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Design, Synthesis and Molecular Docking Studies of Novel Isoxazole Analogs as HIV-1 and TB Inhibitors

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ABSTRACT

HIV is a major threat to public health care systems since the virus can mutate rapidly and develop drug-resistant variants. Chemotherapy is more complicated when accompanied by opportunistic infections like TB. Toward this objective, a series of benzimidazolyl isoxazoles were synthesized and molecular docking studies were performed using PyRx virtual screening tool in the active site of two different proteins namely HIV-1 reverse transcriptase (PDB code 1RT2) and Mycobacterium tuberculosis-CYP450 (PDB code 1EA1) to study the binding mode of these analogs. The hydrophobicity and ADME properties were found to be critical for activity. The results reveal that all the compounds can be used orally as good drug candidates and the docking scores were comparable to the standard compounds. compound C1 was found as the highest active against both the proteins.
Keywords: Reverse Transcriptase, Docking, PDB, ADME, Lipinski rule.

I. INTRODUCTION

HIV is known to be the most dangerous virus which is a severe global public health issue. According to the AIDS statistics 2016, 36.7 million people were affected of which, around 30% do not know their status. In 2016, there was a decline of roughly 3million new infections but the progress is still not fast enough to meet the global targets [1]. HIV destroys CD4 T cells in the body, thereby weakening the immune system. The virus can mutate rapidly resulting in rapid development of drug-resistant variants. Severe weakening of the immune system paves way to develop various opportunistic infections. People usually die of opportunistic diseases, particularly tuberculosis. Around one in three AIDS related deaths is caused due to tuberculosis. Combination of HIV with M. Tuberculosis has increased the pressure on current ARV therapy due to drug-drug interactions and the rapid emergence of drug-resistance strains [2].

Antiretroviral therapy is a great success and reduced mortality associated with HIV infection but the drugs used cause severe side effects. ARV toxicity and drug-drug interactions further complicates the treatment. Life-long therapy is inconvenient since the use of these drugs has been reported to produce morphologic and metabolic abnormalities. The major classes of antiretroviral drugs used are Nucleoside reverse transcriptase inhibitors (NRTIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs) and Protease Inhibitors (PIs) [3]. NNRTIs exhibit potent activity compared to NRTIs and PIs. They do not interfere with the polymerase activity and have lower toxicity thresholds. Highly Active Antiretroviral Therapy (HAART) can only increase the life expectancy of the patients. None of the newly emerging drugs have shown to eradicate the virus and complete cure of the disease is not yet possible. Hence, there is a need for continuing research in this prospect.

The aim of this research is to investigate the interaction mechanism of novel benzimidazolyl isoxazoles with HIV-1 reverse transcriptase and M.tuberculosis protein cytochrome P450 enzyme 14-alpha-demethylase through molecular docking approach.

II. METHODS AND MATERIAL

General procedure for the synthesis of C(1-4)
A mixture of chalcone **(3a-d)** (0.001mol) and hydroxyl amine hydrochloride (0.002mol) was refluxed in ethanol (5ml) for 6 hours in the presence of sodium acetate. The reaction mixture was then poured into

10ml of ice-cold water. The precipitate formed was filtered, dried and recrystallised from ethanol.

5-phenyl-3-(benzimidazol-2-yl)-isoxazole (C1)

Yield: 79 % ; m.p. 257-160 ^{o}C; ^{1}H NMR ($CDCl_3$) δ ppm: 7.52-7.73(m, 4H, ArH), 7.26-7.51(m, 5H, ArH), 11.45(s, 1H, NH), 5.48(s, 1H, CH); Anal. Calcd for $C_{16}H_{11}N_3O$ (261.21): C 73.55, H 4.24, N 16.08. Found: C 73.87, H 3.99, N 16.32.

5-(4-methoxyphenyl)-3-(benzimidazol-2-yl)-isoxazole (C2)

Yield: 71 % ; m.p. 257-160 ^{o}C; ^{1}H NMR ($CDCl_3$) δ ppm: 7.51-7.73(m, 4H, ArH), 7.17-7.49(m, 4H, ArH), 11.77(s, 1H, NH), 5.40(s, 1H, CH), 3.59(s, 3H, OCH_3); Anal. Calcd for $C_{17}H_{13}N_3O_2$ (291.46): C 70.09, H 4.24 , N 14.42. Found: C 70.45, H 4.26, N 14.68.

5-(4-chlorophenyl)-3-(benzimidazol-2-yl)-isoxazole (C3)

Yield: 88 % ; m.p. 257-160 ^{o}C; ^{1}H NMR ($CDCl_3$) δ ppm: 7.52-7.73(m, 4H, ArH), 7.16-7.52(m, 4H, ArH), 11.7(s, 1H, NH), 5.51(s, 1H, CH); Anal. Calcd for $C_{16}H_{10}ClN_3O$ (295.83): C 64.98, H 3.41, N 14.21. Found: C 64.7, H 3.17, N 14.08.

5-(4-methylphenyl)-3-(benzimidazol-2-yl)-isoxazole (C4)

Yield: 66 % ; m.p. 257-160 ^{o}C; ^{1}H NMR ($CDCl_3$) δ ppm: 7.42-7.73(m, 4H, ArH), 6.76-7.39(m, 4H, ArH), 11.83(s, 1H, NH), 5.60(s, 1H, CH), 2.34(s, 3H, CH_3); Anal. Calcd for $C_{17}H_{13}N_3O$ (275.18): C 74.17, H 4.76, N 15.26. Found: C 75.18, H 74.01, N 4.79.

Molecular docking study

The compounds which obey Lipinski rule of five were selected and docking studies were done using AutoDock Vina in PyRx virtual screening tool [4]. Autodock Vina presented in ranked nine best docking modes based on the value of the lowest binding affinity (kcal/mol) according to thermodynamics law and delta Gibbs free energy (ΔG) [5]. PyRx is a powerful visualization engine which makes it a valuable tool for Computer-aided drug design.

Protein structure preparation

The X-ray crystallographic structure of the HIV-1 RT (PDB code: 1RT2) and the Mycobacteruim protein (PDB code: 1EA1) was obtanied from the Brookhaven protein data bank [6]. All water molecules and ligands were removed from the complex. Partial atomic charges were computed by OPLS_AA force field and the protein structure was optimized. The optimized structure was then saved as PDB file and used for docking simulation. The co-crystallized ligands TNK 651 and fluconazole defines a grid for 1EA1 and 1RT2 respectively.

Preparation of ligands

The 2D structure of the compounds C1-C4 were built and then converted to 3D with the help of ACD\ChemSketch 1.1. The structures were optimized using Avagadro package. The ligands were analyzed for their hydrophobicity (7] since protein molecules are hydrophobic in nature and most interactions in the binding sites are hydrophobic. Hydrophilic drugs are usually poorly absorbed.

Validation of the docking protocol

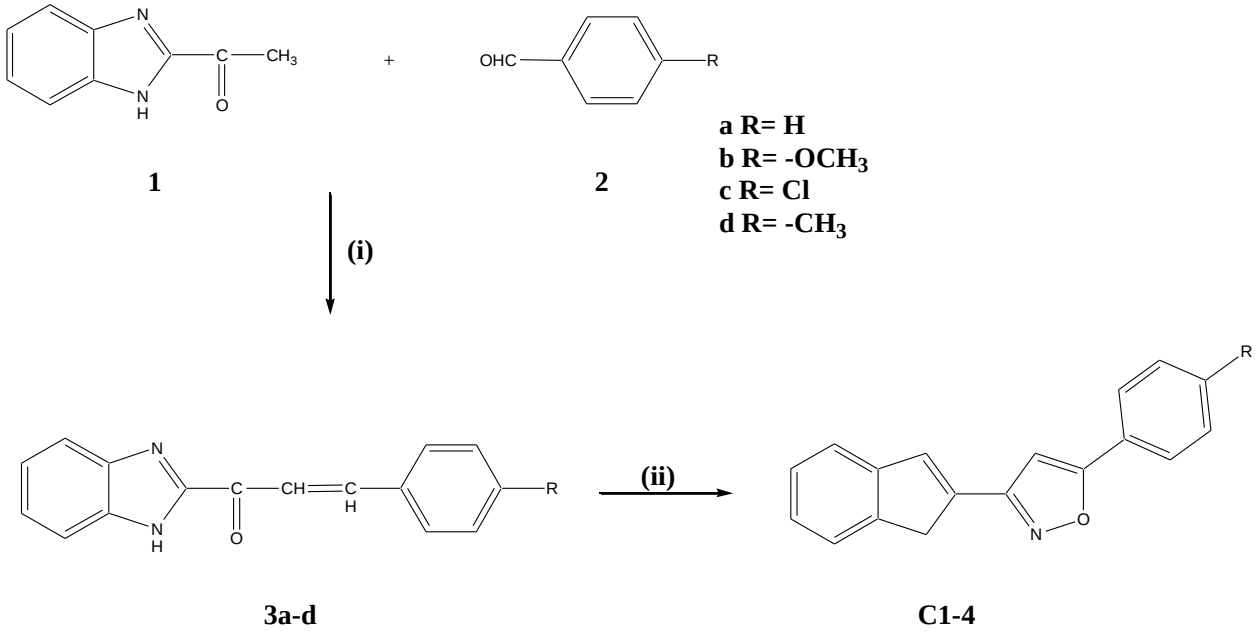
The accuracy of the docking procedure is evaluated by redocking the co-crystallized ligand with the corresponding protein. The score determined should resemble the one found by X-ray crystallography. TNK 651 was extracted from 1RT2 and redocked into it. PyRx has successfully reproduced the reported docking scores and the experimentally determined binding sites of the ligand into 1RT2. Similar was the case of redocking Fluconazole with 1EA1. The general structure of the reference compounds TNK-651 and fluconazole mentioned in **Fig. 1**. The docked complex of compound C1 in the active site of 1RT2 and 1EA1 is depicted in **Fig. 2**.

III. RESULTS AND DISCUSSION

Chemistry

The target compounds were synthesized according to the reaction sequences as illustrated in scheme 1. Chalcones **(3a-d)** were synthesized by reacting 2-acetyl benzimidazole with an aromatic aldehyde in the presence of sodium hydroxide by conventional Claisen-Schmidt condensation. The reaction between chalcones (3a-c) with hydroxylamine hydrochloride in

ethanol led to synthesis of novel isoxazole derivatives. The structures of the novel compounds were confirmed by their physical and spectral data. Cyclization of (3a-d) by treatment with hydroxylamine hydrochloride in the presence of sodium acetate in absolute ethanol afforded 5-aryl-3-(benzimidazol-2-yl)-isoxazole (C1-4). The analogs were prepared in good yield



Scheme 1 : Reagents and conditions (i)EtOH, 10% NaOH, stir, rt, 8hrs (ii) $NH_2OH.HCl$, $NaOCOCH_3$, EtOH, reflux, 6 h.

Computational studies

A series of isoxazole analogs were designed and selected for the molecular docking studies in the NNIBP of reverse transcriptase (PDB ID 1RT2) and 14-alpha-demethylase (PDB ID 1EA1) by PyRx virtual screening tool. The docking score of the compounds are summarized in **Table 1**. Of the four compounds synthesized, highest docking score is observed for C1. The intermolecular hydrogen bonds of compound C1 in the NNIBP of 1RT2 and 1EA1 is shown as black dotted lines in **Fig. 2**. The ligand interaction studies of compound C1 in the active site of 1RT2 showed three H-bond interactions, NH with ASP127, ALA265 and ASP 127, the third one between oxygen and THR 268. In the active site of 1EA1, three hydrogen bonds, one between O and TPF 470 and the other two between N and ASP 127 is observed.

FIGURE 1

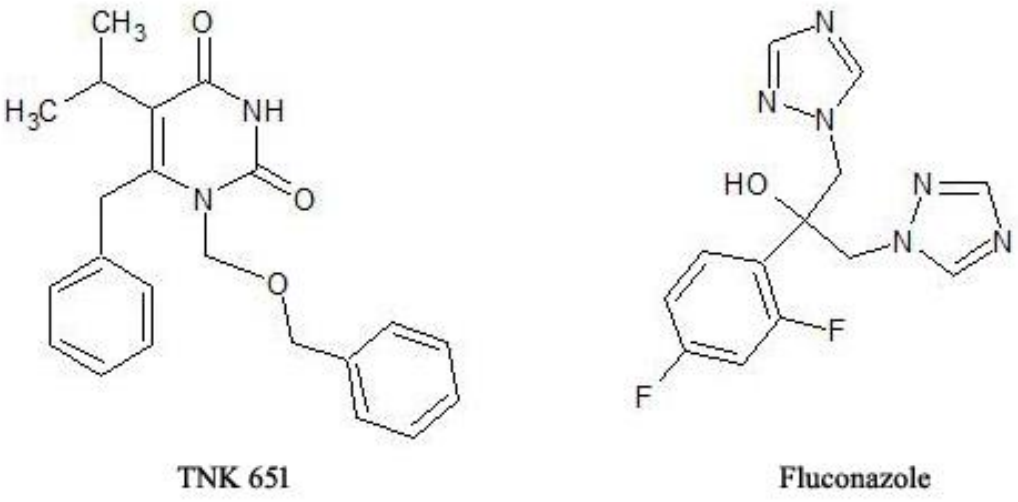
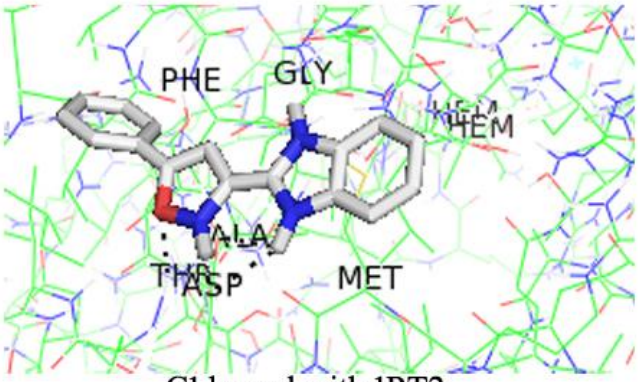


TABLE I. DOCKING SCORES OF ISOXAZOLE ANALOGS WITH 1RT2 and 1EA1

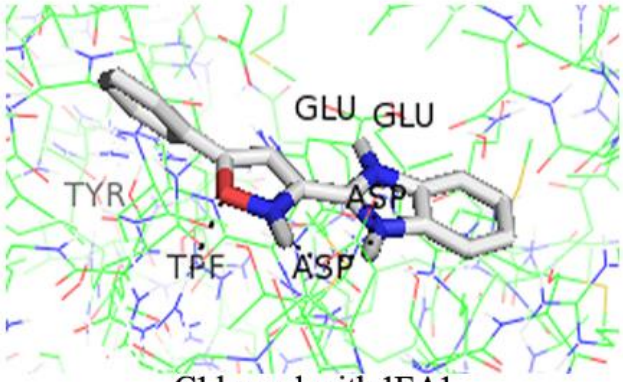
Compound	**R**	**Docking Score Kcal/mol**		**Hydrogen Bonding Interactions**	
		1RT2	**1EA1**	**1RT2**	**1EA1**
C1	H	-7.8	-7.8	THR268, ASP127, ALA265	TPF470, ASP127
C2	$-OCH_3$	-7.7	-7.7	MET124, PHE140, LEU144	HEM490, EN403, MET254, LEU105
C3	Cl	-7.3	-7.4	ASP127, MET124, TRP128	0
C4	$-CH_3$	-7.5	-7.5	0	ALA350

The most active of the synthesized compounds C3 showed lowest interaction energy that is -7.5 Kcal/mol for 1RT2. The compounds C4 showed lowest interaction energy that is -8.2 Kcal/mol for 1EA1. The amino acid residues such THR268, ASP127, ALA265, MET124, PHE140, LEU144, TRP128 and ALA350 form hydrogen bonding interactions with the benzimidazolyl isoxazole core.

FIGURE 2



C1 bound with 1RT2



C1 bound with 1EA1

ADME properties

All the synthesized compounds were computed for the prediction of ADME properties which describes the pharmacokinetics of a drug. The values obtained is presented in **Table 2**. It is observed that the compounds exhibited a good % HIA (Human intestinal absorption) ranging from 92.95 to 93.16 %. Furthermore, all compounds obey Lipinski's rule of five.

TABLE 2

Compound	%HIA >80%	TPSA 7 to 200Å	No.of rot. Bonds <15	MW <500	Log p	Lipinski violation
C1	92.95	54.71	2	261	3.31	0
C2	93.17	63.94	3	291	3.32	0
C3	93.76	54.71	2	275	3.62	0
C4	93.16	54.71	2	275	3.62	0

IV. CONCLUSION

In summary, we have synthesized new benzimidazole based isoxazole derivatives. Molecular docking study of the synthesized isoxazole derivatives towards the active site of enzyme cytochrome P450 and HIV-1 Reverse Transcriptase using PyRx virtual screening tool. Compound C1 with the highest docking score showed three hydrogen bonding interactions with both 1RT2 and two with 1EA1. The ADME properties of the derivatives show that these compounds can be used as good oral drug candidates. It is concluded from the binding mode analysis that these novel compounds with electron withdrawing and electron donating substituents can be exploited for the development of novel HIV and TB inhibitors and provides a strong platform for new structure based drug design.

V. ACKNOWLEDGEMENT

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VI. REFERENCES

[1] UNAIDS(2017) 'Fact sheet 2017'

[2] Swastika Ganguly, Geeta Yadav. 2013. Molecular docking studies of novel benzimidazole analogs as HIV-1-RT inhibitors with broad spectrum chemotherapeutic properties; Int.J.Drug Design. Discovery. 4(4): 1193-1213.

[3] G. Reiner, Villavicencio, B. Junie, Billones. 2009. Computational studies of dipyridodiazepinones as Human Immunodeficiency Virus (HIV) Reverse Transcriptase inhibitors. Philippine.J.Sci. 138 (1): 105-113.

[4] Untiwa Ounthaisong, Prasan Tangyuenyongwatana. 2017 . Cross docking study of flavanoids against tyrosinase enzymes using PyRx 0.8 virtual screening tool. TJPS, 41, 189-192.

[5] D.Vivitri. Prasasty, Ignatia Eveline, Ekaristy, J. Noya. 2017. Potential natural inhibitor bioactivities against high-risk HPV-16, HPV-18 and HPV-52 of E6 oncoprotein through molecular docking. (2)2: 39-45

[6] Y. Chen, B. Shoichet, R. Bonnet. 2005. Structure, function, and inhibition along the reaction coordinate of CTX-M β-lactamases. J.Am.Chem.Soc. 127(15): 5423-5434.

[7] Sainath Namthabad, Estari Mamidala. 2014. Molecular docking of HIV-1 protease using alkaloids from Tinospora cordifolia. Int. J. Res.App. 1(1): 12-16