Synthesis and anti-HIV evaluation of novel 1,3-disubstituted thieno[3,2-c][1,2,6]thiadiazin-4(3H)-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(3H)-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(3H)-one 2,2-dioxide (5f) with a 50% effective concentration (EC50) 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-2H,4H-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 N2 position and a cyanomethyl chain linked to the N4 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 N4 cyanomethyl with a substituted benzyl group led to the discovery of a new precursor
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 \( 5a-\text{m} \). 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,\(^1\) 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 thiadiazin-4(3H)-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 \( N \)-dimethylformamide (DMF) solvent, achieved the target 1,3-disubstituted TTDDs \( 5a-\text{m} \) (Scheme 1).

The starting material \( N \)-benzylsulfamoyl chloride \( 1 \) was prepared by the modified Kloeck and Leschinsky procedure.\(^1\) 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.
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.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>CH_2R</th>
<th>HIV-1 (IC_{50})</th>
<th>CC_{50} (μM)</th>
<th>SI</th>
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<tr>
<td>4</td>
<td>H</td>
<td>&gt;373.9</td>
<td>373.9</td>
<td>&lt;1</td>
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<tr>
<td>5a</td>
<td>Benzyl</td>
<td>&gt;37.5</td>
<td>37.5</td>
<td>&lt;1</td>
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<tr>
<td>5b</td>
<td>2-Cl-Benzyl</td>
<td>4.8</td>
<td>&gt;298.6</td>
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<tr>
<td>5c</td>
<td>2-Br-Benzyl</td>
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<td>&gt;270.0</td>
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<tr>
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<td>52.1</td>
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<td>&gt;8.2</td>
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<td>AZTd</td>
<td></td>
<td>0.0007</td>
<td>35.6</td>
<td>50,587</td>
</tr>
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</table>

*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.*
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 N1, N1-disubstituted TTDDs, N1-monosubstituted derivative 4 was inactive, which is accordan with the results obtained for the SAR in the TTD series. Apparently, the molecule that lacked the N1-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. 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 N1, N1-disubstituted benzyl series (5a-k), the activities of the substituents at N1-benzyl group were found in the following declining trends: the ortho and meta substituted benzyl (5b-f) > 2,4-Cl2-benzyl (5g) > para substituted benzyl (5h-k). The results demonstrated that the N1-o- or substituted benzyl groups were still effective substituents, as previously reported for the SAR of the TTDs, and further indicated that the introduction of m-substituted benzyl group in the N1 position could also result in the congeners endowed with the equivalent potency and selectivity. Whereas the N1 para (p) substituted benzylics, such as 2,4-dichlorobenzyl 5g, p-halogenated benzyl 5h-i, p-cyanobenzyl 5j and p-nitrobenzyl 5k, had reduced or abolished activities in contrast to the ortho and meta monosubstituted benzyl analogues, 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 Trp229. In particular, it affected π-π interaction of the phenyl ring with the Tyr181 side chain. 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]thiaziazin-4(3H)-one 2,2-dioxides (TTDDs), which were structurally confirmed by IR, 1H NMR, 13C NMR and MS spectral analysis and evaluated for their anti-HIV (HIV-1 IC50 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 EC50 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. 1H NMR (600 MHz) and 13C 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 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 CH2Cl2 (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 was 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-1H-thieno[3,2-c][1,2,6]thiaziazin-4(3H)-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 sulfamate 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. 1H NMR (DMSO-d6) 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, CH2); 13C NMR (DMSO-d6) δ: 158.67
4.1.3. General procedure for the preparation of 1,3-disubstituted TTDDs (5a–m).

To a solution of the 3-benzyl-1H-thieno[3,2-c][1,2,6]thiadiazin-4(3H)-one (5a).

Reagents: Compound 1,3-dibenzyl-thieno[3,2-c][1,2,6]thiadiazin-4(3H)-one 2,2-dioxide (5a).

Reagents: Composite 1,3-dibenzyl-thieno[3,2-c][1,2,6]thiadiazin-4(3H)-one 2,2-dioxide (5b).

Reagents: Compound 1-(2-Chloro)benzyl-3-benzyl-thieno[3,2-c][1,2,6]thiadiazin-4(3H)-one 2,2-dioxide (5c).

Reagents: Compound 1-(2-Bromo)benzyl-3-benzyl-thieno[3,2-c][1,2,6]thiadiazin-4(3H)-one 2,2-dioxide (5d).

Reagents: Compound 1-(3-Chloro)benzyl-3-benzyl-thieno[3,2-c][1,2,6]thiadiazin-4(3H)-one 2,2-dioxide (5e).

Reagents: Compound 1-(3-Cyanobenzyl)-3-benzyl-thieno[3,2-c][1,2,6]thiadiazin-4(3H)-one 2,2-dioxide (5f).

Reagents: Compound 1-(2,4-Dichloro)benzyl-3-benzyl-thieno[3,2-c][1,2,6]thiadiazin-4(3H)-one 2,2-dioxide (5g).

(C=O), 137.22(C-1), 135.28, 128.52(C3), 127.97(C3), 127.55, 121.41(C-7), 44.30(CH2–N3); IR (KBr, cm–1): 3274(N–H), 3094(Ar–H), 1645(C=C), 1490(Ar–C–C), 1373, 1179(SO2); ESI-MS: m/z 295.4 [M + 1].
4.1.3.8. 1-(4-Chloro)benzyl-3-benzyl-thieno[3,2-c][1,2,6]thiadiazin-4(3H)-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); 1H NMR (DMSO-d6): δ: 8.22 (d, 1H, J = 5.3 Hz, thiophene), 7.42–7.14 (m, 10H, benzene and thiophene), 5.15 (s, 2H, CH2); 4.98 (s, 2H, CH2); 13C NMR (DEPT) (DMSO-d6): 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 (CH2–N1), 45.71 (CH2–N2); IR (KBr, cm–1): 3112 (Ar–H), 1672 (C=O), 1535 (Ar–C, C), 1376, 1173 (SO2); 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(3H)-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); 1H NMR (DMSO-d6): δ: 8.22 (d, 1H, J = 5.3 Hz, thiophene), 7.45–7.09 (m, 10H, benzene and thiophene), 5.14 (s, 2H, CH2), 4.98 (s, 2H, CH2); 13C NMR (DEPT) (DMSO-d6): 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 (CH2–N1), 45.71 (CH2–N2); IR (KBr, cm–1): 3111(Ar–H), 1673 (C=O), 1536 (Ar–C, C), 1374, 1171 (SO2); 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(3H)-one 2,2-dioxide (5j). Reagents: Compound 4 (1.2 g, 4 mmol), 4-cyanobenzyl chloride (0.91 g, 4 mmol). Conditions: 80 °C, 6 h. Purification: recrystallization. Yield 0.80 g (47%) as a white solid: mp 94–96 °C (EtOH); 1H NMR (DMSO-d6): δ: 8.24 (d, 1H, J = 5.3 Hz, thiophene), 7.41–7.36 (m, 5H, benzene), 7.35, 5.04 (s, 2H, CH2), 3.41 (3H); 13C NMR (DEPT) (DMSO-d6): δ: 157.66 (C=O), 145.34(C-1), 136.56, 136.27, 127.80, 127.94(2C), 127.92(2C), 121.06, 120.95, 117.24, 45.75 (CH2–N2), 37.06 (CH3–N2); IR (KBr, cm–1): 3102 (Ar–H), 1668 (C=O), 1540 (Ar–C, C), 1368, 1175 (SO2); ESI-MS: m/z 393.5 [M + 1].

4.1.3.11. 1-(4-Nitro)benzyl-3-benzyl-thieno[3,2-c][1,2,6]thiadiazin-4(3H)-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); 1H NMR (DMSO-d6): 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, CH2), 5.00 (s, 2H, CH2); 13C NMR (DEPT) (DMSO-d6): δ: 157.46 (C=O), 143.28(C-1), 143.27(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 (CH2–N1), 45.78 (CH2–N2); IR (KBr, cm–1): 3098 (Ar–H), 1684 (C=O), 1520 (Ar–C, C), 1351 (NO2), 1380, 1177 (SO2); ESI-MS: m/z 430.4 [M + 1].

4.1.3.12. 1-Cyromethyl-3-benzyl-thieno[3,2-c][1,2,6]thiadiazin-4(3H)-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); 1H NMR (DMSO-d6): 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, CH2); 5.07 (s, 2H, CH2); 13C NMR (DMSO-d6): 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 (CH2–N2), 39.24 (CH2–N3); IR (KBr, cm–1): 3091 (Ar–H), 1686 (C=O), 1527 (Ar–C, C), 1384, 1185 (SO2); ESI-MS: m/z 334.5 [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 × 10^4 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 (EC50) was defined as the compound concentration required to protect 50% of the virus-infected cells against viral cytopathicity. The 50% cytotoxic concentration (CC50) 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 EC50 and CC50 values for at least two separate experiments are presented.

Acknowledgments

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References and notes