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Design, synthesis and molecular docking of pyrazolo [3,4d] thiazole hybrids as potential anti-HIV-1 NNRT inhibitors

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ABSTRACT

A series of pyrazolo[3.4,*d*]thiazole hybrids **6** were synthesized from 5-arylidene-2-imino-3-(4-arylthiazol-2-yl)-thiazolidin-4-ones **5.** The 5-arylidene-2-imino-3-(4-arylthiazol-2-yl)-thiazolidin-4-ones **5** were synthesized from 2-amino-4-arylthiazoles **1** and 2-chloro-acetamido-4-arylthiazoles **2** via the formation of 2-imino-3-(4-substituted-arylthiazol-2-yl)-thiazolidin-4-ones **3** using substituted aldehydes **4**. The 5-acrylidene derivative **5** on cyclisation with phenyl hydrazine give the pyrazolo [3, 4, d] thiazole derivatives **6**. The obtained pyrazolo [3.4, *d*]thiazole derivatives were studied as anti-HIV-1 NNRT inhibitors. It was found that these compounds might have potent RT inhibition activity.

1. Introduction

Most of the all commercially available agrochemicals and medicinal drug belong to the group of heterocycles [1]. Most of these active ingredients bear either one or more monoheterocycclic moieties or a benzannulated bicyclic core. There are only a few highly active compound classes known, which contain a bicyclic scaffold made up of two different hetero-cycles. Examples for such rare hetero-bicyclic active ingredients are the cephalosporin antibiotics [2], such as cefalexin, the imidazothiazole anthelminthics[3], such as levamisole, and the triazolopyrimidine herbicides[4], such as florasulam.

Due to the lack of general reaction methods, lengthy synthesis pathways, in availability of complex intermediates etc., heterobicyclic compounds amongst the group of biologically active compound classes are under representation. In addition, a heterobicyclic scaffold should be an appropriate core to link several pharmacophoric substituent with the right exit-bond vectors into the three-dimensional space for perfect binding at the target-site of the enzyme [5].

The presence of a heterocyclic ring is an event that occurs commonly in many natural and synthetic drugs which functions as core of drug molecules. In particular, fused five and six membered heterocyclic systems are scaffolds of many efficacious drugs. Among these, pyrazole is a versatile five-membered ring capable with varied biological activity on humans such as antimicrobial [6], anti-inflammatory [7], anticancer [8], antiviral [9], anticonvulsant and antidepressant [10] and Anti-HIV [11]. The compounds containing thiazole ring play a prominent role in various biological process. Thiazoles were reported to posses anti-microbial [12,13,14,15], analgesic[16], anti-inflammetry[17], anti-convulsant[18], cardiotonic[19], anti-cancer[20], anti-tubercular[21] and anthelmintic [22] activity. The biological activity of substituted thiazoles were possess (S—C═N) toxophoric unit. Thiazoles have lipid solubility with hydrophilicity. Thiazoles are easily metabolized by biological reactions and are non-carcinogenic in nature. The emergence of drug-resistant strains due to the rapid mutability of the virus, the treatment of viral infectious diseases becomes an important challenge [23,24]. Earlier antiviral researches has primarily paying attention on the development of nucleoside analogues but in recent times, non-nucleoside derivatives [25–27] have also received considerable attention. Among the non-nucleoside analogues, some novel pyrazoles; A[28,29], thiazolones; B[30] and thiazole derivatives especially BILS 179 BS; C [31] were reported to demonstrate a high antiviral activity against hepatitis A virus, hepatitis C virus (HCV) and HSV, respectively.

Hence, heterobicyclic compounds containing thiazole and pyrazole found to be important class of heterocycles [32,33] particularly applicable for new materials [34] and dyes [35]. Thiazoles and Pyrazoles are displaying a large number of mild to potent pharmacological activities [36] due to their highly versatile ring systems. Therefore, we decided to synthesize a novel fused heterobicycles containing pyrazole and thiazole scaffolds. Based on the above observations and our interest in the development of new bioactive molecules, in this article we are describing the design and synthesis of novel pyrazolothiazoles with excellent anti-HIV activity.

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2. Result and discussiion

2.1. Chemistry

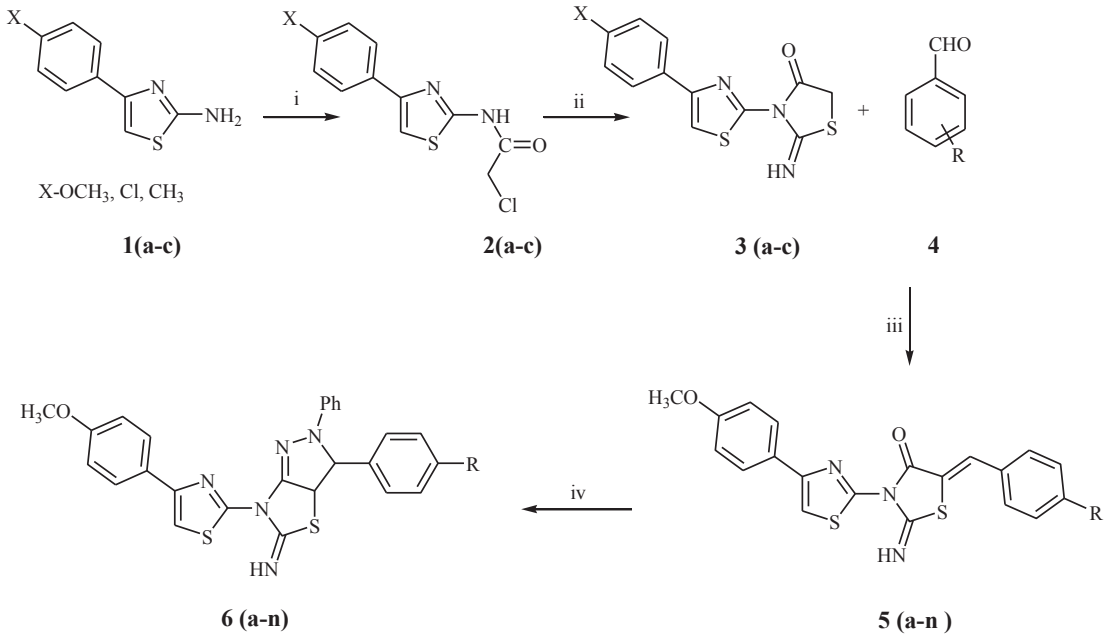
Here in we synthesized a pyrazolothiazole derivatives starting from substituted 2-amino-4-aryl-thiazoles using a series of reactions and to study their NNRT inhibitor activity. We have synthesized pyrazolo [3.4, *d*] thiazoles **6** from thiazol-2-yl substituted 2-imino-thiazolidin-4-ones **3** and a series of their 5-arylidene derivatives **5** via the 2-chloro-acetamido-4-arylthiazoles **2**. The 2-amino-4-arylthiazoles **1** were refluxed with chloroacetyl chloride in toluene in the presence of potassium carbonate to produce the corresponding 2-chloro-acetamido-4-arylthiazoles **2**. The 2-chloro-acetamido-4-arylthiazoles latter was treated with potassium thiocyanate in refluxing acetone to get the related 2-imino-3-(4-arylthiazol-2-yl)-thiazolidin-4-ones **3**. Condensation of 2-imino-3-(4-arylthiazol-2-yl)-thiazolidin-4-ones **3** with different aromatic aldehydes **4** in acetic acid using sodium acetate gave the orange color crystals of 5-arylidene-2-imino-3-(4-arylthiazol-2-yl)-thiazolidin-4-ones derivatives **5.** In the last step of the series of reactions to get a target molecule the 5-acrylidene derivatives **5** on cyclisation with phenyl hydrazine in acetic acid gives the pyrazolo [3,4,d] thiazole derivatives **6** as outlined in scheme 1. The detailed general synthesis procedure of the compounds is mentioned in the experimental section. Exploration of the substrate scope for the synthesis of 3-(substituted phenyl)-6-(4-(4-substitutedphenyl) thiazol-2-yl)-3, 3a-dihydro-2-phenyl-2H-pyrazolo [3, 4-d] thiazol-5(6H)-imine derivatives is as shown in Table 1.

The structures of the synthesized compounds were assigned based on IR, ^{1}H NMR, ^{13}C NMR and GCMS spectral data. The conversion of the primary amine group of 1 (a-c) into the 2-chloro-acetamido-4-arylthiazoles **2** were confirmed by melting points and IR, ^{1}H and ^{13}C NMR. Differences in melting point and IR, ^{1}H and ^{13}C NMR data, can easily be observed to distinguish the above transformation. The peak at 3550–3414 cm^{-1} and 1690–1694 cm^{-1} in IR indicates transformation of primary amine to the acetamide of **2**. ^{1}H NMR also shows the additional $-CH_2-$ protons singlet at 4.23–4.99 δppm, which indicates the chloroacetylation of amine. The 2-chloro-acetamido-4-arylthiazoles **2** on treatment with KSCN gives 2-imino-3-(4-arylthiazol-2-yl)-thiazolidin-4-ones **3**, which is demonstrated by the appearance the IR band at 1730–1718 cm^{-1} and 1572–1581 cm^{-1} due to C=O of cyclic amide and C=N of imine. The proton attached to the nitrogen of imine (NH=C) appears at 12.00–12.10 δppm, similarly it is also confirmed by ^{13}C NMR spectral data. The diapearance of signal due to $-CH_2-$ protons of thiazolidinone ring and appearance of new signals at 8.56–9 δppm due to ⩾C=C<u>H</u> in proton NMR and additional stretching band due to ⩾C=CH shows the condensation of 2-imino-3-(4-arylthiazol-2-yl)-thiazolidin-4-ones **3** with aromatic aldehydes had been takes place, with the formation of 5-arylidene-2-imino-3-(4-arylthiazol-2-yl)-thiazolidin-4-ones derivatives **5.** In the IR spectra of compounds **6**, the amide carbonyl band was absent, which clearly confirmed that a cyclocondensation with phenyl hydrazine had been taken place. Besides, the C=N bands of **6** were observed in the 1596–1602 cm^{-1} region. Absence of signal in ^{1}H NMR of **6** due to ⩾C=C<u>H</u> proton at 8.56–9 δppm and appearance of two doublets for single proton at 2.51–3.20 δppm and 4.00–4.90 δppm indicates the formation of 3,3a-dihydro-2-phenyl-2H-pyrazolo[3,4-d] thiazole bicyclic ring. The^{13}C NMR spectra and presence of a molecular ion peak at respective *m/z* value of the products in the GC–MS further confirmed the structure of **6**.

Various substituted aromatic aldehydes with the both electron donating and withdrawing groups have been used for the condensation of thiazolidinones. The electron-withdrawing group on aromatic aldehydes resulted in excellent yields. However, good yields are obtained for aromatic aldehyde with electron donating groups (Table 1). Therefore, the method in Scheme 1 not only expands the synthetic capacity for the formation of pyrazolo [3, 4, d] thiazoles, but also opens an effective way for accessing some valuable compounds.

2.2. Molecular docking studies

Docking score of compound **6k** and **6f** was found to be good around −7.849 and −7.594 respectively. All molecules from phenyl-2H-pyrazolo [3,4-d]thiazol-5(6H)-imine series were be docked into the non-nucleoside inhibitor binding pocket (NNIBP) of HIV-1 RT. As illustrated in Fig. 1(a) and (b) and native ligand TMC 278 in Fig. 1(c) and (c), the 4-phenyl-thiazole and phenyl substituted pyrazolothiazole moiety of compound **6k** and **6f** of phenyl-2H-pyrazolo [3,4-d]thiazol-5(6H)-imine series interacts through hydrophobic interactions into the hydrophobic binding pocket, surrounded by the aromatic portion of Tyr 181, Tyr



Scheme 1. *Reagent and Conditions*: (i) K_2CO_3, Toluene, chloroacetylchloride; (ii) KSCN, dry acetone, reflux, 3 h; (iii) NaOAC, EtOH, reflux, 3 h at 120 °C, 70–85%; (iv) $PhNH_2NH_2$, Glycial acetic acid, sodium acetate, refluxed, 6 h.

Table 1
Exploration of the substrate scope for the synthesis of 5-arylidene-2-imino-3-(4-arylthiazol-2-yl)-thiazolidin-4-ones[a] and pyrazolo [3, 4, d] thiazole derivatives.[b]

Entry	X	R	5(a-n)[a]	Time (h)	M.P. (°C)	Yield %	6(a-n)[b]	Time (h)	M. P. (°C)	Yield[c] %
1	$-OCH_3$	H	5a	3	260	70	6a	7	225	80
2	$-OCH_3$	2-Cl	5b	3	210	74	6b	7	220	78
3	$-OCH_3$	3-NO_2	5c	2.5	272	80	6c	6.5	228	88
4	$-OCH_3$	2-Br	5d	3	224	84	6d	7.5	240	82
5	$-OCH_3$	4-Br	5e	2.5	220	87	6e	7	252	75
6	$-OCH_3$	4-CH_3	5f	3	216	72	6f	7.5	248	79
7	$-OCH_3$	4-Cl	5g	3	290	76	6g	7	245	84
8	−Cl	H	5h	3	198	68	6h	7.5	208	68
9	−Cl	2-Cl	5i	3	244	65	6i	7.5	205	86
10	−Cl	3-NO_2	5j	2.5	230	78	6j	6.5	215	87
11	−Cl	2-Br	5k	2.5	249	67	6k	7	195	84
12	$-CH_3$	4-Br	5l	3	285	86	6l	7.5	236	70
13	$-CH_3$	4-CH_3	5m	3	266	63	6m	7.5	201	62
14	$-CH_3$	4-Cl	5n	3	233	74	6n	7	261	72

[a] 2-imino-3-(4-arylthiazol-2-yl)-thiazolidin-4-ones(1 mmol), substituted aldehydes (1 mmol), acetic acid, sodium acetate (2 mmol) reflux 3 h.
[b] 5-arylidene derivatives (1 mmol), acetic acid (10 ml), sodium acetate (1gm), phenyl hydrazine (1 ml) reflux 7 h.
[c] Isolated Yield.

188, Phe227, Trp 229, Val 106, Val 179, Ile180, Leu100, Leu 234, Pro95, Val 381 and Ile 382. From the two dimensional Fig. 1(a) and (b) and three dimensional view Fig. 2(a) and (b), it is observed that Lys 101 and Lys 103 is juxtaposed for better interaction with the phenyl-2H-pyrazolo [3,4-d]thiazol-5(6H)-imine series (see Table 2).

Abbreviations: VAL, valine; LEU, leucine; GLY, glycine; ASP, aspartate; SER, serine; ALA, alanine; LYS, lysine; ILE, isoleucine; HIE, histidine epsilon H; MET, methionine; THR, threonine.

The Chloro and Methoxy phenyl- thiazolyl nucleus moiety at of compounds **6k** and **6f** makes π-π interaction into the hydrophobic binding pocket, surrounded by the aromatic side chains of portion of Trp229 residue. The proton on nitrogen of the imine on pyrazolothiazole moiety of compounds **6k** and **6f** form the hydrogen bond interactions with the backbone N—H of Ile 180 residue. The aryl group of toluene on pyrazolothiazole ring of the compound **6f** also makes π-cation interaction with the hydrophobic binding pocket, surrounded by the aromatic side chains of portion of lys101 residue. The decrease in activity of compounds **6i** and **6e** of the phenyl-2H-pyrazolo [3,4-d] thiazol-5(6H)-imine series was due to lack of π-π interaction of the methoxy phenyl-thiazolyl nucleus into the hydrophobic binding pocket, surrounded by the aromatic side chains of portion of Trp229 residue and nonexistence of π- cation interaction with the hydrophobic binding pocket, surrounded by the aromatic side chains of portion of lys101 residue (Fig. 3). Only it forms the hydrogen bond interactions with the backbone N—H of Ile 180 residue.

Docking score of compound **6k, 6f, 6i** and **6e** was found to be around −7.849, −7.597, −5.899 and −5.755 respectively, while of native ligand was found to be −13.413 which confirms that **6 k** and **6f** compounds might have potent RT inhibition activity. Further, *in silico* binding studies suggested that inhibitors possessing π-π interaction of the phenyl-thiazolyl nucleus into the hydrophobic binding pocket, surrounded by the aromatic side chains of portion of Trp229 residue and π-cation interaction with the aromatic side chains of portion of lys103 residue improves the inhibitor selectivity for RT and thus helps in further drug design attempts to obtain potent phenyl-2H-pyrazolo [3,4-d]thiazol-5(6H)-imine derivatives.

2.3. *In vitro anti-HIV assay*

According to the docking study of synthesized compounds, some of it showed the high inhibition activity and some with low activity. From

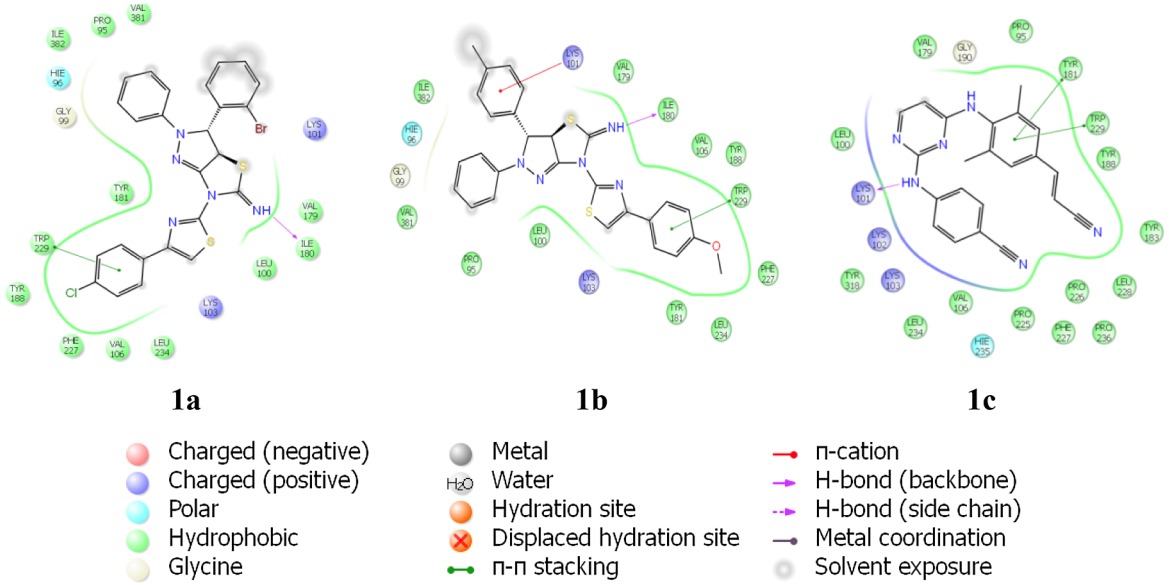


Fig. 1. Two-dimensional view of the binding interaction of the most active compounds, 6k (9a), 6f (9b) with active site of HIV-1 reverse transcriptase (RT) in complex with TMC278 and native ligand TMC 278 (9c) with HIV-1 reverse transcriptase (RT).

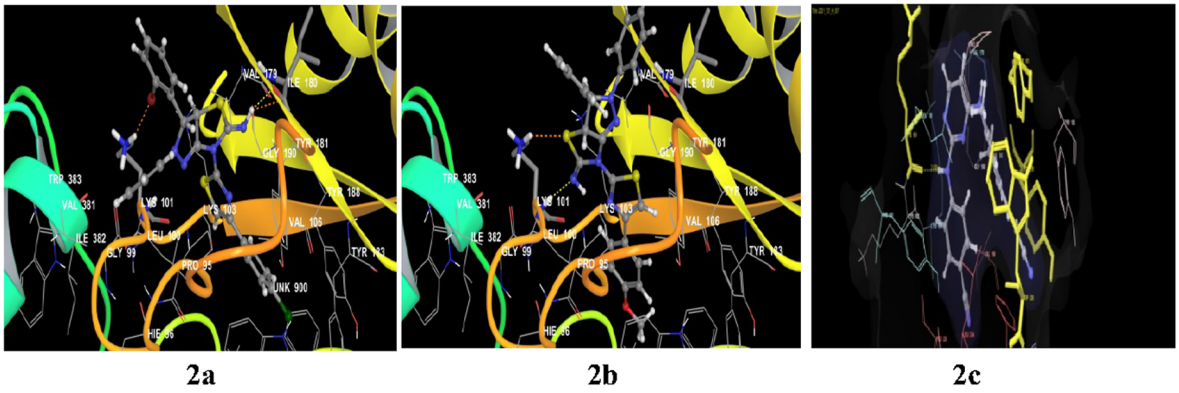


Fig. 2. Three dimensional view of the binding interaction of the most active compounds, 6k (10a), 6f (10b) with active site of HIV-1 reverse transcriptase (RT) in complex with TMC278 and native ligand TMC278 (10c) with HIV-1 reverse transcriptase (RT).

Table 2
Docking score of Novel 2-Phenyl-3-(4H-1, 2, 4-triazol-4-yl) thiazolidin-4-ones due binding interaction with active site of HIV-1 reverse transcriptase (RT) in complex with TMC278.

Title	docking score	XP GScore	glide gscore	glide evdw	glide ecoul	glide energy	glide einternal	glide emodel	XP HBond
2ZD1	−13.413								
6k	−7.849475	−7.849475	−7.849475	−39.963416	−5.594786	−45.558202	3.063549	−74.041264	−0.7
6f	−7.547273	−7.547273	−7.547273	−40.234612	−5.271893	−45.506505	3.471281	−72.118526	−0.7
6a	−7.083975	−7.083975	−7.083975	−45.83853	−4.364333	−50.202862	3.685489	−54.501316	−0.668876
6b	−6.971286	−6.971286	−6.971286	−40.197659	−5.043644	−45.241303	4.000247	−72.504164	−0.7
6c	−6.645447	−6.645447	−6.645447	−44.311642	−3.81061	−48.122253	3.297892	−71.984905	−0.338102
6h	−6.567393	−6.567393	−6.567393	−44.699404	−4.367326	−49.06673	5.312677	−71.321814	−0.217418
6n	−6.458644	−6.458644	−6.458644	−44.988028	−3.95854	−48.946568	2.183347	−64.385089	−0.303922
6d	−6.355003	−6.355003	−6.355003	−40.021352	−5.528269	−45.54962	5.213494	−73.415471	−0.7
6l	−6.339996	−6.339996	−6.339996	−43.593921	−3.082626	−46.676548	3.814228	−60.746038	−0.467302
6m	−6.118304	−6.118304	−6.118304	−42.126854	−3.153137	−45.279991	5.491032	−72.121264	−0.494984
6g	−6.095082	−6.095082	−6.095082	−44.404501	−4.00595	−48.410451	2.397994	−58.876431	−0.244943
6j	−5.936749	−5.936749	−5.936749	−43.448215	−3.146492	−46.594707	5.780947	−67.419327	−0.36247
6i	−5.899335	−5.899335	−5.899335	−45.094918	−1.342442	−46.43736	12.47373	−68.521009	0
6e	−5.755529	−5.755529	−5.755529	−42.031885	−3.924049	−45.955933	4.108433	−70.495738	−0.24965

the above conclusion, we studied in vitro anti-HIV assay for particular compounds to verify their activity. The HIV-RT inhibition assay was performed by using an RT assay kit (Roche), and the procedure for assaying RT inhibition was performed as described in the kit protocol (Roche Kit) [43]. The compounds presented in this study namely phenyl-2H-pyrazolo [3,4-d]thiazol-5(6H)-imine derivatives **(6k, 6f, 6a, 6g, 6i and 6e)** were evaluated for anti-HIV-1 activity by using enzymatic (RT) and cell based assays. The HIV-1 RT inhibition activity range for these compounds showed from 60 to 94% inhibition at 100 μg/ml concentrations. The compounds **6k** and **6f** showed highest inhibitory activity both in docking as well cell based study (90.57%, 89.80%) respectively, were **6i** and **6e** shows low activity (62.77% and 57.85%) respectively, whereas the control NNRTI marketed drug nevirapine showed 99.15% inhibition at 100 μg/ml concentration. The enzyme assay results demonstrated that the compound **6k** and **6f** were more potent than remaining derivatives comparing against reverse transcriptase enzyme. Subsequently, the inhibitory activity of HIV-1 viral replication was also assessed by cell-based assay. The results are summarized in Table 3 along with standard nevirapine as reference drug. In the cell based assay, the compounds **6k** and **6f** were the most potent inhibitors of HIV-1 replication against HIV-1 IIIB (EC_{50} = 0.74 and 1.08 μg/ml respectively; the selectivity index (SI) = 57.70 and 32.00 respectively; CC_{50} with HIV-1 IIIB = 42.7 and 38.4 μg/ml respectively) and HIV-1 ADA5 (EC_{50} = 1.08 and 0.34 μg/ml;the selectivity index (SI) = 39.25 and 131.76 respectively; CC_{50} = 43.6; 41.7 μg/ml respectively). Some other compounds, **6i** and **6e** showed low anti-HIV-1 potency (EC50 = 1.04, 1.12 and 4.6, 5.5 μg/ml) against HIV-1 IIIB and (EC50 = 0.86, 0.95 and 6.3, 5.6 μg/ml) against HIV-1 ADA5 strains, respectively.

3. Experimental:

3.1. General details

All solvents were used as commercial anhydrous grade without further purification. Aluminium sheets 20 × 20 cm, Silica gel 60 F_{254}, Merck grade was used for thin layer chromatography to determine progress of reaction. Melting points were determined in open capillary tube and are uncorrected. IR, ^1H and ^{13}C NMR spectra were recorded on a Brucker AV-300 MHz spectrometer in $CDCl_3$ solvent. Mass spectra were taken on Polaris-Q Thermoscintific MS.

3.2. General procedure for the preparation of 2-chloro-acetamido-4-sbstituted arylthiazoles (2a-c)

A mixture of 2-amino 4-substitutedarylthiazoles (10 mmol) and chloroacetylchloride (15 mmol) was refluxed in dry toluene (20 ml) in the presence of anhydrous K_2CO_3 (10 mmol) for 8 h. The reaction mixture was then filtered while hot and the filtrate was evaporated to dryness under vacuum. The residual mass was washed with water, dried and crystallized from ehanol to obtain white crystals with better yield.

2-chloro-N-(4-(4-methoxyphenyl) thiazol-2-yl) acetamide (2a): White solid, **M.P. (158 °C):**

IR (cm^{-1}): 3414, 3043, 2928, 2844, 2042, 1692, 1615, 1567, 1493, 1452, 1408, 1325, 1254, 1171, 1100, 1027, 970, 911, 533, 495. **^1H NMR (300 MHz, $CDCl_3$):** δ 3.85 (s, 3H), 4.23 (s, 2H), 6.96 (d, 2H), 7.05 (s, 1H), 7.73–7.76 (d, 2H), 10.01 (s, 1H, NH);**^{13}C NMR (75 MHz, $CDCl_3$):** δ 42.83, 56.00, 102.00, 113.76, 125.46, 129.15, 148.20, 161.74, 165.00, 166.43.

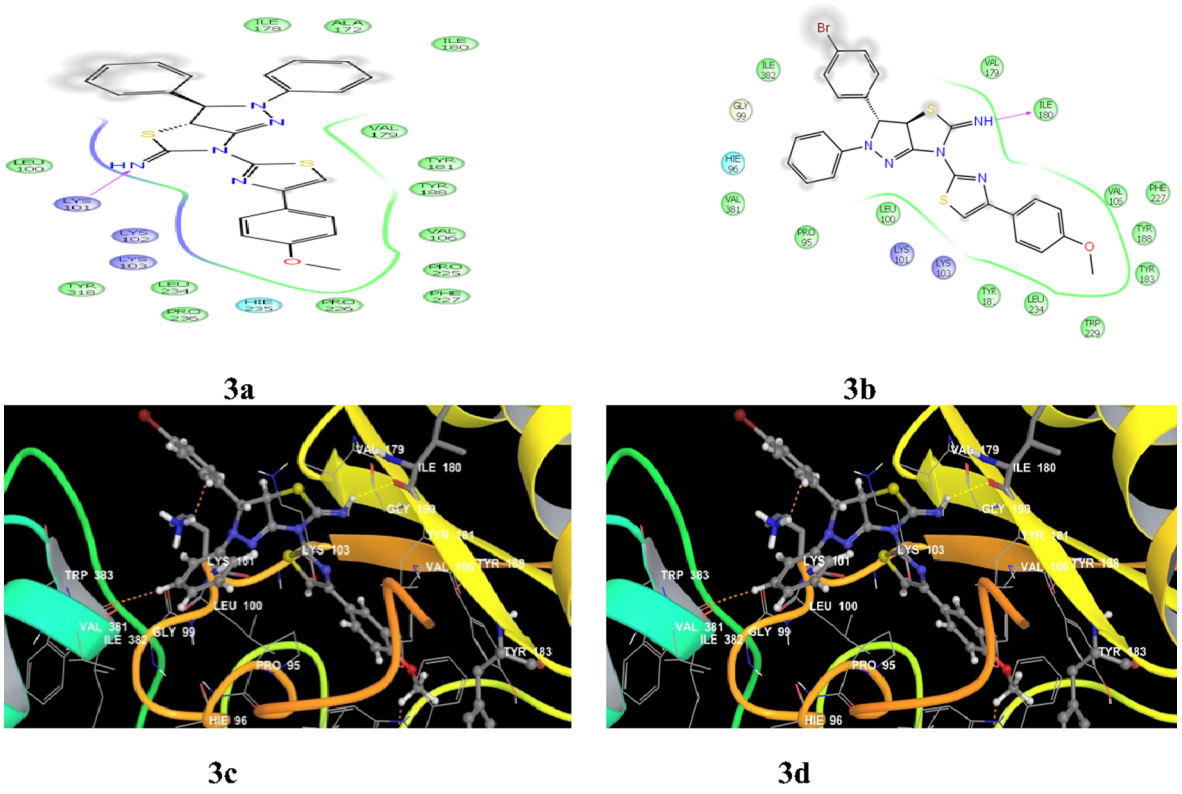


Fig. 3. Two (3a, 3b) and three-dimensional (3c and 3d) view for decrease in activity of compound 6i and 6e respectively.

Table 3
Anti-HIV-1 activity, cytotoxicity and selectivity index in HIV-1IIIB, ADAS5 and HIV-1 RT kit assay for compounds.

Compound	X	R	Anti-HIV-1 activity						% Inhibition (HIV-RT kit assay) (100 µg/ml)
			EC_{50}[b] (µg/ml)		CC_{50}[c] (µg/ml)		SI[d]		
			HIV-1IIIB	ADA5	HIV-1 IIIB	ADA5	HIV-1 IIIB	ADA5	
6k	Cl	2-Br	0.74 ± 0.02	1.08 ± 0.04	42.7 ± 0. 04	42.4 ± 0.06	57.70	39.25	90.57
6f	OCH_3	4-CH3	1.2 ± 0.31	0.34 ± 0.02	38.4 ± 0.34	44.8 ± 0.06	32.00	131.76	89.80
6a	OCH_3	H	0.9 ± 0.04	0.35 ± 0.12	39.8 ± 0.07	46.5 ± 0.36	44.22	132.85	84.67
6g	OCH_3	4-Cl	1.42 ± 0.05	1.24 ± 0.08	52.9 ± 0.13	48.3 ± 0.24	37.25	38.95	69.84
6i	Cl	3-No_2	1.04 ± 0.12	0.86 ± 0.09	4.6 ± 0.16	6.3 ± 0.36	4.42	7.32	62.77
6e	Cl	2-Cl	1.12 ± 0.14	0.95 ± 0.01	5.5 ± 0.16	5.6 ± 0.05	4.91	5.89	57.85
Nevirapine			0.05	0.05	76.12	76.15	1522.51	1522.51	99.15

[a]Data represent the mean of two and three independent assays for EC_{50} and CC_{50}, respectively.
[b] EC_{50} is the 50% effective concentration required to reduce HIV-1 induced cytopathic effect of HIV-1 IIIB and HIV-1 ADA5.
[c] The CC_{50} is the 50% cytotoxic concentration for HIV-1 IIIB and HIV-1 ADA5.
[d] Selectivity index ratio CC_{50}/EC_{50}.

2-chloro-N-(4-(4-chlorophenyl) thiazol-2-yl) acetamide (2b): White solid, **M.P.(162–164 °C).**

IR (cm^1): 3415, 3107, 2986, 2941, 1908, 1690, 1646, 1541, 1479, 1443, 1401, 1314, 1259, 1164, 1082, 902, 838, 802, 754, 700, 598, 516, 466; **^{1}H NMR (300 MHz, $CDCl_3$):** δ 4.29 (s, 2H), 7.18 (s, 1H), 7.37–7.40 (t, 2H), 7.75–7.78 (q, 2H), 9.77 (s, 1H, NH); **^{13}C NMR (75 MHz, $CDCl_3$):** δ, 42.94, 100.50, 128.45, 130.45, 135.40, 148.10, 165.56, 166.46.

2-chloro-N-(4-p-tolylthiazol-2-yl) acetamide (2c): White solid, **M.P. (150 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** δ 2.83 (s, 3H), 4.99 (s, 2H), 6.53 (s, 1H), 6.89–6.92 (d, 2H), 7.69–7.71 (d, 2H), 10.26 (s, 1H, NH); **^{13}C NMR (75 MHz, $CDCl_3$):** δ 24.69, 42.00, 100.21, 127.47, 129.00, 130.12, 38.23, 148.24, 164.43, 165.45.

3.3. General procedure for synthesis of 2-imino-3-(4-substituted arylthiazol-2-yl)-thiazolidin-4-ones (3a-c)

A mixture of 2-chloro-acetamido-4-sbstituted arylthiazoles (1 mmol), KSCN (2 mmol) and dry acetone (30 ml) was refluxed for 3 h. Excess of acetone was removed in vacuum and residue was stirred with water (50 ml). The solid product was filtered, washed with water and dried. The thiazolidinone was obtained by recrystalization from ethanol with affordable quantitative yield.

2-imino-3-(4-(4-methoxyphenyl) thiazol-2-yl) thiazolidin-4-one (3a): Yellowish solid, **M.P. (185 °C): IR (cm^{-1}):** 3414, 3119, 3071, 2964, 2842, 2034, 1730, 1695, 1572, 1481. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 3.79 (s, 3H), 4.02 (s, 2H), 6.99–7.01(d, 2H), 7.04 (s, 1H), 7.87–7.89 (d, 2H), 12.10 (s, 1H, NH);**^{13}C NMR (75 MHz, $CDCl_3$):** δ 29.37, 56.03, 101.43, 116.08, 124.48, 130.41, 147.79, 153.25, 161.24, 163.46, 172.54, 173.55.

3-(4-(4-chlorophenyl)thiazol-2-yl)-2-iminothiazolidin-4-ne (3b): Yellowishsolid, (**M.P.189 °C): IR (cm^{-1}):** 3415, 3102, 2922, 2804, 2057, 1718, 1581, 1474, 1405, 1325, 1285, 1254, 1209, 1167, 1082, 1010, 930, 900, 827, 773, 742, 688, 613, 511. **^{1}H-NMR (300 MHz, $CDCl_3$):** δ 4.12 (s, 2H), 7.02–7.09 (d, 2H), 7.64 (s, 1H), 7.77–7.82 (d, 2H), 12.06 (s, 1H, NH); **^{13}C NMR (75 MHz, $CDCl_3$):** δ 29.06, 103.45, 128.02, 130.41, 132.14, 1354.51, 147.99, 163.25, 172.54, 174.04.

*3.4. General procedure for synthesis of 5-arylidene-2-imino-3-(4-substituted arylthiazol-2-yl)-thiazolidin-4-ones (**5a-n**)*

A mixture of 2-imino-3-(4-substituted arylthiazol-2-yl)-thiazolidin-4-ones (1 mmol), substituted benzaldehyde(1 mmol) in acetic acid was refluxed for 3 h at 120 °C on addition of sodiumacetate (2 mmol). Then orange colored crystals were appeared. It was filtered washed with ethanol then with water to afford analytically pure product.

5-(3-nitrobenzylidene)-2-imino-3-(4-(4-methoxyphenyl) thiazol-2-yl)thiazolidin-4-one(5c): Orange colored solid, **M.P. (270–272 °C): IR (cm^{-1}):** 3472, 3415, 3089, 3001, 2949, 2807, 2035, 1722, 1687, 1610, 1533, 1481, 1346, 1324, 1249, 1163, 1107, 1071, 1029, 899, 866, 831, 740, 669, 612, 522, 493, 466; **^{1}H NMR (300 MHz, $CDCl_3$):** δ 3.82 (s, 3H), 7.06–7.08 (d, 2H), 7.70 (s, 1H), 7.83–7.88(t, 2H), 7.96–7.99 (d, 2H), 8.15–8.18 (d, 1H), 8.31–8.34 (d, 1H), 8.56(s, 1H), 12.87(s, 1H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ 56.22, 100.44, 115.98, 116.53, 121.28, 124.48, 126.02, 130.41, 141.14, 142.33, 147.79, 148.21, 151.24, 161.46, 166.51, 173.30.

5-(4-chlorobenzylidene)-2-imino-3-(4-(4-methoxyphenyl) thiazol-2-yl)thiazolidin-4-one(5g): Orange colored solid, **M.P. (290 °C): IR (cm^{-1}):** 3415, 3122, 2924, 2848, 2034, 1725, 1691, 1608, 1576, 1485, 1409, 1334, 1284, 1248, 1171, 1070, 1028, 898, 832, 780, 741, 693, 612, 518, 466. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 3.82 (s, 3H), 6.99–7.01 (d, 2H), 7.38 (s, 1H), 7.72–7.76 (t, 2H), 7.90–8.00 (d, 2H), 8.25–8.27 (d, 2H), 8.57 (s, 1H), 11.89 (s, 1H);**^{13}C NMR (75 MHz, $CDCl_3$):** δ 100.30, 116.51, 127.02, 128.04, 128.48, 130.41, 132.14, 133.28, 134.513, 135.23, 142.15, 147.74, 153.25, 166.24, 172.30.

5-(4-bromobenzylidene)-2-imino-3-(4-p-tolylthiazol-2-yl)thiazolidin-4-one(5l): Light Yellowish solid, **M.P. (285 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** δ 2.53 (s, 3H), 6.99–7.01 (d, 2H), 7.09–7.12 (d, 1H), 7.34 (s, 2H), 7.65 (s, 2H), 7.72 (s, 2H), 7.89 (s, 1H), 12.11 (s, 1H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ 25.30, 100.90, 116.58, 122.20, 127.31, 129.58, 130.02, 132.33, 134.59, 135.35, 138.70, 141.50, 149.32, 153.54, 167.29, 173.16.

*3.5. General procedure for synthesis of pyrazolo [3, 4, d] thiazole derivatives (**6a-n**)*

A mixture 5-arylidene derivative (1 mmol) in glacial acetic acid (10 ml), sodium acetate (1 g) and phenyl hydrazine (1 ml) were heated for 7 h. The mixture was filtered when hot to remove any insoluble material, cooled, and then water was added and boiled for few minutes, then it was cooled to afford the crude product, which was purified by column chromatography from *n*-hexane-ethyl acetate (2:1).

3,3a-dihydro-6-(4-(4-methoxyphenyl)thiazol-2-yl)-3-(3-nitrophenyl)-2-phenyl-2H-pyrazolo- [3,4-d] thiazol-5(6H)-imine (6c): Yellowish solid, **M.P. (228 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** δ 3.19–3.20 (d, 1H), 3.82 (s, 3H), 4.82–4.90 (d, 1H), 6.50–6.53 (t, 2H), 6.71–6.78 (d, 2H), 7.06–7.08 (d, 2H), 7.38–7.43 (t, 1H), 7.77 (s,1H), 7.83–7.88 (t, 2H), 7.96–7.99(d, 2H), 8.15–8.18 (d, 1H), 8.31–8.34(d, 1H), 12.87 (s,1H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ 44.44, 54.22, 55.66, 100.41, 113.15, 117.28, 120.43, 125.04, 126.02, 130.51, 132.14, 143.59, 146.44, 148.10, 150.06, 155.11, 161.37, 163.06, 174.00; **MS:** *m/z* 588 (M^+). *Anal.*Calcd for $C_{26}H_{20}N_6O_3S_2$ (528.61): C, 59.08; H, 3.81; N, 15.90; O, 9.08; S, 12.13. Found: C, 59.04; H, 3.78; N, 15.88; O, 9.06; S, 12.11.

3-(4-chlorophenyl)-3,3a-dihydro-6-(4-(4-methoxyphenyl) thiazol-2-yl)-2-phenyl-2H-pyrazolo [3,4-*d*] thiazol-5(6H)-imine (6g): Light Yellowish solid, (**M.P.245 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** δ 3.09–3.10 (d, 1H), 3.53 (s, 3H), 4.11–4.08 (d, 1H), 6.05–6.02 (m, 1H), 6.11–6.10 (d, 2H), 6.54–6.53 (d, 2H), 6.70–6.67 (d, 2H), 7.01–6.99 (d, 1H), 7.12–7.09 (d, 2H), 7.33 (s, 2H), 7.65 (s, 2H), 7.72(s, 1H), 12.11(s,1H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ 44.59, 54.30, 55.07, 100.34, 113.02, 115.33, 117.20, 125.02, 126.02, 128.70, 130.50, 132.12, 141.98, 143.43, 148.20, 155.20, 161.33, 163.06, 173.00; **MS:** *m/z* 517. *Anal.*Calcd for $C_{26}H_{20}ClN_5OS_2$ (518.05): C, 60.28; H, 3.89; Cl, 6.84; N, 13.52; O, 3.09; S, 12.38. Found: C, 60.25; H, 3.84; Cl, 6.80; N, 13.48; O, 3.05; S, 12.35.

3-(4-bromophenyl)-3,3a-dihydro-2-phenyl-6-(4-p-tolylthiazol-2-yl)-2H-pyrazolo [3,4-d] thiazol-5(6H)-imine (6l): Yellowish solid, **M.P. (236 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** δ 2.53 (s, 3H), 3.01–3.09 (d, 1H), 4.08–4.11 (d, 1H), 6.02–6.11 (m, 1H), 6.53–6.54 (d, 2H), 6.67–6.70 (d, 2H), 6.99–7.01 (d, 2H), 7.09–7.19 (d, 1H), 7.33 (s, 2H), 7.55(s, 2H), 7.63(s, 2H), 7.72(s, 1H), 12.10 (s, 1H); **^{13}C NMR (75 MHz, $CDCl_3$):** δ 24.52, 44.44, 54.03, 101.49, 111.30, 117.28, 122.04, 129.062, 129.37, 130.41, 132.14, 134.51, 138.02, 143.21, 145.08, 147.79, 153.25, 162.24, 169.55, 173.46; **MS:** *m/z* 546. *Anal.*Calcd for $C_{26}H_{20}BrN_5S_2$ (546.5): C, 57.14; H, 3.69; Br, 14.62; N, 12.81; S, 11.73. Found: C, 57.12; H, 3.65; Br,14.58; N, 12.78; S, 11.72.

3,3a-dihydro-6-(4-(4-methoxyphenyl)thiazol-2-yl)-2,3-diphenyl-2H-pyrazolo [3,4-*d*] thiazol-5(6H)-imine (6a): Yellowish solid, **M.P. (225 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** δ 3.20–3.09 (d, 1H), 3.89 (s, 3H), 4.90–4.82 (d, 1H), 6.51–6.50 (d, 2H), 6.78–6.71 (d, 2H), 7.08–7.06 (d, 2H), 7.43–7.38 (d, 1H), 7.777 (s, 1H), 7.88–7.83 (d, 2H), 7.99–7.96(d, 2H), 8.18–8.15(d, 1H), 8.34–8.31(d, 2H), 11.87(s, 1H); *Anal.*Calcd for $C_{26}H_{21}N_5OS_2$ (483.61) C, 64.57; H, 4.38; N, 14.48; O, 3.31; S, 13.26. Found: C,64.55; H, 4.35; N,14.46; O,3.29; S, 13.24.

3-(2-chlorophenyl)-3,3a-dihydro-6-(4-(4-methoxyphenyl) thiazol-2-yl)-2-phenyl-2H-pyrazolo [3,4-*d*] thiazol-5(6H)-imine (6b): Yellowish solid, **M.P. (220 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** δ 3.72 (s, 3H), 4.40–4.39 (d, 1H), 4.90–4.89 (d, 1H), 6.50 (s, 1H), 6.53–6.51 (d, 2H), 6.78–6.71(d, 1H), 7.08–7.06 (d, 2H), 7.43–7.41 (d, 1H), 7.88–7.86 (d, 2H), 7.99–7.96(d, 2H), 8.18–8.15(d, 1H), 8.34–8.31(d, 1H), 12.77(s, 1H); *Anal.*Calcd for $C_{26}H_{20}ClN_5OS$ (5 1 8): C, 60.28; H, 3.89; Cl, 6.84; N, 13.52; O, 3.09; S, 12.38. Found: C, 60.25; H, 3.87; Cl, 6.82; N, 13.49; O, 3.06; S, 12.35.

3-(2-bromophenyl)-3,3a-dihydro-6-(4-(4-methoxyphenyl) thiazol-2-yl)-2-phenyl-2H-pyrazolo [3,4-*d*] thiazol-5(6H)-imine (6d): Yellowish solid, **M.P. (240 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** δ 3.20–3.15 (d, 1H), 3.72 (s, 3H), 4.83–4.77 (d, 1H), 6.43 (s, 1H), 6.51–6.50 (d, 2H), 7.08–7.06(d, 2H), 7.43–7.38 (m, 1H), 7.77 (s, 1H), 7.88–7.86 (d, 2H), 7.99–7.96(d, 2H), 8.18–8.15(d, 1H), 8.39–8.31(d, 1H), 12.90(s, 1H); *Anal.*Calcd for $C_{26}H_{20}BrN_5OS_2$ (562.05): C, 55.52; H, 3.58; Br, 14.21; N, 12.45; O, 2.84; S, 11.40. Found: C, 55.50; H, 3.55; Br, 14.17; N, 12.43; O, 2.82; S, 11.38.

3-(4-bromophenyl)-3,3a-dihydro-6-(4-(4-methoxyphenyl) thiazol-2-yl)-2-phenyl-2H-pyrazolo [3,4-*d*] thiazol-5(6H)-imine (6e): Yellowish solid, **M.P. (252 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** δ 3.11–3.08 (d, 1H), 3.33 (s, 3H), 4.09–4.01 (d, 1H), 6.03–6.02 (d, 1H), 6.06–6.05 (d, 2H), 6.11–6.10 (d, 2H), 6.54–6.53 (d, 2H), 6.70–6.67 (d, 1H), 7.01–6.99 (d, 2H), 7.12–7.09 (d, 2H), 7.65 (s, 2H), 7.72 (s, 1H), 12.33 (s, 1H); *Anal.*Calcd for $C_{26}H_{20}BrN_5OS_2$ (562.5): C, 55.52; H, 3.58; Br, 14.21; N, 12.45; O, 2.84; S, 11.40. Found: C, 55.50; H, 3.55; Br, 14.20; N, 12.40; O, 2.81; S, 11.38.

3,3a-dihydro-6-(4-(4-methoxyphenyl)thiazol-2-yl)-2-phenyl-3-p-tolyl-2H-pyrazolo [3,4-*d*] thiazol-5(6H)-imine(6f): Yellowish solid, **M.P. (248 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** δ3.82(s, 3H), 4.27–4.25 (d, 1H), 6.47–6.45 (d, 1H), 6.64 (s, 1H), 6.99–6.94 (d, 2H), 7.27–7.25 (d, 2H),7.38 (d, 2H), 7.72 (s, 1H), 7.76–7.74 (d, 2H), 8.00–7.98 (d, 2H), 8.27–8.25 (d, 2H), 8.57 (s, 1H), 11.89 (s, 1H); *Anal.*Calcd for $C_{27}H_{23}N_5OS_2$ (493.63): C, 65.17; H, 4.66; N, 14.07; O, 3.22; S, 12.89.

Found: C,65.14; H, 4.62; N, 14.03; O, 3.20; S, 12.84.

3-(2-bromophenyl)-6-(4-(4-chlorophenyl)thiazol-2-yl)-3,3a-dihydro-2-phenyl-2H-pyrazolo [3,4-*d*] thiazol-5(6H)-imine(6k): Yellowish solid, **M.P. (195 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** *δ* 3.73–3.72 (d, 1H), 4.83–4.77 (d, 1H), 6.43 (d, 1H), 6.51–6.50 (d, 2H), 6.78–6.71 (d, 1H), 7.089–7.086 (d, 2H), 7.43–7.41 (m, 1H), 7.77 (s, 1H), 7.88–7.83 (s, 2H), 7.99–7.96 (d, 2H), 8.18–8.15 (d, 1H), 8.39–8.31 (d, 1H), 12.93 (s, 1H); *Anal.*Calcd for $C_{25}H_{17}BrClN_5S_2$ (566.92): C, 52.96; H, 3.02; Br, 14.09; Cl, 6.25; N, 12.35; S, 11.31. Found: C, 52.94; H, 3.00; Br, 14.05Cl, 6.23; N, 12.33; S, 11.28.

3,3a-dihydro-2-phenyl-3-p-tolyl-6-(4-p-tolylthiazol-2-yl)-2H-pyrazolo [3,4-*d*] thiazol-5(6H)-imine(6m): Yellowish solid, **M.P. (201 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** *δ*2.82 (s, 3H), 4.27–4.25 (s, 1H), 4.647–4.645 (s, 1H), 6.75 (s, 1H), 6.99–6.94 (d, 2H), 7.27–7.25 (d, 2H), 7.388–7.389 (d, 2H), 7.72 (s, 1H), 7.76–7.74 (d, 2H), 8.00–7.98 (d, 2H), 8.27–8.25 (s, 2H), 8.57 (s, 1H), 11.72 (s, 1H); *Anal.*Calcd for $C_{27}H_{23}N_5S_2$ (481.63): C, 67.33; H, 4.81; N, 14.54; S, 13.32. Found: C, 67.30; H, 4.78; S, 13.25.

3-(4-chlorophenyl)-3,3a-dihydro-2-phenyl-6-(4-p-tolylthiazol-2-yl)-2H-pyrazolo [3,4-d] thiazol-5(6H)-imine(6n): Yellowish solid, **M.P. (261 °C): ^{1}H NMR (300 MHz, $CDCl_3$):** *δ*2.82 (s, 3H), 4.27–4.25 (s, 1H), 4.647–4.645 (s, 1H), 6.75 (s, 1H), 6.99–6.94 (d, 2H), 7.27–7.25 (d, 2H), 7.388–7.389 (d, 2H), 7.72 (s, 1H), 7.76–7.74 (d, 2H), 8.27–7.98 (d, 2H), 8.647–8.645 (d, 2H), 8.72 (s, 1H), 12.29 (s, 1H); *Anal.*Calcd for $C_{26}H_{20}ClN_5S_2$ (502.05): C, 62.20; H, 4.02; Cl, 7.06; N, 13.95; S, 12.77. Found: C, 62.16; H, 4.00; Cl, 7.04; N, 13.94; S, 12.74.

3.6. Molecular docking studies: material and methods

To guide the lead optimization strategy and rationalize the SARs, modeling study was performed to examine the possible binding conformations of our newly synthesized compounds and their interaction mode with RT; using Glide [37]. Structure-based docking studies were carried out to investigate the intermolecular interaction between the ligand and the targeted enzyme. The coordinates of the non-nucleoside binding site were taken from the crystal structure of HIV-1 reverse transcriptase (RT) in complex with TMC278 (Rilpivirine) (PDB code: 2ZD1) [38]. Docking study of all the synthesized molecules was carried out with enzyme reverse transcriptase PDB ID: 2ZD1. The ligands were prepared by using LigPrep [39]. The protein was refined using the protein preparation wizard present in Maestro 9.3[40]. All the water molecules were deleted. H-atoms were added to the protein, including the protons necessary to define the correct ionization and tautomeric states of the amino acid residues. Prime interface module incorporated in Maestro was used to add the missing residues of the side chain. Each structure minimization was carried out with the impact refinement module, using the OPLS-2005 force field to alleviate steric clashes potentially existing in the structures. Minimization was terminated when the energy converged or the root mean square deviation reached a maximum cutoff of 0.30 Å. To find out active site grid was prepared using grid generation panel of glide with the default settings. Grid is prepared for defining the binding site of native ligand on the receptor. The ligand was selected to define the position and size of the active site [41,42]. Glide XP docking was used for docking purposes.

4. Conclusion

A series of new class of Phenyl-2H-pyrazolo [3,4-*d*]thiazol-5(6H)-imine derivatives were synthesized and evaluated as potent inhibitors of human immunodeficiency virus type-1 (HIV-1). Based upon the preliminary molecular docking studies of these new pyrazolo[3.4,*d*] thiazole hybrids, some structural requirements for high potency against HIV-1 were rationalized, which confirms that these compounds might have potent RT inhibition activity. In the series of pyrazolo[3.4,*d*] thiazoles, compound **6 k** and **6f** identified as potent inhibitor against the strains HIV-1 IIIB and HIV-1 ADA5. The decrease in activity of compound **6i and 6e** of pyrazolo[3.4,*d*]thiazole derivative was due to lack of π-π interaction of the methoxy phenyl-thiazolyl nucleus into the hydrophobic binding pocket, surrounded by the aromatic side chains of portion of Trp229 residue and nonexistence of π- cation interaction with the hydrophobic binding pocket, surrounded by the aromatic side chains of portion of lys101 residue. This study suggested that inhibitors possessing π-π interaction in to hydrophobic binding pocket with aromatic side chain of Trp229 residue and existence of π- cation interaction with lys101 residue improves the inhibitor selectivity for RT.

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Appendix A. Supplementary material

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