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N-hydroxy substituted 2-aryl acetamide analogues: a novel class of HIV-1 integrase inhibitors

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Abstract

An in silico method has been used to discover *N*-hydroxy substituted 2-aryl acetamide analogues as a new class of HIV-1 integrase inhibitors. Based on the molecular requirements of the binding pocket of catalytic active site, two molecules (compounds **2** and **4b**) were designed as fragments. These were further synthesized and biologically evaluated. In vitro potency along with docking studies highlighted compound **4b** as an active fragment which was further used to synthesize new leads as HIV-1 integrase inhibitors. Finally, six promising compounds (compounds **5b**, **5c**, **5e, 6-2c, 6-3b** and **6-5b**) were identified by integrase inhibition assay (>50% inhibition). Based on in-vitro anti-HIV-1 activity in a reporter gene based cell assay system, compounds 5d, 6s and 6k were found as novel HIV-1 integrase inhibitors due to its better selectivity index. Additionally, docking study revealed the importance of H-bond as well as hydrophobic interactions with Asn155, Lys156 and Lys159 which were required for their anti-HIV-1 activity.

Key words: AIDS, HIV-1 integrase, ZINC database**,** Molecular modeling

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Introduction

Worldwide, more than 36.7 million people are infected with human immunodeficiency virus (HIV) and approximately 2.1 million new cases are reported each year (UNAIDS Fact sheet, 2016, www.unaids.org/en/resources/fact-sheet). Highly active antiretroviral therapy (HAART) mainly consisting of HIV protease and reverse transcriptase inhibitors, introduced in the mid-to-late 1990, has largely improved patient quality of life as well as longevity [1, 2]. However, drug resistance and toxicity are the main problems during HIV treatment [3]. Integrase enzyme is one of the widely explored targets for designing diverse molecules against HIV-1[4]. This enzyme acts as an intermediate to transfer the viral DNA into the host genome [5]. Mg^{+2} and Mn^{+2} are the two important co-factors in the catalytic site of integrase enzyme [5]. Moreover, Mg^{+2} is a preferred divalent co-factor for integration [6]. This co-factor is used for the 3'-end processing and strand transfer steps. Integrase inhibitor can play an essential role by the process of inhibition of Mg^{+2} chelation in the catalytic site of the enzyme to induce the functional impairment of integrase activity [7].

It has been seen that diverse natural and synthetic molecules are being explored as integrase inhibitors to stop the viral progression [8-10]. Most of the drugs like Raltegravir, Elvetegravir and Dolutegravir make a co-ordinate bond with divalent ions (e.g. Mg^{+2}) to stop the 3'-end processing followed by strand transfer inhibition [11]. Historical structure-activity relationship (SAR) of all integrase inhibitors indicate that two structural segments are crucial for their activity: (1) a hydrophobic benzyl moiety that can bind to a hydrophobic site at one end of the integrase catalytic active site and (2) a planner chelating triad for binding with Mg^{+2} ion in hydrophilic zone of the enzyme active site. Generally all active molecules reported so far contain at least one of these features, while diketo and catechol based inhibitors contain both hydrophobic and magnesium binding features [6, 8]. Based on this development, a multitude of different scaffolds have been utilized to develop new inhibitors for this enzyme. However, due to mutations in the loop region (amino acid residues 140 to 149) of the catalytic site, most of the current drugs like Raltegravir and other related molecules are facing serious drug resistance problem [11,12]. Therefore, there is an urgent requirement to develop new active molecules/scaffolds as integrase inhibitors. The central theme of this study is the de novo design, synthesis and biological evaluation of new scaffold as HIV-1 integrase inhibitors.

In the present study, we have used an *in silico* approach to identify a new fragment namely, *N*-hydroxyl-2-arylacetimidamide which contains all the required features for integrase inhibition (*vide supra*) which was further exploited to prepare *in silico* ZINC database. The prepared database is used to identify new *N*-hydroxy substituted 2-arylacetamide as an anti-HIV-1 integrase inhibitor. Synthesis and biological evaluation of the identified best docked

molecule and its analogues thereof to confirm the potential anti-HIV-1 activity of the scaffold has been described. Furthermore, molecular docking studies were performed for all synthesized molecules to explain the observed structure-activity relationship. Finally, the lead is identified after consideration of its good anti HIV-1 activity profile along with its good selectivity index (SI).

Materials and Methods

In silico screening

An *in silico* screening method was used to identify a new class of molecules based on binding scores. To do that, *de novo* designed fragments were prepared which were used for getting a ZINC database containing 290 compounds. This database [13] was advanced to a primary filtration of the 290 compounds through a molecular docking method using SYBYLX 1.3 [14]. For docking experiments, a co-crystal of HIV-1 integrase protein was selected (PDB ID: **1QS4**) [15] and prepared by adding hydrogen atoms, fixing backbone amides and applying Gastregial Huckle charges. Next, the energy of the protein was minimized by the Powell method using the Tripos force field with a distance-dependent dielectric constant of 1.0 and a non-bonding interaction cut-off of 8.0 Å and iterations up to 1000 (convergence criteria 0.001 kcal/mol Å). Using ligand-based option procedures, the binding pocket was generated in the Protomol module of SYBYLX 1.3. The energy-minimized standard inhibitors and synthesized compounds were docked using the Surflex-Dock-Geom X docking mode into the pockets of selected target. The best docking poses of the compounds were selected based on the total, crash and polar scores. The best docked conformation was used for site-directed residue interaction analysis and visualization in Pymol [16] and MOE [17].

General synthesis procedures

Syntheses were done through general procedures as shown in Schemes 1 and 2 [18, 19].

(i) ***Synthesis of Compound 2***: 4-chlorophenyl acetohydroxamic acid (**2**) was prepared in a single-step reaction (Scheme 1) [18]. 4-chlorophenylacetic acid was treated with cyanuric chloride (TCT) (0.3 equiv) and *N*-methylmorpholine (NMM) (1.0 equiv) in dichloromethane (DCM). Additionally, dimethylamino pyridine (DMAP) was added as a catalyst (0.1 equiv), followed by addition of hydroxylamine hydrochloride (1.1 equiv). Stirring was continued at room temperature for 6-8 h, until 4-chloro phenylacetic acid was completely consumed as monitored by TLC. The reaction mixture was filtered and washed with 0.1 (N) HCl and brine. The desired product was recovered simply by removing the solvent (DCM) at reduced pressure. The pure form of the product was obtained with high yield value. The triazine by-products were easily removed by simple aqueous workup using 0.1N HCl solution and brine. Finally, spectroscopic analyses were done to confirm the structures.

(ii) ***Synthesis of Compounds 5a-5e & 6-(1a-5c)***: The synthesis of **5a-e** and **6-(1a-5c)** was performed using the corresponding *N*-hydroxy2-arylacetimidamide derivatives (4a-e) as starting material, and according to previously reported method [19](Scheme 2). Initially,

compounds 4a-e were synthesized by condensation of different substituted benzene acetonitriles (1 mmol), with hydroxylamine hydrochloride (1.1 mmol) and sodium methoxide (1.1 mmol) in methanol at reflux temperature for 12 h. Afterwards, the compound was purified by the silica gel column chromatography using appropriate solvent system (1:10: : methanol:chloroform).

Then, reaction of compounds **4a-e** (1mmol) with 3-nitrobenzoyl chloride (1.2 mmol) in dichloromethane (30 ml) using 1.2 mmol of triethayl amine as base at 0 °C led to the formation of corresponding *N*-(3-nitrobenzoyloxy)-2-aryl acetimidamide derivatives **(5a-e)**. In the same condition of reaction, condensation of **4a-e** with different aryl sulphonylchloride gave the *N*-arylsulphonyloxy 2-aryl acetamide derivatives **6-(1a-5c)**. In both cases, reactions were stirred for 1-2 h. The reaction was monitored by TLC (40% AcOEt/hexane). After completion of reaction, the organic layer was washed with brine solution and treated with anhydrous sodium sulphate (Na_2SO_4). After separating the organic layer, was evaporated under reduced pressure to get (**5a-5e**) and **6-(1a-5c**) with good yield values (65-85%). The crude product was purified by silica gel column chromatography using methanol/chloroform gradient. Spectroscopic analyses were carried out to characterize all the compounds.

In vitro assay for HIV-1 integrase inhibition

The HIV-1 integrase inhibitory activity of the compounds was determined by the commercial kit (XpressBio, Life Science Products, MD, USA) as per the manufacturer's instructions. Raltegravir (2 μM; Santa Cruz Biotechnology, Dallas, Texas, USA) was used as positive reference control [20].

In vitro anti-HIV-1 activity in reporter gene cell-based assay

TZM-bl cells [HeLa cell line expressing high levels of CD4, HIV-1 co-receptors CCR5 and CXCR4 with β-galactosidase and luciferase as reporter genes under HIV-1 LTR promoter] were maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich Inc., St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Biological Industries, Kibbutz beit Haemek, Israel) and an antibiotic-antimycotic cocktail [Penicillin (100 units/ml), Streptomycin (100 μg/ml) and Amphotericin B (250 ng/ml); Pen-Strep-Ampho sol, Biological Industries]. TZM-bl cells (5.0×10^4/well) were seeded in 24-well cell culture plate (Greiner Bio-One, GmbH, Frickenhausen, Germany) and cultured overnight at 37°C in presence of 75% relative humidity and 5% CO_2. In separate vials, HIV-1 NL4.3 (CXCR4 using virus) at a multiplicity of infection (MOI) of 0.05 was treated with various synthetic compounds or solvent for 1 h at 37ºC [21]. Subsequently, pretreated viruses were added in duplicates to TZM-bl cells and cultured for 4 h. Nevirapine (Sigma Aldrich Inc.) was used as a positive reference control whereas negative control comprised of cells without HIV infection. After incubation, the cells were washed once with cold 50 mM PBS, pH 7.4 to remove the cell-free virus followed by addition of fresh culture medium with or without the synthetic compounds. Cells were further incubated for 48 h, washed twice with PBS and

lysed with 1X lysis buffer (Promega Corporation, Madison, USA) by freeze-thaw. The supernatant was analyzed for luciferase activity by BrightGlo Luciferase Assay kit (Promega Corporation) in white opti-plate and luminescence was read using Fluorimeter (BMG Labtech GmbH, Offenberg, Germany) at a spectral range of 240 nm to740 nm. The results were expressed as percentage inhibition, calculated by taking the luminescence in experimental group divided by the luminescence in infected cells in absence of test compound multiplied by hundred. Percent inhibition was calculated by subtracting the above value from hundred.

Cytotoxicity assay

The cytotoxicity of the synthetic compounds on TZM-bl cells was assessed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma Aldrich Inc.] assay [22]. In brief, TZM-bl cells were seeded (8 x 10^3/well) in 96-well cell culture plates (Greiner Bio-One) and grown overnight at 37°C in presence of 75% relative humidity and 5% CO_2. After 24 h, synthetic compounds were added in increasing concentrations followed by further incubation for 48 h. Negative control included cells treated with solvent/medium. After incubation, cell viability was assessed by adding 20 µl MTT (5 mg/ml in PBS) per well and incubated at 37°C for 3 h followed by addition of MTT solvent (100 µl/well; absolute isopropanol, 0.04 N HCl). The absorbance (OD) was read at 540 nm with reference filter at 630 nm. Experiments were performed in triplicates and percent viability was calculated by dividing the OD obtained in treatment group by OD of the untreated cell control multiplied by hundred.

Result and Discussion

Virtual Screening analysis

To increase the available drug repertoire for 'HAART' therapy, integrase enzyme is explored as a crucial target for small molecule inhibitors. In the catalytic domain of the integrase enzyme, loop regions are used to allow conformational changes that are required for 3´processing of the viral DNA and strand transfer (STF) reactions, which are the two key steps of the integration process. Due to the presence of loop regions (connecting area of α helix and β sheets) in the catalytic core domain (CCD), diverse flexible molecules can be examined to explore the binding site. To identify new molecules for this target, we employed an in silico screening method by using online ZINC database. The ZINC database was prepared using a *de novo* designed active fragment, compound **4b,** due to its better binding score (total score 4.38, Figure 1) compared to compound **2** (total score 3.81, Figure 1). The ZINC database containing 290 molecules was further filtered by molecular docking method to identify the most potent molecules from the database. Based on the total score, crash score, polar score and C score ten molecules were selected (Table 1, Figure 2) from the database. Most of them were showing good H-bond interactions (ZINC 02935679, ZINC 08829370, ZINC 08683725) with important amino acids such as Asn155, Lys156 and Lys159 present in

the active site of integrase (Table 1, Figure 2). Additionally, ZINC 08829370 and ZINC 02974425 were showing CH-π interactions respectively with Phe121 and Phe139 (Supporting Information, Figure S1). This led to improved binding scores (ZINC 08829370; total score 8.26, ZINC 02974425; total score 8.61) but also reduce the crash score value near to zero which indicated correct penetration of these molecules into the binding pocket of integrase enzyme. From this docking analysis, it was clear that ZINC 08829370 provided the most promising binding scores (total score 8.26; crash score -0.31; polar score 7.04 and C score 5). It also displayed the important H-bond interactions with the important amino acids Glu152, Lys156 and Lys159. Therefore, we synthesized this molecule and its analogues to explore their structure-activity relationships as HIV-1 integrase inhibitor.

Synthesis and biological evaluations

Newly designed compounds (**2** and **4a-4e**) were synthesized by two different approaches (Schemes 1 and 2). Both compounds were obtained through single-step one-pot multi components reactions with high yields (>90%). Reactions were completed in 6-12 hrs. Again, compound **4c** was used to synthesize ZINC 08829370 (compound **5c**) and its different derivatives by base-catalysed (TEA) elimination reaction. The desired products were obtained in pure form by silica gel column chromatography.

Synthesized compounds (**2** and **4b**) were tested for in vitro integrase inhibitory activity to find out the active one. Here, only compound **4b** was considered for in vitro assay since it showed good docking score as well as maximum structural similarity with compound **2**. Percentage inhibition data at 50 µg/ml concentration confirmed compound **4b** to be a potent integrase inhibitor (Table 2). After identifying compound **4b** as a hit molecule, other related compounds were identified based on *in silico* screening. Top score based screened molecule (ZINC 08829370) was synthesized (compound **5c**) to examine its inhibitory activity against integrase enzyme as well as infection by HIV-1 using in-vitro gene reporter cell based assay. The percent inhibition data pointed towards a good integrase inhibition (Table 2) along with anti HIV-1 activity (Table 3). This data provide a new scaffold that was used to explore the activity of other analogues and to scrutinize their ability as an integrase inhibitor.

All synthesized analogues of ZINC 08829370 (**5c**) were screened for integrase inhibition activity. Molecules with greater than 50% integrase inhibitions were tested further in an in-vitro reporter gene cell (TZM-bl) based assay for anti HIV-1 activity. Furthermore, the cytotoxicity assay was also carried out to estimate the selectivity index of the molecules. It has been observed that compounds with halogen substitutions (compounds **5a**, **5b, 6-1a, 6-5b** etc.) at R position showed moderate to good inhibition against integrase enzyme (Table 2) whereas para-hydroxyl group substituted compounds at 4^{th} position of a benzyl moiety resulted in poor inhibitions (compounds **6-4a**, **6-4b** and **6-4c**, Table 2). R^1 substitution at meta and para position of benzene ring gave promising activity against integrase as well as HIV-1(compounds **5c**, **5b**, **5e**, **6-2c** and **6-5b**, Tables 2, 3, Figure 3). In most of the cases, bulky groups like acetamide and methoxy substituted aryl groups were showing less to moderate activities against integrase (compounds **6-1d**, **6-1e**, **6-3c**, **6-5c** etc.). More bulky

groups like 1,3,5-triisopropylbenzene (compound **6-1c**) were less favourable at R^1 position. In vitro percent inhibition data further indicated that hydrophobic substitution on the aryl moiety at R^1 position decreased the inhibitory activities (compounds **6-1c**, **6-2a**, **6-3a**, **6-4a** etc). The biological data also highlighted the importance of the linker of all synthesized molecules. It may be inferred from the integrase inhibitory activity data that imine (=NH) group at Y position plays an important role for biological activity (compound **4b**, Table 2). Similarly, linker at X position of all synthesized compounds influenced the activity. Substitution with both carbonyl (C=O) and sulphonyl groups (SO_2) exhibit reasonable anti HIV-1 integrase activity (compounds **5b, 5c, 6-1a, 6-2c, 6-5b** etc.) compared to Raltegravir. Furthermore, the improved cytotoxicity profile of some compounds (**6-2c, 6-5b**, Tables 2, 3) offered safe selectivity index.

Docking analysis of synthesized compounds

After biological evaluations the docking studies was further utilized to explain the SAR by docking the synthesized compounds in the integrase binding pocket (PDB ID: **1QS4**). Docking analysis indicated that total, crash and polar scores play a significant role for compound's binding affinity towards integrase enzyme. C score pointed out the overall rank of the docked molecules. In most of the cases it has been observed that less active compounds showed crash score values far away from zero (compounds **2**, **6-1c**, **6-1f**, **6-2a** and **6-4b**), which represents their inadequate penetration quality into the binding domain. Therefore, these molecules provided poor binding scores (Supporting information, Table S1). Similarly, some of the active molecules gave crash score values near to zero, which verified their well penetrating characteristic into the binding site of integrase. Thus, better crash score value might improve the total binding scores (Supporting information, Table S1).

Interestingly, molecular substitution with hydrophobic and halo groups at R^1 position (compounds **6-1c**, **6-1f, 6-2a**) were found to exhibit poor crash and total scores, which were reflected in their decreased activity against integrase. At the same time, polar groups containing molecules (compounds **5b**, **5e**, **6-2c**, **6-5b** etc.) influenced the total and crash scores, which were signified through good anti HIV-1 integrase activity (Supporting information, Table S1). The present results highlight the importance of polar interactions instead of hydrophobic interactions due to the polar nature of the binding site of the integrase enzyme. Furthermore, Figure 4 revealed that strong H-bond interactions with important amino acids (Glu152, Asn155, Lys156 and Lys159) led to the improved activity of the molecules (compounds **4b**, **6-2c**, **6-5b** etc.). Moreover, H-bond interactions with other amino acids such as Thr 66, Glu 148 etc. were not so significant for anti-integrase activity (compounds **2**, **6-1c** etc.).

Conclusion

In the present study, *in silico* based new *N*-hydroxy substituted 2-aryl acetamide analogues were designed, synthesized, and evaluated for activity, which exhibited moderate to good inhibitory activity against HIV-1 integrase enzyme. Selected molecules, having better inhibitory activities against integrase (>50%) were tested for their anti-HIV-1 activity in TZM-bl cell based assay. Cytotoxicity profiles of selected molecules were estimated to identify the potent lead molecule. Among the tested compounds **5c**, **5e**, **6-2c** and **6-5b** showed potent anti-HIV-1 activity with an IC_{50} value of 5.94, 14.36, 8.17 & 5.87 µg/ml in TZM-bl cell line respectively. Furthermore, molecular docking study revealed that polar interaction of the molecules with Asn155, Lys156, and Lys159 were significant for their inhibitory activity against HIV-integrase. These docking analyses showed a similar binding mode of **6-5b** and Raltegravir with HIV-1 integrase enzyme. Therefore, compound **6-5b** may serves as conceptually new lead to design and prepare novel analogues to improve potency of this new class of integrase strand transfer inhibitors for the treatment of HIV-1 infection.

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Conflict of interest

The authors declare that no competing interests exist.

Supporting Information

Supporting information includes (**1**) all experimental data of synthesized compounds (**2**) docking poses of selected top ten molecules from ZINC database (Figure S1) and (**3**) Docking scores of all synthesized compounds along with their H-bond interaction sites (Table S1).

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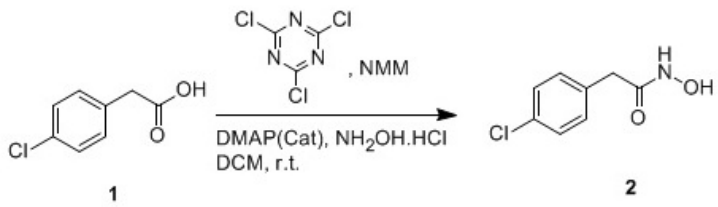
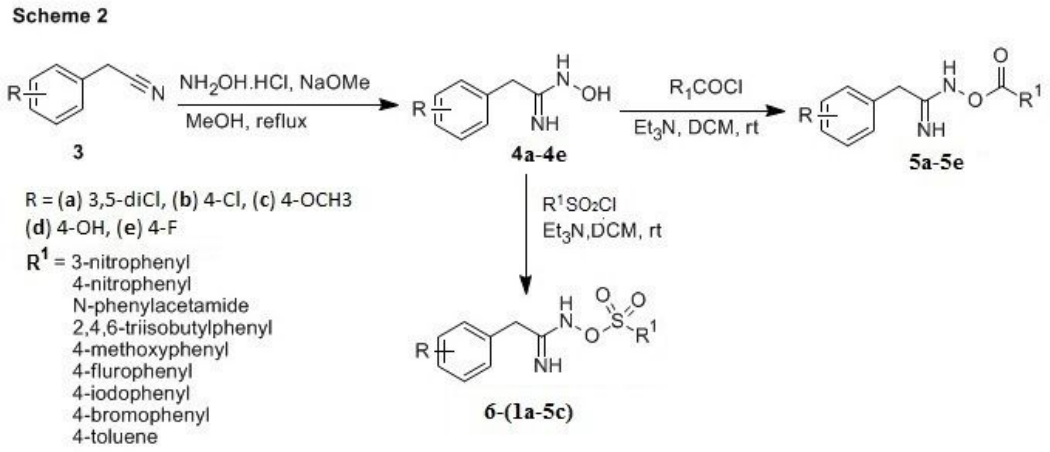
Scheme 1



Scheme 1: Synthesis of hydroxamic acid analogue.



Scheme 2: General scheme for synthesis of *N*-hydroxy substituted 2-aryl acetamide analogues.

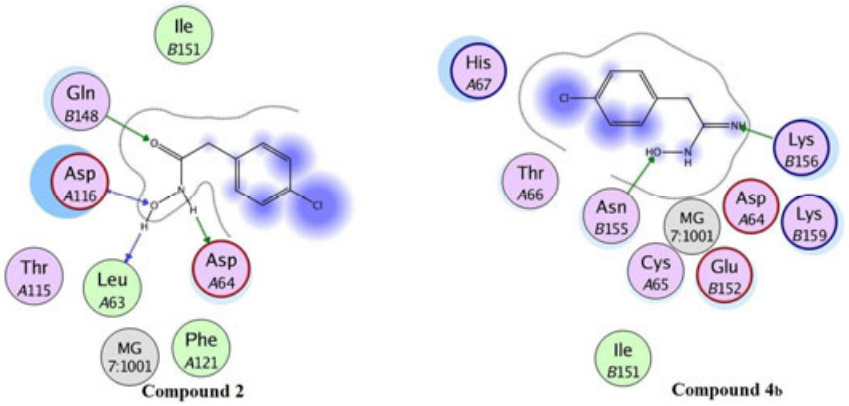


Figure 1: Two dimension figures of docked **2** and **4b** compounds with their binding residues of HIV-1 integrase enzyme. Green and pink colour of the binding residues indicates the H-bond acceptor and H-bond donor sites respectively. Violet colour near to the aromatic ring of the molecules illustrates hydrophobic interactions with surrounding amino acids of the binding domain.

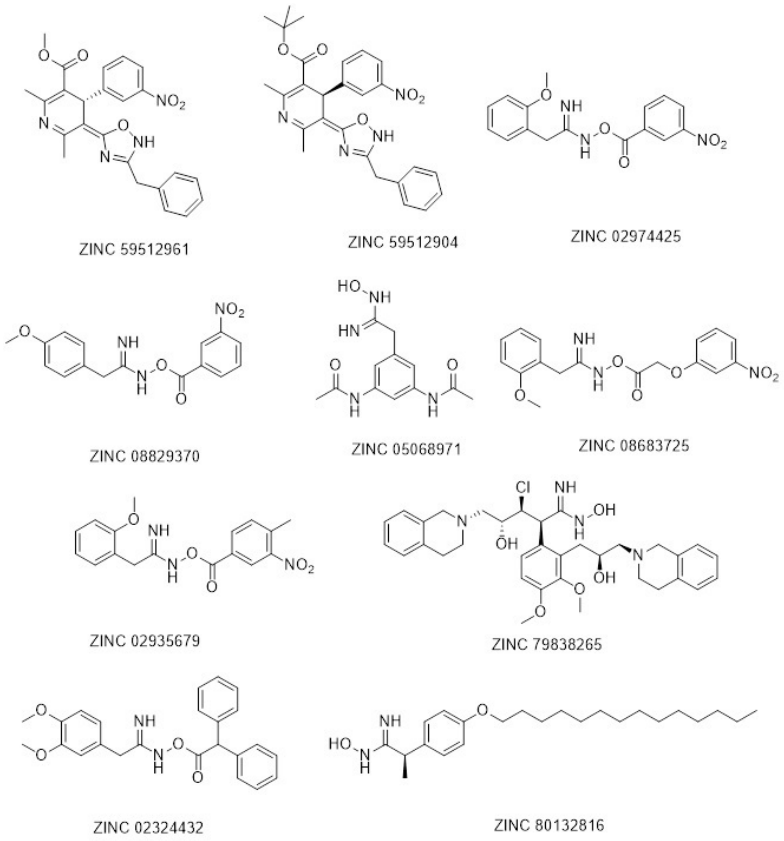


Figure 2: Structures of selected top ten molecules from ZINC database.

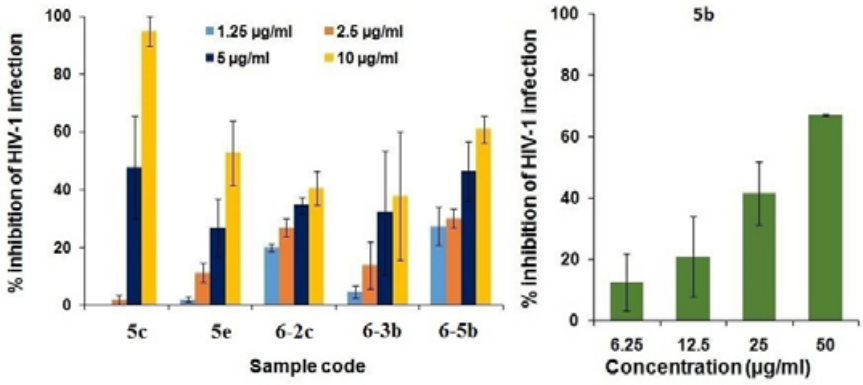


Figure 3: Left hand panel shows anti-HIV-1 activity of compounds **5c**, **5e**, **6-2c**, **6-3b** and **6-5b** whereas right hand panel shows anti-HIV-1 activity of compound **5b** at the tested concentrations of the compounds.

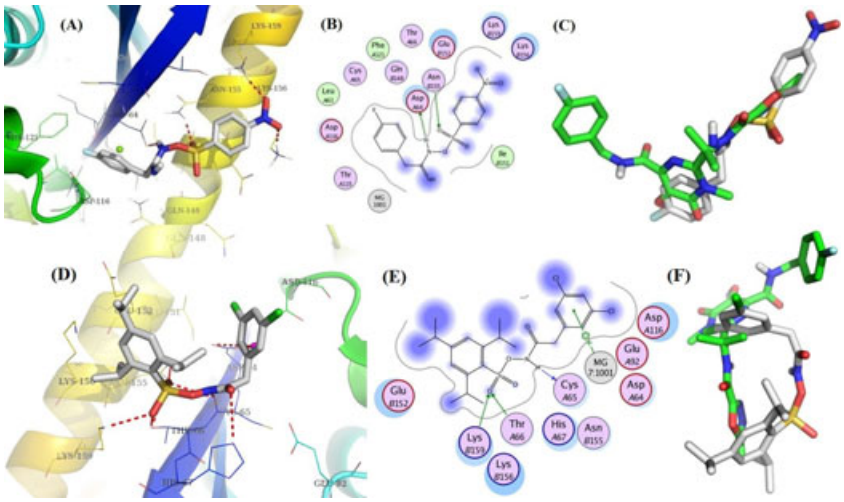
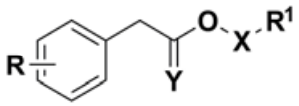


Figure 4: Binding mode of compound **6-5b** (active) and **6-1c** (inactive) with HIV-1 integrase (PDB ID: **1QS4**). Panels (A)/(B) and (D)/(E) showed 3D and 2D conformations of compound **6-5b** and **6-1c** along with their interacting nearby important binding residues. Panels (C) and (F) showed superimpositions of compound **6-5b** (white) and **6-1c** (white) with standard drug Raltegravir (green) respectively.

Table 1: Binding scores of top ten ZINC molecules along with H-bond interaction sites.

Sl. No	ZINC ID	Total Score	Crash Score	Polar Score	C Score	H-bond interaction
1	ZINC 59512961	7.00	-0.52	3.80	4	Asn155, Lys156, Lys159
2	ZINC 59512904	7.67	-0.63	4.47	4	Asn155, Lys156, Lys159
3	ZINC 02974425	8.61	-0.58	6.78	4	Asp64, Asn155, Lys156 Lys159
4	ZINC 08829370	8.26	-0.31	7.04	5	Glu152, Lys156, Lys159
5	ZINC 05068971	8.16	-0.82	6.58	4	Asp64, Lys65, Asn155
6	ZINC 08683725	7.20	-0.96	3.32	4	Glu152, Asn155, Lys156 Lys159
7	ZINC 02935679	8.81	-0.99	7.43	4	Glu152, Lys156, Lys159
8	ZINC 79838265	9.27	-3.61	6.06	5	Asp64, Cys65,Thr66, Glu152, Lys156, Lys159
9	ZINC 02324432	8.00	-1.23	4.26	5	Asp64, Lys65, Asn155 Lys156
10	ZINC 80132816	8.12	-1.72	3.36	5	Lys65, Thr66, Asn155

Table 2: In vitro anti HIV-1 integrase activity of all synthesized compounds.



Code	R	R^1	X	Y	% inhibition of integrase (50 μg/mL)	Code	R	R^1	X	Y	% inhibition of integrase (50 μg/mL)
2	4-Cl	-	-	O	NS	**6-1f**	3,5-diCl	4-fluro phenyl	SO_2	NH	23.3
4b	4-Cl	**-**	-	NH	31.3	**6-1g**	3,5-diCl	4-iodo phenyl	SO_2	NH	NS[a]
5a	3,5-diCl	3-nitro phenyl	CO	NH	37.8	**6-1h**	3,5-diCl	4-bromo phenyl	SO_2	NH	NS[a]
5b	4-Cl	3-nitro phenyl	CO	NH	56.1	**6-2a**	4-Cl	4-methyl phenyl	SO_2	NH	NS[a]
5c	4-OCH_3	3-nitro phenyl	CO	NH	52.10	**6-2b**	4-Cl	4-nitro phenyl	SO_2	NH	29.57
5d	4-OH	3-nitro phenyl	CO	NH	NS[a]	**6-2c**	4-Cl	4-methoxy phenyl	SO_2	NH	79.92
5e	4-F	3-nitro phenyl	CO	NH	56.8	**6-3a**	4-OCH_3	4-methyl phenyl	SO_2	NH	26.1
6-1a	3,5-diCl	4-nitro phenyl	SO_2	NH	37.9	**6-3b**	4-OCH_3	4-nitro phenyl	SO_2	NH	56.4
6-1b	3,5-	4-methyl	SO_2	NH	21.7	**6-3c**	4-OCH_3	4-methoxy	SO_2	NH	NS[a]

	diCl	phenyl						phenyl			
6-1c	3,5-diCl	2,4,6-tributyl phenyl	SO_2	NH	NS[a]	**6-4a**	4-OH	4-methyl phenyl	SO_2	NH	NS[a]
6-1d	3,5-diCl	4-phenyl acetamide	SO_2	NH	16.40	**6-4b**	4-OH	4-nitro phenyl	SO_2	NH	NS[a]
6-1e	3,5-diCl	4-methoxy phenyl	SO_2	NH	NS[a]	**6-4c**	4-OH	4-methoxy phenyl	SO_2	NH	NS[a]
6-5a	4-F	4-methyl phenyl	SO_2	NH	NS[a]	**6-5c**	4-F	4-methoxy phenyl	SO_2	NH	24.5
6-5b	4-F	4-nitro phenyl	SO_2	NH	88.01	**Raltegravir** (reference)					100

NS[a] : Not significant.

Table 3: Anti-HIV-1 activity, inhibition of HIV-1 integrase activity and cytotoxicity of selected synthetic compounds along with their docking scores.

Code	IC_{50} value of in vitro anti HIV-1 integrase activity of synthetic compounds[a] (μg/mL)	In vitro cytotoxicity and anti-HIV-1 activity of synthetic compounds in TZM-bl cells based assay			Total Score
		IC_{50} value for anti-HIV-1 activity[b] (μg/mL)	CC_{50} value by MTT assay on TZM-bl cells[c] (μg/mL)	S.I.[d]	
5b	18.68	36.34 ± 12.19	251.39 ± 26.34	11.26	7.94
5c	21.7	5.94 ± 0.39	28.32 ± 9.47	4.76	8.12
5e	17.93	14.36 ± 3.51	104.04 ± 9.16	11.35	7.75
6-2c	1.59	8.17 ± 0.28	339.58 ± 53.33	37.03	7.33
6-3b	13.50	22.52 ± 13.95	271.63 ± 22.78	12.06	7.28
6-5b	1.55	5.87 ± 1.87	172.25 ± 11.82	29.34	8.68
Raltegravir		**0.22**	**379.96**	**1727**	**9.43**

[a] *IC_{50}: Concentration of the compounds resulting in 50% inhibition in the HIV-1 integrase enzyme activity determined using a commercial kit*

[b] *IC_{50}: The concentration of the compounds that resulted in 50% inhibition in HIV infection. All data presented are averages of three independent experiments performed in duplicate*

[c] *CC_{50}: The cytotoxic concentration of the compounds that resulted in the reduction of viable cells by 50%. All data presented are averages of three independent experiments performed in duplicate*

[d] *S.I.: Selectivity Index is CC_{50}/IC_{50}*