SYNTHESIS AND DOCKING STUDIES OF NOVEL THIAZOLE INCORPORATED BENZIMIDAZOLE ANALOGUES AS POTENT ANTI-RETROVIRAL AND ANTI-TUBERCULAR AGENTS

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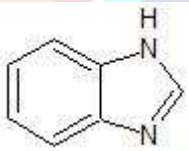
Abstract: Tuberculosis is the major cause of death in persons living with HIV infection. The discovery of drugs with non-competetive inhibition of the viral protein is a major challenge. In the present study, a series of benzimidazolyl thiazoles were synthesized and screened for their toxicity and ADME parameters. Molecular docking studies were performed by PyRx- Python prescription 0.8 in the active site of two different enzymes HIV1-RT (PDB ID:1RT2) and Mycobacterium tuberculosis-CYP51 (PDB ID:1EA1). All the compounds showed good docking scores compared to the standard drugs as well as good oral absorption.

***Keywords*: Benzimidazole, ADME, Lipinski rule, LD_{50}, Docking**

I. INTRODUCTION

Benzimidazole is one of the privileged medicinal scaffold which appears as an integral part in natural compounds and generated great attention because of their interesting biological activity. This bicyclic compound consists of the fusion of benzene and imidazole.



Benzimidazole

Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications, they are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio(Ansari et al 2009). Looking at the importance of benzimidazole and thiazole nucleus, it was thought that it would be worthwhile to design and synthesize some new benzimidazole derivatives bearing thiazole moiety and screen them for potential biological activities. Benzimidazole ring displays an important heterocyclic pharmacophor in drug discovery. These compounds carrying different substituent's in the benzimidazole structure are associated with a wide range of biological activities including anti-cancer, anti-viral, anti-bacterial, antifungal, anti-helmintic, anti-inflammatory, antihistaminic, proton pump inhibitor, anti-oxidant, Anti-hypertensive and anti-coagulant properties(Tuncbilek et al 2009). Recent observations suggest that substituted benzimidazoles and heterocyclic, show easy interactions with the biopolymers, possess potential activity with lower toxicities in the chemotherapeutic approach(Haugwitz 1982).

Tuberculosis comes under one of the greatest infectious disease which causes mortality worldwide. It is caused by the intracellular pathogens Mycobacterium sp. One of the major concerns is that it is the most common HIV-related opportunistic infection, thus caring of the patients infected with both the diseases is a major challenge(Dterling et al 2010, Abdool Karim et al 2010, Breen et al 2004). More than half a million people die from HIV-associated tuberculosis annually(http:/whqlibdoc.who.int). Lethal combination of tuberculosis (TB) and human immunodeficiency virus (HIV) infection for nearly the past 3 decades has posed a major threat to the international community's effort to achieve the health related United Nations Millennium Development Goals for TB and HIV infection(Elzinga et al 2004). After so many years of its discovery, HIV is still a major health and socioeconomic issue, specifically in developing countries(Ghosh 1986).

Two main categories of HIV RT inhibitors have been discovered to date. The first category of inhibitors is the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), which bind to the enzymatic site of RT in a competitive manner with natural nucleotides and thereby terminate DNA synthesis after their incorporation into the growing DNA chain. The second category of inhibitors are the non-nucleoside reverse transcriptase inhibitors (NNRTIs), a group of structurally and chemically diverse compounds that noncompetitively and selectively bind to the unique allosteric hydrophobic non nucleoside inhibitory binding pocket (NNIBP) causing non-competitive inhibition of the viral polymerase(Beale et al 2011). The poor pharmacokinetics, unsatisfactory side effects and the rapid appearance of drug resistance of the clinically

approved anti-HIV drugs compelled the medicinal chemist to develop novel nonnucleoside reverse transcriptase inhibitors or modify the existing nonnucleoside reverse transcriptase inhibitors(Martins et al 2008, Sweeny et al 2008, Daar et al 2007).

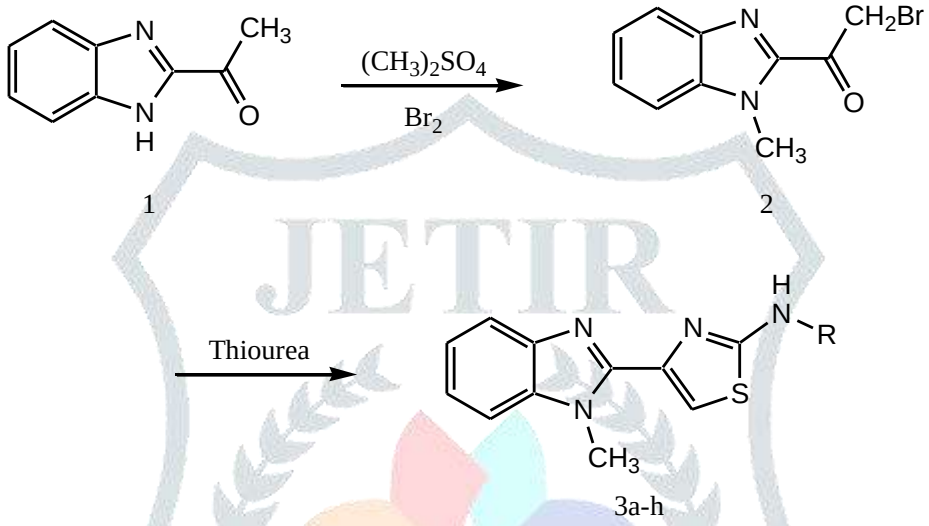
Thus, the main objective of the current investigation is to find out the binding mode analysis of designed benzimidazole derivatives with the HIV-1 reverse transcriptase protein (PDB ID-1RT2) and Cytochrome P450 14-alpha-demethylase of M. tuberculosis (PDB ID 1EA1) especially to analyse the amino acids present in the active binding site of reverse transcriptase, the type and number of binding interactions along with prediction of ADME parameters of the designed compounds. Furthur, the toxicity of the designed analogs has also been performed by using the online software 'protox'.

II. MATERIALS AND METHODS

2.1 General synthesis of benzimidazolyl thiazole analogues (3a-h)

Chemistry:

The new 2-substituted thiazoles containing 1-methyl benzimidazole derivatives **3a-h** were synthesized from acetyl benzimidazole via bromination and nucleophilic substitution by corresponding thioureas. The schematic representation of the synthesis of 2-substituted-4-(1-methyl benzimidazol-2-yl)thiazole derivatives was illustrated in **scheme 1**.



Scheme 1: Synthesis of thiazole derivatives (3a-h)

R_{3a}- Phenyl	R_{3e}- methyl
R_{3b}- p-chloro phenyl	R_{3f}- ethyl
R_{3c}- p-methoxy phenyl	R_{3g}- butyl
R_{3d}- p-ethoxy phenyl	R_{3h}- isopropyl

2-phenylamino-4-(1-methylbenzimidazol-2-yl)thiazole, 3a

Yield 63.3%. m.p 198-201ºC. IR(cm^{-1}): 3325, 3341 (N-H), 3090 (aromatic C-H); ^{1}H NMR ($CDCl_3$): δ 7.37-7.48(m, 4H, ArH), 4.1(s, 3H, N-CH_3), 7.21-7.29(m, 4H, ArH), 7.05(t, 8.4Hz, 1H, ArH), 7.8(s, 1H, H-5 of thiazole), 8.54(s, 1H, NH); MS: m/z 307 (M^+). Anal.calcd for $C_{12}H_{12}N_4S$: C, 66.64; H, 4.61; N, 18.29. Found: C, 66.25; H, 4.36; N, 18.88.

2-(4-chlorophenylamino)-4-(1-methylbenzimidazol-2-yl)thiazole, 3b

Yield 62.4%. m.p 250-252ºC. IR(cm^{-1}): 3548, 3437 (N-H), 3040 (aromatic C-H); ^{1}H NMR ($CDCl_3$): δ .33-7.5(m, 4H, ArH), 4.11(s, 3H, N-CH_3), 7.26-7.3(m, 4H, ArH), 7.88(s, 1H, H-5 of thiazole), 8.89(s, 1H, NH); MS: m/z 341 (M^+). Anal.calcd for $C_{12}H_{12}N_4S$: C, 59.91; H, 3.84; N, 16.44. Found: C, 59.86; H, 3.52; N, 16.69.

2-(4-methoxyphenylamino)-4-(1-methylbenzimidazol-2-yl)thiazole, 3c

Yield 69.5%. m.p 231-234ºC. IR(cm^{-1}): 3466, 3454, 3427 cm^{-1} (N-H), 3047 cm^{-1} (aromatic C-H); ^{1}H NMR ($CDCl_3$): δ 7.52-7.55(m, 4H, ArH), 4.35(s, 3H, N-CH_3), 7.70-7.72(m, 4H, ArH), 7.95(s, 1H, H-5 of thiazole), 8.73(s, 1H, NH), 3.75(s, 3H, OCH_3); MS: m/z 337 (M^+). Anal.calcd for $C_{12}H_{12}N_4S$: C, 64.26; H, 4.79; N, 16.65. Found: C, 64.64; H, 4.77; N, 16.98.

2-(4-ethoxyphenylamino)-4-(1-methylbenzimidazol-2-yl)thiazole, 3d

Yield 66.8%. m.p 212-214ºC. IR(cm^{-1}): 3533, 3481, 3437 cm^{-1} (N-H), 3047 cm^{-1} (aromatic C-H), 2852, 2743 cm^{-1} (aliphatic C-H); ^{1}H NMR ($CDCl_3$): δ 7.30-7.48(m, 4H, ArH), 4.0(s, 3H, N-CH_3), 7.22-7.29(m, 4H, ArH), 7.9(s, 1H, H-5 of thiazole), 8.05(s, 1H, NH), 1.46(t, 7Hz, 3H, CH_3), 3.98(q, 7.2Hz, 2H, CH_2); MS: m/z 351 (M^+). Anal.calcd for $C_{12}H_{12}N_4S$: C, 65.12; H, 5.18; N, 15.99. Found: C, 65.17; H, 5.02; N, 15.60.

2-methylamino-4-(1-methylbenzimidazol-2-yl)thiazole, 3e

Yield 60.7%. m.p 186-189ºC. IR(cm^{-1}): 3556, 3454, 3427 cm^{-1} (N-H), 3047 cm^{-1} (aromatic C-H), 2852, 2743 cm^{-1} (aliphatic C-H); ^{1}H NMR ($CDCl_3$): δ 7.04-7.25(m, 2H, ArH), 7.51-7.74(m, 2H, ArH), 4.13(s, 3H, N-CH_3), 7.42(s, 1H, H-5 of thiazole), 4.8(s, 1H, NH), 2.85(s, 3H, CH_3); MS: m/z 245 (M^+). Anal.calcd for $C_{12}H_{12}N_4S$: C, 58.99; H, 4.95; N, 22.93. Found: C, 59.14; H, 4.59; N, 22.82.

2-ethylamino-4-(1-methylbenzimidazol-2-yl)thiazole, 3f

Yield 62.3%. m.p 197-205°C. IR(cm^{-1}): 3502, 3445, 3411 cm^{-1} (N-H), 3049 cm^{-1} (aromatic C-H), 2701 cm^{-1} (aliphatic C-H); ^1H NMR ($CDCl_3$): δ 7.31-7.48(m, 2H, ArH), 7.43-7.61(m, 2H, ArH), 4.23(s, 3H, N-CH_3), 7.58(s, 1H, H-5 of thiazole), 4.54(s, 1H, NH), 3.12(q, J=7.4Hz, 1H, CH_2), 1.22(t, 3H, CH_3); MS: m/z 259 (M^+). Anal.calcd for $C_{13}H_{14}N_4S$: C, 60.44; H, 5.46; N, 21.90. Found: C, 60.84; H, 5.49; N, 21.90.

2-isopropylamino-4-(1-methylbenzimidazol-2-yl)thiazole, 3g

Yield 65.3%. m.p 186-189°C. IR(cm^{-1}): 3508, 3446 cm^{-1} (N-H), 3074 cm^{-1} (aromatic C-H), 2925 cm^{-1} (aliphatic C-H); ^1H NMR ($CDCl_3$): δ 7.12-7.27(m, 2H, ArH), 7.29-7.86(m, 2H, ArH), 4.16(s, 3H, N-CH_3), 7.88(s, 1H, H-5 of thiazole), 4.65(s, 1H, NH), 2.35(q, 1H, CH), 1.29(d, 6H, $2CH_3$); MS: m/z 273 (M^+). Anal.calcd for $C_{14}H_{16}N_4S$: C, 61.77; H, 5.99; N, 20.11. Found: C, 61.92; H, 5.81; N, 20.23.

2-butylamino-4-(1-methylbenzimidazol-2-yl)thiazole, 3h

Yield 80.8%. m.p 154-156°C. IR(cm^{-1}): 3566cm^{-1} (N-H), 3070 cm^{-1} (aromatic C-H), 2490 cm^{-1} (aliphatic C-H); ^1H NMR ($CDCl_3$): δ 7.51-7.57(m, 2H, ArH), 7.70-7.73(m, 2H, ArH), 4.15(s, 3H, N-CH_3), 7.67(s, 1H, H-5 of thiazole), 4.8(s, 1H, NH), 1.24-1.51(m, 9H, $CH_2CH_2CH_3$); MS: m/z 290 (M^+). Anal.calcd for $C_{15}H_{18}N_4S$: C, 62.91; H, 6.33; N, 19.56. Found: C, 62.25; H, 6.17; N, 19.74.

2.2 Molecular docking studies

2.2.1 Ligand preparation

The 3-dimensional structure of the compounds were generated using ACD/Chemsketch version C30E41, a powerful all-purpose chemical drawing and graphics package which includes 2D and 3D structure viewing, cleaning and functionality for naming structures.

2.2.2 ADME prediction

The four processes involved when a drug is taken are absorption, distribution, metabolism and elimination or excretion (ADME). The ADME properties of the proposed analogs were generated by PreADMET. The most well known rule relating the chemical structures to their biological activities is Lipinski's rule(Lipinski et al 1997), it is called the 'rule of five'. According to Lipinski's rule of five, a moleculeis said to be orally active when its molecular weight(MW) (MW) < 500g/Mol, hydrophylicity (LogP) < 5, hydrogen bond donor (HBD) < 5, hydrogen bondacceptor (HBA) < 10 and the number of rotatable bonds <5(Lipinski 2004). PreADMET contains drug-likeness prediction module based on these rules.

2.2.3 Toxicity prediction

Computational toxicity studies are having an important role in the reduction of the number of animal experiments, time and cost. Protox is one of the suitable web servers to evaluate the similarity of compounds with known toxic things and toxic fragments. In addition, the web server gives the information about the possible binding affinity of drugs to the different toxicity targets by using various protein–ligand pharmacophore based models(Drwal et al 2014). The compounds 1a-h were uploaded in sdf format for predicton of LD_{50} and the toxicity class.

2.2.4 Protein preparation

The x-ray crystallographic structure of HIV1-RT protein (PDB ID: 1RT2) Mycobacterium tuberculosis (PDB ID: 1EA1) were obtained from Protein Data Bank (RCSB) (http://www.rcsb.org/pdb). The protein was then optimized using 'PyMol' molecular graphics system. All water molecules and metal atoms were removed from the protein for docking studies.

2.2.5 Validation of docking protocol

The aim of molecular docking is to give a prediction of the ligand-receptor complex structure using computation methods. All Docking calculations were performed using 'PyRx' virtual screening tool. PyRx performed the complete systematic search of the conformational orientation and positional space of the docked ligand and eliminated unwanted conformations using scoring followed by energy optimization. The reliability of the docking protocol was studied by redocking TNK651 into the active site of 1RT2 and floconazole into 1EA1.

III. RESULTS AND DISCUSSION

TB is the most common opportunistic infection in HIV-positive individuals, and its treatment is complicated by interactions with antiretrovirals. In the present investigation, a series of benzimidazolyl thiazole derivatives were designed and used for molecular docking studies on the active sites of HIV1-1RT2 and Mycobacterium tuberculosis- 1EA1.

All the designed molecules are obeying Lipinski rule of five, thereby proving its drug-likeliness (**Table 1**). This character that would make it a likely orally active drug. The physicochemical characteristics expressed by various descriptors like the optimum value of rotatable bonds, polar surface area, etc relate to optimal pharmacokinetics (ADME) of the drug in the human body. The partition coefficient (log p) has a strong influence on ADME properties of the drug. It is used to predict the lipophilic efficiency of a compound which in turn is a measure of solubility of the drug. The resultsof human intestinal absorption (HIA) and log p denotes that all of the designed analogs are coming to the prescribed range (**Table 2**).

Table 1: **Lipinski's Rule of Five**

Compound	Mol.Wt **<500**	HB Donar **<5**	HB Acceptor **<10**	Log p **<5**	Mol.Refractivity **40 to 130**
3a	306	1	3	4.06	90.23
3b	320	1	3	4.369	94.96

3c	348	1	4	4.583	101.05
3d	336	1	4	4.069	96.78
3e	242	0	3	2.649	69.71
3f	282	0	3	3.595	83.47
3g	270	0	3	3.429	78.94
3h	256	0	3	3.039	74.33

Mol.Wt- Molecular weight; HB- Hydrogen Bond; lop p- Partition coefficient;
Mol Refractivity- Molecular Refractivity

Table 2: Toxicity and ADME parametres

Compound	**LD50 mg/kg**	**Toxicity**	**No.of rot.bonds <15**	PSA **7 to 200 Å**	HIA **>80%**
3a	3100	Class 4	3	70.98	96.4
1b	3100	Class 4	3	70.98	96.79
3c	3100	Class 4	5	70.98	96.48
3d	3100	Class 4	4	80.21	96.44
3e	3100	Class 4	3	70.98	96.37
3f	3100	Class 4	5	70.98	96.28
3g	3100	Class 4	3	70.98	96.21
3h	3100	Class 4	3	70.98	96.34

No.of rot.bonds- Number of rotatable bonds; PSA- Polar Surface Area; HIA- Human Intestinal Absorption

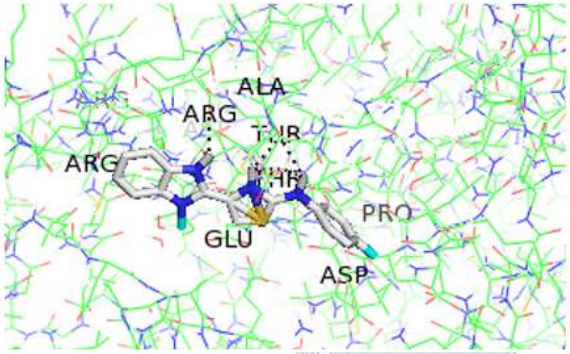
LD_{50} is defined as the dose at which 50% of the tested animal die. LD50 data is helping todetermine the effective dose of a compound and gives the level of compound's acute toxicity.The acute toxicity data predicted by protox showed that all the selected compounds had high LD50 values that falls under the toxicity class 4, according to the GHS, United Nations guidelines (UN GHS, 2005).

Docking allow us to characterize the behaviour of small molecules in the binding site of target proteins. The more the negative value of the energy of binding the better is affinity of the molecule to the receptor. Understanding the ligand binding modes and the corresponding intermolecular interactions that stabilize the ligand-receptor complex is essential for the success of virtual screening approaches in structure-based drug design. The designed benzimidazolyl thiazole derivatives with the highest docking score (**3b**) has shown a good binding affinity towards the binding pocket site of 1RT2 and 1EA1 enzymes. The dock score of the designed analogues were summarized in **Table 3**. The derivatives with aromatic substituents showed highest docking scores than that of alkyl sustituents. 1b has the highest docking score against both the proteins (-7.5 kcal/mol). The docked complex of compound 1b in the active site of1RT2 and 1EA1 was visualized in **Fig 1.**

Table 3: Docking scores of the synthesized compounds

Compounds	**Docking Score Kcal/mol**		**Hydrogen Bonding Interactions**	
	1RT2	**1EA1**	**1RT2**	**1EA1**
3a	-7.2	-7.2	ARG123, ASP127, MET124	ASP127, GLU121, LEU127
3b	-7.5	-7.5	ARG123, ASP127, MET124	THR147, SER201, ASP127 ARG123, ASP127,
3c	-7.1	-7.1	HEM460, ASP127, MET124	THR147
3d	-7.3	-7.3	MET235, ARG158, ARG123, ASP127	ARG158, ARG123, ASP127, MET124
3e	-6.3	-6.3	LEU324, MET124, HIS450	ALA131, ASP127, LEU105
3f	-6.3	-6.8	ASP138	PHE140, GLU142, ASP133
3g	-6.5	-6.5	ARG271, MET124, ASP648	THR200, LEU322, PRO26

3h	-6.4	-6.5	GLU94	HIS253, ALA260, THR470



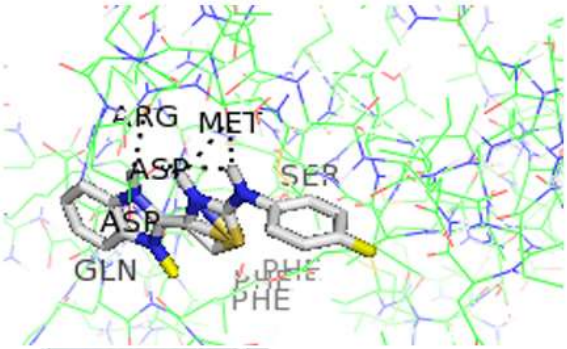


Fig 1: 3b bound with 1RT2 3b bound with 1EA1

The docking scores of the synthesized ligands are almost similar when bound with 1RT2 and 1EA1.The binding modes of the protein-ligand interactions were compared with the experimentally determined binding mode interactions of TNK651 and Floconazole with 1RT2 and 1EA1. Almost all the ligands had docking interactions simlar to that of the co-crystallised ligands TNK651 and Fluconazole. The scores are higher compared to the reference drug Floconazole except 3e which has the same binding affinity as that of Fluconazole (-6.3 kcal/mol).

The binding mode comparison shows that the compounds 3a-c form similar binding modes in terms of utilizing same binding residue in docking with 1RT2 and 1EA1, ie, ASP127 for hydrogen bond formation. However, the docking of other compounds 3d-h showed different binding for 1RT2 and 1EA1, thereby supporting the concominant usage of these drugs for HIV and TB co-infection.

IV. CONCLUSION

Docking studies were performed on benzimidazolyl thiazole derivatives 3a-h towards two targets, 1RT2 (HIV1-Reverse Transcriptase) and 1EA1 (Cytochrome P450 14 alpha-sterol demethylase of Mycobacterium tuberculosis). Good binding affinity is observed for all the eight compounds towards both the targets. In the case of 1EA1, seven of the eight synthesized compounds showed higher docking scores than the standard drug Floconazole. The assessment of Lipinski's rule and other ADME parameters assures oral activity of the compounds. The compound with p-cloro phenyl substituent (3b) contributes to the highest dockig score (-7.5 kcal/mol). Compounds 3d-h with different binding strategies have proved to be better analogues for retroviral tubercular co-infection. These compounds may be considered to be a novel scaffold in the discovery of an ideal anti-HIV agent and for combating other opportunistic infections.

V. ACKNOWLEDGEMENT

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