	ENZYME INHIBITOR
S_1A	-0.37	-0.57	0.01	-0.94	-0.83	-0.30
S_2H	-0.29	-0.51	0.07	-0.73	-0.75	-0.23
S_3M	-0.37	-0.61	-0.01	-0.85	-0.78	-0.33
S_4C	-0.34	-0.56	0.01	-0.91	-0.81	-0.33
S_5N	-0.47	-0.57	-0.12	-0.91	-0.83	-0.38

with only one hydrogen bond donor and two compounds (S_2H, S_4C) were noted with 2 hydrogen bond donor. Number of rotatable bonds in most of the compounds are 2. All the designed compounds (S_1A-S_5N) showed zero violation of Lipinski's rule of five, which indicates good bioactivity and bioavailability. The complete data are presented in Table 1.

Biological activity

Analysis of various biological activities of the compounds were carried out, which includes G-Protein Coupled Receptor activity, Ion channel, Kinase inhibitor activity, Nuclear receptor Ligand, Protease Inhibitor activity and Enzyme inhibitor activity. It showed that the G-Protein Coupled Receptor activity are ranges from -0.47 to -0.29; Kinase inhibitor activity are ranges from -0.12 to 0.07; Protease inhibitor activity are ranges from -0.83 to -0.75; and the enzyme inhibitors activity are from -0.38 to -0.23 for the designed compounds. The biological activity of the compounds are presented in Table 2.

Docking

Docking of designed ligands (S_1A- S_5N) with Topoisomerase IV were performed using ARGUS LAB 4.0. Based on the literature, it has been found that Isatin and Thiazole linked acetophenone derivatives can be used to target Topoisomerase IV.

Table 3. : Docking results of five drugs against Topoisomerase IV

Compound Code	Docking score Kcal/mol
S_1A	-8.05
S_2H	-5.70
S_3M	-7.32
S_4C	-6.71
S_5N	-8.37
Standard (Isoniazid)	-5.83

Compound code	R
S_1A	H
S_2H	OH
S_3M	OCH_3
S_4C	Cl
S_5N	NO_2

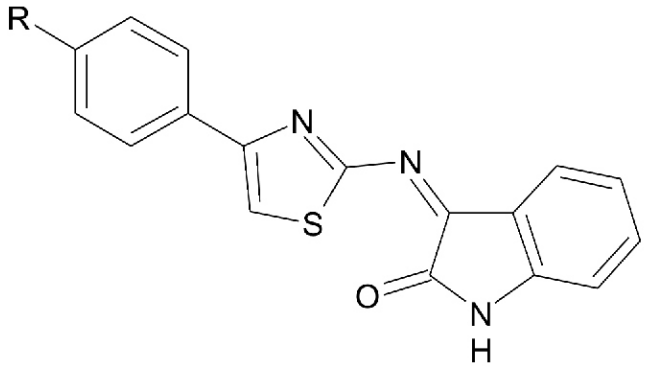


Figure 3 : Structure of Designed Ligands

The energy values were calculated using Argus lab. The ligand S_1A and S_5N showed more binding energy values which is greater than isoniazid drug score value. The complete docking data are presented in Table 3.

DISCUSSION

Lipinski's rule of five is generally used to evaluate drug likeness or to determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion (ADME). However, the rule does not predict if a compound is pharmacologically active [14].

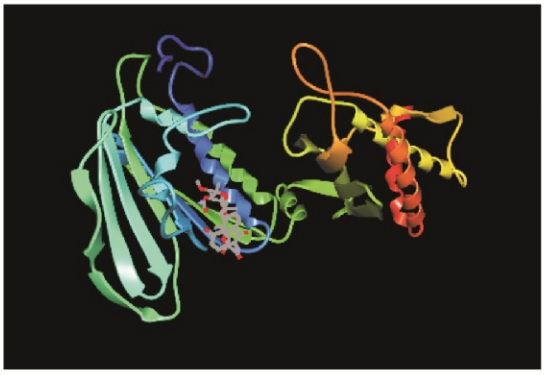
The polar surface area (PSA) of a molecule is the surface sum over all polar atoms, primarily oxygen and nitrogen, also including their attached hydrogens.[15] PSA is used for the optimisation of a drug's ability to permeate cells. Molecules with a polar surface area of greater than 140 angstroms squared tend to be poor at permeating cell membranes. For molecules to penetrate the bloodbrain barrier (and thus act on receptors in the central nervous system), a PSA less than 90 angstroms squared is usually needed.[16,17] It has been shown to correlate well with drug transport properties, such as intestinal absorption, or blood-brain barrier penetration [18]. In our study, all the designed compounds (S_1A- S_5N) showed zero violation of Lipinski's rule of five, which indicates good bioactivity and bioavailability.

Docking analysis of the designed ligands (S_1A- S_5N) with Topoisomerase IV showed that Isatin and Thiazole linked acetophenone derivatives can be used to target Topoisomerase IV. Among all the designed ligands, the ligand S_1A and S_5N showed more binding energy values (8.05 and -8.37 kcal/mol), which is greater than isoniazid drug score value -5.83 kcal/mol.

CONCLUSION

In *Mycobacterium tuberculosis*, Topoisomerase IV (3LPS) is a key condensing enzyme responsible to pass one double strand DNA through another double strand of DNA thereby changing the linking number of DNA, and has emerged as an attractive new

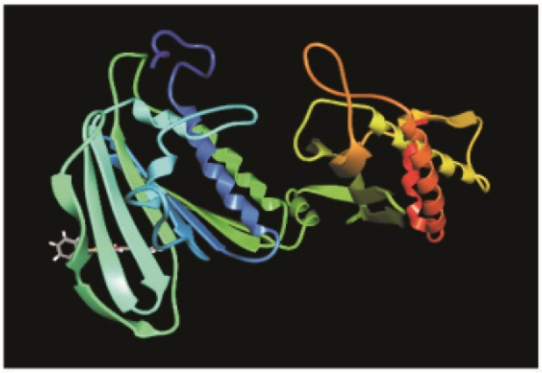
ENZYME TOPOISOMERASE IV



SOURCE RCSB - PROTEIN DATA BANK
PDB CODE - 3LPS

Figure 2

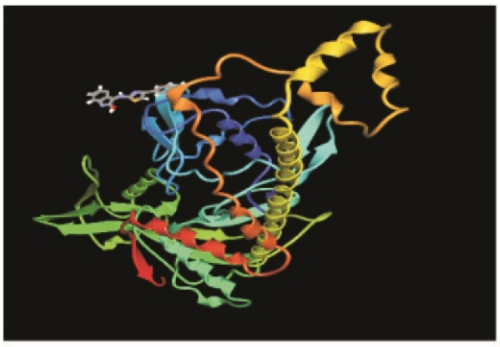
MOLECULAR DOCKING OF S_1A



DOCKING SCORE (-8.05)

Figure 3

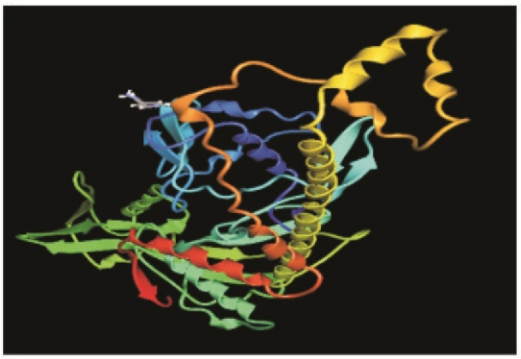
MOLECULAR DOCKING OF S_5A



DOCKING SCORE (-8.37)

Figure 4

MOLECULAR DOCKING OF IZONIAZID (STANDARD)



DOCKING SCORE (-5.83)

Figure 5

target for novel anti-tuberculosis agents in recent years. It was observed that Topoisomerase IV when docked with the compounds, give good scores, also showed good result for ligand S_1A and S_5N. The predicted potency of the five compounds with unknown potency showed that three ligands had very low activity value which ensures the potentiality of the compounds as good anti-tubercular drugs. In future, research on synthesis of these Thiazole derivatives and screen for their *in-vitro* anti mycobacterial activity are warranted.

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