Bioorganic & Medicinal Chemistry 21 (2013) 7091–7100

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluation of N_2,N_4-disubstituted-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6] thiatriazine derivatives as HIV-1 NNRTIs

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ARTICLE INFO

Article history:
Received 10 August 2013
Revised 3 September 2013
Accepted 4 September 2013
Available online 14 September 2013

Keywords:
HIV
Reverse transcriptase
NNRTIs
Thienothiadiazine
Pyrrolothiatriazine
Heterocycle
Drug design

ABSTRACT

A series of N_2,N_4-disubstituted-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine derivatives (PTTDs) was designed and synthesized by a facile route. The biological assay results showed that five most potent compounds displayed inhibitory activity against HIV-1 at low micromolar concentrations (EC_{50} = 5.1–8.9 μM). Structure–activity relationship analysis indicated that N_2-(3-halogenated-benzyl) analogues were more potent than N_2-(unsubstituted-benzyl) analogues. The N_4-substitutions contributed to the antiviral activity in the following order: 2-/3-cyano substituted benzyl > 2-/3-halogenated benzyl > non-substituted benzyl > 4-halogenated benzyl. Docking studies of the representative compound revealed the binding conformation of these compounds and provided critical insights for the further development of PTTD analogues.

1. Introduction

Non-nucleoside reverse transcriptase inhibitors (NNRTIs), which target an allosteric pocket of reverse transcriptase (RT), represent an important component of drug combination therapy for the treatment of HIV-infected patients.[1–3] Up to date, five NNRTIs have been approved for clinical application by the FDA: nevirapine (NVP),[4] delavirdine (DLV),[5] efavirenz (EFV),[6] etravirine (ETR),[7] and rilpivirine (RPV).[8] First generation of NNRTIs, NVP and EFV suffer from severe side-effects during long term use, as well as an inevitable emergence of HIV-1-resistant strains.[3] Although the second generation of NNRTIs, ETR, and RPV perform better against a variety of variants bearing clinically prevalent mutations,[9–11] the development of novel NNRTI families are still demanded to address issues of side effects, poor solubility, cross-resistance, and virologic failure.[12,13]

In 1998, a class of 2,4-disubstituted-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine derivatives (TTDs) was discovered as potent NNRTIs, which can effectively inhibit the replication of wild-type HIV-1 strains as well as a variety of mutant HIV-1 strains. Prototype compounds **QM96521**, **QM96539**, **QM96639**, and **QM96652** were endowed with EC_{50} values in the low micromolar range in HIV-1 infected MT-4 cell cultures (Fig. 1).[14–16] Initial structure–activity relationship (SAR) analysis revealed that dual substitutions containing π-electrons at the N_2 and N_4 sites of TTDs were necessary for antiviral activity. Particularly, compounds with a benzyl or 3-halogenated benzyl moieties connected at the N_2 position combined with a cyanomethyl group or a halogenated benzyl moiety substituted at the N_4 position showed optimal activities. With the aim to develop novel heterobicyclic compounds structurally related to TTDs, our team has synthesized a collection of novel five-membered heterocycle-fused thiadiazines, viz. pyrazolo[4,5-*e*][2,1,3]thiadiazines (PTDDs) and 7-methylpyrazolo[4,5-*e*][1,2,4]thiadiazines (PTDs),[17–21] generally with diminished or even annihilated anti-HIV activity.

Compared with TTDs, the physicochemical properties (calculated by molinspiration software)[22–24] of PTDDs and PTDs display no significant difference with the exception of the lower lipophilicity (expressed by log *P*), which affects permeability, hydrophobic ligand–receptor interactions, and toxicity of bioactive compounds (Fig. 1).[25] This results indicated a preference for hydrophobicity over hydrophilicity at the central heterobicyclic moiety in term

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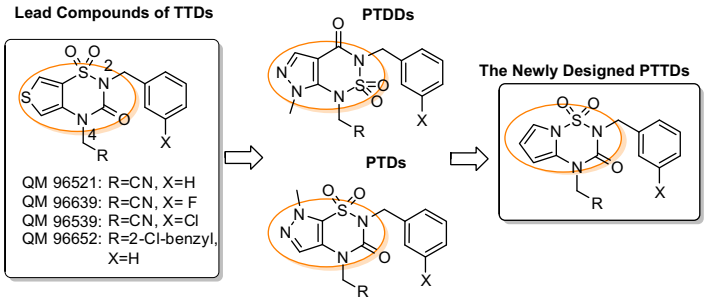
http://dx.doi.org/10.1016/j.bmc.2013.09.009



X=H, R=2-Cl-benzyl	EC_{50} (μM)	miLogP	natoms	MW	nON	nOHNH	nViol	nrotb
TTD analogue	0.01μM	4.05	27.0	418.93	5	0	0	4
PTD analogue	>300	3.17	28.0	416.89	7	0	0	4
PTDD analogue	>20	2.73	28.0	416.89	7	0	0	4
PTTD analogue	-	3.68	27.0	401.88	6	0	0	4
Acceptable range		< 5		<500	<10	<5		

EC_{50}: for the anti-HIV-1 activity; miLog P: molinspiration predicted Log P; natoms: no. of atoms; MW: molecular weight; nON: no. of hydrogen bond acceptors; nOHNH: no.of hydrogen bond donors; nViol: number of violations; nrotb: no. of rotatable bonds.

Figure 1. Structures and representative physicochemical properties of TTDs, PTDs, PTDDs, and PTTDs (physicochemical properties was predicted by a computational molinspiration method).

of anti-HIV-1 inhibition. Based on these findings, we further designed a series of 2,4-disubstituted-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine derivatives (PTTDs) based on the general principle of bioisosteric replacement (Fig. 1). These PTTDs are demonstrated to possess similar lipophilicity with TTDs by a computational Molinspiration method, thus to maximally simulate molecular properties of TTDs. Additionally, the optimal substitutions at the N_2 and N_4 position of TTDs elucidated by SAR studies were retained in the new compounds.[26,27] To further exploit the nature and the pattern of substitutions on the N_4-benzyl ring, the nitrile group, which is ubiquitous in many highly potent NNRTIs,[28] such as UK-453061,[29] MK-4965,[30] IDX-899,[31] and TMC-125,[10] was also introduced to the N_4-benzyl moiety of PTTD to investigate its influence on the antiviral activity. Herein, we report the synthesis and anti-HIV activity of these PTTD analogues, as well as the molecular modeling analysis of the representative compound within the RT binding pocket.

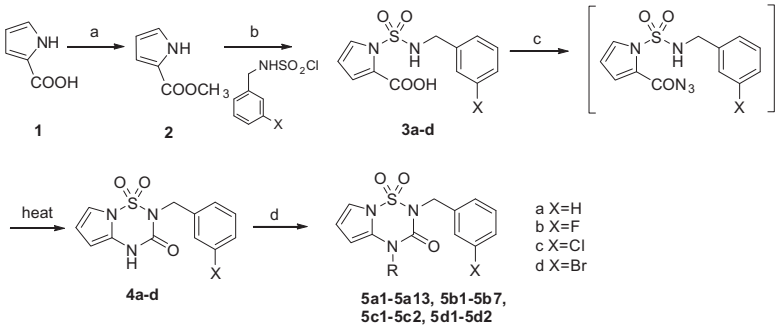
2. Results and discussion

2.1. Chemistry

The synthetic route for PTTDs is outlined in Scheme 1. The commercially available pyrrole-2-carboxylic acid (**1**) was firstly converted to its methyl ester (**2**) using $SOCl_2$ in methanol. Then, the methyl ester (**2**) was treated with *N*-benzylsulfamoyl chlorides[5] in the presence of sodium hydride at 0 °C to afford methyl 1-(*N*-benzylsulfamoyl)-1*H*-pyrrole-2-carboxylates, which underwent the subsequent hydrolysis reaction in a one-pot procedure at room temperature to give intermediate 1-(*N*-benzylsulfamoyl)-1*H*-pyrrole-2-carboxylic acids (**3a–d**). Next, the carboxylic acids (**3a–d**) were converted to corresponding acyl azides using diphenylphosphoryl azide (DPPA)[32] in the presence of triethylamine, which were subjected to thermally Curtius rearrangement at 70 °C to form N_2-substitued pyrrolo[1,2-*b*][1,2,4,6]thiatriazines (**4a–d**). Further alkylation of intermediates (**4a–d**) at their N_4 positions with different benzylhalides or chloroacetonitrile in the presence of sodium hydride afforded the desired N_2,N_4-disubstituted pyrrolo[1,2-*b*][1,2,4,6]thiatriazine derivatives (**5a–d**). Spectral data of all compounds are in full agreement with the proposed structures. To the best of our knowledge, this is the first report about the synthesis of this unique pyrrolo[1,2-*b*][1,2,4,6]thiatriazine ring system.

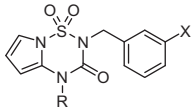
2.2. Biological activity

The newly synthesized N_2,N_4-disubstituted pyrrolo[1,2-*b*][1,2,4,6]thiatriazine analogues were evaluated for their anti-HIV activity and cytotoxicity in MT-4 cell cultures infected with wild-type HIV-1 strain (III_B) or HIV-2 strain (ROD), respectively. The biological evaluation results, expressed as 50% inhibitory concentration (EC_{50}), 50% cytotoxic concentration (CC_{50}), and SI (selectivity, given by the CC_{50}/EC_{50} ratio) values, are listed in Table 1. The FDA-approved drug nevirapine (NVP) and lamivudine (3TC), and zalcitabine (ddC) were used as reference drugs. For comparison, previously reported antiviral data of **QM96521** and **QM96652** were also included in Table 1.[16]



Scheme 1. Synthetic route of PTTDs. Reagents and conditions: (a) $SOCl_2$, MeOH, 30 °C, rt; (b) NaH, THF, 0 °C then aq NaOH, room temperature, rt; (c) DPPA, Et_3N, dioxane, 70 °C; (d) DMF, NaH, RCl or RBr, 50 °C.

Table 1
Anti-HIV activity and cytotoxicity of N_2,N_4-disubstituted-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine derivatives (PTTDs) (**5a–d**)[a]



Compd	X	R	HIV-1 (III_B)			HIV-2 (ROD)		
			EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]	EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]
5a1	H	Benzyl	43	196	5	>267	267	<1
5a2	H	2-CN-benzyl	5.1	>318	>63	>318	>318	×1
5a3	H	3-CN-benzyl	6.5	>318	>49	>318	>318	×1
5a4	H	2-Cl-benzyl	23	291	12	>311	>311	×1
5a5	H	2-Br-benzyl	30	228	8	>226	226	<1
5a6	H	3-Cl-benzyl	40	196	5	>239	239	<1
5a7	H	2,4-Di-Cl-benzyl	>218	218	<1	>273	273	<1
5a8	H	4-F-benzyl	>190	190	<1	>150	150	<1
5a9	H	4-Me-benzyl	>241	241	<1	>241	241	<1
5a10	H	CH_2CN	>187	187	<1	>180	180	<1
5a11	H	Cinnamyl	>318	>318	×1	>318	>318	×1
5a12	H	Allyl	>802	>802	> or ×1	>802	>802	< or ×1
5a13	H	3-Methylbut-2-en-1-yl	25	226	9	>226	226	<1
5b1	F	Benzyl	52	288	6	>324	>324	×1
5b2	F	2-CN-benzyl	5.6	>305	>55	>305	>305	×1
5b3	F	2-Cl-benzyl	11	231	21	>262	262	<1
5b4	F	2-Br-benzyl	29	>269	>9	>269	>269	×1
5b5	F	3-Cl-benzyl	7.2	208	29	>212	212	<1
5b6	F	2,4-Di-Cl-benzyl	>249	249	<1	>275	>275	×1
5b7	F	CH_2CN	34	172	5	>187	187	<1
5c1	Cl	2-CN-benzyl	8.9	>293	>33	>293	>293	×1
5c2	Cl	2,4-Di-Cl-benzyl	>251	251	<1	>265	>265	×1
5d1	Br	2-Cl-benzyl	18	>260	>14	>260	>260	×1
5d2	Br	2,4-Di-Cl-benzyl	>243	>243	×1	>243	>243	×1
QM96625[d]			0.1	>119	>119			
QM96521[d]			0.9	503	559			
NVP[e]			0.11 ± 0.09	>15	>133			
3TC[e]			1.8 ± 0.6	87	>47	5.9 ± 5.2	>87	>15
ddC[e]			1.4 ± 0.05	>95	>68			

[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cell cultures against HIV-1-induced cytotoxicity, as determined by the MTT method.
[b] CC_{50}: concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.
[c] SI: selectivity index (CC_{50}/EC_{50}).
[d] The synthesis and antiviral properties of these lead compounds were previously described.[16]
[e] These data were obtained from the same laboratory (Rega Institute for Medical Research, KU Leuven, Belgium).

According to the biological results, 15 PTTDs analogues were found to be active against HIV-1 (III_B) with EC_{50} values in the range of 5.1–52 μM, but none of the compounds was active against HIV-2 (ROD). The optimal activity against HIV-1 (III_B) was observed with compound **5a2** and **5b2**, with EC_{50} values of 5.1 μM and 5.6 μM, and SI values of >63 and >55, respectively, inferior to those of the lead NNRTI compounds **QM96625**, **QM96521** and **NVP**, but in the same order of magnitude as that of the NNRTI reference drugs **ddC** and **3TC**. These results indicated that the pyrrolo[1,2-*b*][1,2,4,6]thiatriazine ring (PTTD) is a promising bioisosteric moiety of thieno[3,4-*e*][1,2,4]thiadiazine ring in the NNRTI lead compounds.

As shown in Table 1, the substitution pattern of the N_4 benzyl moiety of PTTDs played a determinant role in their HIV-1 inhibitory potency. Analysis of the anti-HIV-1 activity of PTTDs featuring the same N_2-benzyl group (**5a1**–**5a13**) revealed that the presence of a CN group at the 2- or 3-position of the benzene ring of the N_4-benzyl group furnished two most potent compounds **5a2** and **5a3** (EC_{50} = 5.1 μM, SI >63; EC_{50} = 6.5 μM, SI >55, respectively). Compounds (**5a4–5a6**) bearing a halogen atom (Cl or Br) at the 2- or 3-position of the N_4-benzyl ring exhibited higher activities compared with corresponding non-substituted compound **5a1**. However, the presence of a substitution at the 4-position of the N_4-benzyl ring abolished their anti-HIV-1 activity, which was exemplified by N_4-(2,4-di-Cl-benzyl) analogue **5a7**, N_4-(4-F-benzyl) compound **5a8**, and N_4-(4-Me-benzyl) analogue **5a9**. These results indicated that the activities of PTTDs were significantly affected by the electronic nature or steric hindrance of the *para*-substitution at the N_4-benzyl ring. In summary, the substitutions at the N_4 position contributed to the antiviral activity of PTTDs in the following order: 2-/3-cyano substituted benzyl >2-/3-halogenated benzyl > non-substituted benzyl > 4-substituted benzyl. The same conclusion was also drawn from the SAR analysis of the analogues featuring the same N_2-(3-F-benzyl) group (**5b1**–**5b7**): N_4-(2-CN-benzyl) analogue **5b2** showed the best activity; N_4-(2-/3-halogenated benzyl) compounds (**5b3**–**5b5**) exhibited better activity than non-substituted compound **5b1**; N_4-(2,4-di-Cl-benzyl) compound **5b7** was completely inactive. In conclusion, the SAR pattern of N_4-benzyl substitutions of PTTDs was roughly consistent with that of TTDs.[26,33]

In addition to different benzyl groups, other groups containing π-electrons, including cyanomethyl, cinnamyl, allyl, and a 3-methylbut-2-en-1-yl group, were also introduced to the N_4 position of PTTDs to further explore the diversity of the N_4-susbtitution. Unfortunately, only **5a13** and **5b7** bearing a 3-methylbut-2-en-1-yl group and a cyanomethyl group respectively at the N_4 position

exhibited moderate potency (EC_{50} = 25 μM and 34 μM, respectively).

From the SAR point of view, introduction of halogens to the 3-position of the N_2-benzyl ring generally brought a marked improvement on the anti-HIV-1 activity and selectivity. For instance, all the active N_2-(3-F-benzyl) compounds (**5b1**–**5b5**) showed improved anti-HIV-1 activity and higher SI values than corresponding N_2-benzyl compounds. However, all analogues (**5a6**, **5b6**, **5c6**) bearing a cyano group at the N_4 benzyl ring exhibited similar antiviral activities (EC_{50} = 5.1, 5.6, 8.9 μM, respectively), regardless of the substitution patterns of the N_2-benzyl groups. To some extent, these results further confirmed that the cyano group, as the 'privileged fragment' of NNRTIs, could often retain optimal biological activity compared with other counterparts.

2.3. Inhibition of HIV-1 RT

To confirm the drug target of PTTDs, the representative compounds (**5a2** and **5b2**) endowed with the highest anti-HIV-1 potency in MT-4 cell cultures were further evaluated for their in vitro HIV-1 RT inhibitory activities using a RT assay Kit. As illustrated in Table 2, compounds **5a2** and **5b2** exhibited moderate anti-RT activity at micromolar concentrations. Although the anti-RT activity of PTTD analogues **5a2** and **5b2** was less potent than the lead compound NVP, these biological results proved that the newly PTTD derivatives exerted the anti-HIV-1 activity by inhibiting RT, to some extent.

2.4. Molecular modeling analysis

Molecular modeling of the lead compound Q96652 and the representative PTTD compound **5b2** by docking into the NNRTI binding pocket (NNIBP) of HIV-1 RT was performed using Surflex-Dock module of SYBYL-X 1.1 to predict the binding mode and rationalize the SAR conclusion. Coordinates of the NNIBP were taken from the crystal structure of the RT complex with efavirenz (PDB code: IFK9),[34] which also contains a fused heterocyclic scaffold.

The docking results suggested that PTTD **5b2** was fairly superposable to the TTD lead compound **Q966525**, and both of them have similar binding orientations and conformation with that of efavirenz. As illustrated in Figure 2, the pyrrolothiatriazine ring of **5b2**, which overlapped with the thienothiadiazine ring of **Q966525**, was sandwiched between the side chains of Val106 and Try318, as expected, and involved in a series of hydrophobic contacts. In this case, pyrrolothiatriazine had been shown to act as essential pharmacophore elements, favorably contributing to ligand binding. The observation supports our initial presumption that the lipophilic properties of the central heterobicyclic ring has significant influence on the biological activity, indicating that pyrrolothiatriazine could serve as a suitable surrogate for the thienothiadiazine ring of TTDs.

Additionally, pyrrolothiatriazine serving as the core building block, oriented its N_2 and N_4 substituents in the appropriate conformation. As the extension of pyrrolothiatriazine, the N_2-(3-F-benzyl) group was tolerated by the entrance channel defined by the residues of Lys103, Glu138 and Val179. The fluoro atom was found to be engaged in a halogen bond with the backbone *NH* moiety of the K101 residue, which may contribute substantially to the free energy of binding for the inhibitor. The N_4-2-CN-benzyl groups of **5b2** which acted as the cyclopropyl–propynyl group of efavirenz and were positioned in the top sub-pocket, apparently developed extensive van der Waals and π–π stacking interactions with adjacent aromatic residues of Tyr181, Tyr188, and Trp229. It was well known that introduction of a cyano group at the phenyl ring located in this Tyr181–Tyr188–Trp229 π-rich region furnished many highly potent compounds, such as UK-453061,[29] MK-4965,[30] and IDX-899.[31] This is also true for PTTD analogues, which was validated by the most potent compounds **5a2** and **5b2** that were characterized by a cyano group at the N_4-benzyl ring. This observation further proved the cyano group to be a 'privileged fragment' in NNRTIs.[28] It seems possible through introduction of the 3,5-diCN group at the N_4-benzyl ring which is known to be able to make compensatory contacts with the conserved residue Trp229, to improve the resistance profiles of PTTDs. This drug design strategy should be considered in the further optimization of PTTD compounds. In addition, it was noticed that the 4-position of the N_4-benzyl group pointed to the indole ring of Trp229, with a minimal distance of 2.86 Å. Therefore, introduction of substitutions to the 4-position of the N_4-benzyl ring was likely to cause a steric hindrance, thus to destroy the favorable binding mode, which may explain the disappointing activity observed with all the N_4-(4-substitued benzyl) analogues.

Thus, the docking simulation helped to interpret the binding conformation and to identify the potential key pharmacophore elements of PTTDs, which are in agreement with the SAR and provide guide for the design of more potent NNRTIs with this scaffold.

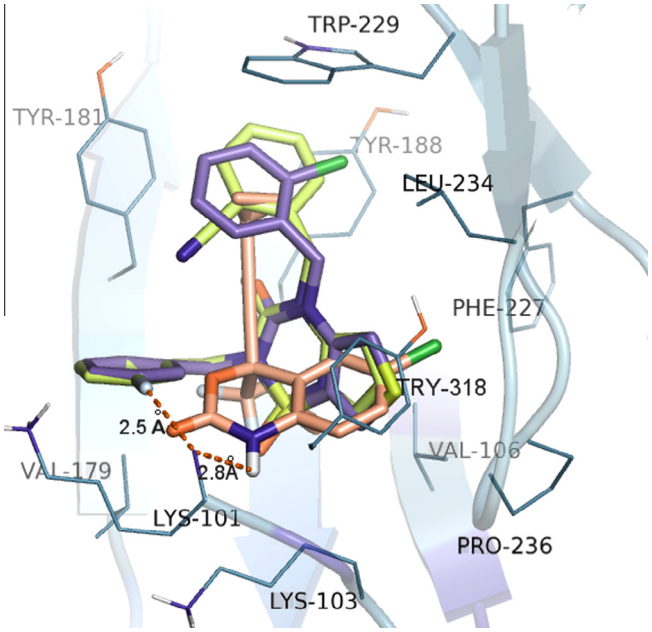


Figure 2. Superposition of the TTD lead compound **Q96652** (purple), **PTTD** analogue **5b2** (green) and efavirenz (pink) in the binding pocket of RT. (Docking result was shown by PyMOL. Proposed hydrogen bonds and halogen bonds are indicated with dashed lines in red.)

Table 2
In vitro inhibitory activity of representative PTTD compounds against HIV-1 RT

Compound	5a2	5b2	NVP
[a]IC_{50} (μM)	56.9	58.3	6.2

[a] IC_{50}: inhibitory concentration required to inhibit biotin deoxyuridine triphosphate (biotin-dUTP) incorporation into the HIV-1 RT by 50% (RT kit, Roche).

3. Conclusion

In summary, based on the analysis of SAR and predicted physicochemical properties of TTD derivatives, a novel series of 2,4-disubstituted-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine derivatives (PTTDs) was designed as potential NNRTIs based on the principle of bioisosteric replacement. A facile methodology for the

synthesis of 1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine as an original heterobicyclic ring system was reported for the first time. Subsequent biological evaluation for their anti-HIV-1 activities revealed that most of the synthesized compounds were effective to inhibit the replication of HIV-1 at micromolar concentrations. The preliminary SAR and molecular modeling studies of these unique inhibitors provide valuable information for rational structural optimization. Further structural modifications are warranted to develop novel PTTD analogues with higher potency against mutant variants and lower cytotoxicity.

4. Experimental

4.1. Chemistry

The key reactants including pyrrole-2-carboxylic acid, benzylamines, benzyl halides, alkyl halides, and DPPA were purchased from Adamas-beta Co. Ltd. and used without further purification. Thin layer chromatography was performed on pre-coated HUANGHAI® $HSGF_{254}$, 0.15–0.2 mm TLC-plates. Flash column chromatography was performed on columns packed with silica gel 60 (200–300 mesh). NMR spectra were recorded on INOVA-600 (600 MHz) or Bruker AVANCE-300 (300 MHz) NMR-spectrometer, using solvents as indicated ($CDCl_3$ or DMSO-d_6) with tetramethylsilane (TMS) as internal standard. Chemical shifts were reported in δ values (ppm), signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet), and *J* values were reported in hertz (Hz). Mass spectrometry was performed on a LC Autosampler Device: Standard G1313A instrument. Melting points were determined on a micromelting point apparatus RY-1 (produced by Tian Jin Analytical Instrument Factory) and are uncorrected.

4.1.1. The preparation of methyl 1*H*-pyrrole-2-carboxylate (2)

To a solution of pyrrole-2-carboxylic acid (**1**) (16.65 g, 0.15 mol) in 10 mL of MeOH was added 50 mL of $SOCl_2$ dropwise within 30 min at 0 °C. Subsequently, the reaction mixture was stirred at 35 °C for 24 h. Then, the solvent was evaporated in vacuo, and the residue was purified by column chromatography using ethyl acetate/petroleum ether as eluent, giving intermediate **2** as a white solid, yield 85%, mp: 72–73 °C.

4.1.2. General procedure for the preparation of 1-(*N*-benzylsulfamoyl)-1*H*-pyrrole-2-carboxylic acids (3a–d)

To an anhydrous THF suspension of sodium hydride (60% dispersion in mineral oil, 1.80 g, 0.045 mol) was added methyl 2-pyrrolecarboxylate (**2**) (1.88 g, 0.015 mol) by portions at 0 °C. The resulting mixture was stirred for 30 min and then corresponding *N*-benzylsulfamoyl chlorides (0.03 mol) dissolved in anhydrous THF was added dropwise at 0 °C. The mixture was stirred for 12 h at room temperature. Then, water was cautiously added to quench the reaction, followed by addition of 30 mL of 5 N aqueous NaOH solution. This mixture was stirred for another 12 h. Then, the organic layer was evaporated in vacuo and the remaining aqueous layer was acidified with concentrated hydrochloric acid to adjust to pH 9. The resulting precipitate was filtered-off and purified by column chromatography using ethyl acetate/petroleum ether as eluent to give intermediates **3a–d**.

4.1.2.1. 1-(*N*-Benzylsulfamoyl)-1*H*-pyrrole-2-carboxylic acid (3a). Reagents: methyl 2-pyrrolecarboxylate (**2**) (1.88 g, 0.015 mol) and *N*-benzylsulfamoyl chloride (6.17 g, 0.030 mol). Product as a white solid, yield 82%, mp: 124–126 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.21 (s, 2H, CH_2), 6.18 (t, *J* = 3.30 Hz, 1H, pyrrole-H), 6.92 (dd, *J* = 3.60 Hz, *J* = 1.80 Hz, 1H, pyrrole-H), 7.17–7.25 (m, 5H, Ar-H), 7.32 (dd, *J* = 3.00 Hz, 1.8 Hz, 1H, pyrrole-H), 8.31 (s, 1H, NH), 12.84 (bs, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 47.5 (CH_2), 109.4, 122.6, 122.4, 127.9, 128.1, 128.7, 129.7, 136.7, 161.2 (C=O); ESI-MS: 281.5 $[M+H]^+$.

4.1.2.2. 1-(*N*-(3-Fluorobenzyl)sulfamoyl)-1*H*-pyrrole-2-carboxylic acid (3b). Reagents: methyl 2-pyrrolecarboxylate (**2**) (1.88 g, 0.015 mol) and *N*-(3-fluororobenzyl)sulfamoyl chloride (6.71 g, 0.030 mol). Product as a white solid, yield 56%. Mp: 120–121 °C. ESI-MS: 295.5 $[M+H]^+$.

4.1.2.3. 1-(*N*-(3-Chlorobenzyl)sulfamoyl)-1*H*-pyrrole-2-carboxylic acid (3c). Reagents: methyl 2-pyrrolecarboxylate (**2**) (1.88 g, 0.015 mol) and *N*-(3-chlorobenzyl)sulfamoyl chloride (7.20 g, 0.030 mol). Product as a white solid, yield 59%. Mp: 115–117 °C. ESI-MS: 315.4 $[M+H]^+$, 317.3 $[M+H]^+$.

4.1.2.4. 1-(*N*-(3-Bromobenzyl)sulfamoyl)-1*H*-pyrrole-2-carboxylic acid (3d). Reagents: methyl 2-pyrrolecarboxylate (**2**) (1.88 g, 0.015 mol) and *N*-(3-bromobenzyl) sulfamoyl chloride (8.54 g, 0.030 mol). Product as a white solid, yield 59%. Mp: 110–112 °C. ESI-MS: 361.3 $[M+H]^+$, 359.4 $[M+H]^+$.

4.1.3. General procedure for the preparation of N_2-benzyl-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazines (4a–d)

To a solution of intermediates **3a–d** (0.010 mol) and diphenylphosphoryl azide (DPPA) (3.3 g, 0.012 mol) in anhydrous 1,4-dioxane was added triethylamine (1.2 g, 0.012 mol). The reaction mixture was stirred at 70 °C for 12 h. Then, the solvent was removed under reduced pressure. Further, the residue was purified by column chromatography using ethyl acetate/petroleum ether as eluent to afford N_2-benzyl-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazines (**4a–d**).

4.1.3.1. N_2-Benzyl-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (4a). Reagents: **3a** (2.77 g, 0.01 mol) and DPPA (3.30 g, 0.012 mol). Product as a white solid, yield 58%. Mp: 128–130 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 5.02 (s, 2H, CH_2), 5.66 (dd, *J* = 3.60 Hz, 1.50 Hz, 1H, pyrrole-H), 6.42 (t, *J* = 3.40 Hz, 1H, pyrrole-H), 7.13 (dd, *J* = 3.30 Hz, 1.50 Hz, 1H, pyrrole-H), 7.27–7.38 (m, 5H, Ar-H), 11.92 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 45.9 (CH_2), 93.3, 110.8, 114.4, 136.4, 128.3, 128.9, 128.4, 129.0, 147.9 (C=O); ESI-MS: 278.3 $[M+H]^+$.

4.1.3.2. N_2-(3-Fluorobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (4b). Reagents: **3b** (2.98 g, 0.01 mol) and DPPA (3.30 g, 0.012 mol). Product as a white solid, yield 25%. Mp: 122–124 °C. ESI-MS: 296.3 $[M+H]^+$.

4.1.3.3. N_2-(3-Chlorobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6] thiatriazine (4c). Reagents: **3c** (3.15 g, 0.01 mol) and DPPA (3.30 g, 0.012 mol). Product as a white solid, yield 29%. Mp: 117–119 °C. ESI-MS: 312.3 $[M+H]^+$, 314.2 $[M+H]^+$.

4.1.3.4. N_2-(3-Bromobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6] thiatriazine (4d). Reagents: **3d** (3.59 g, 0.01 mol) and DPPA (3.30 g, 0.012 mol). Product as a white solid, yield 30%. Mp: 115–117 °C. ESI-MS: 358.2 $[M+H]^+$, 356.3 $[M+H]^+$.

4.1.4. General procedure for the preparation of N_2,N_4-disubstitued-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6] thiatriazines (5a1–5a13, 5b1–5b7, 5c1–5c2, 5d1–5d2)

To a solution of the **3a–d** (2 mmol) in dry DMF (10 mL) was added sodium hydride (60% dispersion in mineral oil, 96 mg, 2.4 mmol) at 0 °C. After stirring for 15 min, the alkyl halide (2.4 mmol) was added at 0 °C. The resulting mixture was stirred at 75 °C for 12 h. Thereafter, the solvent was evaporated off in

vacuo. The residue was purified by flash column chromatography using dichloromethane/petroleum ether as eluent to give target compounds.

4.1.4.1. N_2-Benzyl-N_4-benzyl-1,1,3-trioxo-*2H*,*4H*-pyrrolo[1,2-*b*][1,2,4,6] thiatriazine (5a1). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with benzyl bromide (0.41 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid, yield 34%, mp: 113–117 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.10 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 6.00 (d, J = 3.00 Hz, 1H, pyrrole-H), 6.45 (t, J = 3.00 Hz, 1H, pyrrole-H), 7.15 (t, J = 7.50 Hz, 1H, Ar-H), 7.22–7.27 (m, 4H, Ar-H and pyrrole-H), 7.32–7.38 (m, 6H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 47.2 (CH_2), 49.7 (CH_2), 94.9, 111.0, 113.3, 126.8, 127.9, 128.4, 128.7, 128.8, 128.9, 134.9, 135.2, 148.7 (C=O); ESI-MS: 368.2 $[M+H]^+$.

4.1.4.2. N_2-Benzyl-N_4-(*o*-cyanobenzyl)-1,1,3-trioxo-2*H*,*4H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a2). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with 2-cyanobenzylchloride (0.37 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 27%, mp: 149–153 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.10 (s, 2H, CH_2), 5.31 (s, 2H, CH_2), 6.02 (dd, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 6.48 (t, J = 1.80 Hz, 1H, pyrrole-H), 7.28 (d, J = 8.40 Hz, 1H, Ar-H), 7.30 (d, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 7.32 (d, J = 7.20 Hz, 1H, Ar-H), 7.36–7.40 (m, 4H, Ar-H), 7.50 (t, J = 7.20 Hz, 1H, Ar-H), 7.67 (t, J = 7.20 Hz, 1H, Ar-H), 7.90 (d, J = 7.80 Hz, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 47.3 (CH_2), 47.5 (CH_2), 94.8, 111.3, 111.5, 113.3, 116.9, 126.2, 128.4, 128.5, 128.7, 128.8, 129.2, 133.1, 133.6, 134.9, 138.6, 148.8 (C=O); ESI-MS: 393.2 $[M+H]^+$.

4.1.4.3. N_2-Benzyl-N_4-(*m*-cyanobenzyl)-1,1,3-trioxo-2*H*,*4H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a3). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with 3-cyanobenzyl bromide (0.47 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 32%, mp: 122–124 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.12 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 6.00 (dd, J = 3.60 Hz, 1.20 Hz, 1H, pyrrole-H), 6.46 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.28 (dd, J = 3.00 Hz, 1.20 Hz, 1H, pyrrole-H), 7.30 (t, J = 6.60 Hz, 1H, Ar-H), 7.37–7.41 (m, 4H, Ar-H), 7.56–7.61 (m, 2H, Ar-H), 7.77–7.78 (m, 2H, 2H, Ar-H); ^{13}C NMR (75 Hz, $CDCl_3$) δ: 47.5 (CH_2), 48.9 (CH_2), 94.7, 111.5, 113.1, 113.2, 118.3, 128.6, 128.7, 128.8, 129.4, 129.8, 130.4, 131.1, 131.8, 134.8, 136.6, 148.7 (C=O); ESI-MS: 393.5 $[M+H]^+$.

4.1.4.4. N_2-Benzyl-N_4-(*o*-chlorobenzyl)-1,1,3-trioxo-2*H*,*4H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a4). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with 2-chlorobenzyl chloride (0.39 g, 2.4 mmol) and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 27%, mp: 122–124 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.11 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 5.93 (dd, J = 3.60 Hz, 1.20 Hz, 1H, pyrrole-H), 6.45 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.12 (d, J = 7.20 Hz, 1H, pyrrole-H), 7.28–7.31 (m, 2H, Ar-H), 7.32–7.34 (m, 2H, Ar-H), 7.36–7.40 (m, 4H, Ar-H), 7.51 (d, J = 7.80 Hz, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 47.0 (CH_2), 49.3 (CH_2), 94.7, 111.2, 113.2, 126.8, 127.4, 128.4, 128.7, 128.8, 128.9, 129.6, 129.7, 132.0, 132.7, 135.1, 148.6 (C=O); ESI-MS: 402.4 $[M+H]^+$, 404.4 $[M+H]^+$.

4.1.4.5. N_2-Benzyl-N_4-(*o*-bromobenzyl)-benzyl-1,1,3-trioxo-2*H*, *4H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a5). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with 2-bromobenzyl chloride (0.60 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 20%, mp: 115–117 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.11 (s, 2H, CH_2), 5.13 (s, 2H, CH_2), 5.9 (dd, J = 3.60 Hz, 1.20 Hz, 1H, pyrrole-H), 6.5 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.06 (d, J = 7.80 Hz, 1H, Ar-H), 7.25 (t, J = 7.20 Hz, 1H, Ar-H), 7.30 (dd, J = 3.60 Hz, 1.20 Hz, 1H, pyrrole-H), 7.32 (t, J = 7.20 Hz, 2H, Ar-H), 7.36–7.40 (m, 4H, Ar-H), 7.68 (d, J = 7.20 Hz, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 47.3 (CH_2), 49.6 (CH_2), 94.8, 111.3, 113.3, 122.5, 126.8, 128.0, 128.5, 128.7, 128.9, 129.2, 129.6, 133.0, 133.5, 135.0, 148.6 (C=O); ESI-MS: 446.2 $[M+H]^+$, 448.2 $[M+H]^+$.

4.1.4.6. N_2-Benzyl-N_4-(*m*-chlorobenzyl)-1,1,3-trioxo-2*H*,*4H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a6). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with 3-chlorobenzyl chloride (0.39 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 26%, mp: 155–158 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.12 (s, 2H, CH_2), 5.14 (s, 2H, CH_2), 6.01 (dd, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 6.46 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.23 (d, J = 7.20 Hz, 1H, Ar-H), 7.27 (dd, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 7.32–7.39 (m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 47.3 (CH_2), 49.2 (CH_2), 94.7, 111.2, 113.2, 124.8, 127.0, 128.2, 128.5, 128.8, 129.6, 130.2, 134.8, 135.0, 137.0, 148.7 (C=O); ESI-MS: 402.3 $[M+H]^+$, 404.3 $[M+H]^+$.

4.1.4.7. N_2-Benzyl-N_4-(2,4-dichlorobenzyl)-1,1,3-trioxo-2*H*,*4H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a7). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with 2,4-dichlorobenzyl chloride (0.47 g, 2.4 mmol), and then purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 25%, mp: 122–124 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.10 (s, 2H, CH_2), 5.15 (s, 2H, CH_2), 5.95 (dd, J = 3.60 Hz, 1.20 Hz, 1H, pyrrole-H), 6.45 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.17 (d, J = 8.40 Hz, 1H, Ar-H), 7.29 (dd, J = 3.60 Hz, 1.20 Hz, 1H, pyrrole-H), 7.32–7.34 (m, 1H, Ar-H), 7.36–7.38 (m, 4H, Ar-H), 7.41 (dd, J = 7.80 Hz, 2.10 Hz, 1H, Ar-H), 7.70 (d, J = 1.80 Hz, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 46.6 (CH_2), 47.4 (CH_2), 94.7, 111.4, 113.3, 127.7, 127.9, 128.5, 128.7, 128.8, 129.4, 129.6, 130.7, 133.4, 134.2, 134.9, 148.6 (C=O); ESI-MS: 436.3 $[M+H]^+$, 438.2 $[M+H]^+$, 437.2 $[M+H]^+$.

4.1.4.8. N_2-Benzyl-N_4-(*p*-fluorobenzyl)-1,1,3-trioxo-2*H*,*4H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a8). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with 4-fluorobenzyl chloride (0.35 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 22%, mp: 120–122 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.01 (s, 2H, CH_2), 5.11 (s, 2H, CH_2), 6.02 (dd, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 6.5 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.18 (t, J = 9.00 Hz, 2H, Ar-H), 7.26 (d, J = 1.20 Hz, 1H, pyrrole-H), 7.32–7.40 (m, 6H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 47.3 (CH_2), 49.1 (CH_2), 94.8, 111.2, 113.2, 115.8 ($^2J_{C-F}$ = 22 Hz), 128.4, 128.6, 128.7, 128.8 ($^3J_{C-F}$ = 6 Hz), 129.7, 130. 7 ($^4J_{C-F}$ = 3 Hz), 135.1, 148.64 (C=O), 162.4 ($^1J_{C-F}$ = 245 Hz); ESI-MS: 386.4 $[M+H]^+$.

4.1.4.9. N_2-Benzyl-N_4-(*p*-methylbenzyl)-1,1,3-trioxo-2*H*,*4H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a9). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with 4-methylbenzyl chloride (0.34 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid, yield 75%, mp: 86–88 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.31 (s, CH_3, 3H), 5.00 (s, 2H,

CH_2), 5.11 (s, 2H, CH_2), 5.55 (dd, J = 3.48 Hz, 1.52 Hz, 1H, pyrrole-H), 6.25 (t, J = 3.48 Hz, 1H, pyrrole-H), 6.89 (dd, J = 3.40 Hz, 1.52 Hz, 1 H, pyrrole-H), 7.09–7.14 (m, 4H, Ar-H), 7.30–7.36 (m, 3H, Ar-H), 7.47 (d, J = 2.60 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.1 (CH_3), 47.2 (CH_2), 49.6 (CH_2), 94.8, 110.9, 113.2, 126.8, 128.3, 128.7, 128.8, 129.5, 129.9, 131.9, 135.2, 148.6 (C=O); ESI-MS: 382.5 $[M+H]^+$.

4.1.4.10. N_2-Benzyl-N_4-cyanolmethyl-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a10). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with chloroacetonitrile (0.18 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 63%, mp: 131–134 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.10 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.23 (dd, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 6.57 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.32–7.34 (m, 1H, Ar-H), 7.35 (dd, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 7.37–7.41 (m, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 33.6 (CH_2), 48.0 (CH_2), 94.4, 112.3, 113.1, 113.3, 128.0, 128.7, 128.8, 129.0, 134.3, 148.0 (C=O); ESI-MS: ESI-MS: 317.2 $[M+H]^+$.

4.1.4.11. N_2-Benzyl-N_4-cinnamyl-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a11). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with cinnamyl chloride (0.37 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a colorless oil, yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.61 (dd, J = 6.00 Hz, 1.40 Hz, 2H, CH_2), 5.09 (s, 2H, CH_2), 5.72 (dd, J = 3.56 Hz, 1.56 Hz, 1H, pyrrole-H), 6.18-6.23 (m, 1H, –CH=), 6.33 (t, J = 3.44 Hz, 1H, pyrrole-H), 6.61 (d, J = 15.96 Hz, 1H, – CH=), 6.93 (dd, J = 3.40 Hz, 1.56 Hz, 1H, pyrrole-H), 7.25–7.34 (m, 8H, Ar-H), 7.34 (d, J = 1.40 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 47.1 (CH_2), 48.5 (CH_2), 94.3, 111.0, 113.3, 121.7, 126.6, 128.2, 128.4, 128.6, 128.7, 128.9, 129.9, 133.9, 135.1, 135.9, 148.1 (C=O). ESI-MS: 394.5 $[M+H]^+$.

4.1.4.12. N_2-Benzyl N_4-allyl-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a12). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with allyl chloride (0.18 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a colorless oil, yield 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.45 (d, J = 5.36 Hz, 1H, CH_2), 5.07 (s, 2H, CH_2), 5.24–5.28 (m, 2H, CH_2=), 5.65 (dd, J = 3.44 Hz, 1.44 Hz, 1H, pyrrole-H), 5.79–5.89 (m, 1H, – CH=), 6.33 (t, J = 3.48 Hz, 1H, pyrrole-H), 6.92 (dd, J = 3.36 Hz, 1.48 Hz, 1H, pyrrole-H), 7.28–7.34 (m, 3H, Ar-H), 7.36 (d, J = 2.04 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 47.1 (CH_2), 48.7 (CH_2), 94.2, 111.0, 113.2, 118.4, 128.3, 128. 7, 128.8, 129.9, 130.5, 135.1, 148.1 (C=O); ESI-MS: 318.5 $[M+H]^+$.

4.1.4.13. N_2-Benzyl-N_4-(4-(3-methylbut-2-en-1-yl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5a13). This compound was obtained from reaction of compound **4a** (0.55 g, 2 mmol) with 1-chloro-3-methyl-2-butene (0.25 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a yellow oil, yield 62% ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.77 (s, 3H, CH_3), 1.72 (s, 3H, CH_3), 4.44 (d, J = 6.44 Hz, 2H, CH_2), 5.06 (s, 2H, CH_2), 5.18 (m, 1H, – CH=), 5.60 (dd, J = 3.52 Hz, 1.60 Hz, 1H, pyrrole-H), 6.33 (t, J = 3.52 Hz, 1H, pyrrole-H), 6.92 (dd, J = 3.40 Hz, 1.56 Hz, 1H, pyrrole-H), 7.23–7.36 (m, 3H, Ar-H), 7.46–7.48 (m, 2H, Ar-H); ESI-MS: 346.3 $[M+H]^+$.

4.1.4.14. N_2-(*m*-Fluorobenzyl)-N_4-benzyl-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5b1). This compound was obtained from reaction of compound **4b** (0.59 g, 2 mmol) with benzyl bromide (0.41 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 32%, mp: 101–106 °C. ^{1}H NMR (300 MHz, DMSO) δ: 5.10 (s, 2H, CH_2), 5.17 (s, 2H, CH_2), 6.00 (d, J = 3.00 Hz, 1H, pyrrole-H), 6.46 (t, J = 3.00 Hz, 1H, pyrrole-H), 7.15 (t, J = 7.20 Hz, 1H, Ar-H), 7.23 (t, J = 8.70 Hz, 2H, Ar-H), 7.26 (d, J = 2.40 Hz, 1H, pyrrole-H), 7.32–7.38 (m, 6H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 46.5 (CH_2), 49.8 (CH_2), 95.1, 111.1, 113.4, 115.4 ($^2J_{C-F}$ = 21 Hz), 115.7 ($^2J_{C-F}$ = 17 Hz), 124.4 ($^4J_{C-F}$ = 3 Hz), 128.8, 128.0, 128.9, 129.8, 130.3 ($^3J_{C-F}$ = 8 Hz), 134.8, 137.5 ($^3J_{C-F}$ = 8 Hz), 148.6 (C=O), 162.9 ($^1J_{C-F}$ = 245 Hz); EI-MS: 386.4 $[M+H]^+$.

4.1.4.15. N_2-(*m*-Fluorobenzyl)-N_4-(*o*-cyanobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5b2). This compound was obtained from reaction of compound **4b** (0.59 g, 2 mmol) with 2-cyanobenzylchloride (0.37 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 37%, mp: 162–165 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.09 (s, 2H, CH_2), 5.29 (s, 2H, CH_2), 6.00 (dd, J = 3.60 Hz, 1.20 Hz, 1H, pyrrole-H), 6.4 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.13 (td, J = 8.40 Hz, 2.40 Hz, 1H, Ar-H), 7.17–7.21 (m, 2H, Ar-H), 7.28 (dd, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 7.29 (d, J = 7.80 Hz, 1H, Ar-H), 7.37–7.41 (m, 1H, Ar-H), 7.47 (t, J = 7.80 Hz, 1H, Ar-H), 7.65 (td, J = 7.80 Hz, 1.20 Hz, 1H, Ar-H), 7.87 (d, J = 7.80 Hz, 1H, Ar-H); ^{13}C NMR (75 Hz, $CDCl_3$) δ: 46.8 (CH_2), 47.4 (CH_2), 95.0, 111.3, 111.6, 113.4, 115.6 ($^2J_{C-F}$ = 23 Hz), 115.7 ($^2J_{C-F}$ = 21 Hz), 116.9, 124.4 ($^4J_{C-F}$ = 3 Hz), 126.6, 128.5, 129.1, 130.4 ($^3J_{C-F}$ = 8 Hz), 133.1, 133.6, 137.2 ($^3J_{C-F}$ = 8 Hz), 138.5, 148.7 (C=O), 161.30 ($^1J_{C-F}$ = 202 Hz); ESI-MS: 411.4 $[M+H]^+$.

4.1.4.16. N_2-(*m*-Fluorobenzyl)-N_4-(*o*-chlorobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5b3). This compound was obtained from reaction of compound **4b** (0.59 g, 2 mmol) with 2-chlorobenzyl chloride (0.39 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 30%, mp: 113–114 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 4.46 (s, 2H, CH_2), 4.50 (s, 2H, CH_2), 5.95 (d, J = 1.80 Hz, 1H, pyrrole-H), 6.46 (t, J = 2.40 Hz, 1H, pyrrole-H), 7.16–7.24 (m, 4H, Ar-H), 7.29–7.35 (m, 3H, Ar-H and pyrrole-H), 7.42–7.45 (m, 1H, Ar-H), 7.51 (d, J = 7.80 Hz, 1H, Ar-H); ^{13}C NMR (75 Hz, $CDCl_3$) δ: 46.6 (CH_2), 47.1 (CH_2), 95.0, 111.4, 113.5, 115.5 (d, $^2J_{C-F}$ = 18 Hz), 115.6 (d, $^2J_{C-F}$ = 18 Hz), 124.4 (d, $^4J_{C-F}$ = 3 Hz), 126.8, 127.4, 129.0, 129.5, 129.8, 130.3 (d, $^3J_{C-F}$ = 8 Hz), 131.9, 132.7, 137.4 (d, $^3J_{C-F}$ = 8 Hz), 148.5 (C=O), 162.8 (d, $^1J_{C-F}$ = 245 Hz); ESI-MS: 420.3 $[M+H]^+$, 422.3 $[M+H]^+$.

4.1.4.17. N_2-(*m*-Fluorobenzyl)-N_4-(*o*-bromobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5b4). This compound was obtained from reaction of compound **4b** (0.59 g, 2 mmol) with 2-bromobenzyl chloride (0.60 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 29%, mp: 113–116 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.11 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 5.52 (dd, J = 3.60 Hz, 1.50 Hz, 1H, pyrrole-H), 6.29 (t, J = 3.60 Hz, 1H, pyrrole-H), 6.93 (dd, J = 3.30 Hz, 1.50 Hz, 1H, pyrrole-H), 7.00–7.06 (m, 2H, Ar-H), 7.11–7.37 (m, 6H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 46.6 (CH_2), 49.6 (CH_2), 95.1, 111.4, 113.5, 115.5 (d, $^2J_{C-F}$ = 18 Hz), 115.8 (d, $^2J_{C-F}$ = 18 Hz), 122.5, 124.4 (d, $^4J_{C-F}$ = 3 Hz), 126.7, 128.0, 129.3, 129.5, 130.3 (d, $^3J_{C-F}$ = 8 Hz), 133.0, 133.4, 137.4 (d, $^3J_{C-F}$ = 8 Hz), 148.5 (C=O), 162.8 (d, $^1J_{C-F}$ = 245 Hz). ESI-MS: 464.3 $[M+H]^+$, 466.3 $[M+H]^+$.

4.1.4.18. N_2-(*m*-Fluorobenzyl)-N_4-(*m*-chlorobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5b5). This compound was obtained from reaction of compound **4b** (0.59 g,

2 mmol) with 3-chlorobenzyl chloride (0.39 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 31%, mp: 122–124 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.13 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 5.95 (dd, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 6.46 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.16–7.24 (m, 4H, Ar-H), 7.29–7.35 (m, 3H, Ar-H and pyrrole-H), 7.42–7.46 (m, 1H, Ar-H), 7.51 (dd, J = 7.20 Hz, 1.20 Hz, 1H); ^{13}C NMR (75 Hz, $CDCl_3$) δ: 46.6 (CH_2), 47.1 (CH_2), 95.0, 111.4, 113.5, 115.5 (d, $^2J_{C-F}$ = 18 Hz), 115.7 (d, $^2J_{C-F}$ = 19 Hz), 124.4 (d, $^4J_{C-F}$ = 2 Hz), 126.9, 127.4, 129.0, 129.5, 129.8, 130.3 (d, $^3J_{C-F}$ = 8 Hz), 131.9, 132.7, 137.4 (d, $^3J_{C-F}$ = 8 Hz), 148.5 (C=O), 161.25 (d, $^1J_{C-F}$ = 245 Hz); ESI-MS: 420.3 $[M+H]^+$, 422.3 $[M+H]^+$.

4.1.4.19. N_2-(*m*-Fluorobenzyl)-N_4-(2,4-dichlorobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5b6). This compound was obtained from reaction of compound **4b** (0.59 g, 2 mmol) with 2,4-dichlorobenzyl chloride (0.47 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 30%, mp: 109–116 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.08 (s, 2H, CH_2), 5.13 (s, 2H, CH_2), 5.9 (dd, J = 3.00 Hz, 1.80 Hz, 1H, pyrrole-H), 6.4 (t, J = 3.00 Hz, 1H, pyrrole-H), 7.13–7.20 (m, 4H, Ar-H), 7.27 (dd, J = 3.00 Hz, 1.80 Hz, 1H, pyrrole-H), 7.35–7.42 (m, 2H, Ar-H), 7.47 (d, J = 1.80 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 46.6 (CH_2), 46.7 (CH_2), 94.9, 111.5, 113.4, 115.5 ($^2J_{C-F}$ = 20 Hz), 115.7 ($^2J_{C-F}$ = 21 Hz), 124.4 ($^4J_{C-F}$ = 3 Hz), 127.8 ($^3J_{C-F}$ = 10 Hz), 129.3, 129.6, 130.3, 130.6, 131.0, 133.4, 134.3, 137.3 ($^3J_{C-F}$ = 10 Hz), 148.5 (C=O), 162.8 ($^1J_{C-F}$ = 255 Hz); ESI-MS: 454.3 $[M+H]^+$, 456.3 $[M+H]^+$.

4.1.4.20. N_2-(*m*-Fluorobenzyl)-N_4-cyanomethyl-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5b7). This compound was obtained from reaction of compound **4b** (0.59 g, 2 mmol) with chloroacetonitrile (0.18 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 30%, mp: 122–127 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.73 (s, 2H, CH_2), 5.06 (s, 2H, CH_2), 5.87 (dd, J = 3.60 Hz, 1.50 Hz, 1H, pyrrole-H), 6.43 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.00 (dd, J = 3.60 Hz, 1.50 Hz, 1H, pyrrole-H), 7.06 (dd, J = 8.40 Hz, 2.40 Hz, 1H, Ar-H), 7.17–7.22 (m, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 7.30–7.37 (m, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 47.2 (CH_2), 47.3 (CH_2), 94.6, 112.4, 113.1, 113.5, 115.7 ($^2J_{C-F}$ = 21 Hz), 115.9 ($^2J_{C-F}$ = 23 Hz), 124.5 ($^4J_{C-F}$ = 3 Hz), 127.9, 130.4 ($^3J_{C-F}$ = 8 Hz), 136.6 ($^3J_{C-F}$ = 8 Hz), 147.9 (C=O), 162.8 ($^1J_{C-F}$ = 245 Hz); ESI-MS: 335.4 $[M+H]^+$.

4.1.4.21. N_2-(*m*-Chlorobenzyl)-N_4-(*o*-cyanobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5c1). This compound was obtained from reaction of compound **4c** (0.62 g, 2 mmol) with 2-cyanobenzylchloride (0.37 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 38%, mp: 203–205 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.11 (s, 2H, CH_2), 5.32 (s, 2H, CH_2), 6.03 (dd, J = 3.60 Hz, 1.20 Hz, 1H, pyrrole-H), 6.5 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.31–7.32 (m, 2H, Ar-H and pyrrole-H), 7.35–7.43 (m, 3H, Ar-H), 7.45 (s, 1H, Ar-H), 7.51 (t, J = 7.80 Hz, 1H, Ar-H), 7.68 (t, J = 7.80 Hz, 1H, Ar-H), 7.90 (d, J = 1.80 Hz, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 46.7 (CH_2), 47.4 (CH_2), 95.0, 111.3, 111.6, 113.5, 116.9, 126.6, 127.0, 128.5, 128.8, 128.9, 129.1, 130.1, 133.1, 133.6, 134.6, 136.8, 138.4, 148.7 (C=O); ESI-MS: 427.3 $[M+H]^+$, 429.4 $[M+H]^+$.

4.1.4.22. N_2-(*m*-Chlorobenzyl)-N_4-(2,4-dichlorobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5c2). This compound was obtained from reaction of compound **4c** (0.62 g, 2 mmol) with 2,4-dichlorobenzyl chloride (0.47 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 28%, mp: 164–168 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 5.11 (s, 2H, CH_2), 5.16 (s, 2H, CH_2), 5.97 (dd, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 6.47 (t, J = 3.60 Hz, 1H, pyrrole-H), 7.21 (d, J = 7.80 Hz, 1H, Ar-H), 7.21 (dd, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 7.35 (d, J = 7.20 Hz, 1H, Ar-H), 7.40–7.44 (m, 4H, Ar-H), 7.70 (d, J = 2.40 Hz, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 46.6 (CH_2), 46.7 (CH_2), 95.0, 111.5, 113.4, 127.0, 127.8, 127.9, 128.8, 128.9, 129.3, 129.6, 130.0, 130.6, 133.4, 134.3, 134.6, 136.9, 148.5 (C=O); ESI-MS: 470.2 $[M+H]^+$, 472.2 $[M+H]^+$, 473.2 $[M+H]^+$.

4.1.4.23. N_2-(*m*-Bromobenzyl)-N_4-(*o*-chlorobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5d1). This compound was obtained from reaction of compound **4d** (0.71 g, 2 mmol) with 2-chlorobenzyl chloride (0.39 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 31%, mp: 103–105 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.08 (s, 2H, CH_2), 5.19 (s, 2H, CH_2), 5.55 (dd, J = 3.60 Hz, 1.50 Hz, 1H, pyrrole-H), 6.29 (t, J = 3.60 Hz, 1H, pyrrole-H), 6.94 (dd, J = 3.60 Hz, 1.50 Hz, 1H, pyrrole-H), 7.03 (dd, J = 9.30 Hz, 2.40 Hz, 1H, Ar-H), 7.17–7.26 (m, 3H, Ar-H), 7.38–7.48 (m, 3H, Ar-H), 7.63 (s, 1H, Ar-H), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 46.4 (CH_2), 47.1 (CH_2), 95.0, 111.4, 113.5, 122.7, 126.8, 127.4, 129.0, 129.5, 129.8, 130.3, 131.7, 131.8, 131.9, 132.7, 137.3, 148.5 (C=O); ESI-MS: 480.1 $[M+H]^+$, 482.2 $[M+H]^+$.

4.1.4.24. N_2-(*m*-Bromobenzyl)-N_4-(2,4-dichlorobenzyl)-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine (5d2). This compound was obtained from reaction of compound **4d** (0.71 g, 2 mmol) with 2,4-dichlorobenzyl chloride (0.47 g, 2.4 mmol), and purified by flash column chromatography using dichloromethane/petroleum ether as eluent. Product as a white solid: yield 25%, mp: 113–117 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.07 (s, 2H, CH_2), 5.14 (s, 2H, CH_2), 5.53 (dd, J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 6.30 (t, J = 3.60 Hz, 1H, pyrrole-H), 6.94 (J = 3.60 Hz, 1.80 Hz, 1H, pyrrole-H), 7.00 (s, 1H, Ar-H), 7.12–7.40 (m, 3H, Ar-H), 7.42 (d, J = 2.10 Hz, 1H, Ar-H), 7.45–7.48 (m, 1H, Ar-H), 7.62 (t, J = 1.80 Hz, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 46.5 (CH_2), 46.7 (CH_2), 94.9, 111.6, 113.5, 122.7, 127.5, 127.8, 127.9, 129.2, 129.6, 130.3, 130.6, 131.7, 131.8, 133.4, 134.3, 137.1, 148.5 (C=O); ESI-MS: 516.1 $[M+H]^+$, 514.1 $[M+H]^+$, 518.2 $[M+H]^+$.

4.2. Anti-HIV activity assays

The target compounds were evaluated for their antiviral activities against wild-type HIV-1 strain (HIV-III_B), double mutant HIV-1 III_B strain RES056 (K103N+Y181C) and HIV-2 strain (ROD) in MT-4 cell cultures using the MTT method as previously described.[35] Briefly, stock solutions (10 × final concentration) of test compounds were added in 25 μL volumes to two series of triplicate wells to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial fivefold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays by adding 100 μL medium to the 25 μL stock solution and transferring 25 μL of this solution to another well that contained 100 μL medium using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1(IIIB)[36] or HIV-2 (ROD)[37] stock (50 μL) at 100–300 $CCID_{50}$ (50% cell culture infectious dose-50%) or culture medium was added to either the infected or mock-infected wells of the microtiter plate. Mock-infected cells were used to evaluate the effect of the test compounds on uninfected cells in order to assess their

cytotoxicity. Exponentially growing MT-4 cells were centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, and 50-μL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the viability of the mock-infected MT-4 cell cultures by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

4.3. Inhibition of HIV-1 RT

A RT assay kit produced by Roche was used for the RT inhibition assay. All the reagents for performing the RT reaction are contained in the kit and the ELSIA procedures for RT inhibition assay was performed as described in the kit protocol.[38,39]

Briefly, the reaction mixture containing HIV-1 reverse transcriptase (RT) enzyme, reconstituted template and viral nucleotides (digoxigenin (DIG)-dUTP, biotin-dUTP and dTTP) in the incubation buffer with or without inhibitors was incubated for 1 h at 37 °C. Then, the reaction mixture was transferred to a streptavidine-coated microtitre plate (MTP) and incubated for another 1 h at 37 °C. The biotin-labeled dNTPs that was incorporated in cDNA chain in the presence of RT were bound to streptavidine. The unbound dNTPs were removed by rinsing using washing buffer followed by the addition of anti-DIG-POD working solution into the MTPs. After incubation for 1 h at 37 °C, the DIG-labeled dNTPs incorporated in cDNA were bound to the anti-DIG-POD antibody. The unbound anti-DIG-PODs were removed and the peroxide substrate (ABST) solution was added to the MTPs. Incubation of the reaction mixture at 25 °C until a green color was sufficiently developed for detection. The absorbance of the sample was determined at O.D. 405 nm using microtiter plate ELISA reader. The percentage inhibitory activity of RT inhibitors was calculated by formula as given below:

$$\%\text{Inhibition} = [\text{O.D. value with RT but without inhibitors} - \text{O.D. value with RT and inhibitors}]/[\text{O.D. value with RT and inhibitors} - \text{O.D. value without RT and inhibitors}]$$

The IC_{50} values corresponded to the concentrations of the inhibitors required to inhibit biotin-dUTP incorporation by 50%.

4.4. Molecular simulation

RT crystal structures (PDB code: IFK9), were obtained from the Protein Data Bank. The docking calculations were conducted with the surflex-docking module of Sybyl X-1.1 and the docking results were shown by the software of PyMOL. Briefly, prior to docking, the representative PTTD analogue **5b2** and TTD lead compound Q96652 for docking were optimized for 1000-generations until the maximum derivative of energy became 0.05 kcal/(mol * Å), using the Tripos force field. The protein was prepared by extracting the ligand substructures, removing all the water molecules and other useless small molecules from the complex of HIV-1 RT with efavirine, in parallel with adding polar hydrogen atoms to the protein and assigning Gasteiger–Huckel charges to the ligand and protein. After the SFXC file was generated, the optimized compounds **5b2** and **Q96652** were surflex-docked into the binding pocket of NNRTIs, with the relevant parameters set as defaults, such as the starting conformations per molecule of 0, the angstroms to expand search grid of 6, the max number of rotatable bonds per molecule of 100, and the maximum number of poses per ligand of 20. The binding interaction energy was calculated based on the van der Waals, electrostatic, and torsional energy terms defined in the Tripos force field. The best docking conformation of compounds **5b2** and **Q96652** with the highest score was shown by PyMOL(http://www.pymol.org/), in overlap with the ligand efavirine. The secondary structure of RT are shown in cartoons, and only the key residues for interactions with the inhibitors were shown in sticks and labeled. The potential hydrogen-bondings were presented by dashed lines.

Acknowledgments

We thank K. Erven, K. Uyttersprot and C. Heens for technical assistance with the anti-HIV assays. The financial support from the National Natural Science Foundation of China (NSFC Nos. 81273354, 81102320, 30873133, 30772629, 30371686), Key Project of NSFC for International Cooperation (No. 30910103908), Research Fund for the KU Leuven (GOA 10/014), Doctoral Program of Higher Education of China (No. 20110131130005), and Shandong Provincial Natural Science Foundation (No. ZR2009CM016) is gratefully acknowledged.

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