British Journal of Pharmaceutical and Medical Research Vol.02, Issue 02, Pg.492-501, March-April 2017

Available Online at http://www.bjpmr.org

BRITISH JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

Cross Ref DOI : https://doi.org/10.24942/bjpmr.2017.93 Volume 02, Issue 02, March-April 2017

Research Article

Virtual Screening Of Nucleotide Reverse Transcriptase Inhibitors For Anti-HIV Activity

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ARTICLE INFO

Article History:
Received on 10th March, 2017
Peer Reviewed on 21st March 2016
Revised on 14th April, 2017
Published on 30th April, 2017

Keywords:
Human immunodeficiency virus (HIV), AIDS, Nucleotide Reverse Transcriptase, *In-silico,* Molecular docking.

ABSTRACT

Human immunodeficiency virus (HIV) is lentivirus (group retrovirus) that causes AIDS. AIDS is the condition in the human where progressive failure of the immunity system occurs, the available treatment do not successfully cure the AIDS patient completely. Effective inhibition of nucleotide reverse transcriptase activity is prominent clinically viable approach for treatment of AIDS. In order to enhance the therapeutic option against the HIV virus and to discover more effective lead we examined different heterocyclic derivative which have wide spectrum of *in silico* activity by using rational drug discovery approach and molecular docking. Molecules were \docked against NRT enzyme which was downloaded from protein data bank (PDB) the ligand library molecule was generated. Only few molecule have shown inhibition activity against NRT (3KK1), the non-competitive binding of the lead ligand to the cavity four, in the target occurs enzyme mainly by Van Der Waal and hydrophobic interaction, the ligand molecule interact with following amino acid of the receptor LYS65, LYS 66, LYS70, LYS219, ARG72, ASP110, ASP186, ASP218, HIS221 and LEU228. All these interaction are one of the catalytically pocket of HIV-1 NRT. It is expected that these interaction could be critical for inhibition of HIV-1RT activity.

INTRODUCTION:

AIDS disease caused by the retrovirus human immunodeficiency virus(HIV) and characterized by immunosuppressant that lead to opportunistic infection secondary neoplasm and neurological manifestations, explosion of research amid at understanding HIV and finding new lead molecule which is more effective for treatment of HIV .There are two genetically different but related forms of HIV called as HIV-1 and HIV-2. HIV-1 is the most common type associated with AIDS in United States, Europe and central Africa. Reverse transcriptase and integrase, the viral core is surrounded by a matrix protein called P17 which lies underneath the virion envelop, studding the viral envelope are two viral glycoprotein gp120and gp41 which are critical for HIV infection of cell .The HIV -1 RNA genome contains the gac, pol and env genes which code for viral proteins, CD4 molecule is a high affinity receptor for HIV this explain the selective tropism of the virus.

NRT Enzyme

HIV-1 NRT enzymes are asymmetric heterodimer which is 1000 amino acid long. The NRT enzyme composed of two targeted subunit, P66 and P51 both subunits derive by cleavage by viral protease from gag and pol poly protein that is synthesized from unsliced viral RNA P66 and P51 share a common amino acid terminus, P66 is 560 amino acid long and P51 is 440 amino acid long. This is the main enzyme which is responsible for HIV-1activity in CD4+ cell, NRT enzyme. The enzyme converts RNA to double stranded DNA. The enzyme required RNA and DNA as template in reverse transcriptase of viral genomic RNA. Deoxyribo-nucleotide triphosphates dATP, dCTP, dGTP, TTP are required for synthesis of DNA that complement sequence to RNA template. The enzyme also controls the replications of the virus's genetic material via its polymerase activity it convert the viral single stranded RNA into an integration competent do-double stranded DNA[6].

Materials and Methods

PDB selection and validation [20]

The three dimensional structure of NRT enzyme of HIV-1 it was obtained from protein data bank by accessing through (www.rcsb.org),RCSB PDB an information portal to 120057 biological macromolecule structure protein date bank. 3KK1 reverse transcriptase-DNA complex with nucleotide inhibitor Gs-9148-diphosphate bond in nucleotide site. The molecule released in 2010 the X-ray diffraction method was fallowed and have residue count 1060, by employing macromolecule tool in the discovery software VLife MDS4.4 the macromolecule contain four cavity, the ligand which were selected were docked against cavity number four which contain 34 amino acid residue, the water molecules were removed from the NRT enzyme macromolecule by using software Vlife MDS 4.4 the resolution of the macromolecule 3KK1 is 2.70Aº.

The following figure show the 3KK1 receptor with the cavity number four and amino acid residue which present in the cavity which is obtained by employing Vlife MDS4.4 software for computer aided and drug design.

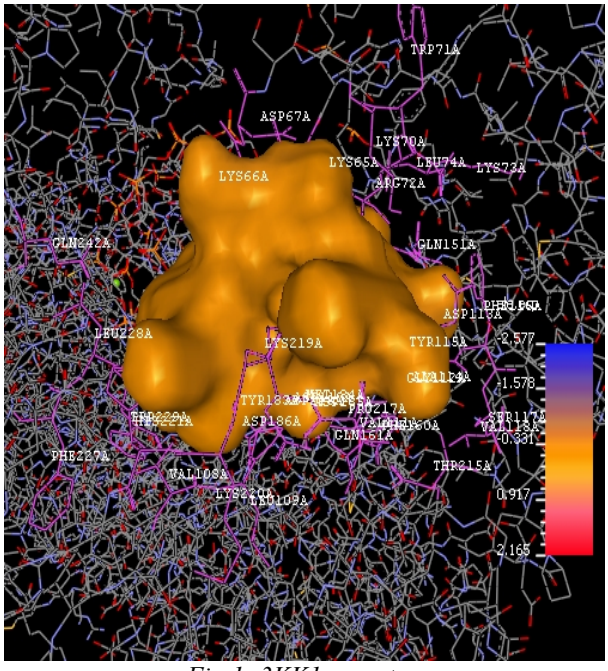


Fig 1: 3KK1 receptor

Experimental

In silico docking [2,3,19]

In-silico ADME predictions were obtained from www.bmrd.org. VLife MDS 4.4 Drug Design software was used for docking simulation on windows os. Marvin beans were used to draw structure and convert to 2D to 3D in mol files using Vlife MDS4.4. The 3D structure was stabilized by minimizing energy using molecular mechanics followed by Merck molecular force field (MMFF).Library of 15 ligand molecules were prepared and docking score compared with standard Abacavir with docking score of -63.12.

Log P predictions

Mole file of the top scoring molecules were selected and subject to predict Log P value, using mol inspiration software available online, the selected molecules were uploaded www.molinspiration.com.

ADME Predictions

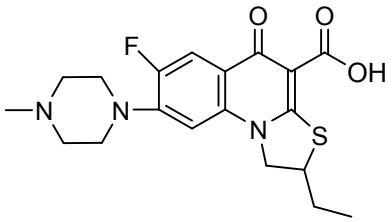
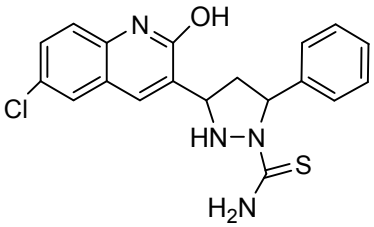
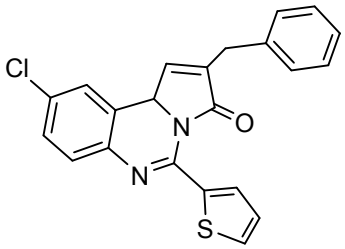
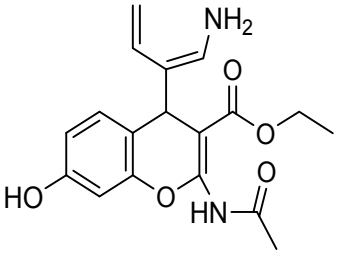
The mole file of the selected molecule were subjected to ADME prediction by using following online software preadmet.bmdrc.kr

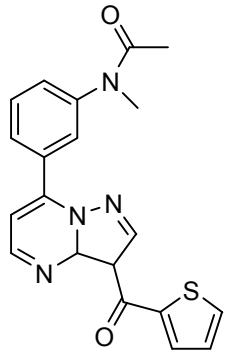
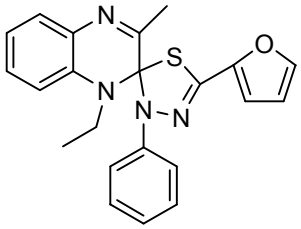
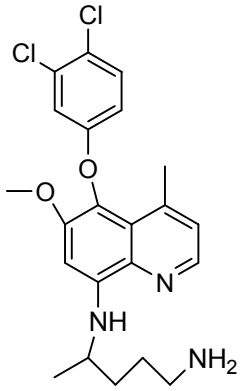
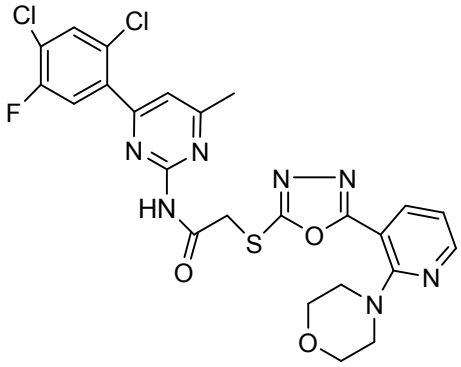
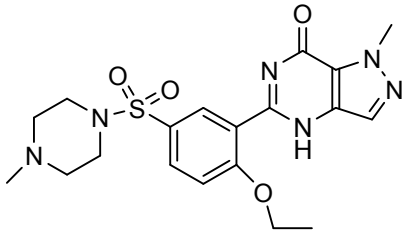
Table 1: It shows in silico ADME Prediction data for selected compound

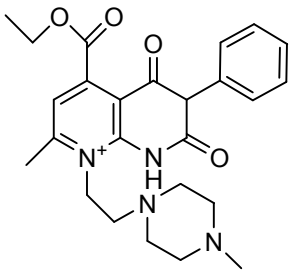
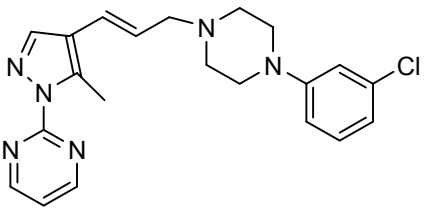
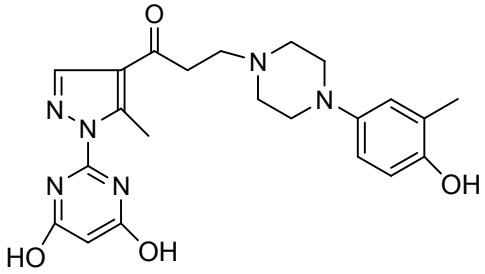
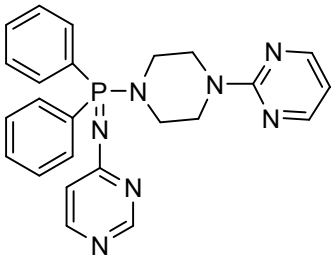
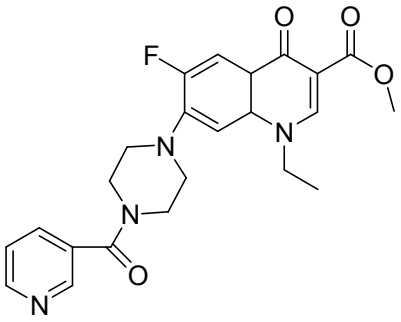
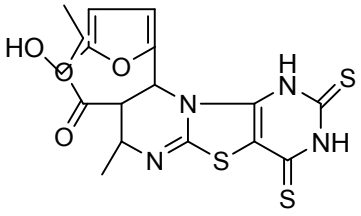
Sr.no.	Compound	logP	BBB	PPB	P-gp inhibition	Caco2	PWS	HIA
1	ST 05	5.598	0.488	98.28	+	29.06	0.027	97.00
2	ST 07	2.742	0.208	86.18	+	17.67	0.315	88.77
3	ST 14	5.432	0.043	56.91	Non	52.70	1679.8	97.70
4	ST 03	4.301	4.219	100.0	+	55.25	0.508	97.51
5	ST 15	1.794	0.038	74.79	+	16.15	6.149	94.53

@Log P value, Blood Brain Barrier (BBB), Plasma Protein Binding (PPB), Caco2 cell permeability, pure water solubility (PWS), Human Intestinal Absorption (HIA).P pg inhibition.

Table No. 2: Library of Molecules

Sr. No.	Compound	Structure	Docking Score
1	ST1		-77.92
2	ST2		-74.42
3	ST3		-72.24
4	ST4		-76.58

5	ST5		-89.95
6	ST6		-72.32
7	ST7		-82.08
8	ST8		-73.56
9	ST9		-69.85

10	ST10		-78.16
11	ST11		-69.80
12	ST12		-71.43
13	ST13		-69.73
14	ST14		-80.94
15	ST15		-73.11
16	Abacavir	-	-63.12

Toxicity prediction

The mole file of the selected molecule was subjected to the drug likeliness and toxicity study by using preadmet.bmdrc.kr the drug likeliness mainly considered with rule five and WDI like rule. The toxicity study by using same PreADMET software by considering carcinogenicity, algae-at and hERG-inhibition as show in table 3.

Table No. 3: Toxicity Prediction of prioritized molecules

Sr. No.	Code	LRF	hERG inhibition	Carcino Rat	Carcino Mouse	Ames test	Algae-at
1	ST 05	Suitable	High risk	Positive	Positive	Mutagen	0.0082
2	ST 07	Suitable	Medium risk	Negative	Negative	Mutagen	0.0154
3	ST 14	Suitable	Medium risk	Positive	Positive	Mutagen	0.0122
4	ST 03	Suitable	Medium risk	Negative	Positive	Mutagen	0.0126
5	ST 15	Suitable	Medium risk	Positive	Negative	Mutagen	0.0171

CYP-450 interaction

Table No. 4: Interaction of prioritized molecules with CYP-450

Sr. No.	Code	CYP-450 2C19 I	CYP-450 2C9 I	CYP-450 2D6 I	CYP-450 2C9 S	CYP-450 3A4 I	CYP-450 3A4 S
1	ST 05	Non	Non	Non	Non	Non	Non
2	ST 07	Non	Inhibitor	Non	Non	Inhibitor	Substrate
3	ST 14	Non	Non	Non	Non	Non	Substrate
4	ST 03	Non	Non	Non	Non	Non	Substrate
5	ST 15	Non	Non	Non	Weak	Non	Substrate

(I: Inhibitions: Substrate)

Interaction of prioritized molecules with 3KK1 receptor:

The interaction between the lead molecule and the receptor 3KK1 through hydrogen bonding, hydrophobic bond, van der Waal force and charge bonding which mean that those molecules have Non-competitive inhibition reversible binding to the receptor even though the show high inhibition activity on docking the tables below show the interaction of priorities lead molecule with atomic distance and amino acid Involved and the atom which involve in the binding from the lead molecule.

Table No. 5: Interaction of ST 05

Sr. No.	Residue atom	Ligand atom	Distance	Interaction type
1	LYS66 503C	36H	3.271	VDW
2	LYS66 512C	12C	3.887	VDW
3	ARG67 522C	25O	3.710	VDW
4	ARG72 569C	30C	3.729	VDW
5	ARG72 569C	47H	3.009	VDW
6	ARG72 571N	30C	3.383	VDW
7	ARG72 571N	47H	3.013	VDW
8	ASP110 881C	20C	3.839	VDW
9	ASP110 881C	21C	3.560	VDW
10	ASP110 883O	21C	3.621	VDW
11	ASP110 884O	21C	3.269	VDW
12	ASP186 1506C	22C	3.887	VDW
13	ASP186 1506C	39H	3.107	VDW
14	ASP186 1508O	22C	3.393	VDW
15	ASP218 1770O	4C	3.314	VDW
16	ASP218 1770O	32H	2.966	VDW
17	LYS2219 1775N	5C	3.536	VDW
18	LYS219 1781C	20C	3.603	VDW
19	LYS219 1782C	12C	3.460	VDW
20	LYS219 1782C	13C	3.762	VDW
21	LYS219 1783N	12C	3.530	VDW
22	LYS219 1783N	13C	3.576	VDW

Table No. 6: Interaction of ST 07

Sr. No.	Residue atom	Ligand atom	Distance	Interaction type
1	LYS65 503C	4N	3.423	VDW
2	LYS66 512C	2C	3.574	VDW
3	LYS66 514C	7C	3.619	VDW
4	LYS66 516N	47H	3.221	VDW
5	ASP218 1769C	31F	3.610	VDW
6	ASP218 1770O	25C	3.359	VDW
7	ASP218 1770O	32O	3.306	VDW
8	LYS219 1775N	18C	3.702	VDW
9	LYS219 1775N	31F	3.084	VDW
10	LYS219 1780C	16C	3.832	VDW
11	LYS219 1781C	14C	3.794	VDW

Table No.7: Interaction of ST 14

Sr. No.	Residue atom	Ligand atom	Distance	Interaction type
1	LYS64 493O	27C	3.361	VDW
2	LYS65 500C	26C	3.507	VDW
3	LYS65 500C	27C	3.500	VDW
4	LYS65 501C	25C	3.757	VDW
5	LYS65 501C	27C	3.817	VDW
6	LYS65 501C	49H	2.948	VDW
7	LYS66 508N	1C	3.327	VDW
8	ARG72 569C	28C	3.749	VDW
9	ARG72 570N	27C	3.461	VDW
10	ARG72 570N	51H	2.849	VDW
11	ASP110 881C	30C	3.757	VDW
12	ASP110 882C	53H	3.259	VDW
13	ASP186 1508O	54H	2.806	VDW
14	LYS219 1776C	14C	3.789	VDW
15	LYS219 1776C	15C	3.651	VDW
16	LYS219 1776C	19O	3.613	VDW
17	HIS221 1799N	19O	3.433	VDW
18	HIS221 1799N	55H	2.944	VDW
19	HIS221 1800C	32C	3.805	VDW
20	HIS221 1800C	58H	3.091	VDW
21	HIS221 1801C	15C	3.091	VDW
22	HIS221 1801C	18C	3.840	VDW

Table No. 8: Interaction of ST 03

Sr. No.	Residue atom	Ligand atom	Distance	Interaction type
1	LYS66 508N	2C	3.352	VDW
2	LYS66 508N	3C	3.387	VDW
3	LYS66 508N	28H	2.809	VDW
4	LYS66 509C	2C	3.736	VDW
5	LYS66 509C	23CI	3.925	VDW
6	LYS66 509C	28H	3.310	VDW
7	LYS66 512C	3C	3.534	VDW
8	LYS66 512C	10C	3.661	VDW
9	LYS66 512C	12C	3.794	VDW
10	LYS66 514C	1C	3.805	VDW

11	LYS66 514C	10C	3.608	VDW
12	VAL108 867C	41H	3.039	VDW
13	ASP186 1507O	27C	3.512	VDW
14	ASP186 1508O	26C	3.427	VDW
15	ASP186 1508O	27C	3.653	VDW
16	LYS219 1780C	32C	3.157	VDW
17	LYS219 1781C	32H	3.248	VDW
18	LYS219 1783N	12C	3.619	VDW
19	LEU228 1857C	27C	3.862	VDW
20	LEU228 1857C	42H	3.862	VDW
21	LEU228 1858O	26C	3.233	VDW
22	TRP229 1864C	42H	3.018	VDW

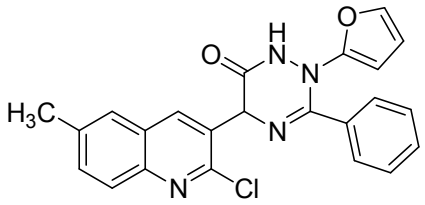
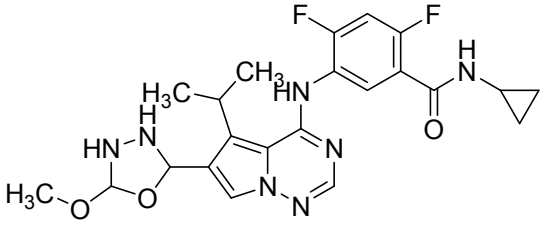
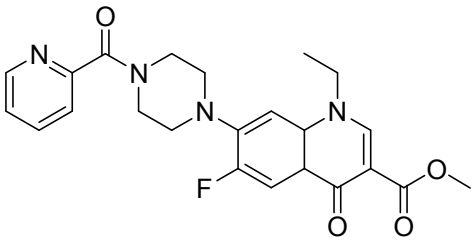
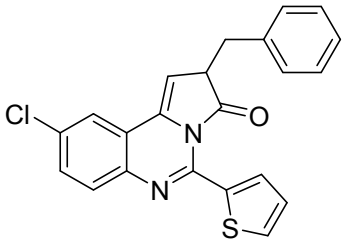
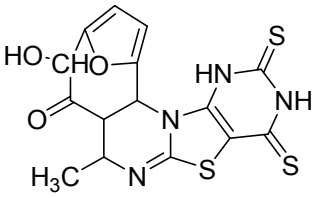
Table No. 9: Interaction of ST 15

Sr. No.	Residue atom	Ligand atom	Distance	Interaction type
1	LYS65 503C	21S	3.503	VDW
2	LYS65 503C	33H	3.164	VDW
3	LYS65 505C	21S	3.754	VDW
4	LYS66 508N	12N	3.517	VDW
5	LYS66 508N	33H	3.025	VDW
6	LYS66 512C	11C	3.575	VDW
7	LYS66 512C	12N	3.425	VDW
8	LYS66 512C	13C	3.744	VDW
9	LYS66 513C	37H	3.333	VDW
10	ASP67 517N	38H	3.233	VDW
11	ASP67 518C	20O	3.402	VDW
12	ASP67 522C	20O	3.717	VDW
13	VAL108 867C	40H	3.399	VDW
14	LYS219 1776C	35H	3.172	VDW
15	LYS219 1779C	18C	3.800	VDW
16	LYS219 1780C	14C	3.520	VDW
17	LYS219 1781C	1C	3.640	VDW
18	LYS219 1781C	14C	3.481	VDW
19	LYS219 1782C	1C	3.505	VDW
20	LYS219 1782C	10N	3.264	VDW
21	LYS219 1783N	29H	2.957	VDW
22	HIS221 1799N	40H	3.074	VDW

RESULT AND DISCUSSION

Around 15 molecules were docked and screened best score prior to actual synthesis. Changes were made in substituent's position carried out. Alkyl/phenyl substitution on various heterocyclic ring results in increased score and activity and it was found that it may results in increased anti-HIV activity. All the molecules were found to be fitting the actual binding pocket of standard molecule Abacavir (-63.12). Molecules were prioritized based on inhibitory and ADME property. ST05, ST07, ST14, ST03, ST15 molecules were prioritized for actual synthesis.

Table no. 10: Prioritized molecules for synthesis

Sr.no.	Code	Structure	Molecular Formula	Docking Score
1	ST 05		C23H17ClN4O2	-89.95
2	ST 07		C22H25F2N7O3	-82.08
3	ST 14		C24H27FN4O3	-80.94
4	ST 03		C22H15CLN2OS	-72.24
5	ST 15		C17H18N4O4S3	-73.11

CONCLUSION

Most of ligands were found to be interacting with the amino acid residues of the active sites. Various heterocyclic compound showed *in-silico* inhibition activity but the most interesting heterocyclic ring 1,2,4- triazine derivative after structure activity relationship for the drugs which is actually use and available in the market like; Acyclovir , Ganciclovir , Ribavir , Lamivudine and Didanosine most of these molecule contain the ether bridge either in the form of ring or aliphatic side chain. The present work leads to the development of various heterocyclic derivatives as an anti-HIV "Hit" by *In-Silico* design. Compound ST 05,ST 07, ST 14, ST 03 and ST 15 were found to be active *In Silico* as compared with Abacavir (-63.12) which is used as standard and can be considered as useful template for anti-HIV lead development.

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How to cite this article:
Ibrahim K. Mahmood, Amit G.Nerkar and Ajay M. Sisode ***Virtual Screening Of Nucleotide Reverse Transcriptase Inhibitors For Anti-HIV Activity***. Br J Pharm Med Res , Vol.02, Issue 02, Pg.492-501, March-April 2017.

Source of Support: Nil **Conflict of Interest:** None declared.