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Synthesis and anti-HIV evaluation of novel 1,3-disubstituted thieno[3,2-*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxides(TTDDs)

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Abstract—A series of novel 1,3-disubstituted thieno[3,2-*c*] [1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxides (TTDDs), designed as non-nucleoside reverse transcriptase inhibitors (NNRTIs), was synthesized, structurally confirmed by spectral analysis and evaluated for their anti-HIV-1 activities by inhibition of HIV-1(IIIB)-induced cytopathogenicity in MT-4 cell culture. The results showed that TTDD analogues exhibited marked potency as anti-HIV-1 agents. The most active and selective compound was 1-(3-cyano)benzyl-3-benzyl-thieno[3,2-*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxide (**5f**) with a 50% effective concentration (EC_{50}) of 4.0 μM and a selectivity index (SI) of >76. The structure–activity relationship (SAR) is discussed.

1. Introduction

The introduction of highly active anti-retroviral therapy (HAART) has dramatically decreased the morbidity and mortality from the infection by HIV, the causative agent of acquired immunodeficiency syndrome (AIDS). However, the AIDS prevalence remains one of the world's most serious health problems, causing millions of deaths each year.[1] The principal chemotherapeutic agents that have been used in the clinic to block the replication of HIV are the reverse transcriptase inhibitors (RTIs), protease inhibitors (PIs) and a fusion inhibitor. The HIV-1 reverse transcriptase (HIV-1 RT)-catalyzed step in the life cycle of HIV is an attractive target for anti-AIDS drug development. Three classes of HIV-1 RTIs are currently available: nucleoside (and nucleotide) reverse transcriptase inhibitors (NRTIs, NtRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). NNRTIs are structurally diverse compounds specifically targeting at an allosteric site of HIV-1 RT, approximately 10–15 Å from the polymerase active site, causing a distortion of the catalytic aspartate triad.[2,3] The efficacy of NNRTIs, and of the other anti-AIDS agents, has been limited by the emergence of drug-resistant viral strains and possible side effects.[4–6] Therefore, new NNRTIs with more potent activity and lesser toxicity are still needed.

In recent studies aimed at the discovery of new NNRTIs, Dr. S. Vega and his colleagues reported that a series of 2,4-disubstituted-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazines (TTDs) effectively inhibited, at the reverse transcription step, the replication of a variety of HIV-1 strains, including strains that are resistant to AZT (azidothymidine, zidovudine), but not HIV-2 (ROD).[7,8] The prototype compounds **QM96521**, **QM96539** and **QM96639** (Fig. 1), containing a benzyl or 2-halogenated benzyl moiety at the N_2 position and a cyanomethyl chain linked to the N_4 position, were found to selectively inhibit HIV-1 (III-B) replication in MT and CEM cell cultures. The cross-resistance pattern of these compounds against other NNRTI-resistant mutant HIV-1 strains and molecular modelling of the HIV-1 RT binding site were both found to be similar to that of nevirapine. Furthermore, molecular modification by replacement of the N_4 cyanomethyl with a substituted benzyl group led to the discovery of a new precursor

Keywords: HIV-1 NNRTIs; Thieno[3,2-*c*][1,2,6]thiadiazine; Synthesis.
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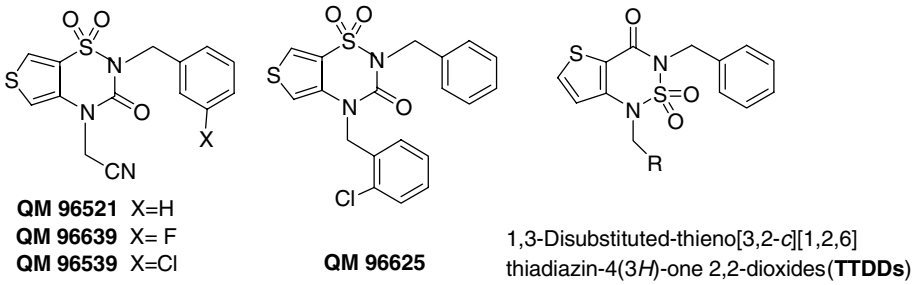


Figure 1. Structures of lead compounds TTDs and newly designed TTDDs.

QM96625 (N_2-benzyl, N_4-2-chlorobenzyl, EC_{50} = 0.1 μM, SI >1190. Fig. 1).[7]

Initial structure–activity relationship analysis together with the molecular modelling disclosed that double substitutions containing π-electrons at the N_2 and N_4 sites of TTDs were necessary for preserving anti-HIV-1 activity. Their structure is in perfect accord with the 'butterfly-like' three-dimensional (3D) model proposed by Schafer et al.[10]

In continuing research of the new HIV-1 NNRTIs and study of the structure–activity relationships of TTD analogues, we designed a series of novel 1,3-disubstituted thieno[3,2-*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxides (TTDDs) based on the general principle of bioisosteric replacement in medicinal chemistry, since TTDDs can be considered as the bioisosteres of TTDs. In the newly designed TTDD analogues, the heterocyclic system of thieno[3,2-*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxide served as a scaffold. The benzyl group at N_3 position, benzyl, substituted benzyl, cyanomethyl and other groups containing π-electrons at N_1 position, which proved essential for anti-HIV activity in TTD analogues, were respectively introduced in the TTDD nuclear ring, so as to arrange molecules in 'butterfly-like' orientation, which is thought to be the determinant for potent anti-HIV-1 activity of the NNRTIs.[9] Here, we report the synthesis of novel TTDD analogues and their anti-HIV activity.

2. Results and discussion

2.1. Chemistry

TTDD acyclonucleosides and two TTDD 1,3-dibenzyl substituted analogues had been synthesized previously and reported as potent human cytomegalovirus (HCMV) inhibitors,[11–13] in which thieno[3,2-*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxide heterocycle was prepared starting from methyl 3-amino-2-thiophene carboxylate **2** with sulfamoyl ($ClSO_2NH_2$) followed by two alkylations at N_1 and N_3 position, respectively.[11] Two weaknesses were found in this process: the first-step alkylation at N_1 position usually produced a mixture of N_1- and N_1, N_3-disubstituted products; the second-step alkylation at N_3 position was found not only difficult to achieve probably due to the steric hindrance, but also the side product of O_4-substituted compound was formed concomitantly with the TTDDs.[13]

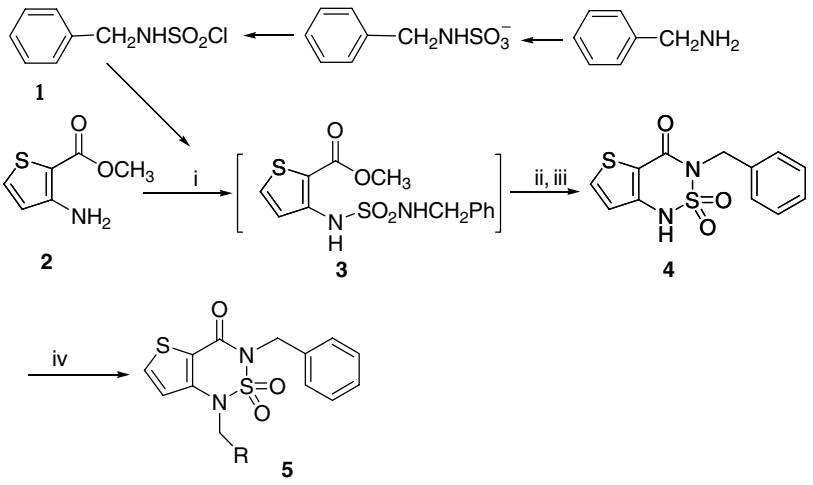
In order to avoid producing a N_1,N_3- and N_1,O_4-disubstituted TTDDs mixture, an unambiguous synthetic pathway was planned for the preparation of the newly designed TTDDs **5a**–**m**. Thus, we chose 3-substituted TTDD **4** as the starting material, which was prepared from methyl 3-amino-2-thiophene carboxylate **2** according to the Cohen and Klarberg procedure,[14] but with an improvement by one-pot reaction. In brief, to an anhydrous toluene solution of the compound **2**, *N*-benzylsulfamoyl chloride **1** dissolved in toluene was added dropwise at room temperature. The obtained solution mixture was stirred for 4 h at 60 °C and cooled to room temperature, then neutralized with 5% aq KOH to adjust pH at 11, forming the solution mixture of thiophene sulfamide derivative **3**. The ring-closure reaction was performed by continuing stirring of the two-phase solution at room temperature for 16 h without separation of compound **3**. The key intermediate of 3-benzyl thieno[3,2-*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxide (**4**) was precipitated after the water layer was separated and treated with conc. HCl to adjust pH at 1, and purified by recrystallization from ethanol in 45% of total yield.

Alkylation of **4** with cyanomethyl, benzyl or substituted benzyl halides, in the presence of equal molar of sodium hydride (NaH) and N, N-dimethylformamide (DMF) solvent, achieved the target 1,3-disubstituted TTDDs **5a**–**m** (Scheme 1).

The starting material *N*-benzylsulfamoyl chloride **1** was prepared by the modified Kloek and Leschinsky procedure.[15] To a cooled (0 °C) solution of benzylamine in CH_2Cl_2 was added chlorosulfonic acid cautiously with vigorous stirring. The resulting suspension was stirred for 0.5 h at room temperature and then filtered. The collected solids of *N*-benzylsulfamate were dissolved in toluene and treated with phosphorus pentachloride. The solution was refluxed for 1 h and the solid was filtered off. The filtrate was concentrated in vacuo, and the syrupy residue (*N*-benzylsulfamoyl chloride **1**) thus obtained was used in the next synthetic step without further purification.

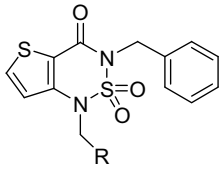
2.2. Anti-HIV evaluation

The activity and cytotoxicity of the newly designed and synthesized TTDDs **4** and **5** were tested in MT-4 cells for inhibition of HIV-1-induced cytopathogenicity. The results are listed in Table 1. The precursors QM96521 and QM96625 were used as reference



Scheme 1. Reagents: (i) toluene/*N*-benzylsulfamoyl chloride; (ii) H_2O/5%KOH; (iii) H_2O/HCl; (iv) DMF/NaH/RCH_2Cl.

Table 1. Structure, anti-HIV-1 activity and cytotoxicity of 1,3-disubstituted thieno[3,2-*c*][1,2,6] thiadiazin-4(3*H*)-one 2,2-dioxide (TTDD) analogues **4** and **5a**–**m**



Compound	CH_2R	HIV-1 (III_B)		
		EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]
4	H	>373.9	373.9	<1
5a	Benzyl	>37.5	37.5	<1
5b	2-Cl-Benzyl	4.8	>298.6	>62
5c	2-Br-Benzyl	7.3	>270.0	>37
5d	2-CN-Benzyl	5.1	>305.5	>59
5e	3-Cl-Benzyl	5.9	>298.6	>51
5f	3-CN-Benzyl	4.0	>305.5	>76
5g	2,4-Cl_2-benzyl	52.1	247.1	5
5h	4-Cl-Benzyl	>8.2	8.2	<1
5i	4-Br-Benzyl	>6.6	6.6	<1
5j	4-CN-Benzyl	>8.5	8.5	<1
5k	4-NO_2-Benzyl	>268.0	268.0	<1
5l	CH_2CN	>5.7	5.7	<1
5m	CH_3	71.0	202.4	3
QM96521[d]	CH_2CN	0.9	502.7	559
QM96625[d]	2-Cl-Benzyl	0.1	>119.0	>1190
Nevirapine[d]		0.03	683	22,767
AZT[d]		0.0007	35.6	50,587

[a] EC_{50}: 50% effective concentration, or concentration of compound required to achieve 50% protection of MT-4 cell from HIV-1 induced cytotoxicity, as determined by the MTT method.

[b] CC_{50}: 50% cytotoxic concentration, or 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] Values for anti-HIV-1 activities reported in Refs.7–9. All data represent mean values for at least two separate experiments.

compounds for comparative purposes; AZT (Zidovudine) and nevirapine were used as the reference drugs. The new TTDDs derivatives were confirmed to be both potent and selective HIV-1 inhibitors.

Compounds **5b**–**f** emerged as the most active HIV-1 inhibitors with EC_{50} values in the range of 4–7 μM and the selective indexes (SI, ratio of CC_{50} for cell growth to EC_{50} for virus replication) in the range between 37 and 76. The most active and selectivity TTDD derivative was compound **5f** (N_1- 3-Cl-benzyl, N_3-benzyl) with an EC_{50} value of 4.0 μM and selective index SI >76. Compounds **5g** and **5m** proved slightly active against HIV-1, while compounds **4** and **5k** were totally

inactive. Other compounds displayed no selectivities because of the high cytotoxicity, compounds **5h–j** and **5l** being cytotoxic for MT-4 cells at concentrations lower than 10 μM. In addition, all synthesized compounds were screened for activity against HIV-2 (strain ROD), but none of the compounds was found to inhibit HIV-2 replication.

These biological data led us to take into consideration the structure–activity relationship (SAR) analysis of these compounds. The active anti-HIV-1 agents were the N_1, N_3- disubstituted TTDDs, N_3-monosubstituted derivative **4** was inactive, which is accordant with the results obtained for the SAR in the TTD series. Apparently, the molecule that lacked the N_1-substituent did not meet the structural requirements of the 'butterfly-like' conformation, which seems particularly important in other known NNRTIs, i.e. TIBO, α-APA, nevirapine and thiazolidones.[16] The essential substituents are arylmethyl groups such as benzyl, or substituted benzyl. Other groups having no π-electron character, such as alkyl (**5m**), lost or decreased the potency and selectivity against HIV-1 replication.

In the N_1, N_3-disubstituted benzyl series (**5a–k**), the activities of the substituents at N_1-benzyl group were found in the following declining trends: the ortho and meta substituted benzyl (**5b–f**) >2,4-Cl_2-benzyl (**5g**) > para substituted benzyl (**5h–k**). The results demonstrated that the N_1-*o*-substituted benzyl groups were still effective substituents, as previously reported for the SAR of the TTDs,[7] and further indicated that the introduction of *m*-substituted benzyl group in the N_1 position could also result in the congeners endowed with the equivalent potency and selectivity. Whereas the N_1para (*p*) substituted benzyls, such as 2,4-dichlorobenzyl **5g**, *p*-halogenatedbenzyl **5h–i**, *p*-cyanobenzyl **5j** and *p*-nitrobenzyl **5k**, had reduced or abolished activities in contrast to the ortho and meta monosubstituted benzyl anologues, which might reflect a spatial restriction in the target site of the HIV-1 enzyme. This feature can be used in the design of new TTDDs.

The newly designed TTDD compounds presented decreased activities, leading us to hypothesize that the changed positions of the heteroatoms N and S in the TTDD heterocycle, giving rise to the changed direction of 'butterfly wing', affected the binding orientation in the aromatic-rich non-nucleoside binding site of HIV-1 RT, surrounded by the aromatic side chains, such as Tyr181, Tyr188, Phe227 and Trp 229. In particular, it affected π–π interaction of the phenyl ring with the Tyr181 side chain.[5,7] Molecular modelling of TTDDs by AutoDocking analysis is underway.

3. Conclusions

In summary, we designed and synthesized a series of novel 1,3-disubstituted thieno[3,2-*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxides (TTDDs), which were structurally confirmed by IR, ^{1}H NMR, ^{13}C NMR and MS spectral analysis and evaluated for their anti-HIV (HIV-1 III_B and HIV-2 ROD) activities by inhibition of HIV-induced cytopathogenicity in MT-4 cell cultures. The results showed that TTDD analogues exhibited high potency as anti-HIV-1 agents. The most active and selective compound was **5f** with an EC_{50} value of 4.0 μM and a SI of > 76. TTDD analogues may seem promising for further activity optimization studies.

4. Experimental

4.1. Chemistry

Melting points were determined on a Gallenkamp capillary apparatus and are uncorrected.^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were obtained on a Bruker Avance-600 instrument in the indicated solvent. Chemical shifts are expressed in δ units with tetramethylsilane (TMS) as internal reference. Infrared spectra (IR) were recorded with a Nexus 470FT-IR Spectrometer. Mass spectra were recorded on a LC Autosampler Device: Standard G1313A instrument. All compounds were routinely checked by TLC on pre-coated silica gel G plates with fluorescent indicator at 254 nm, which were prepared in our laboratory. Developed plates were visualized by UV light. Solvents were of reagent grade and, when necessary, were purified and dried by standard methods. Concentration of the reaction solutions involved the use of rotary evaporator under reduced pressure.

4.1.1. *N*-Benzylsulfamoyl chloride (1). To a 0 °C cooled and stirred solution of benzylamine (10.7 g, 0.1 mol) in CH_2Cl_2 (100 mL) was added chlorosulfonic acid (3.49 g, 0.03 mol) cautiously. The resulting suspension was stirred for 0.5 h at room temperature and then filtered. The collected solids of *N*-benzylsulfamate were dissolved in toluene (50 mL) and treated with phosphorus pentachloride (6.24 g, 0.03 mol). A mild exothermic reaction took place. The solution was refluxed for 1 h and the solid was filtered off. The filtrate was concentrated in vacuo and *N*-benzylsulfamoyl chloride 1 was obtained as syrupy residue.

4.1.2. 3-Benzyl-1*H*-thieno[3,2-*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxide (4). To an anhydrous toluene solution of the 3-amino-2-thiophene carboxylate (**2**) (2.30 g, 0.015 mol), *N*-benzylsulfamoyl chloride (**1**) dissolved in toluene was added dropwise at room temperature. The obtained mixture solution was stirred for 4 h at 60 °C and cooled to room temperature, then neutralized with 5% aq KOH to adjust pH at 11, forming the mixture solution of thiophene sulfamide derivative **3**. The ring-closure reaction was performed without separation of compound **3** by continuing stirring of the two-phase solution at room temperature for 16 h. The intermediate **4** was precipitated after the water layer was separated and treated with conc. HCl (pH 1), and purified by recrystallization from ethanol in 45% of total yield. mp 180–182 °C.^{1}H NMR (DMSO-d_6) δ: 8.02 (d, 1H, J = 5.2 Hz, thiophene), 7.35–7.26 (m, 5H, benzene), 7.25 (m, 1H, NH), 6.93 (d, 1H, J = 5.1 Hz, thiophene), 4.97 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 158.67

(C=O), 137.22(C-1), 135.28, 128.52(3C), 127.97(3C), 127.55, 121.41(C-7), 44.30 (CH_2–N_3); IR (KBr, cm^{-1}): 3274 (N_1-H), 3094 (Ar–H), 1645 (C=O), 1490 (Ar, C–C), 1373, 1179 (SO_2); ESI-MS: *m/z* 295.4 [M + 1].

4.1.3. General procedure for the preparation of 1,3-disubstituted TTDDs (5a–m). To a solution of the 3-benzyl-1*H*-thieno[3,2-*c*] [1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxide (**4**) (1.2 g, 4 mmol) in dry DMF (15 mL), under N_2, was added sodium hydride (60% dispersion in mineral oil, 0.24 g, 6 mmol) by portions, maintaining the temperature below 10 °C. After 15 min, the alkyl halide (6 mmol) was added and the reaction mixture was stirred at 50–80 °C for 16–24 h. The solvent was evaporated in vacuo to dryness and the crude oil washed with 5% aq KOH, extracted with CH_2Cl_2, evaporated in vacuo and the crude product was recrystallized from the appropriate solvent.

4.1.3.1. 1,3-Dibenzyl-thieno[3,2-*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxide (5a). Reagents: Compound **4** (1.2 g, 4 mmol), benzyl bromide (1.0 g, 6 mmol). Conditions: 60 °C, 10 h. Purification: recrystallization. Yield 0.65 g (42.3%) as a white solid: mp 96–98 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.21 (d, 1H, J = 5.4 Hz, thiophene), 7.43–7.13 (m, 11H, benzene and thiophene), 5.16 (s, 2H, CH_2), 4.97 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 157.59 (C=O), 143.43(C-1), 136.54(C-1), 136.12, 134.57, 128.83(2C), 128.75(2C), 128.55,128.23(4C), 128.03, 121.32, 118.72(C-7), 54.02 (CH_2–N_1), 45.80 (CH_2–N_3); IR (KBr, cm^{-1}): 3104 (Ar–H), 1666 (C=O), 1539 (Ar, C–C), 1371, 1173 (SO_2); ESI-MS: *m/z* 385.2 [M + 1].

4.1.3.2. 1-(2-Chloro)benzyl-3-benzyl-thieno[3,2-*c*][1,2,6]-thiadiazin-4(3*H*)-one 2,2-dioxide (5b). Reagents: Compound **4** (1.2 g, 4 mmol), 2-chlorobenzyl chloride (1.0 g, 6 mmol). Conditions: 80 °C, 18 h. Purification: recrystallization. Yield 0.50 g (30%) as a white solid: mp 83–84 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.16 (d, 1H, J = 5.3 Hz, thiophene), 7.49 (d, 1H, J = 7.9 Hz benzene H-3), 7.36–7.27 (m, 8H, benzene), 7.09 (d, 1H, J = 5.3 Hz, thiophene), 5.20 (s, 2H, CH_2), 5.05 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 157.64 (C=O), 143.60(C-1), 136.42(C-1), 136.11, 132.50, 132.40, 130.23, 129.93, 129.74, 128.7 (2C), 128.15(2C), 127.98, 127.72, 121.04, 118.50(C-7), 51.82 (CH_2–N_1), 45.98 (CH_2–N_3); IR (KBr, cm^{-1}): 3109 (Ar–H), 1668 (C=O), 1538 (Ar, C–C), 1373, 1168 (SO_2); ESI-MS: *m/z* 419.2 [M + 1].

4.1.3.3. 1-(2-Bromo)benzyl-3-benzyl-thieno[3,2-*c*][1,2,6]-thiadiazin-4(3*H*)-one 2,2-dioxide (5c). Reagents: Compound **4** (1.2 g, 4 mmol), 2-bromobenzyl bromide (1.5 g, 6 mmol). Conditions: 60 °C, 8 h. Purification: recrystallization. Yield 0.6 g (32%) as a red solid: mp 103–105 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.16 (d, 1H, J = 5.3 Hz, thiophene), 7.67 (d, 1H, J = 7.8 Hz benzene H-3), 7.36–7.23 (m, 8H, benzene), 7.03 (d, 1H, J = 5.3 Hz, thiophene), 5.16 (s, 2H, CH_2); 5.06 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 157.72 (C=O), 143.70(C-1), 136.49(C-1), 136.20, 134.16, 133.27, 130.45, 129.57, 128.75(2C), 128.43, 128.22(2C), 128.04, 122.50, 120.97, 118.53(C-7), 54.23 (CH_2–N_1), 46.09 (CH_2–N_3); IR (KBr, cm^{-1}): 3108 (Ar–H), 1669 (C=O), 1538 (Ar, C–C), 1373, 1167 (SO_2); ESI-MS: *m/z* 463.2 [M^+], 465.2 [M + 2].

4.1.3.4. 1-(2-Cyano)benzyl-3-benzyl-thieno[3,2-*c*][1,2,6] thiadiazin-4(3*H*)-one 2,2-dioxide (5d). Reagents: Compound **4** (1.2 g, 4 mmol), 2-cyanobenzyl chloride (0.91 g, 6 mmol). Conditions: 80 °C, 18 h. Purification: recrystallization. Yield 0.9 g (55%) as a white solid: mp 102–103 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.22 (d, 1H, J = 5.4 Hz, thiophene), 7.89 (d, 1H, J = 7.8 Hz benzene H-3), 7.65–7.30 (m, 8H, benzene), 7.22 (d, 1H, J = 5.3 Hz, thiophene), 5.30 (s, 2H, CH_2), 5.01 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 157.57 (C=O), 143.63(C-1), 138.47(C-1), 136.61, 136.06, 133.79, 129.31, 128.71 (3C), 128.11 (3C), 127.98, 121.27, 119.01 (CN), 117.11(C-7), 111.05(C-2), 52.77 (CH_2–N_1), 46.06 (CH_2–N_3); IR (KBr, cm^{-1}): 3110 (Ar–H), 2231 (CN), 1656 (C=O), 1535 (Ar, C–C), 1382, 1176 (SO_2); ESI-MS: *m/z* 410.5 [M + 1].

4.1.3.5. 1-(3-Chloro)benzyl-3-benzyl-thieno[3,2-*c*][1,2,6]-thiadiazin-4(3*H*)-one 2,2-dioxide (5e). Reagents: Compound **4** (1.2 g, 4 mmol), 3-chlorobenzyl chloride (1.0 g, 6 mmol). Conditions: 80 °C, 18 h. Purification: recrystallization. Yield 0.75 g (45%) as a white solid: mp 108–109 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.23 (d, 1H, J = 5.3 Hz, thiophene), 7.41–7.28 (m, 9H, benzene), 7.10 (d, 1H, J = 5.3 Hz, thiophene), 5.18 (s, 2H, CH_2), 4.99 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 157.53 (C=O), 143.39(C-1), 137.28(C-1), 136.78, 136.08, 133.42, 130.80, 128.75(2C), 128.52, 128.12(2C), 128.03(2C), 126.65, 121.05, 118.50(C-7), 53.13 (CH_2–N_1), 45.86 (CH_2–N_3); IR (KBr, cm^{-1}): 3112 (Ar–H), 1660 (C=O), 1535 (Ar, C–C), 1375, 1174 (SO_2); ESI-MS: *m/z* 419.3 [M + 1].

4.1.3.6. 1-(3-Cyano)benzyl-3-benzyl-thieno[3,2-*c*][1,2,6]-thiadiazin-4(3*H*)-one 2,2-dioxide (5f). Reagents: Compound **4** (1.2 g, 4 mmol), 3-cyanobenzyl chloride (0.91 g, 6 mmol). Conditions: 80 °C, 24 h. Purification: recrystallization. Yield 0.80 g (49%) as a white solid: mp 83–85 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.25 (d, 1H, J = 5.3 Hz, thiophene), 7.80 (d, 1H, J = 7.3 Hz benzene H-6), 7.66 (s, 1H, benzene H-2), 7.48–7.28 (m, 8H, benzene and thiophene), 5.23 (s, 2H, CH_2); 4.99 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 157.43 (C=O), 143.24(C-1), 136.89(C-1), 136.45, 136.00, 132.75, 132.33, 131.68, 130.15, 128.72 (3C), 128.05 (3C), 121.05 (CN), 118.52(C-7), 111.70(C-3), 52.86 (CH_2–N_1), 45.76 (CH_2–N_3); IR (KBr, cm^{-1}): 3107 (Ar–H), 2231(CN), 1658 (C=O), 1534 (Ar, C–C), 1375, 1172 (SO_2); ESI-MS: *m/z* 410.5 [M + 1].

4.1.3.7. 1-(2,4-Dichloro)benzyl-3-benzyl-thieno[3,2-*c*]-[1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxide (5g). Reagents: Compound **4** (1.2 g, 4 mmol), 2,4-dichlorobenzyl chloride (1.17 g, 6 mmol). Conditions: 80 °C, 24 h. Purification: recrystallization. Yield 0.95 g (52%) as a white solid: mp 112–114 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.18 (d, 1H, J = 5.3 Hz, thiophene), 7.67–7.28 (m, 8H, benzene), 7.15 (d, 1H, J = 5.3 Hz, thiophene), 5.18 (s, 2H, CH_2); 5.03 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 157.61 (C=O), 143.54(C-1), 136.48(C-1), 136.07, 133.91, 133.51, 131.79, 131.28, 129.43, 128.68(2C), 128.12 (2C), 127.98, 127.88, 121.22, 118.70(C-7), 51.51 (CH_2–N_1), 45.97 (CH_2–N_3); IR (KBr, cm^{-1}): 3095 (Ar–H), 1652 (C=O), 1536 (Ar, C–C), 1375, 1185 (SO_2); ESI-MS: *m/z* 453.3 [M^+].

4.1.3.8. 1-(4-Chloro)benzyl-3-benzyl-thieno[3,2-*c*][1,2,6]-thiadiazin-4(3*H*)-one 2,2-dioxide (5h). Reagents: Compound **4** (1.2 g, 4 mmol), 4-chlorobenzyl chloride (1.0 g, 6 mmol). Conditions: 80 °C, 18 h. Purification: recrystallization. Yield 0.60 g (36%) as a white solid: mp 77–79 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.22 (d, 1H, J = 5.3 Hz, thiophene), 7.42–7.14 (m, 10H, benzene and thiophene), 5.15 (s, 2H, CH_2); 4.98 (s, 2H, CH_2); ^{13}C NMR (DEPT) (DMSO-d_6) δ: 157.48 (C═O), 143.22(C-1), 136.64(C-1), 136.02, 133.57, 133.16, 130.07(2C), 128.78(2C), 128.66 (2C), 128.17(2C), 127.98, 121.24, 118.75(C-7), 53.26 (CH_2–N_1), 45.71 (CH_2–N_3); IR (KBr, cm^{-1}): 3112 (Ar–H), 1672 (C═O), 1535 (Ar, C–C), 1376, 1173 (SO_2); ESI-MS: *m*/*z* 419.2 [M + 1].

4.1.3.9. 1-(4-Bromo)benzyl-3-benzyl-thieno[3,2-*c*][1,2,6] thiadiazin-4(3*H*)-one 2,2-dioxide (5i). Reagents: Compound **4** (1.2 g, 4 mmol), 4-bromobenzyl chloride (1.5 g, 6 mmol). Conditions: 60 °C, 8 h. Purification: recrystallization. Yield 0.50 g (27%) as a white solid: mp 91–93 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.22 (d, 1H, J = 5.3 Hz, thiophene), 7.45–7.09 (m, 10H, benzene and thiophene), 5.14 (s, 2H, CH_2), 4.98 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 157.48 (C═O), 143.22(C-1), 136.66(C-1), 136.01, 133.99, 131.71(2C), 130.36(2C), 128.67(2C), 128.17(2C), 127.97, 121.78, 121.21, 118.69 (C-7), 53.29 (CH_2–N_1), 45.71 (CH_2–N_3); IR (KBr, cm^{-1}): 3111(Ar–H), 1673 (C═O), 1536 (Ar, C–C), 1374, 1171 (SO_2); ESI-MS: *m*/*z* 463.2 [M$^+$], 465.2 [M + 2].

4.1.3.10. 1-(4-Cyano)benzyl-3-benzyl-thieno[3,2-*c*][1,2,6]-thiadiazin-4(3*H*)-one 2,2-dioxide (5j). Reagents: Compound **4** (1.2 g, 4 mmol), 4-cyanobenzyl chloride (0.91 g, 6 mmol). Conditions: 80 °C, 24 h. Purification: recrystallization. Yield 0.50 g (31%) as a white solid: mp 118–119 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.24 (d, 1H, J = 5.3 Hz, thiophene), 7.74–7.73 (q, 2H, benzene H-4, H-5), 7.41–7.28 (m, 8H, benzene and thiophene), 5.27 (s, 2H, CH_2), 4.99 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 157.45 (C═O), 143.29(C-1), 140.38(C-1), 136.90, 136.00, 132.74 (2C), 128.79(2C), 128.69(2C), 128.18(2C), 128.02, 120.92, 118.62 (CN), 118.35(C-7), 111.20(C-4), 53.21 (CH_2–N_1), 45.76 (CH_2–N_3); IR (KBr, cm^{-1}): 3113 (Ar–H), 2226 (CN),1686 (C═O), 1533 (Ar, C–C), 1366, 1177 (SO_2); ESI-MS: *m*/*z* 410.5 [M + 1].

4.1.3.11. 1-(4-Nitro)benzyl-3-benzyl-thieno[3,2-*c*][1,2,6]-thiadiazin-4(3*H*)-one 2,2-dioxide (5k). Reagents: Compound **4** (1.2 g, 4 mmol), 4-nitrobenzyl bromide (0.86 g, 6 mmol). Conditions: 60 °C, 8 h. Purification: recrystallization. Yield 0.80 g (47%) as a yellow solid: mp 147–149 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.24 (d, 1H, J = 5.3 Hz, thiophene), 8.12–8.10 (m, 2H, benzene H-3, H-5), 7.43–7.29 (m, 8H, benzene and thiophene), 5.32 (s, 2H, CH_2), 5.00 (s, 2H, CH_2); ^{13}C NMR (DEPT) (DMSO-d_6) δ: 157.46 (C═O), 147.39(C-1), 143.28(C-1), 142.37(C-4), 136.95, 136.01, 129.15(2C), 128.66(2C), 128.25(2C), 128.00, 123.93 (2C), 120.95, 118.43(C-7), 52.98 (CH_2–N_1), 45.78 (CH_2–N_3); IR (KBr, cm^{-1}): 3098 (Ar–H), 1684 (C═O), 1520 (Ar, C–C), 1351 (NO_2), 1380, 1177 (SO_2); ESI-MS: *m*/*z* 430.4 [M + 1].

4.1.3.12. 1-Cynomethyl-3-benzyl-thieno[3,2-*c*][1,2,6]-thiadiazin-4(3*H*)-one 2,2-dioxides (5l). Reagents: Compound **4** (1.2 g, 4 mmol), chloroacetonitrile (0.45 g, 6 mmol). Conditions: 80 °C, 24 h. Purification: recrystallization. Yield 0.80 g (60%) as a white solid: mp 148–150 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.35 (d, 1H, J = 5.3 Hz, thiophene), 7.53–7.35 (m, 5H, benzene), 7.33 (d, 1H, J = 7.14 Hz, thiophene), 5.24 (s, 2H, CH_2); 5.07 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ: 157.30 (C═O), 142.11(C-1), 137.25, 135.75, 128.71(2C), 128.11(2C), 128.09, 122.01, 120.93(C-7), 114.69 (CN), 46.45 (CH_2–N_3), 39.24 (CH_2–N_1); IR (KBr, cm^{-1}): 3091 (Ar–H), 1686 (C═O), 1527 (Ar, C–C), 1384, 1185 (SO_2); ESI-MS: *m*/*z* 334.5 [M + 1].

4.1.3.13. 1-Methyl-3-benzyl-thieno[3,2-*c*][1,2,6]thiadiazin-4(3*H*)-one 2,2-dioxides (5m). Reagents: Compound **4** (1.2 g, 4 mmol), $(CH_3)_2SO_4$ (0.76 g, 6 mmol). Conditions: 60 °C, 8 h. Purification: recrystallization. Yield 0.30 g (24.3%) as a white solid: mp 94–96 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 8.24 (d, 1H, J = 5.3 Hz, thiophene), 7.41–7.36 (m, 5H, benzene), 7.35, 5.04 (s, 2H, CH_2), 3.41 (3H); ^{13}C NMR (DMSO-d_6) δ: 157.66 (C═O), 145.34(C-1), 136.56, 136.27, 128.70, 127.94(2C), 127.92(2C), 121.06, 120.95, 117.24, 45.75 (CH_2–N_3), 37.06 (CH_3-N_1); IR (KBr, cm^{-1}): 3102 (Ar–H), 1666 (C═O), 1540 (Ar, C–C), 1368, 1175 (SO_2); ESI-MS: *m*/*z* 309.5 [M + 1].

4.2. Anti-HIV activity assays

The anti-HIV activity and cytotoxicity were evaluated against HIV-1 strain IIIB and HIV-2 (ROD) in MT-4 cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.[17] MT-4 cells were suspended in culture medium at 1×10^5 cells/ mL and infected with HIV at a multiplicity of infection (MOI) of 0.02. Immediately after viral infection, 100 μL of the cell suspension was placed in each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. Stock solutions of the test compounds were prepared in DMSO at a concentration of 10 mg/ml. After 4 days of incubation at 37 °C, the number of viable cells was determined using the MTT method. Compounds were tested in parallel for cytotoxic effects in uninfected MT-4 cells.

The 50% effective antiviral concentration (EC_{50}) was defined as the compound concentration required to protect 50% of the virus-infected cells against viral cytopathicity. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration required to reduce the viability of mock-infected cells by 50%. The symbol '>' is used to indicate the highest concentration at which the compounds were tested and still found to be non-cytotoxic. Average EC_{50} and CC_{50} values for at least two separate experiments are presented.

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