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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, biological evaluation, and molecular modeling studies of new 1,3,4-oxadiazole- and 1,3,4-thiadiazole-substituted 4-oxo-4*H*-pyrido[1,2-*a*]pyrimidines as anti-HIV-1 agents

Z. Hajimahdi · A. Zarghi · R. Zabihollahi · M. R. Aghasadeghi

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Abstract A new series of 4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine derivatives containing 1,3,4-oxadiazole and 1,3,4-thiadiazole rings as a part of the metal chelation motif were synthesized and evaluated for their in vitro anti-HIV-1 activity. Most of the tested compounds displayed moderate inhibitory properties against HIV-1 virus (NL4-3) in Hela cell cultures. Compounds **11e** and **11b** exhibited the highest activity among the synthesized compounds with inhibition rate of 51 and 48 % at concentration of 100 μM, respectively. Molecular docking study using the later crystallographic data available for PFV integrase (IN) showed that the designed compounds bind into the active site of IN such that the keto oxygen atom at position of C-4 and nitrogen atom of thiadiazole or oxadiazole ring moiety chelate the Mg^{2+} ion. Our results also showed that all tested compounds presented no significant cytotoxicity at concentration of 100 μM. Therefore, these compounds can provide a very good basis for the development of new hits.

Keywords 4-Oxo-4*H*-pyrido[1,2-*a*]pyrimidines · Oxadiazoles · Thiadiazoles · Anti-HIV-1 activity · Molecular modeling

Z. Hajimahdi · A. Zarghi (✉)
Department of Medicinal Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, P.O. Box 14155-6153, Tehran, Iran
e-mail: azarghi@yahoo.com

R. Zabihollahi · M. R. Aghasadeghi
Hepatitis and AIDS Department, Pasteur Institute of Iran, Tehran, Iran

Introduction

Human immuno-deficiency virus type 1 (HIV-1) is the etiological agent that causes acquired immuno-deficiency syndrome (AIDS). HIV infection is a life-threatening health problem necessitating discovery of novel targets and new lead molecules. Studies in HIV biology have provided important information about the main steps of the virus life cycle which consists of viral entry, reverse transcription, integration, gene expression, virion assembly, budding, and maturation. HIV-1 encodes three enzymes that are essential for retroviral replication: reverse transcriptase (RT), protease, and integrase (IN) (Moyle *et al.*, 2008). Although drugs targeting RT and protease are in wide use and have shown effectiveness particularly when employed in combination, these highly active antiretroviral therapies (HAART) still show important limitations. These are costs, occurrence of various side effects due to drug toxicity and most importantly, the loss of drug effectiveness over time caused by development of resistance, including multidrug resistance and cross-resistance (Barbaro *et al.*, 2005). HIV-1 IN plays a central role in the insertion of viral DNA into the genome of host cells, which first catalyzes removal of the terminal dinucleotide from each 3′ end of the viral DNA (3′ processing) and subsequently mediates joining of the 3′ end of the viral DNA to the host DNA (strand transfer). This enzymatic process is dependent on an active site containing dual Mg^{2+} metal ions held in place with a highly conserved triad of carboxylate amino acid residues (asp64/asp116/glu152) commonly referred to as a DD(35)E triad (Colicelli and Goff, 1988; Miller *et al.*, 1997; Pommier *et al.*, 2005). HIV-1 IN represents a crucial target for antiretroviral drugs, because it has no counterpart in mammalian cells. A plethora of HIV-1 IN inhibitors were discovered in the last decade but only one compound, Raltegravir, was FDA

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approved in October 2007. This is the unique currently used clinical drug targeting IN, which is administered as a new addition to HAART regimens. Only two other compounds are in clinical development: GS-9137 (Elvitegravir) is in the late stages of clinical development (Shimura *et al.*, 2008), whereas S/GSK-1349572 is in phases I and II clinical trials (Min *et al.*, 2010). Raltegravir's clinical use has already evidenced the development of viral resistances (Charpentier *et al.*, 2008). This evidently shows that there is a strong need to discover novel IN targeting hits with different structural scaffolds. In this research, aimed at the discovery of new compounds as anti-HIV-1 agents, we selected the HIV-1 IN inhibitors as a lead to design new analogs. Among numerous attempts to develop IN inhibitors, the keto-enol acid class (often referred to as the diketo acid class) of compounds has been most aggressively developed because of the marked antiretroviral activities exhibited (Pommier *et al.,* 2005). Since the keto-enol acid compounds often exhibit adverse hepatic effects (Kirschberg and Parrish, 2007), there is an attempt to find new bioiosters for keto-enol acid motif. The carboxylic acid in the keto-enol acid motif can be replaced with not only well-known bioisosters of a carboxylic acid group, such as triazole and tetrazole (Herr, 2002), but also by a basic aromatic heterocycle bearing a lone pair donor atom, such as a pyridine ring (Zhuang *et al.*, 2003) and oxadiazole (**1**, Fig. 1) (Johns *et al.,* 2009). On the other hand, our literature studies showed that bicyclic pyrido[1,2-*a*]pyrimidin-4-one derivatives are promising anti-HIV agents due to their ability to inhibit HIV-1 IN (Donghi *et al.*, 2009) (**2**, Fig. 1). Based upon this, we decided to design and synthesize a new series of pyrido[1,2-*a*]pyrimidin-4-one derivatives possessing 1,3,4-oxadiazole and 1,3,4-thiadiazole rings as a part of the metal chelation motif. According to HIV-1 IN inhibitors structure–activity relationship, we incorporated different substituted phenyl rings to evaluate the effect of these substituents on anti-HIV-1 activity. We also performed docking studies to predict the interaction of newly synthesized compounds into the active site of IN and their probable mechanism of action.

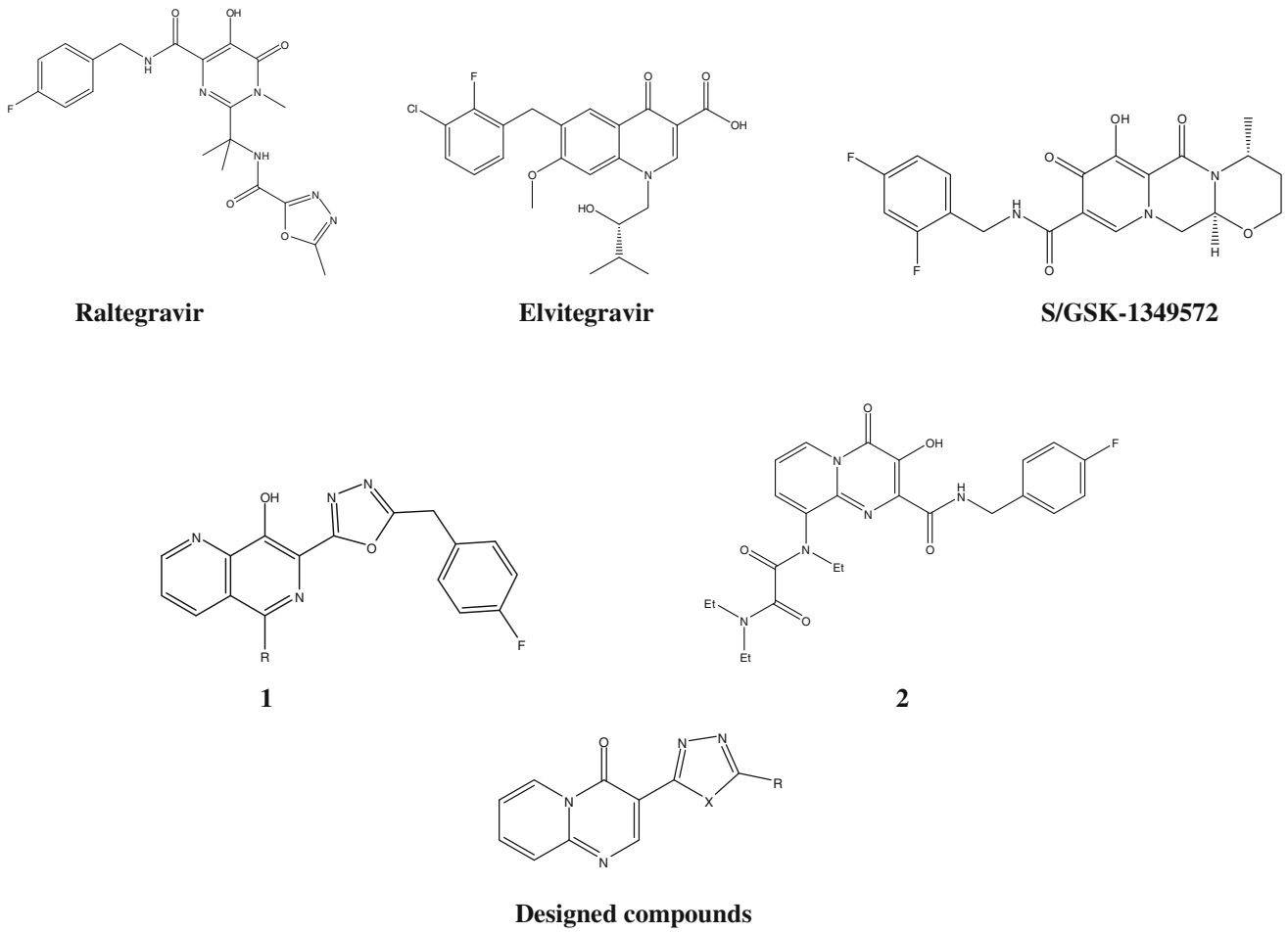
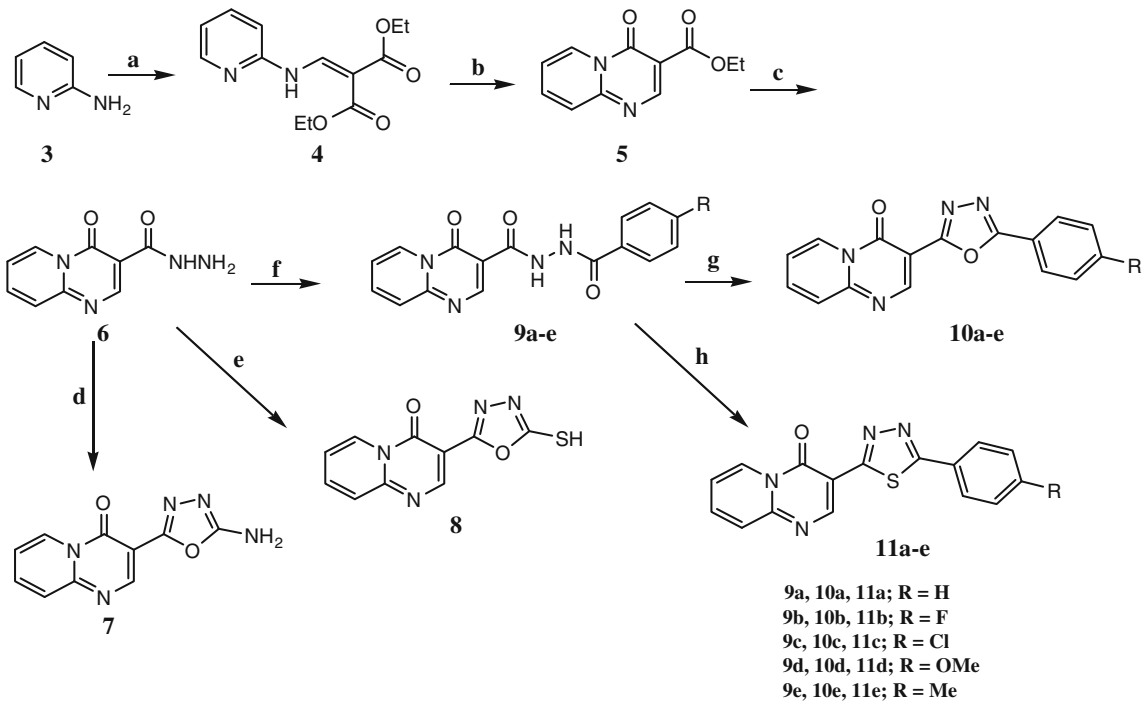


Fig. 1 HIV-1 IN inhibitors (Raltegravir, Elvitegravir, S/GSK-1349572), lead compounds (**1**, **2**), and our designed scaffold



Scheme 1 Reagents and conditions: *a* EMME, 120 °C, 1 h; *b* 2-chlorobenzoic acid, Ph_2O, 250 °C, 1 h; *c* $NH_2NH_2.OH$, DMF, rt, 30 min; *d* BrCN, MeOH, reflux, 2 h; *e* CS_2, KOH, MeOH, reflux, 4 h; *f* 4-substituted benzoyl chloride, DMF, rt, 6 h; *g* P_2O_5, toluene, reflux, 5 h; *h* LR, THF, reflux, 24 h

Result and discussion

Chemistry

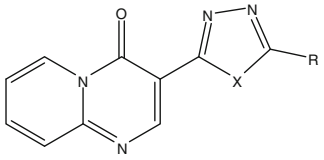
The target 4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine derivatives were synthesized via the route outlined in Scheme 1. Oxadiazole- and thiadiazole-substituted 4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine derivatives were prepared starting from the 2-aminopyridine (**3**) in five steps. Condensation of **3** with ethoxymethylenemalonate diethyl ester yielded aminomethylene malonate (**4**). The compound (**4**) was converted to ethyl 4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylate (**5**) in Ph_2O containing catalytic 2-chlorobenzoic acid at 250 °C. Building upon the literatures, we found that the typical reaction time for optimal yields for this series transformation should be within 1–2 h. The unwanted byproducts will come into being under too higher temperature beyond 1 h. Also, we found the catalyst 2-chlorobenzoic acid did limited effects to shorten the reaction time and improve the yields. Compound (**5**) was subsequently treated with hydrazine in DMF to form the corresponding hydrazide intermediate (**6**). Hydrazide intermediate (**6**) was converted to target molecule containing 5-amino-1,3,4-oxadiazole (**7**) by heating with BrCN in methanol. The reaction of hydrazide (**6**) with CS_2 in methanol gave compound (**8**). To synthesize phenyl oxadiazoles and thiadiazoles, compound (**6**) reacted with 4-substituted benzoyl chlorides in DMF to give acylated compounds (**9a–e**). Compounds (**10a–e**) were synthesized by dehydrocyclization of diacylhydrazines (**9a–e**) using P_2O_5 in toluene. Dehydrosulfurization of diacylhydrazines (**9a–e**) by the Lawesson's reagent (LR) in THF led to formation of derivatives containing 1,3,4-thiadiazole ring (**11a–e**). All compounds were stable and kept in dry place at 25 °C. The structure of the synthesized compounds was confirmed by IR, ^{1}HNMR, ESI–MS, and CHN analysis.

Anti-HIV-1 activity

The anti-HIV activity of the all compounds was measured by determining their ability to general inhibition of the replication of HIV-1 in Hela cell cultures. For comparative purposes, nucleoside RT inhibitor, AZT, was assayed in the same cells. The results are listed in Table 1.

All the compounds displayed no significant cytotoxicity at concentration of 100 μM. Most of the tested compounds produced inhibitory effects at 100 μM concentration ranging from 11 to 51 %. However, in all cases, the measured activities were lower than that of AZT. The results demonstrate that the anti-HIV-1 activity seems to have a dependence on the existence of a hydrophobic group at C-5 of oxadiazole and thiadiazole rings. The 1,3,4-oxadiazole analogs (**7** and **8**) containing $–NH_2$ and –SH groups resulted in no considerable activity (0–11 %). These results

Table 1 Anti-HIV activity of synthesized compounds



Compounds	X	R	100 μM	
			(%) inhibition rate of P_{24} expression	% cell viability
7	O	$-NH_2$	–	96
8	O	–SH	11	100
10a	O	Phenyl	–	98
10b	O	4-Fluorophenyl	18	100
10c	O	4-Chlorophenyl	26	94
10d	O	4-Methoxyphenyl	–	100
10e	O	4-Methylphenyl	29	94
11a	S	Phenyl	33	100
11b	S	4-Fluorophenyl	48	92
11c	S	4-Chlorophenyl	39	95
11d	S	4-Methoxyphenyl	28	97
11e	S	4-Methylphenyl	51	93
AZT			100	100

indicated that chelation of the 2 Mg^{2+} cations by 1,3,4-oxadiazole and 1,3,4-thiadizole heterocycles as components of the chelation motif are not strong enough to allow for a strong anti-HIV-1 activity by these compounds. However, the introduction of *para*-substituted phenyl at C-5 position of the 1,3,4-oxadiazole ring (**10b**, **10c**, and **10e**), led to increased anti-HIV-1 activity. Our results also showed that the compounds containing thiadiazole ring (**11a–e**) are more potent than the corresponding ones with oxadiazole ring (**10a–e**). It was also found that the introduction of methyl or fluoro group at the *para* position of the benzene ring appears to considerably benefit anti-HIV-1 activity as compounds **11e** and **11b** exhibited significantly higher potency (51 and 48 %, respectively). These findings conform to the common pharmacophore of HIV-1 IN inhibitors in which a fluorobenzyl group with a specific spatial arrangement to a chelator is a required structural determinant.

Molecular modeling

We performed detailed docking studies to predict interaction of synthesized compounds into the active site of HIV-1 IN. In the absence of key, relevant crystal structures, molecular modeling has been used extensively to assist the understanding of the binding of inhibitors to the IN active site and to aid in the design of novel inhibitors.

To date, no X-ray structure of the entire HIV-1 IN for interaction with its cognate DNA with or without substrate has been solved. Recently, Hare *et al.* (2010) solved the crystal structures of full-length prototype foamy virus (PFV) IN DNA complexes with various HIV-1 IN inhibitors, exhibiting two-metal ions in the strand transfer active site (Mg^{2+} or Mn^{2+}). This breakthrough now allows for a better understanding of the binding mode of IN strand transfer inhibitors. As shown by Hare *et al.*, PFV IN can be considered as a good model for the development of HIV-1 IN strand transfer inhibitors. The secondary structures of PFV IN (PDB: 3L2T or 3OYA) and HIV-1 IN catalytic core domain (PDB:1BL3) have highly conserved architectures, with a calculated RMSD of 1.04 Å. On the basis of these considerations, we decided to undertake docking studies of our molecules based on the 3L2T X-ray crystallographic structure of PFV IN.

Docking of the most potent compounds (**11b** and **11e**) in the newly built model was performed with the AUTODOCK 4.2 program. All these compounds showed a similar interfacial-binding mode in which the keto oxygen atom at

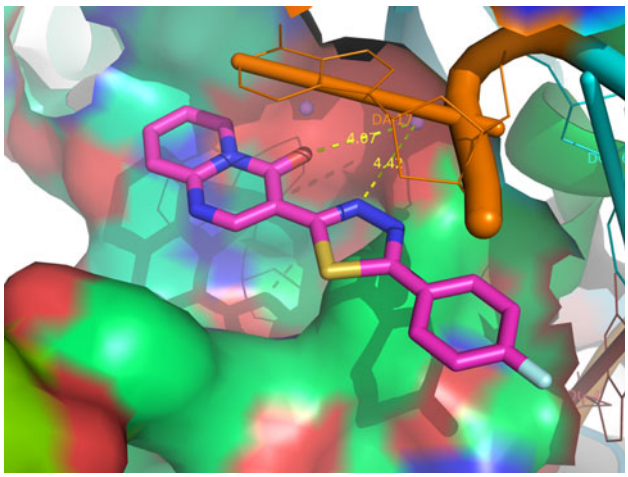


Fig. 2 Binding model of compound **11b** in the active site of HIV-1 IN

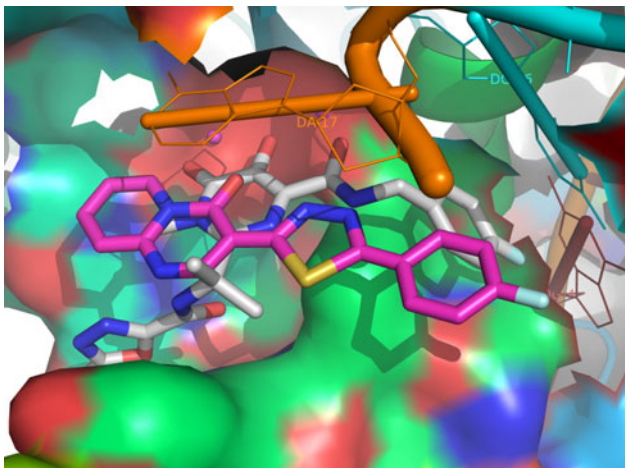


Fig. 3 Superimposition of compound **11b** on the raltegravir molecule

position of C-4 and nitrogen atom of thiadiazole ring moiety chelate the Mg^{2+} ion (see docking result of **11b**, Fig. 2). The displaced 3′ adenosine terminal base (A17) was involved in a π-stacking interaction with the 4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine ring. The *p*-fluoro-phenyl group fitted within a tight pocket formed by cytosine 16 (C16) and guanine 4 (G4). Further docking study revealed that **11b** and raltegravir positioned a similar orientation in the active site of HIV-1 IN (Fig. 3). These docking results were in agreement with the common structure–activity relationships of HIV-1 IN inhibitors.

Conclusion

This study indicates that (i) 1,3,4-oxadiazole- and 1,3,4-thiadiazole-substituted 4-oxo-4*H*-pyrido[1,2-*a*]pyrimidines are suitable scaffold to design anti-HIV agents; (ii) all the target compounds were completely safe and exhibited no cytotoxicity at concentration of 100 μM; (iii) most of the compounds displayed moderate HIV-1 inhibition rate at concentration of 100 μM; (iv) the most active compounds (**11e** and **11b**) exhibited activity against HIV-1 virus (NL4-3) with inhibition values of 51 and 48 %, respectively; (v) docking study revealed that the anti-HIV activity of these compounds might involve a metal chelating mechanism.

Experimental

Materials

All reagents purchased from the Aldrich (USA) or Merck (Germany) Chemical Company and were used without further purifications.

General

Melting points (mp) were determined using a Thomas Hoover capillary apparatus (Philadelphia, USA). Infrared spectra were acquired on a Perkin-Elmer 1420 ratio recording spectrometer. A Bruker FT-500 MHz instrument (Brucker Biosciences, USA) was used to acquire ^{1}HNMR spectra; chloroform-D and DMSO-d_6 used as solvents. Coupling constant (*J*) values are estimated in hertz (Hz) and spin multiples are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). The mass spectral measurements were performed on an 6410 Agilent LCMS triple quadrupole mass spectrometer (LCMS) with an electrospray ionization (ESI) interface.

*Diethyl 2-((pyridin-2-ylamino)methylene)malonate (**4**)*

A mixture of 2-aminopyridine **3** (1 eq.) and diethyl ethoxymethylenemalonate (EMME) (1 eq.) was heated at 120 °C for 1 h. The mixture was then evaporated to dryness to give a residue which was triturated with cyclohexane to give a solid which was filtered and dried to afford compound **4**. Yield, 89 %; white powder; mp: 60–62 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1680–1730 (C=O), 3300 (N–H); LC–MS (ESI) *m*/*z*: 286.8 (M+23, 100).

*Ethyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carboxylate (**5**)*

A biphenyl ether solution of **4** containing catalytic 2-chlorobenzoic acid was heated by microwave irradiation (250 °C)

for 1 h. After cooling, the reaction mixture was filtered and the precipitate **5** was washed with *n*-hexane and water. Yield, 97 %; cream powder; mp: 99–100 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1730, 1750 (C=O); LC–MS (ESI) *m/z*: 219.1 (M+1, 100).

*4-Oxo-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (**6**)*

A mixture of **5** (1 g, 4.5 mmol) and hydrazine hydrate (3 ml, 45 mmol) in DMF (5 ml) was stirred in room temperature for 30 min. After reaction completion, the solid product obtained was filtered, washed with diethyl ether to give **6**. Yield, 70 %; cream powder; mp: 150 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1730 (C=O), 2500–3400 (O–H), 3310 (N–H), 3370 (NH_2); LC–MS (ESI) *m/z*: 205.1 (M+1, 100).

*3-(5-Amino-1,3,4-oxadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**7**)*

To a solution of **6** (0.6 g, 3 mmol) in methanol, BrCN (3 g, 30 mmol) was added and the solution was refluxed for 2 h. After completion of the reaction (monitored by TLC), the solvent was evaporated under reduced pressure and the residue was extracted with EtOAc. The organic layer was washed with water (2 × 10 ml) and finally dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting crude compound was purified by crystallization in ethanol. Yield, 27 %; yellow powder; mp: 235 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1735 (C=O), 2400–3400 (O–H), 3100–3300 (NH_2); ^{1}HNMR (DMSO-d_6, 500 MHz): δ 7.13 (br s, 1H, oxadiazole N–H), 7.59–7.62 (t, 1H, H_6, $J = 8.1$ Hz), 7.89 (d, 1H, H_8, $J = 8.7$ Hz), 8.19–8.22 (t, 1H, H_7, $J = 7.2$ Hz), 8.27 (br s, 1H, oxadiazole N–H), 8.78 (s, 1H, H_2), 9.17 (d, 1H, H_5, $J = 6.9$ Hz); LC–MS (ESI) *m/z*: 230.1 (M+1, 100); Anal. Calcd. for $C_{10}H_7N_5O_2$: C, 52.40; H, 3.08; N, 30.56. Found: C, 52.26; H, 3.31; N, 30.69.

*3-(5-Mercapto-1,3,4-oxadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**8**)*

A mixture of **6** (0.8 g, 4 mmol), KOH (1.1 g, 20 mmol), and carbon disulfide (3 ml) in ethanol (50 ml) was refluxed on a steam bath for 4 h. The solution was then concentrated, cooled, and acidified with dilute HCl. The solid mass that separated out was filtered, washed with ethanol, dried, and recrystallized from ethanol. Yield, 20 %; yellow powder; mp: 145 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1680 (C=O), 2600–3400 (O–H), 3250 (N–H); ^{1}HNMR (DMSO-d_6, 500 MHz): δ 7.12–7.15 (t, 1H, H_6, $J = 6.4$ Hz), 7.27 (d, 1H, H_8, $J = 8.1$ Hz), 7.77–7.80 (t, 1H, H_7, $J = 7.3$ Hz), 8.34 (s, 1H, H_2), 9.13 (d, 1H, H_5, $J = 12.4$ Hz), 11.36 (s, 1H, oxadiazole N–H); LC–MS (ESI) *m/z*: 247.1 (M+1, 100); Anal. Calcd. for $C_{10}H_6N_4O_2S$: C, 48.78; H, 2.46; N, 22.75. Found: C, 48.99; H, 2.70; N, 22.68.

General procedure for preparation of *N′*-(4-substituted benzoyl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**9a–e**)

A mixture of **6** (0.3 g, 1.5 mmol) and 4-substituted benzoyl chlorides (0.2 ml, 1.5 mmol) was dissolved in dry DMF (10 ml) and stirred in room temperature for 4 h. The reaction mixture was slowly poured over crushed ice and kept for 1 h. The solid thus separated out was filtered, washed with water, and dried to give compounds (**9a–e**) (yield, 40–70 %).

*N′-Benzoyl-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (**9a**)*

Yield, 40 %; white powder; mp: 110 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1690 (C=O), 2500–3200 (O–H), 3200, 3440 (N–H); LC–MS (ESI) *m/z*: 309.0 (M+1, 100).

*N′-(4-Fluorobenzoyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (**9b**)*

Yield, 70 %; white powder; mp: 170–172 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1690 (C=O), 2500–3200 (O–H), 3200 (N–H); LC–MS (ESI) *m/z*: 327.1 (M+1, 100).

*N′-(4-Chlorobenzoyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (**9c**)*

Yield, 60 %; white powder; mp: 208–210 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1685 (C=O), 2500–3200 (O–H); LC–MS (ESI) *m/z*: 343.1 (M+1).

*N′-(4-Methoxybenzoyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (**9d**)*

Yield, 60 %; white powder; mp: 146–149 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1700 (C=O), 2500–3300 (O–H); LC–MS (ESI) *m/z*: 339.1 (M+1, 100).

*N′-(4-Methylbenzoyl)-4-oxo-4H-pyrido[1,2-a]pyrimidine-3-carbohydrazide (**9e**)*

Yield, 63 %; white powder; mp 235 °C; IR (KBr disk): υ (cm^{-1}) 1400-1600 (aromatic), 1685 (C=O), 2500–3200 (O–H), 3250 (N–H); LC–MS (ESI) *m/z*: 322.9 (M+1, 100).

General procedure for preparation of 3-(5-(4-substituted benzoyl)-1,3,4-oxadiazol-2-yl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**10a–e**)

To a solution of compounds (**9a–e**) (1 g, 3 mmol) in toluene, P_2O_5 (3 g, 20 mmol) was added and the solution was refluxed for 5 h. After completion of the reaction (monitored by TLC), the solvent was evaporated under reduced pressure and the residue was extracted with EtOAc. The organic layer was washed with $NaHCO_3$ (10 %, 2 × 10 ml) and water (10 ml), and finally dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting crude compound was purified by column chromatography (yield, 10–20 %).

*3-(5-Phenyl-1,3,4-oxadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**10a**)*

Yield, 10 %; cream powder; mp: 180 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1714 (C=O); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 7.39–7.42 (m, 1H, H_6), 7.51–7.55 (m, 3H, phenyl H_3, H_4 & H_5), 7.87 (d, 1H, H_8, $J = 8.6$ Hz), 7.97–8.00 (m, 1H, H_7), 8.18–8.20 (m, 2H, phenyl H_2 & H_6), 9.24 (s, 1H, H_2), 9.34 (d, 1H, H_5, $J = 6.3$ Hz); LC–MS (ESI) *m/z*: 291.1 (M+1, 100); Anal. Calcd. for $C_{16}H_{10}N_4O_2$: C, 66.20; H, 3.47; N, 19.30. Found: C, 66.36; H, 3.29; N, 19.38.

*3-(5-(4-Fluorophenyl)-1,3,4-oxadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**10b**)*

Yield, 10 %; cream powder; mp: 24 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1705 (C=O); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 7.21–7.23 (t, 2H, 4-fluorophenyl H_3 & H_5, $J = 8.8$ Hz), 7.39–7.42 (t, 1H, H_6, $J = 6.9$ Hz), 7.87 (d, 1H, H_8, $J = 8.5$ Hz), 7.97–7.99 (t, 1H, H_7, $J = 7.3$ Hz), 8.18–8.21 (dd, 2H, 4-fluorophenyl H_2 & H_6), 9.23 (s, 1H, H_2), 9.34 (d, 1H, H_5, $J = 7.1$ Hz); LC–MS (ESI) *m/z*: 309.1 (M+1, 100); Anal. Calcd. for $C_{16}H_9FN_4O_2$: C, 62.34; H, 2.94; N, 18.17. Found: C, 62.48; H, 3.11; N, 18.29.

*3-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**10c**)*

Yield, 20 %; cream powder; mp: 238 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1696 (C=O); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 7.40–7.43 (t, 1H, H_6, $J = 6.9$ Hz), 7.50 (d, 2H, 4-chlorophenyl H_3 & H_5, $J = 7.0$ Hz), 7.87 (d, 1H, H_8, $J = 8.7$ Hz), 7.98–8.01 (t, 1H, H_7, $J = 7.3$ Hz), 8.13 (d, 2H, 4-chlorophenyl H_2 & H_6, $J = 8.7$ Hz), 9.23 (s, 1H, H_2), 9.33 (d, 1H, H_5, $J = 6.9$ Hz); LC–MS (ESI) *m/z*: 347.0 (M+1); Anal. Calcd. for $C_{16}H_9ClN_4O_2$: C, 59.18; H, 2.79; N, 17.25. Found: C, 59.31; H, 2.66; N, 17.10.

*3-(5-(4-Methoxyphenyl)-1,3,4-oxadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**10d**)*

Yield, 10 %; cream powder; mp: 245 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1705 (C=O); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 3.89 (s, 3H, OCH_3), 7.03 (d, 2H, 4-methoxyphenyl H_3 & H_5, $J = 8.8$ Hz), 7.40–7.43 (t, 1H, H_6, $J = 6.9$ Hz), 7.93 (d, 1H, H_8, $J = 8.7$ Hz), 7.98-8.01 (t, 1H, H_7, $J = 7.2$ Hz), 8.12 (d, 2H, 4-methoxyphenyl H_2 & H_6, $J = 8.7$ Hz), 9.21 (s, 1H, H_2), 9.34 (d, 1H, H_5, $J = 7.1$ Hz); LC–MS (ESI) *m/z*: 321.1 (M+1, 100); Anal. Calcd. for $C_{17}H_{12}N_4O_3$: C, 63.75; H, 3.78; N, 17.49. Found: C, 64.01; H, 3.99; N, 17.55.

*3-(5-p-Tolyl-1,3,4-oxadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**10e**)*

Yield, 10 %; cream powder; mp: 184 °C; IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1700 (C=O); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 2.44 (s, 3H, CH_3), 7.33 (d, 2H, 4-methylphenyl H_3 & H_5, $J = 8.01$ Hz), 7.39–7.42 (t, 1H, H_6, $J = 5.8$ Hz), 7.89 (d, 1H, H_8, $J = 8.7$ Hz), 7.97–8.00 (m, 1H, H_7), 8.07 (d, 2H, 4-methylphenyl H_2 & H_6, $J = 8.1$ Hz), 9.22 (s, 1H, H_2), 9.33 (d, 1H, H_5, $J = 7$ Hz); LC–MS (ESI) *m/z*: 327.1 (M+1, 100); Anal. Calcd. for $C_{17}H_{12}N_4O_2$: C, 67.10; H, 3.97; N, 18.41; O, 10.52. Found: C, 67.22; H, 3.68; N, 10.40.

General procedure for preparation of 3-(5-4-substituted benzoyl-1,3,4-thiadiazol-2-yl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (**11a–e**)

To a solution of compounds (**9a–e**, 1 g, 3 mmol) in THF, LR (2 g, 5 mmol) was added and the solution was refluxed for 24 h. After completion of the reaction (monitored by TLC), the solvent was evaporated under reduced pressure and the residue was extracted with EtOAc. The organic layer was washed with HCl solution (20 %, 2 × 10 ml). The aqueous layer was neutralized with NaOH solution (10 %) and then it was extracted with EtOAc. The organic layer was washed with water (10 ml) and finally dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting crude compound was purified by column chromatography (yield, 5–10 %).

*3-(5-Phenyl-1,3,4-thiadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**11a**)*

Yield, 10 %; yellow powder; mp: 190 °C (decomposed);IR (KBr disk): υ (cm^{-1}) 1400-1600 (aromatic), 1670 (C=O);

^{1}HNMR ($CDCl_3$, 500 MHz): δ 7.40–7.43 (m, 1H, H_6), 7.49–7.52 (m, 3H, phenyl H_3, H_4 & H_5), 7.90 (d, 1H, H_8, $J = 8.8$ Hz), 7.94–7.98 (m, 1H, H_7), 8.08–8.10 (m, 2H, phenyl H_2 & H_6), 9.30 (d, 1H, H_5, $J = 7.1$ Hz), 9.70 (s, 1H, H_2); LC–MS (ESI) *m/z*: 307.1 (M+1, 100); Anal. Calcd. for $C_{16}H_{10}N_4OS$: C, 62.73; H, 3.29; N, 18.29. Found: C, 62.51; H, 3.01; N, 18.36.

*3-(5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**11b**)*

Yield, 8 %; yellow powder; mp: 167 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1670 (C=O); ^{1}HNMR ($CDCl_3$, 500 MHz): δ 7.18–7.21 (t, 2H, 4-fluorophenyl H_3 & H_5, $J = 8.6$ Hz), 7.40–7.43 (m, 1H, H_6), 7.90 (d, 1H, H_8, $J = 8.8$ Hz), 7.95–7.98 (m, 1H, H_7), 8.06–8.09 (dd, 2H, 4-fluorophenyl H_2 & H_6), 9.30 (d, 1H, H_5, $J = 7.1$ Hz), 9.68 (s, 1H, H_2); LC–MS (ESI) *m/z*: 325.1 (M+1, 100); Anal. Calcd. for $C_{16}H_9FN_4OS$: C, 59.25; H, 2.80; N, 17.27. Found: C, 59.54; H, 2.68; N, 17.41.

*3-(5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**11c**)*

Yield, 9 %; yellow powder; mp: 250 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1680 (C=O); ^{1}HNMR (CDCl3, 500 MHz): δ 7.42–7.43 (m, 1H, H_6), 7.48 (d, 2H, 4-chlorophenyl H_3 & H_5, $J = 8.5$ Hz), 7.91 (d, 1H, H_8, $J = 8.7$ Hz), 7.97 (m, 1H, H_7), 8.01–8.03 (d, 2H, 4-chlorophenyl H_2 & H_6, $J = 8.5$ Hz), 9.30 (d, 1H, H_5, $J = 7.0$ Hz), 9.68 (s, 1H, H_2); LC–MS (ESI) *m/z*: 341.0 (M+1); Anal. Calcd. for $C_{16}H_9ClN_4OS$: C, 56.39; H, 2.66; N, 16.44. Found: C, 56.25; H, 2.77; N, 16.31.

*3-(5-(4-Methoxyphenyl)-1,3,4-thiadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**11d**)*

Yield, 5 %; yellow powder; mp: 254 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1705 (C=O); ^{1}HNMR (CDCl3, 500 MHz): δ 3.93 (s, 3H, OCH_3), 7.01 (d, 2H, 4-methoxyphenyl H_3 & H_5, $J = 8.8$ Hz), 7.39–7.41 (m, 1H, H_6), 7.89 (d, 1H, H_8, $J = 8.7$ Hz), 7.93-7.96 (m, 1H, H_7), 8.02 (d, 2H, 4-methoxyphenyl H_2 & H_6, $J = 8.8$ Hz), 9.29 (d, 1H, H_5, $J = 7.0$ Hz), 9.68 (s, 1H, H_2); LC–MS (ESI) *m/z*: 337.1 (M+1, 100); Anal. Calcd. for $C_{17}H_{12}N_4O_2S$: C, 60.70; H, 3.60; N, 16.66. Found: C, 60.88; H, 3.97; N, 16.40.

*3-(5-p-Tolyl-1,3,4-thiadiazol-2-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (**11e**)*

Yield, 5 %; yellow powder; mp: 195 °C (decomposed); IR (KBr disk): υ (cm^{-1}) 1400–1600 (aromatic), 1680 (C=O); ^{1}HNMR (CDCl3, 500 MHz): δ 2.51 (s, 3H, CH_3), 7.31 (d, 2H, 4-methylphenyl H_3 & H_5, $J = 9.3$ Hz), 7.39–7.42 (t, 1H, H_6, $J = 6.3$ Hz), 7.89 (d, 1H, H_8, $J = 8.7$ Hz), 7.94–7.98 (m, 3H, H_7 & 4-methylphenyl H_2 & H_6), 9.29 (d, 1H, H_5, $J = 6.7$ Hz), 9.68 (s, 1H, H_2); LC–MS (ESI) *m/z*: 321.1 (M+1, 100); Anal. Calcd. for $C_{17}H_{12}N_4OS$: C, 63.73; H, 3.78; N, 17.49. Found: C, 64.01; H, 4.05; N, 17.53.

In vitro anti-HIV and cytotoxicity assays

The inhibitory effect of compounds against HIV-1 was studied by single-cycle replication assay as previously described (Zabihollahi *et al.*, 2011). In brief, Hela cells (6×10^3 per well of 96-well plate) were infected with single-cycle replicable HIV NL4-3 virions (200 ng P_{24}) in the presence of different concentrations of compounds. Addition of compounds to the cells environment was simultaneous with viral infection. The supernatants were collected 72 h postinfection and evaluated for P_{24} antigen load by capture ELISA (Biomerieux, France). The inhibition rate (%) of P_{24} expression was calculated. The cellular toxicity was evaluated by XTT (sodium 3-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid) proliferation assay (Roche, Germany) according to the kit instruction (Scudiero *et al.*, 1998; Lin *et al.*, 2002). The HIV replication assay plates were directly considered for cytotoxicity assay after determination of P_{24} load.

Molecular modeling (docking) studies

The active compound was selected for docking studies against HIV-1 IN. 3OYA is used for binding mode analysis of HIV-1 IN inhibitory activity. All the compounds were built using Chem Draw and subsequently minimized. The protein structure was prepared for docking using AUTODOCK Tool. Docking was performed by AutoDock 4.0 program using the implemented empirical free energy function and the Lamarckian genetic algorithm (LGA) (Morris *et al.*, 1998). Co-crystallized ligand and all water molecules were removed from crystal protein (3OYA), while a magnesium ion (Mg^{2+}) at the active site of HIV-1 IN was maintained. Polar hydrogens were added and non-polar hydrogens were merged, and finally Kallman united atom charge and atom type parameter was added to 3OYA. Grid map dimensions ($20 \times 20 \times 20$) were set surrounding active site. Lamarckian genetic search algorithm was employed and docking run was set to 50.

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