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Synthesis and Anti-HIV Activity of 1,1,3-Trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazines (TTDs): A New Family of HIV-1 Specific Non-Nucleoside Reverse Transcriptase Inhibitors

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Abstract—The anti-HIV activity of a novel series of 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazines (TTDs) has been described. The compounds were synthesized via Curtius rearrangement of appropriate sulfamoylcarboxy azides which, in turn, were prepared from known starting materials. Several 4-substituted-2-benzyl-derivatives were found to selectively inhibit human immunodeficiency virus type 1 [HIV-1 (III_B)] replication in MT-4 and CEM cells. These TTDs were also effective against other strains of HIV-1 (RF, HE, MN, NDK), including those that are resistant to AZT, but not against HIV-2 (ROD) or simian immunodeficiency virus [SIV(MAC251)] at subtoxic concentrations. Some of the test compounds exhibited antiviral activity against L100I RT mutant virus, but significantly lost antiviral activity against K103N, V106A, E138K, Y181C and Y188H RT mutant viruses. Compounds **6d**, **6f** and **6g** were inhibitory to HIV-1 RT at concentrations that rank between 16.4 and 59.8 μM (nevirapine: $IC_{50} = 4.5$ μM against HIV-1 RT). Inhibition of HIV-1 RT by compound **6g** was purely non-competitive with respect to the natural substrate (dGTP), which is in agreement with the nature of inhibition shown by other NNRTIs such as nevirapine and delarvidine. A structure–activity relationship was established for the anti-HIV activity of these heterocyclic compounds. TTDs represent a new chemical class of non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs).

Introduction

Since the early 1980s, more than 26 million people have been infected with human immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome (AIDS).[1] By the year 2000, the World Health Organization (WHO) estimates that 30–40 million people will have been infected with HIV and that 10 million people will have developed AIDS.[2] Efforts to eradicate the disease have intensified in the last 10 years and, as a consequence, massive resources have been focused on both the study of HIV and the development of antiretroviral agents.[3] The discovery that the virus requires the catalytic activity of several unique enzymes for its life cycle made them ideal therapeutic targets. Research strategies have most particularly targeted inhibition of reverse transcriptase (RT), a multi-purpose enzyme, responsible for the synthesis of double-stranded viral DNA from proviral RNA for subsequent incorporation into host cell chromosome, and therefore essential for the replication of HIV.[4]

Three main classes of RT inhibitors have been hitherto discovered: the 2′,3′-dideoxynucleoside (ddN) and acyclic nucleoside phosphonate (ANP) analogues which compete with natural substrates and function as chain terminators—AZT, ddI, ddC, d4T, 3TC, PMEA, PMPA, etc.—and the non-nucleoside inhibitors (NNRTIs) which bind to an allosteric site on the enzyme at a close distance from its polymerase active site. These latter compounds, in contrast with nucleoside analogues, are highly specific for HIV-1 group M isolates, do not bind to HIV-2 reverse transcriptase and are effective without toxic effects at relatively high concentrations.[5] As in the case of nucleoside congeners, the rapid emergence of NNRTI-resistant viral strains has greatly limited the clinical efficacy of these compounds until the recent encouraging results with combination therapy.[6–9]

Key words: Antivirals; enzyme inhibitors; 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazines (TTDs); HIV-1 reverse transcriptase.
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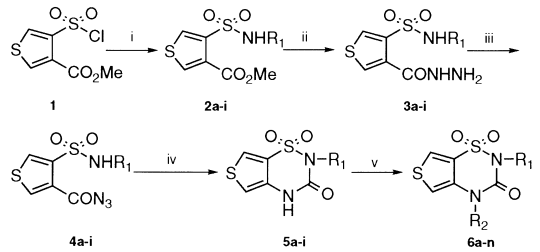
The first compounds of this type discovered were the 1-(2-hydroxyethoxymethyl)-6-(phenylthio)thymine (HEPT)[10,11] and tetrahydroimidazo[4,5,1-*jk*]benzodiazepin-2(1*H*)-one and -thione (TIBO) derivatives.[12,13] Following HEPT and TIBO, several other classes of specific HIV-1 RT inhibitors were disclosed: nevirapine (BI-RG-587),[14,15] pyridinone derivatives (L-696,229 and L-697,661),[16,17] bis(heteroaryl)piperazine (BHAP),[18,19] TSAO derivatives,[20,21] α-anilinophenylacetamides (α-APA),[22] phenylethylthioureathiazole (PETT),[23] oxathiincarboxanilides (although the latter were originally thought not to be targeted at RT)[24] and quinoxaline derivatives.[25] Subsequently, more than 25 novel classes of NNRTIs have been reported. Several overviews, with descriptions of the discovery and development of the majority of these NNRTIs, have appeared recently.[5,6,26–28]

Since we have had a long-time interest in the chemistry and pharmacological properties of heterocyclic sulfonamides,[29–35] we decided to prepare a series of 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazines (here named by the acronym TTDs) and assess them as inhibitors of HIV-1 replication. From the point of view of drug design, such thienothiadiazines can be considered as thiophene bioisosteres of 1,2,4-benzothiadiazines. These latter compounds have been traditionally investigated because of their activities as diuretic, hypoglycaemic, antihypertensive and potassium channels opener agents[36,37] but, recently, some derivatives of this type were also found to be highly potent HIV-1 NNRTIs.[38] The bioisosteric equivalence between benzene and thiophene has been widely demonstrated in many therapeutic fields.[39] In general, the replacement of benzene by thiophene in pharmacologically active molecules gives rise to new compounds with similar or improved potency and selectivity,[40] and our research group has reported different examples of the usefulness of this already classical design approach.[34,35,41–44] Further support for the 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine ring system as the basis for the design of useful NNRTIs is its chemical novelty, since no compound with this structure has hitherto been described. In the present paper we report the synthesis of the TTDs, the results obtained for their anti-HIV evaluation and their structure–function relationship (SAR) with regard to their anti-HIV activity. Further details on their antiretroviral activity, including virus–drug resistance profile and modelling in the active HIV-1 RT drug binding site, have been described previously.[45]

Chemistry

The 2- and 2,4-substituted 1,1,3-trioxo-2*H*,4*H*-thieno-[3,4-*e*][1,2,4]thiadiazines **5** and **6** were prepared according to the synthetic sequence outlined in Scheme 1. The key step for the formation of the bicyclic core fragment, common to all these derivatives, involves the intramolecular ring closure of the methyl sulfamoylthiophencarboxylates (**2**) through a classical Curtius reaction.[46–48] Compounds **2a**–**i** were prepared in good yields following a method described previously,[49] consisting of the reaction of methyl 4-chlorosulfonyl-

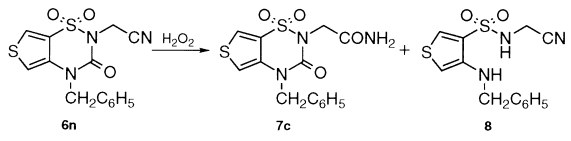


Scheme 1. Reagents: (i) NH_3/CH_2Cl_2 or R_1NH_2/THF; (ii) $N_2H_4{\cdot}H_2O$/EtOH; (iii) 2 N HCl or 2 N HNO_3, $NaNO_2$, H_2O; (iv) Δ/toluene or $CHCl_3$; (v) NaH/DMF, R_2X.

thiophene-3-carboxylate **1** with ammonia or primary amines in the presence of a base such as potassium carbonate (K_2CO_3) or pyridine. The sulfamoylesters **2a**–**i** were readily converted to the sulfamoylcarboxy azides **4a**–**i** by the action of nitrous acid on carboxy hydrazides **3a**–**i**, which, in turn, were obtained from the corresponding **2** by reaction with hydrazine hydrate in ethanol. Sulfamoylcarboxy azides **4a**–**i** were not purified, but they were sufficiently identified by the presence of the N_3 group characteristic band at 2130–2160 cm^{-1} in their IR spectra.

The target thieno[1,2,4]thiadiazines **5a**–**i** were synthesized in generally good yields by spontaneous cyclization of the isocyanates generated in the Curtius thermal rearrangement of sulfamoylcarboxy azides **4a**–**i** in dry toluene, benzene or chloroform. The poorer yields obtained in the ring-closure of carboxy azides **4b** ($R_1 = 2\text{-}ClC_6H_4$) and **4e** ($R_1 = 2\text{-}BrC_6H_4$) (26 and 28%, respectively) could be explained by the steric hindrance produced by the *ortho* substituted anilino moieties attached to these molecules. Compounds **5j** and **5k** were better prepared by *N*-alkylation of the 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine[48] under classical conditions, by treating a solution of the sodium salt of this thiadiazine in *N*,*N*-dimethylformamide (DMF) with one equivalent of 2-chloroacetonitrile and methyl iodide, respectively. Similar conditions (NaH/DMF/alkyl or arylalkyl halide) were successfully employed for the transformation of thiadiazines **5a**–**k** into disubstituted derivatives **6a**–**n**.

Compound **7a** was prepared from **5h** by reaction with benzoyl chloride in dry pyridine. Thienothiadiazines **7b** and **7c** were formed by hydrolysis of their respective cyanomethyl derivatives **6g** and **6n** under basic conditions (Scheme 2). Thus, **7b** was obtained by treating a dimethylsulfoxide solution of **6g** with an excess of 30% hydrogen peroxide in the presence of a catalytic amount of K_2CO_3.[50] Compound **6n** was better hydrolyzed by a modification of this procedure realized under phase



Scheme 2.

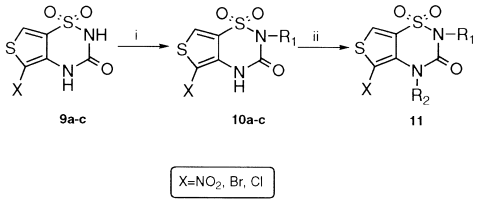
transfer catalysis conditions.[51] In this reaction, in addition to **7c**, another compound was isolated in minor proportion; on the basis of its spectroscopic data, this compound was assigned the open structure **8**.

Thienothiadiazines **10a–c** and **11** (Scheme 3) were prepared from 5-nitro-, 5-bromo- and 5-chloro-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazines **9a–c**[48] following the same sequence of reactions utilized for the synthesis of **5j**, **5k** and **6a–n**. For comparison purposes, the 4-substituted 2-benzyl-benzo[1,2,4]thiadiazine 1,1-dioxides **13a, b** were also prepared by alkylation of **12** (Scheme 4), according to a procedure reported previously.[52] Tables 1–3 summarize the physicochemical and analytical characteristics of all the thienothiadiazine derivatives synthesized.

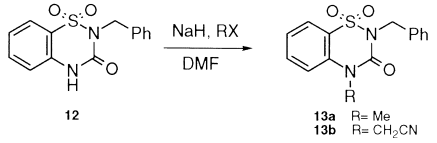
Results and Discussion

The compounds synthesized were initially evaluated for their activity as inhibitors of HIV-1 replication in vitro. The results are summarized in Tables 4 and 5. These biological data led to a series of considerations with regard to the structure–activity relationships for the compounds studied. Since the presence of aromatic substituents on the benzothiadiazine analogues was fundamental for their anti-HIV-1 activity, we first introduced a phenyl or substituted phenyl group at the 2 position of the thieno[3,4-*e*][1,2,4]thiadiazine structure (compounds **5a–g** and **6a, b**). This modification, however, led to products devoid of anti-HIV-1 activity, irrespective of the nature of the R_2 group present at position 4 of the heterocycle. The lack of activity of these derivatives is presumably due to their N-2 phenyl group (R_1), since it does not permit for the molecules to assume the butterfly-like conformation which seems particularly important in other NNRTIs (TIBO, α-APA and nevirapine).[53–56]

The first compounds of this series found to inhibit the HIV-1 replication were **6c** and **d**, with selective indexes (SI) of 196 and >316, respectively. Compounds **6c** and **d** bear at the 2 position a benzyl group which seems to provide a conformation that permits a certain interaction



Scheme 3. Reagents: (ii) NaH/DMF, $C_6H_5CH_2Br$; (iii) NaH/DMF, R_2X.



Scheme 4.

between the aromatic ring of the benzyl group and the HIV-1 RT. Introduction of benzyl, cyanomethyl or other alkyl groups into the 4 position of these 2-benzyl-thienothiadiazines (**6e–i**) also led to significantly active compounds (SI values of 49–578). In the 4-alkyl series, the methyl and ethyl derivatives **6c** and **d** showed the same order of potency, the second being less toxic and therefore more selective. However, the increase of the methylene side chain (**6e**, **h** and **j**) clearly impaired the antiviral activity while not affecting the cytotoxicity profile. The 4-cyanomethyl derivative **6g** proved to be the most potent inhibitor of HIV-1 replication of the series ($EC_{50} = 0.87\,\mu M$), followed by the isostere propargyl derivative **6i** ($EC_{50} = 0.96\,\mu M$), both showing scarce cytotoxicity and therefore good SI values. In contrast, the introduction of a benzoyl or acetamido group (**7a** and **b**), or the absence of substituents at this position (**5h**) resulted in loss of the antiviral activity.

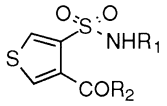
In order to improve the anti-HIV-1 activity of the new molecules, a limited number of changes in their 2 position were also made. These included introduction of substituted benzyl groups (**6k**) or replacement of the benzyl by methyl (**6l**, **m**), cyanomethyl (**6n**) or acetamido (**7c**) groups. None of these alterations, however, led to significantly active products. On the other hand, modification of the 5 position by substitution of thiophene proton with nitro (**10a**, **11**), bromine (**10b**) or chlorine (**10c**) groups did not yield active compounds at non-cytotoxic concentrations. Moreover, neither the benzothiadiazines **12–13** nor the intermediate compounds **2–3** were effective in the HIV-1 antiviral tests.

Compounds **6c**, **d**, **f**, **g** and **i**, which rank among the most potent inhibitors of HIV-1 in cell culture, were evaluated against an extensive panel of mutant virus strains that contained single mutations in their RT that are characteristic for NNRTI resistance (Table 6). As a rule, the compounds keep antiviral activity against L100I RT mutant virus, but significantly lose antiviral activity against K103N, V106A, E138K, Y181C and Y188H RT mutant viruses. Only **6f** kept activity against Y188H RT mutant virus (although it should be recognized that the antiviral activity was close to its cytostatic activity in CEM cells), **6g** lost only six fold activity against E138K RT HIV-1 whereas **6c** had a five to seven fold decreased activity against E138K and Y181C RT mutant viruses.

Four TTD derivatives that rank among the most potent inhibitors of HIV-1 in cell culture were chosen to measure their inhibitory activity against HIV-1 RT (Table 7). Compounds **6d**, **f** and **g**, which were inhibitory to HIV-1 replication in cell culture within the 1.2–1.8 μM range for CEM and 0.87–2.4 μM range for MT-4, were inhibitory to HIV-1 RT at concentrations that rank between 16.4 and 59.8 μM. Nevirapine, that is inhibitory to HIV-1 replication at 0.11 μM (CEM) – 0.03 μM (MT-4), had an IC_{50} of 4.5 μM against HIV-1 RT.

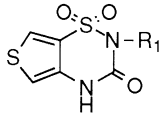
Compound **6g** was evaluated in more detail on its inhibitory activity against HIV-1 RT in the presence of

Table 1. 4-*N*-Substituted sulfamoylthiophene-3-methyl carboxylates **2** and -3-carbohydrazides **3** prepared



Compound no.	R_1	R_2	Yield (%)	mp (°C)	Solvent	Formula	Anal.
2a	Ph	OMe	88	130–132	EtOH	$C_{12}H_{11}NO_4S_2$	C,H,N,S
2b	2-Cl-Ph	OMe	84	127–129	EtOH	$C_{12}H_{10}ClNO_4S_2$	C,H,N,S
2c	3-Cl-Ph	OMe	79	161–163	MeOH	$C_{12}H_{10}ClNO_4S_2$	C,H,N,S
2d	4-Cl-Ph	OMe	95	157–159	MeOH	$C_{12}H_{10}ClNO_4S_2$	C,H,N,S
2e	2-Br-Ph	OMe	70	130–132	MeOH	$C_{12}H_{10}BrNO_4S_2$	C,H,N,S
2f	4-F-Ph	OMe	95	129–131	EtOH	$C_{12}H_{10}FNO_4S_2$	C,H,N,S
2g	4-OMe-Ph	OMe	89	115–116	MeOH	$C_{13}H_{13}NO_5S_2$	C,H,N,S
2h	Bn	OMe	89	73–75	EtOH	$C_{13}H_{13}NO_4S_2$	C,H,N,S
2i	CH_2(2,4-diOMe-Ph)	OMe	50	125–127	EtOH	$C_{15}H_{17}NO_6S_2$	C,H,N,S
3a	Ph	$NHNH_2$	89	222–224	MeOH	$C_{11}H_{11}N_3O_3S_2$	C,H,N,S
3b	2-Cl-Ph	$NHNH_2$	65	226–228	*n*-PrOH–H_2O	$C_{11}H_{10}ClN_3O_3S_2$	C,H,N,S
3c	3-Cl-Ph	$NHNH_2$	94	196–198	EtOH	$C_{11}H_{10}ClN_3O_3S_2$	C,H,N,S
3d	4-Cl-Ph	$NHNH_2$	81	255–257	MeOH	$C_{11}H_{10}ClN_3O_3S_2$	C,H,N,S
3e	2-Br-Ph	$NHNH_2$	87	215–217	MeCN	$C_{11}H_{10}BrN_3O_3S_2$	C,H,N,S
3f	4-F-Ph	$NHNH_2$	70	217–219	EtOH	$C_{11}H_{10}FN_3O_3S_2$	C,H,N,S
3g	4-OMe-PH	$NHNH_2$	86	212–214	MeCN	$C_{12}H_{13}N_3O_4S_2$	C,H,N,S
3h	Bn	$NHNH_2$	91	152–154	EtOH	$C_{12}H_{13}N_3O_3S_2$	C,H,N,S
3i	CH_2(2,4-diOMe-Ph)	$NHNH_2$	87	175–176	EtOH	$C_{14}H_{17}N_3O_5S_2$	C,H,N,S

Table 2. 2-Substituted 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazines **5** synthesized

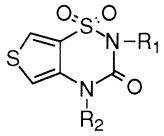


Compound no.	R_1	Yield (%)	mp (°C)	Solvent	IR (KBr) cm^{-1} C=O	IR (KBr) cm^{-1} SO_2	Formula	Anal.
5a	Ph	80	244–245	EtOH	1690	1340,1160	$C_{11}H_8N_2O_3S_2$	C,H,N,S
5b	2-Cl-Ph	26	>250 (d)	EtOH–H_2O	1690	1340,1170	$C_{11}H_7ClN_2O_3S_2$	C,H,N,S
5c	3-Cl-Ph	67	245–247	MeOH	1695	1340,1170	$C_{11}H_7ClN_2O_3S_2$	C,N,H,S
5d	4-Cl-Ph	54	252–254 (d)	MeOH–H_2O	1700	1330,1170	$C_{11}H_7ClN_2O_3S_2$	C,H,N,S
5e	2-Br-Ph	28	263–265	MeOH	1695	1335,1170	$C_{11}H_7BrN_2O_3S_2$	C,H,N,S
5f	4-F-Ph	54	260–262		1695	1335,1170	$C_{11}H_7FN_2O_3S_2$	C,H,N,S
5g	4-OMe-Ph	82	182–183	Toluene	1695	1335,1165	$C_{12}H_{10}N_2O_4S_2$	C,H,N,S
5h	Bn	82	160–162	EtOH–H_2O	1690	1335,1175	$C_{12}H_{10}N_2O_3S_2$	C,H,N,S
5i	CH_2(2,4-diOMe-Ph)	56	163–164	Toluene	1675	1330,1180	$C_{14}H_{14}N_2O_5S_2$	C,H,N,S
5j	Me	75	178–180	EtOH–H_2O	1690	1310,1160	$C_6H_6N_2O_3S_2$	C,H,N,S
5k	CH_2CN	60	185–187	EtOH	1710	1330,1195	$C_{17}H_5N_3O_3S_2$	C,H,N,S

six different concentrations of [2,8-^{3}H]dGTP (i.e. 20, 10, 5, 3, 2 and 1.5 μM) at 30, 15 and 0 (control) μM. A dose-dependent inhibition was observed. When plotted as a Lineweaver–Burk diagram (1/velocity versus 1/substrate), the inhibition was purely non-competitive with respect to the natural substrate (dGTP). This kinetic behaviour is in agreement with the nature of inhibition of HIV-1 RT by other NNRTIs such as nevirapine and delarvidine (data not shown).

In summary, based on the Curtius rearrangement of the 4-(N-substituted-sulfamoyl)thiophene-3-carboxy azides **4**, we have described the synthesis of the novel 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazines (TTDs) **5**, **6**, **7**, **10** and **11**. Several 4-substituted-2-benzyl-derivatives were found to selectively inhibit human immunodeficiency virus type 1 [HIV-1 (III_B)] replication in MT-4 and CEM cells. None of the derivatives without a substituent in the 4 position displayed any anti-HIV activity. These TTDs were also effective against other strains of HIV-1 (RF, HE, MN, NDK), including those that are resistant to AZT, but not against HIV-2 (ROD) or simian immunodeficiency virus [SIV(MAC251)] at subtoxic concentrations. Some of the test compounds kept antiviral activity against L100I RT mutant virus, but significantly lose antiviral activity against K103N, V106A, E138K, Y181C and Y188H RT mutant viruses. Compounds **6d**, **f** and **g** were inhibitory to HIV-1 RT at concentrations that rank between 16.4 and 59.8 μM (nevirapine: $IC_{50} = 4.5$ μM against HIV-1 RT). Inhibition of HIV-1 RT by compound **6g** was purely non-competitive with respect to the natural substrate (dGTP), which is in agreement with the nature of inhibition shown by other NNRTIs such as nevirapine and delarvidine. The 2-benzyl-4-cyanomethyl and 2-benzyl-4-propargyl derivatives **6g** and **i** were the most active of

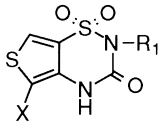
Table 3. 2,4-Disubstituted 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazines **6** and **7** synthesized



Compound no.	R_1	R_2	Yield (%)	mp (°C)	Solvent	EI-MS (*m*/*z*)	Formula	Anal.
6a	3-Cl-Ph	Me	87	177–178	MeOH	328 (M^+)	$C_{12}H_9ClN_2O_3S_2$	C,H,N,S
6b	3-Cl-Ph	Et	93	125–127	MeOH	342 (M^+)	$C_{13}H_{11}ClN_2O_3S_2$	C,H,N,S
6c	Bn	Me	90	158–160	EtOH	308 (M^+)	$C_{13}H_{12}N_2O_3S_2$	C,H,N,S
6d	Bn	Et	91	140–142	EtOH	322 (M^+)	$C_{14}H_{14}N_2O_3S_2$	C,H,N,S
6e	Bn	*n*-Pr	74	134–136	MeOH	336 (M^+)	$C_{15}H_{16}N_2O_3S_2$	C,H,N,S
6f	Bn	Bn	93	128–130	MeOH	384 (M^+)	$C_{19}H_{16}N_2O_3S_2$	C,H,N,S
6g	Bn	CH_2CN	89	182–184	EtOH	333 (M^+)	$C_{14}H_{11}N_3O_3S_2$	C,H,N,S
6h	Bn	Allyl	90	134–136	EtOH	334 (M^+)	$C_{15}H_{14}N_2O_3S_2$	C,H,N,S
6i	Bn	$CH_2C{\equiv}CH$	90	161–163	EtOH	332 (M^+)	$C_{15}H_{12}N_2O_3S_2$	C,H,N,S
6j	Bn	CH_2CH_2CN	45	137–139	EtOH	347 (M^+)	$C_{15}H_{13}N_3O_3S_2$	C,H,N,S
6k	CH_2(2,4-diOMe-PH)	Et	91	167–169	MeOH	382 (M^+)	$C_{16}H_{18}N_2O_5S_2$	C,H,N,S
6l	Me	Bn	95	97–99	MeOH	308 (M^+)	$C_{13}H_{12}N_2O_3S_2$	C,H,N,S
6m	Me	CH_2(2-Cl-Ph)	90	132–134	EtOH	342 (M^+)	$C_{13}H_{11}ClN_2O_3S_2$	C,H,N,S
6n[a]	CH_2CN	Bn	89	119–120	—	333 (M^+)	$C_{14}H_{11}N_2O_3S_2$	C,H,N,S
7a[b]	Bn	COPh	45	131–133	—	398 (M^+)	$C_{19}H_{14}N_2O_4S_2$	C,H,N,S
7b	Bn	CH_2CONH_2	46	228–230	MeCN	351 (M^+)	$C_{14}H_{13}N_3O_4S_2$	C,H,N,S
7c[c]	CH_2CONH_2	Bn	43	238–240	—	351 (M^+)	$C_{14}H_{13}N_3O_4S_2$	C,H,N,S

[a] Purified by column chromatography (hexane:ethyl acetate 3:1).
[b] Purified by column chromatography in dichloromethane.
[c] Purified by column chromatography (chloroform:ethanol 30:1).

Table 4. Anti-HIV-l activity of 2-substituted 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*c*][1,2,4]thiadiazines **5** and **10** in MT-4 and CEM cell lines[a]



Compound no.	R_1	X	HIV-1 (III_B)			
			EC_{50} (μM)		CC_{50} (μM)	SI
			MT-4	CEM	(MT-4)	(MT-4)
5a	Ph	H	> 517.7	53.5	517.7	< 1
5b	*o*-Cl-Ph	H	—	—	—	—
5c	*m*-Cl-Ph	H	> 110.7	> 63.5	110.7	< 1
5d	*p*-Cl-Ph	H	> 266.5	> 12.7	266.5	< 1
5e	*o*-Br-Ph	H	> 695	> 55.7	> 695	1
5f	*p*-F-Ph	H	> 435.8	> 67.0	435.8	< 1
5g	*p*-MeO-Ph	H	> 507.2	> 64.4	507.2	< 1
5h	Bn	H	> 78.8	≥67.9	78.8	< 1
5i	CH_2(2,4-diMeO-Ph)	H	> 705.4	> 282.2	705.4	1
5j	CH_2CN	H	> 411.1	—	410.3	< 1
5k	Me	H	> 99.8	—	> 99.8	< 1
10a	Bn	NO_2	> 5.9	> 2.3	< 5.9	1
10b	Bn	Br	> 418.2	≥53.5	418.2	< 1
10c	Bn	Cl	> 416.7	48.6	416.7	< 1
Nevirapine			0.03	0.11	683	22,767
AZT			0.0007	0.015	35.6	50,857

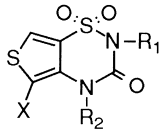
[a] All data represent mean values for at least two separate experiments.

the series and were therefore chosen as lead compounds for the design of more potent molecules.

Experimental

Melting points were determined on a Gallenkamp capillary apparatus and are uncorrected. Elemental analyses were performed with a Heraeus CHN-RAPID instrument at the Centro Nacional de Química Orgánica, CSIC, Madrid. Analytical results which are only indicated by symbols were found within ±0.4% of the theoretical values. ^{1}H NMR spectra were recorded on Varian Gemini 200, Bruker AM-200 and Varian XL-300 spectrometers operating at 200 and 300 MHz in the indicated solvent. Chemical shifts are expressed in δ

Table 5. Anti-HIV-l activity of TTDs **6**, **7** and **11** in MT-4 and CEM cell lines[a]



Compound no.	R_1	R_2	X	HIV-1 (III_B)			
				EC_{50} (μM)		CC_{50} (μM)	SI
				MT-4	CEM	(MT-4)	(MT-4)
6a	*m*-Cl-Ph	Me	H	> 483.6	—	484.5	< 1
6b	*m*-Cl-Ph	Et	H	61.3	—	62.4	< 1
6c	Bn	Me	H	2.7	2.3	527.9	196
6d	Bn	Et	H	2.4	1.5	775.4	> 316
6e	Bn	*n*-Pr	H	6.1	14.9	> 743.1	> 12.1
6f	Bn	Bn	H	2.2	1.8	> 650.3	> 298
6g	Bn	CH_2CN	H	0.87	1.2	502.7	578
6h	Bn	$CH_2CH{=}CH_2$	H	6.3	—	303.8	49
6	Bn	$CH_2C{\equiv}CH$	H	0.96	5.1	> 376.0	> 387
6j	Bn	CH_2CN_2CN	H	4.9	—	130.4	27
6k	CH_2(2,4-diMeO-Ph)	Et	H	> 255.2	> 261.5	255.2	< 1
6l	Me	Bn	H	> 77.8	> 64.8	79.1	< 1
6m	Me	CH_2(*o*-Cl-Ph)	H	> 70	> 11.7	68.8	< 1
6n	CH_2CN	Bn	H	> 291	—	291.3	< 1
7a	Bn	COPh	H	> 133	—	132.3	< 1
7b	Bn	CH_2CONH_2	H	> 401.3	136.5	402.7	< 1
7c	CH_2CONH_2	Bn	H	> 355.7	> 284.5	> 355.7	1
11	Bn	Me	NO_2	> 368.8	—	37.1	< 1
Nevirapine				0.03	0.11	683	22,767
AZT				0.0007	0.015	35.6	50,857

[a] All data represent mean values for at least two separate experiments.

Table 6. Inhibitory activity of TTD derivatives against a series of mutant HIV-1 strains in CEM cell cultures

Compound no.	EC_{50} (μM)[a]						
	HIV-1 (WT)	L100I	K103N	V106A	E138K	Y181C	Y188H
6c	2.3	12.9	> 324	> 324	≥324	> 324	≥324
6d	1.5	10.2	> 310	> 310	≥310	> 310	> 310
6f	1.8	≥10	> 10	≥10	≥10	≥10	7.8
6g	1.2	7.8	> 300	—	7.2	> 300	≥60
6i	5.1	6.0	> 60	—	30.1	36.1	—
Nevirapine	0.11	0.07	1.54	2.44	0.03	864	> 37

[a] 50% effective concentration or compound concentration required to inhibit virus-induced giant cell formation by 50%. All data represent mean values for at least two separate experiments.

Table 7. Inhibitory activity of TTD derivatives against recombinant HIV-1 reverse transcriptase

Compound no.	IC_{50} (μM)[a]
6d	31.0
6f	59.8
6g	16.4
Nevirapine	4.5

[a] 50% inhibitory concentration or compound concentration required to inhibit [2,8-^{3}H]dGTP incorporation into the poly rC.oligo dG template by 50%. 1 μM [2,8-^{3}H]dGTP served as the natural substrate of the enzyme reaction.

units from tetramethylsilane (TMS) as an internal standard. IR spectra were measured with a Shimadzu IR-435 spectrometer. Silica gel/TLC cards (Fluka, silica gel-precoated aluminium cards with fluorescent indicator 254 nm) were used for thin layer chromatography (TLC). Developed plates were visualized by UV light. Flash column chromatography was performed on columns packed with silica gel 60 (230–400 mesh) (Merck).

Chemistry

General procedure for the preparation of derivatives 2a–i. Example: 4-(*N*-phenylsulfamoyl)thiophene-3-methyl carboxylate (2a). To a solution of **1** (2.40 g, 0.01 mol) in THF (10 mL) was added dropwise a solution of aniline (1.82 mL, 0.02 mol) in the same solvent whilst maintaining the temperature below 10°C. The reaction mixture was stirred at 5°C for 1 h and at room temperature for 1 h more. The solvent was evaporated and the residue was treated with water to give a solid which was purified by recrystallization to give **2a**. IR (KBr, cm^{-1}) 3250 (NH); 1695 (C=O); 1345, 1155 (SO_2); 1265 (C−O).

^{1}H NMR (DMSO-d_6) δ 9.70 (bs, 1H, NH); 8.40 (d, 1H, J = 3.3 Hz, thiophene); 8.31 (d, 1H, J = 3.3 Hz, thiophene); 7.22–7.01 (m, 5H, benzene); 3.84 (s, 3H, CH_3).

4-[*N*-(2-Chlorophenyl)sulfamoyl]thiophene-3-methyl carboxylate (2b). IR (KBr, cm^{-1}) 3230 (NH); 1710 (C=O); 1335, 1160 (SO_2); 1250 (C–O). ^{1}H NMR (DMSO-d_6) δ 9.03 (s, 1H, exchange with D_2O, NH); 8.49 (d, 1H, J = 3.3 Hz, thiophene); 8.27 (d, 1H, J = 3.3 Hz, thiophene); 7.44–7.17 (m, 4H, benzene); 3.84 (s, 3H, CH_3).

4-[*N*-(3-Chlorophenyl)sulfamoyl]thiophene-3-methyl carboxylate (2c). IR (KBr, cm^{-1}) 3250 (NH), 1705 (C=O), 1315, 1160 (SO_2); 1250 (C–O). ^{1}H NMR (DMSO-d_6) δ 10.13 (bs, 1H, NH); 8.41 (d, 2H, thiophene); 7.29–7.03 (m, 4H, benzene); 3.84 (s, 3H, CH_3).

4-[*N*-(4-Chlorophenyl)sulfamoyl]thiophene-3-methyl carboxylate (2d). IR (KBr, cm^{-1}) 3180 (NH); 1695 (C=O); 1270, 1155 (SO_2). ^{1}H NMR (DMSO-d_6) δ 9.96 (bs, 1H, exchange with D_2O, NH); 8.41 (d, 1H, J = 3.3 Hz, thiophene); 8.34 (d, 1H, J = 3.3 Hz, thiophene); 7.31–7.09 (m, 4H, benzene); 3.84 (s, 3H, CH_3).

4-[*N*-(2-Bromophenyl)sulfamoyl]thiophene-3-methyl carboxylate (2e). IR (KBr, cm^{-1}) 3250 (NH); 1710 (C=O); 1260, 1150 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.42 (s, 1H, NH); 8.16 (d, 1H, J = 3.3 Hz, thiophene); 8.06 (d, 1H, J = 3.3 Hz, thiophene); 7.66 (dd, 1H, J = 8.1 Hz, J = 1.5 Hz, benzene); 7.45 (dd, 1H, J = 8.1 Hz, J = 1.5 Hz, benzene); 7.25 (m, 1H, benzene); 6.97 (ddd, 1H, J = 8.1 Hz, J = 7.5 Hz, J = 1.5 Hz, benzene); 3.96 (s, 3H, CH_3).

4-[*N*-(4-Fluorophenyl)sulfamoyl]thiophene-3-methyl carboxylate (2f). IR (KBr, cm^{-1}) 3300 (NH); 1695 (C=O); 1150 (SO_2). ^{1}H NMR (DMSO-d_6) δ 9.50 (bs, 1H, NH); 8.38 (d, 1H, J = 3.4 Hz, thiophene); 8.23 (d, 1H, J = 3.4 Hz, thiophene); 7.09–7.05 (m, 4H, benzene); 3.84 (s, 3H, CH_3).

4-[*N*-(4-Methoxyphenyl)sulfamoyl]thiophene-3-methyl car-boxylate (2g). IR (KBr, cm^{-1}): 3300 (NH); 1705 (C=O); 1155 (SO_2); 1245 (C–O). ^{1}H NMR (DMSO-d_6) δ 8.32 (d, 1H, J = 3.4 Hz, thiophene); 8.09 (d, 1H, J = 3.4 Hz, thiophene); 6.94 (dd, 2H, J = 8.9 Hz, J = 2.3 Hz, benzene); 6.75 (dd, 2H, J = 8.9 Hz, J = 2.3 Hz, benzene); 3.85 (s, 3H, CH_3); 3.65 (s, 3H, CH_3).

4-(*N*-Benzylsulfamoyl)thiophene-3-methyl carboxylate (2h). IR (KBr, cm^{-1}): 3300 (NH); 1717 (C=O); 1330, 1150 (SO_2); 1210 (C–O). ^{1}H NMR (DMSO-d_6) δ 8.05 (d, 2H, thiophene); 7.20 (s, 5H, benzene); 6.56 (t, J = 6.9 Hz, NH); 4.16 (d, 2H, J = 6.9 Hz, CH_2).

4-[*N*-(2,4-Dimethoxybenzyl)sulfamoyl]thiophene-3-methyl carboxylate (2i). IR (KBr, cm^{-1}) 3250 (NH); 1705 (C=O); 1320, 1155 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.34 (d, 1H, J = 3.3 Hz, thiophene); 8.08 (d, 1H, J = 3.3 Hz, thiophene); 6.91–6.83 (m, 2H, benzene and NH); 6.34–6.28 (m, 2H, benzene); 4.00 (d, 2H, J = 5.1 Hz, CH_2N); 3.79 (s, 3H, CH_3); 3.68 (s, 3H, CH_3); 3.64 (s, 3H, CH_3).

General procedure for the preparation of sulfamoylcarbohydrazides 3a–i. Example: 4-(*N*-phenylsulfamoyl)thiophene-3-carbohydrazide (3a). A mixture of compound **2a** (1.49 g, 5 mmol) and hydrazine hydrate (98%) (0.72 mL, 0.015 mol) in ethanol (5 mL) was refluxed for 1 h. Upon cooling, the precipitated solid was filtered off, washed with ice cold ethanol and recrystallized to yield **3a**. IR (KBr, cm^{-1}) 3360 (NH); 1660 (C=O); 1335, 1140 (SO_2). ^{1}H NMR (DMSO-d_6) δ 10.20–8.50 (bs, 2H, NH); 8.07 (d, 2H, thiophene); 7.28–7.04 (m, 5H, benzene); 5.10–4.00 (bs, 2H, NH_2).

4-[*N*-(2-Chlorophenyl)sulfamoyl]thiophene-3-carbohydrazide (3b). IR (KBr, cm^{-1}) 3400, 3320 (NH); 1660 (C=O); 1150 (SO_2). ^{1}H NMR (DMSO-d_6) δ 10.00 (bs, 1H, NH); 8.24 (d, 1H, J = 3.2 Hz, thiophene); 8.11 (d, 1H, J = 3.2 Hz, thiophene); 7.44–7.13 (m, 5H, benzene and NH); 6.00–4.00 (bs, 2H, NH_2).

4-[*N*-(3-Chlorophenyl)sulfamoyl]thiophene-3-carbohydrazide (3c). IR (KBr, cm^{-1}) 3300 (NH); 1610 (C=O); 1250, 1110 (SO_2). ^{1}H NMR (DMSO-d_6) δ 9.95 (bs, 1H, NH); 8.23 (d, 1H, J = 3.2 Hz, thiophene); 8.02 (d, 1H, J = 3.2 Hz, thiophene); 7.31–7.06 (m, 5H, benzene and NH); 5.70–4.20 (bs, 2H, NH_2).

4-[*N*-(4-Chlorophenyl)sulfamoyl]thiophene-3-carbohydrazide (3d). IR (KBr, cm^{-1}) 3300 (NH); 1620 (C=O); 1240, 1130 (SO_2). ^{1}H NMR (DMSO-d_6) δ 11.62 (bs, 1H, NH); 7.95 (d, 2H, thiophene); 7.03–6.90 (m, 5H, benzene and NH); 5.70–3.80 (bs, 2H, NH_2).

4-[*N*-(2-Bromophenyl)sulfamoyl]thiophene-3-carbohydrazide (3e). IR (KBr, cm^{-1}) 3400, 3300, 3200 (NH); 1650 (C=O); 1340, 1160 (SO_2). ^{1}H NMR (DMSO-d_6) δ 10.07 (bs, 1H, NH); 8.28 (d, 1H, J = 3.3 Hz, thiophene); 8.11 (d, 1H, J = 3.3 Hz, thiophene); 7.59 (dd, 1H, J = 7.9 Hz, J = 1.4 Hz, benzene); 7.35 (m, 2H, benzene); 7.06 (m, 1H, benzene); 5.80–4.20 (bs, 3H, NH and NH_2).

4-[*N*-(4-Fluorophenyl)sulfamoyl]thiophene-3-carbohydrazide (3f). IR (KBr, cm^{-1}) 3380, 3200 (NH); 1645 (C=O); 1330, 1145 (SO_2). ^{1}H NMR (DMSO-d_6) δ 10.00 (bs, 1H, NH); 8.06 (d, 2H, thiophene); 7.12–7.09 (m, 4H, benzene); 5.30–3.70 (bs, 2H, NH_2).

4-[*N*-(4-Methoxyphenyl)sulfamoyl]thiophene-3-carbohydrazide (3g). IR (KBr, cm^{-1}) 3340 (NH); 1617 (C=O); 1150 (SO_2). ^{1}H NMR (DMSO-d_6) δ 12.00–9.00 (bs, 2H, NH); 7.95 (d, 2H, thiophene); 6.91–6.87 (dd, 2H, J = 8.9 Hz, J = 2.2 Hz, benzene); 6.70–6.66 (dd, 2H, J = 8.9 Hz, J = 2.2 Hz, benzene); 6.50–4.20 (bs, 2H, NH_2); 3.62 (s, 3H, CH_3).

4-(*N*-Benzylsulfamoyl)thiophene-3-carbohydrazide (3h). IR (KBr, cm^{-1}) 3300 (NH); 1660 (C=O); 1320, 1140 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.12 (d, 1H, J = 3.0 Hz, thiophene); 7.95 (d, 1H, J = 3.0 Hz, thiophene); 6.00–4.10 (bs, 4H, NH); 4.03 (s, 2H, CH_2).

4-[*N*-(2,4-Dimethoxybenzyl)sulfamoyl]thiophene-3-carbohydrazide (3i). IR (KBr, cm^{-1}) 3340, 3280, 3200 (NH); 1670 (C=O); 1285, 1150 (SO_2). ^{1}H NMR (DMSO-d_6) δ 9.90 (bs, 1H, NH); 8.10–7.90 (d, 2H, thiophene); 7.30

(bs, 1H, NH); 7.00 (d, 1H, $J=8.0$ Hz, benzene); 6.50–6.30 (m, 2H, benzene); 4.60 (bs, 2H, NH_2); 3.92 (s, 2H, CH_2); 3.70 (s, 3H, CH_3); 3.64 (s, 3H, CH_3).

General procedure for the preparation of sulfamoylcarboxy azides 4a–i. Example: 4-(*N*-phenylsulfamoyl)thiophene-3-carboxy azide (4a). To a solution of compound **3a** (1.19 g, 4 mmol) in 2 N hydrochloric acid (20 mL) was added dropwise a solution of sodium nitrite (0.56 g) in water (2 mL), maintaining the reaction temperature below 10°C. The mixture was stirred at this temperature for 2 h and the insoluble product was filtered, washed with water and dried to yield **4a**, 1.17 g (95%). The compound was pure enough to be used as such in the following step. IR (KBr, cm^{-1}) 3300 (NH); 2150, 1220 (N_3); 1670 (C=O); 1150 (SO_2).

4-[*N*-(2-Chlorophenyl)sulfamoyl]thiophene-3-carboxy azide (4b). Yield: 95%. IR (KBr, cm^{-1}) 3250 (NH); 2150, 1215 (N_3); 1680 (C=O); 1340, 1155 (SO_2).

4-[*N*-(3-Chlorophenyl)sulfamoyl]thiophene-3-carboxy azide (4c). Yield: 96%. IR (KBr, cm^{-1}) 3250 (NH); 2150, 1220 (N_3); 1680 (C=O); 1330, 1160 (SO_2).

4-[*N*-(4-Chlorophenyl)sulfamoyl]thiophene-3-carboxy azide (4d). Yield: 81%. IR (KBr, cm^{-1}) 3300 (NH); 2150, 1220 (N_3); 1670 (C=O); 1380, 1155 (SO_2).

4-[*N*-(2-Bromophenyl)sulfamoyl]thiophene-3-carboxy azide (4e). Yield: 87%. IR (KBr, cm^{-1}) 2150 (N_3); 1680 (C=O).

4-[*N*-(4-Fluorophenyl)sulfamoyl]thiophene-3-carboxy azide (4f). Yield: 95%. IR (KBr, cm^{-1}) 3300 (NH); 2130, 1220 (N_3); 1675 (C=O); 1155 (SO_2).

4-[*N*-(4-Methoxyphenyl)sulfamoyl]thiophene-3-carboxy azide (4g). Yield: 86%. IR (KBr, cm^{-1}) 3300 (NH); 2140, 1210 (N_3); 1675 (C=O); 1155 (SO_2).

4-(*N*-Benzylsulfamoyl)thiophene-3-carboxy azide (4h). Yield: 85%. IR (KBr, cm^{-1}) 3300 (NH); 2150, 1220 (N_3); 1685 (C=O); 1330, 1150 (SO_2).

4-[*N*-(2,4-Dimethoxybenzyl)sulfamoyl]thiophene-3-carboxy azide (4i). Yield: 90%. IR (KBr, cm^{-1}) 3270 (NH); 2150, 1210 (N_3); 1670 (C=O); 1160 (SO_2).

General procedure for the preparation of thienothiadiazines 5a–i. Example: 2-phenyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (5a). A suspension of the carboxy azide **4a** (4.62 g, 0.01 mol) in dry benzene (90 mL) was refluxed for 3 h. The precipitate was filtered off and recrystallized to give **5a**. ^{1}H NMR (DMSO-d_6) δ 11.45 (s, 1H, NH); 8.66 (d, 1H, $J=3.2$ Hz, thiophene); 7.52–7.36 (m, 5H, benzene); 7.10 (d, 1H, $J=3.2$ Hz, thiophene).

2-(*o*-Chlorophenyl)-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*]-[1,2,4]thiadiazine (5b). Compound **4b** (3.0 g, 9 mmol) in dry chloroform (85 mL) was refluxed for 6 h to give **5b**. ^{1}H NMR (DMSO-d_6) δ 11.67 (s, 1H, NH); 8.67 (d, 1H, $J=3.2$ Hz, thiophene); 7.65–7.53 (m, 4H, benzene); 7.16 (d, 1H, $J=3.2$ Hz, thiophene).

2-(*m*-Chlorophenyl)-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*]-[1,2,4]thiadiazine (5c). Compound **4c** (2.30 g, 7 mmol) in dry chloroform (70 mL) was refluxed for 6 h to give **5c**. ^{1}H NMR (DMSO-d_6) δ 11.56 (s, 1H, NH); 8.68 (d, 1H, $J=3.2$ Hz, thiophene); 7.60–7.50 (m, 3H, benzene); 7.40 (dd, 1H, $J=7.4$ Hz, $J=1.7$ Hz, benzene); 7.11 (d, 1H, $J=3.2$ Hz, thiophene).

2-(*p*-Chlorophenyl)-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*]-[1,2,4]thiadiazine (5d). Compound **4d** (2.85 g, 8 mmol) in dry chloroform (94 mL) was refluxed for 2 h to give **5d**. ^{1}H NMR (DMSO-d_6) δ 11.50 (s, 1H, NH); 8.87 (d, 1H, $J=3.2$ Hz, thiophene); 7.59 (AB, 2H, $J=8.6$ Hz, benzene); 7.30 (AB, 2H, $J=8.6$ Hz, benzene); 7.13 (d, 1H, $J=3.2$ Hz, thiophene).

2-(*o*-Bromophenyl)-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*]-[1,2,4]thiadiazine (5e). Compound **4e** (3.0 g, 8 mmol) in dry chloroform (85 mL) was refluxed for 6 h to give **5e**. ^{1}H NMR (DMSO-d_6) δ 11.59 (s, 1H, NH); 8.67 (d, 1H, $J=3.1$ Hz, thiophene); 7.83–7.44 (m, 4H, benzene); 7.13 (d, 1H, $J=3.1$ Hz, thiophene).

2-(*p*-Fluorophenyl)-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*]-[1,2,4]thiadiazine (5f). Compound **4f** (2.96 g, 9 mmol) in dry chloroform (95 mL) was refluxed for 3 h to give **5f**. ^{1}H NMR (DMSO-d_6) δ 11.50 (bs, 1H, NH); 8.66 (d, 1H, $J=3.2$ Hz, thiophene); 7.43–7.33 (m, 4H, benzene); 7.13 (d, 1H, $J=3.2$ Hz, thiophene).

2-(*p*-Methoxyphenyl)-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*]-[1,2,4]thiadiazine (5g). Compound **4g** (1.50 g, 4 mmol) in dry xylene (50 mL) was refluxed for 2 h to give **5g**. ^{1}H NMR (DMSO-d_6) δ 11.35 (bs, 1H, NH); 8.64 (d, 1H, $J=3.3$ Hz, thiophene); 7.28 (AB, 2H, $J=8.9$ Hz, benzene); 7.07 (d, 1H, $J=3.3$ Hz, thiophene); 7.04 (AB, 2H, $J=8.9$ Hz, benzene); 3.80 (s, 3H, CH_3).

2-Benzyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (5h). Compound **4h** (0.5 g, 1 mmol) in dry benzene (13 mL) was refluxed for 7 h to give **5h**. ^{1}H NMR (DMSO-d_6) δ 11.36 (s, 1H, NH); 8.61 (d, 1H, $J=3.2$ Hz, thiophene); 7.30–7.20 (m, 5H, benzene); 7.04 (d, 1H, $J=3.2$ Hz, thiophene); 4.92 (s, 2H, CH_2).

2-(2,4-Dimethoxybenzyl)-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (5i). Compound **4i** (1.0 g, 3 mmol) in dry benzene (50 mL) was refluxed for 6 h to give **5i**. ^{1}H NMR (DMSO-d_6) δ 11.30 (bs, 1H, NH); 8.57 (d, 1H, $J=3.1$ Hz, thiophene); 7.04 (d, 1H, $J=3.1$ Hz, thiophene); 6.92 (d, 1H, $J=8.4$ Hz, benzene); 6.53 (d, 1H, $J=2.2$ Hz, benzene); 6.44 (dd, 1H, $J=8.4$ Hz, $J=2.2$ Hz, benzene); 4.82 (s, 2H, CH_2); 3.77 (s, 3H, CH_3); 3.71 (s, 3H, CH_3).

General procedure for the preparation of thienothiadiazines 5j–k. Example: 2-methyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (5j). To a solution of 1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine[48] (1.0 g, 5 mmol) in dry DMF (25 mL), under N_2, was added

portionwise sodium hydride (60% dispersion in mineral oil) (0.2 g, 5 mmol) maintaining the temperature below 10°C. After 15 min, methyl iodide (0.31 mL, 5 mmol) was added and the reaction mixture was stirred at room temperature for 3 h. The DMF was evaporated in vacuo and the crude residue was recrystallized to yield **5j**. ^{1}H NMR (DMSO-d_6) δ 11.25 (s, 1H, NH); 8.60 (d, 1H, J = 3.2 Hz, thiophene); 7.01 (d, 1H, J = 3.2 Hz, thiophene); 3.13 (s, 3H, CH_3).

2-Cyanomethyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]-thiadiazine (5k). ^{1}H NMR (DMSO-d_6) δ 11.50 (bs, 1H, NH); 8.70 (d, 1H, J = 3.1 Hz, thiophene); 7.11 (d, 1H, J = 3.1 Hz, thiophene); 4.84 (s, 2H, CH_2).

General procedure for the preparation of thienothiadiazines 6a–n. To a solution of the thiadiazine **5** (1 mmol) in dry DMF (15 mL), under Ar, was added slowly sodium hydride (60% dispersion in mineral oil, 0.06 g, 1.5 mmol) maintaining the temperature below 10°C. After 15 min, the alkyl halide (1.5 mmol) was added and the reaction mixture was stirred at 50–70°C for 15 h. The solvent was evaporated to dryness and the crude solid was filtered, washed with water, dried and recrystallized from the appropriate solvent.

Example: 2-(*m*-chlorophenyl)-4-methyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (6a). By reaction of **5c** with methyl iodide at room temperature. IR (KBr, cm^{-1}) 1700 (C=O); 1335, 1180 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.77 (d, 1H, J = 3.2 Hz, thiophene); 7.61–7.37 (m, 5H, benzene and thiophene); 3.43 (s, 3H, CH_3).

2-(*m*-Chlorophenyl)-4-ethyl-1,1,3-trioxo-2*H*,4*H*-thieno-[3,4-*e*][1,2,4]thiadiazine (6b). From thiadiazine **5c** and ethyl iodide at room temperature. IR (KBr, cm^{-1}) 1697 (C=O); 1340, 1175 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.77 (d, 1H, J = 3.1 Hz, thiophene); 7.65–7.37 (m, 5H, benzene and thiophene); 3.98 (c, 2H, J = 7.0 Hz, CH_2); 1.24 (t, 3H, J = 7.0 Hz, CH_3).

2-Benzyl-4-methyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]-thiadiazine (6c). From **5h** and methyl iodide at room temperature. IR (KBr, cm^{-1}): 1680 (C=O); 1330, 1160 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.69 (d, 1H, J = 3.2 Hz, thiophene); 7.39 (d, 1H, J = 3.2 Hz, thiophene); 4.94 (s, 2H, CH_2); 3.39 (s, 3H, CH_3).

2-Benzyl-4-ethyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]-thiadiazine (6d). From **5h** and ethyl bromide. IR (KBr, cm^{-1}): 1677 (C=O); 1330, 1180 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.70 (d, 1H, J = 3.2 Hz, thiophene); 7.50 (d, 1H, J = 3.2 Hz, thiophene); 7.30–7.20 (m, 5H, benzene); 4.95 (s, 2H, CH_2); 3.95 (c, 2H, J = 7.1 Hz, CH_2); 1.19 (t, 3H, J = 7.1 Hz, CH_3).

2-Benzyl-4-propyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]-thiadiazine (6e). From **5h** and 1-bromopropane. IR (KBr, cm^{-1}): 1680 (C=O); 1325, 1190 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.66 (d, 1H, J = 3.2 Hz, thiophene); 7.49 (d, 1H, J = 3.2 Hz, thiophene); 7.30–7.20 (m, 5H, benzene); 4.94 (s, 2H, CH_2); 3.90 (t, 2H, J = 7.0 Hz, CH_2); 1.62 (m, 2H, CH_2); 0.85 (t, 3H, J = 7.0 Hz, CH_3).

2,4-Dibenzyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (6f). From **5h** and benzyl bromide. IR (KBr, cm^{-1}) 1695 (C=O); 1318, 1165 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.69 (d, 1H, J = 3.2 Hz, thiophene); 7.34–7.22 (m, 11H, benzene and thiophene); 5.19 (s, 2H, CH_2); 5.01 (s, 2H, CH_2).

2-Benzyl-4-cyanomethyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (6g). From **5h** and chloroacetonitrile. IR (KBr, cm^{-1}) 1707 (C=O); 1340, 1172 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.78 (d, 1H, J = 3.1 Hz, thiophene); 7.62 (d, 1H, J = 3.1 Hz, thiophene); 7.33–7.29 (m, 5H, benzene); 5.13 (s, 2H, CH_2); 4.98 (s, 2H, CH_2).

4-Allyl-2-benzyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]-thiadiazine (6h). From **5h** and allyl bromide. IR (KBr, cm^{-1}) 1692 (C=O); 1333, 1180 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.71 (d, 1H, J = 3.2 Hz, thiophene); 7.40 (d, 1H, J = 3.2 Hz, thiophene); 7.33–7.27 (m, 5H, benzene); 5.90–5.81 (m, 1H, CH); 5.19 (dd, 1H, J = 5.7 Hz, J = −1.4 Hz, =CH); 5.14 (dd, 1H, J = 12.3 Hz, J = −1.4 Hz, =CH); 4.97 (s, 2H, CH_2); 4.58 (d, 2H, J = 4.9 Hz, CH_2).

2-Benzyl-4-propargyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*]-[1,2,4]thiadiazine (6i). From **5h** and propargyl chloride. IR (KBr, cm^{-1}) 3280 (C≡CH); 1675 (C=O); 1320, 1165 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.74 (d, 1H, J = 3.2 Hz, thiophene); 7.47 (d, 1H, J = 3.2 Hz, thiophene); 7.31 (s, 5H, benzene); 4.96 (s, 2H, CH_2); 4.76 (d, 2H, J = 2.4 Hz, CH_2−C); 3.36 (t, 1H, J = 2.4 Hz, CH).

2-Benzyl-4-cyanoethyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*]-[1,2,4]thiadiazine (6j). From **5h** and 3-chloropropionitrile. Compound **6j** was isolated from the crude solid by column chromatography using CH_2Cl_2 as eluent. IR (KBr, cm^{-1}) 2260 (CN); 1670 (C=O); 1355, 1165 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.73 (d, 1H, J = 3.1 Hz, thiophene); 7.64 (d, 1H, J = 3.1 Hz, thiophene); 7.34–7.27 (m, 5H, benzene); 4.97 (s, 2H, CH_2); 4.25 (t, 2H, J = 6.7 Hz, CH_2); 2.94 (t, 2H, J = 6.7 Hz, CH_2).

2-(2,4-Dimethoxybenzyl)-4-ethyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (6k). From **5i** and ethyl iodide. IR (KBr, cm^{-1}) 1683 (C=O); 1340, 1172 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.64 (d, 1H, J = 3.2 Hz, thiophene); 7.50 (d, 1H, J = 3.2 Hz, thiophene); 6.92 (d, 1H, J = 8.3 Hz, benzene); 6.52 (d, 1H, J = 2.4 Hz, benzene); 6.43 (dd, 1H, J = 8.3 Hz, J = 2.4 Hz, benzene); 4.85 (s, 2H, CH_2); 3.85 (c, 2H, J = 7.0 Hz, CH_2); 3.74 (s, 3H, CH_3O); 3.71 (s, 3H, CH_3O); 1.19 (t, 3H, J = 7.0 Hz, CH_3).

4-Benzyl-2-methyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]-thiadiazine (6l). From **5j** and benzyl bromide. IR (KBr, cm^{-1}) 1685 (C=O); 1320, 1165 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.67 (d, 1H, J = 2.7 Hz, thiophene); 7.33–7.25 (m, 6H, benzene and thiophene); 5.19 (s, 2H, CH_2); 3.22 (s, 3H, CH_3).

4-(*o*-Chlorobenzyl)-2-methyl-1,1,3-trioxo-2*H*,4*H*-thieno-[3,4-*e*][1,2,4]thiadiazine (6m). From **5j** and *o*-chlorobenzyl bromide. IR (KBr, cm^{-1}) 1690 (C=O); 1335, 1165 (SO_2). ^{1}H NMR (DMSO-d_6) δ 8.71 (d, 1H,

$J = 3.0$ Hz, thiophene); 7.54–7.49 (dd, 1H, $J = 7.0$ Hz, $J = 1.8$ Hz, benzene); 7.32–7.25 (m, 3H, benzene and thiophene); 7.04 (d, 1H, $J = 6.4$ Hz, benzene); 5.21 (s, 2H, CH_2); 3.21 (s, 3H, CH_3).

4-Benzyl-2-cyanomethyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*]-[1,2,4]thiadiazine (6n). From **5k** and benzyl bromide. Compound **6n** was isolated from the crude solid by column chromatography using hexane:ethyl acetate 3:1 as eluent. IR (KBr, cm^{-1}) 1695 (C=O); 1330, 1170 (SO_2). ^1H NMR (DMSO-d_6) δ 8.77 (d, 1H, $J = 3.1$ Hz, thiophene); 7.41 (d, 1H, $J = 3.1$ Hz, thiophene); 7.39–7.26 (m, 5H, benzene); 5.23 (s, 2H, CH_2); 4.93 (s, 2H, CH_2).

4-Benzoyl-2-benzyl-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*]-[1,2,4]thiadiazine (7a). To a suspension of **5h** (0.15 g, 0.5 mmol) in dry pyridine was added dropwise benzoyl chloride (0.077 mL, 0.66 mmol), maintaining the temperature below 10°C. The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated to dryness and the crude solid was purified by column chromatography using CH_2Cl_2 as eluent. IR (KBr, cm^{-1}) 1707 (C=O); 1360, 1190 (SO_2). ^1H NMR (DMSO-d_6) δ 8.77 (d, 1H, $J = 3.2$ Hz, thiophene); 8.06 (d, 1H, $J = 3.2$ Hz, thiophene); 7.78–7.54 (m, 5H, benzene); 7.34–7.27 (m, 5H, benzene); 4.92 (s, 2H, CH_2). Anal. ($C_{19}H_{14}N_2O_4S_2$) C, H, N, S.

2-Benzyl-4-(carbamoylmethyl)-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (7b). To a solution of **6g** (0.14 g, 0.4 mmol) in dimethyl sulfoxide (5 mL) containing potassium carbonate (8 mg, 0.06 mmol) was added dropwise a 30% hydrogen peroxide solution (0.06 mL), maintaining the temperature below 10°C. The reaction mixture was stirred at room temperature for 20 h. Ice-water was added and the solid formed was filtered, washed with water, dried and recrystallized. IR (KBr, cm^{-1}) 3480, 3360 (NH); 1697 (C=O); 1680 (C=O); 1307, 1167 (SO_2). ^1H NMR (DMSO-d_6) δ 8.66 (d, 1H, $J = 3.3$ Hz, thiophene); 7.64 (bs, 1H, NH); 7.32–7.25 (m, 6H, benzene and NH); 7.21 (d, 1H, $J = 3.3$ Hz, thiophene CH); 4.94 (s, 2H, CH_2); 4.47 (s, 2H, CH_2).

4-Benzyl-2-(carbamoylmethyl)-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (7c) and 4-(*N*-benzylamino)-3-(*N*-cyanomethyl)sulfamoylthiophene (8). To a solution of **6n** (0.10 g, 0.3 mmol) in dichloromethane (0.2 mL) containing tetrabutylammonium hydrogen sulfate (0.02 g, 0.06 mmol) were added dropwise a 30% hydrogen peroxide solution (0.14 mL) and a 20% aqueous solution of sodium hydroxide (0.11 mL), maintaining the temperature below 10°C. The reaction mixture was stirred at room temperature for 2 h and was then extracted with dichloromethane. The organic layer was washed with a saturated NaCl solution, dried (Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography using chloroform:ethanol 30:1 as eluent. The first fractions yielded **8** (20 mg, 22%) as an oil. ^1H NMR (DMSO-d_6) δ 8.57 (t, 1H, $J = 6.0$ Hz, NH); 8.08 (d, 1H, $J = 3.5$ Hz, thiophene); 7.41–7.23 (m, 5H, benzene); 6.11 (d, 1H, $J = 3.5$ Hz, thiophene); 5.54 (t, 1H, $J = 5.1$ Hz, NH); 4.27 (d, 2H, $J = 5.1$ Hz, CH_2); 4.11 (d, 2H, $J = 6.0$ Hz, CH_2). The slowest moving fractions gave **7c** as a white solid. IR (KBr, cm^{-1}) 3455 (NH_2); 1705, 1687 (C=O); 1335, 1165 (SO_2). ^1H NMR (DMSO-d_6) δ 8.62 (d, 1H, $J = 3.1$ Hz, thiophene); 7.60 (bs, 1H, exchange with D_2O, NH); 7.32–7.23 (m, 7H, benzene, thiophene and NH); 5.18 (s, 2H, CH_2); 4.34 (s, 2H, CH_2).

General procedure for the preparation of thienothiadiazines 10a–c. Example: 2-benzyl-5-nitro-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine (10a). To a solution of 5-nitro-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]-thiadiazine[48] (**9a**) (1.0 g, 4 mmol) in dry DMF, under N_2, was added slowly sodium hydride (60% dispersion in mineral oil) (0.17 g, 4 mmol), maintaining the temperature below 10°C. After 15 min, benzyl bromide (0.49 mL, 4 mmol) was added and the reaction mixture was stirred at 50°C for 23 h. The DMF was evaporated in vacuo and the crude residue was recrystallized to give **10a**, 1.24 g (60%). Mp 187–188°C (EtOH). IR (KBr, cm^{-1}): 3325 (NH); 1705 (C=O); 1585, 1290 (NO_2); 1350, 1170 (SO_2). ^1H NMR (DMSO-d_6) δ 11.43 (bs, 1H, NH); 9.01 (s, 1H, thiophene); 7.34 (m, 5H, benzene); 4.97 (s, 2H, CH_2). Anal. ($C_{12}H_9N_3O_5S_2$) C, H, N, S.

2-Benzyl-5-bromo-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]-thiadiazine (10b). This compound was obtained in an 89% yield from reaction of 5-bromo-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine[48] (**9b**) with benzyl bromide; mp 202–203°C (MeOH). IR (KBr, cm^{-1}) 3150 (NH); 1687 (C=O); 1335, 1165 (SO_2). ^1H NMR (DMSO-d_6) δ 11.37 (bs, 1H, NH); 8.69 (s, 1H, thiophene); 7.31 (s, 5H, benzene); 4.92 (s, 2H, CH_2). Anal. ($C_{12}H_9BrN_2O_3S_2$) C, H, N, S.

2-Benzyl-5-chloro-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]-thiadiazine (10c). This compound was obtained in a 90% yield from the reaction of 5-chloro-1,1,3-trioxo-2*H*,4*H*-thieno[3,4-*e*][1,2,4]thiadiazine[48] (**9c**) with benzyl bromide; mp 192–194°C (EtOH). IR (KBr, cm^{-1}) 3190 (NH); 1690 (C=O); 1340, 1165 (SO_2). ^1H NMR (DMSO-d_6) δ 11.55 (bs, 1H, NH); 8.55 (s, 1H, thiophene); 7.31 (s, 5H, benzene); 4.92 (s, 2H, CH_2). Anal. ($C_{12}H_9ClN_2O_3S_2$) C, H, N, S.

2-Benzyl-4-methyl-5-nitro-1,1,3-trioxo-2*H*,4*H*-thieno-[3,4-*e*][1,2,4]thiadiazine (11). This compound was obtained in an 80% yield from the reaction of **10a** with methyl iodide following the general procedure used for the preparation of thiadiazines **6a**–**n**; mp 167–169°C (EtOH–H_2O). IR (KBr, cm^{-1}) 1695 (C=O); 1340, 1187 (SO_2). ^1H NMR (DMSO-d_6) δ 8.94 (s, 1H, thiophene); 7.33 (s, 5H, benzene); 4.93 (s, 2H, CH_2); 3.44 (s, 3H, CH_3). Anal. ($C_{13}H_{11}N_3O_5S_2$) C, H, N, S.

2-Benzyl-4-methyl-1,1,3-trioxo-2*H*,4*H*-benzo[1,2,4]thiadiazine (13a). This compound was obtained in a 75% yield from the reaction of 2-benzyl-1,1,3-trioxo-2*H*,4*H*-1,2,4-benzothiadiazine (**12**)[52] with methyl iodide; mp 110–111°C (EtOH). ^1H NMR (DMSO-d_6) δ 7.95 (dd, 1H, $J = 7.7$ Hz, $J = 1.5$ Hz, benzene H-8); 7.83 (ddd, 1H, $J = 8.4$ Hz, $J = 7.7$ Hz, $J = 1.5$ Hz, benzene H-6); 7.57 (d, 1H, $J = 8.4$ Hz, benzene H-5); 7.43 (t, 1H, $J = 7.7$ Hz, benzene H-7); 7.31 (s, 5H, benzene); 4.98 (s, 2H, CH_2); 3,47 (s, 3H, CH_3). Anal. ($C_{15}H_{14}N_2O_3S$) C, H, N, S.

2-Benzyl-4-cyanomethyl-1,1,3-trioxo-2*H*,4*H*-benzo[1,2,4]-thiadiazine (13b). This compound was obtained in a 59% yield from the reaction of **12** with 2-chloroacetonitrile. The crude product was purified by column chromatography using CH_2Cl_2 as eluent; mp 141–143°C. ^{1}H NMR (DMSO-d_6) δ 8.02 (dd, 1H, J = 7.8 Hz, J = 1.4 Hz, benzene H-8); 7.93 (ddd, 1H, J = 8.4 Hz, J = 7.8 Hz, J = 1.4 Hz, benzene H-6); 7.66 (d, 1H, J = 8.4 Hz, benzene H-5); 7.52 (t, 1H, J = 7.8 Hz, benzene H-7); 7.34 (s, 5H, benzene); 5.25 (s, 2H, CH_2); 5.01 (s, 2H, CH_2). Anal. ($C_{16}H_{13}N_3O_3S$) C, H, N, S.

Anti-HIV activity assays

The human immunodeficiency virus strain used was HIV-1 (III_B).[57] Anti-HIV activity and cytotoxicity measurements were carried out in parallel. They were based on the viability of MT-4 cells that had been infected with HIV and then exposed to various concentrations of the test compounds. After the MT-4 cells were allowed to proliferate for 5 days, the number of viable cells was quantified by a tetrazolium-based colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) procedure in 96-well microtrays.[58] In all of these assays, viral input (viral multiplicity of infection, MOI) was 0.01, or 100 times the 50% cell culture infective dose ($CCID_{50}$). The 50% antivirally effective 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 several separate experiments are presented as defined above. As a rule, the individual values did not deviate by more than two fold up or down from the EC_{50} and CC_{50} values indicated in the tables. On the other hand, the inhibitory activity of TTDs against wild-type HIV-1 and mutant strains in CEM cell cultures was examined as follows. CEM cells were suspended at 250,000–350,000 cells per mL of culture medium and infected with approximately 100 $CCID_{50}$ (1 $CCID_{50}$ being the 50% cell culture infective dose) of HIV-1 (III_B). Then, 100 μL of the infected cell suspensions was added to 200-μL-microtiter plate wells containing 100 μL of an appropriate dilution of the test compounds. The inhibitory effect of the test compounds on HIV-1-induced syncytium formation in CEM cells was examined on day 4 post infection. The 50% effective concentration (EC_{50}) was determined as the compound concentration required to inhibit syncytium formation by 50%. The kinetics of inhibition of HIV-1 RT by several TTDs were performed as described previously.[59] The template/primer was poly rC.dG and the radiolabelled substrate [2.8-^{3}H]dGTP.

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