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1,2,3-Thiadiazole thioacetanilides as a novel class of potent HIV-1 non-nucleoside reverse transcriptase inhibitors

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ABSTRACT

A novel series of 1,2,3-thiadiazole thioacetanilide (TTA) derivatives have been designed, synthesized and evaluated for its anti-HIV activities in MT-4 cells. Some derivatives proved to be highly effective in inhibiting HIV-1 replication at nanomolar concentrations. Among them, 2-[4-(2,4-dichlorophenyl)-1,2,3-thiadiazol-5-ylthio]-*N*-(2-nitrophenyl)acetamide **7d2** was identified as the most promising compound (EC_{50} = 0.059 ± 0.02 μM, CC_{50} > 283.25 μM, SI > 4883). The structure–activity relationship (SAR) of these novel structural congeners is discussed.

Human immunodeficiency virus type-1 (HIV-1) infection affects close to 40 million individuals worldwide. Since 1981, when the first case reports of individuals dying from a rare opportunistic infection were published, 20 million people have died from this epidemic.[1] Although the introduction of highly active anti-retroviral therapy (HAART) has dramatically decreased the morbidity and mortality resulting from the infection with HIV, the AIDS prevalence remains one of the world's most serious health problems, causing millions of deaths each year.[1] As one of the components of the fist line HAART regimen, non-nucleoside reverse transcriptase inhibitors (NNRTIs) have gained a definitive and important place due to their unique antiviral potency, high specificity and low toxicity. However, NNRTIs currently in clinical use are found having a low genetic barrier to resistance and, therefore, the need for novel NNRTIs active against drug-resistant mutants selected by current therapies is of paramount importance.[2–4]

Recently, from high-throughput screening (HTS) of compound libraries, several interesting sulfanyltriazole- and sulfanyltetrazole-type leads (**A** and **B**) were identified as novel potent HIV-1 NNRTIs, which have a simple, yet distinctively different chemical structure from the other HIV-1 NNRTIs reported in the literature (Fig. 1).[5,6]

Extensive structural modification and bioactivity research demonstrated that most derivatives showed submicromolar activity in cell assay and significant in vitro activity against the WT or double mutant K103N+Y181C strain of HIV-1 RT.[7–9] A number of compounds derived from this triazole/tetrazole scaffold are currently being considered for clinical evaluation. Among them, **VRX-480773** (Fig. 1) was found to inhibit viruses from EFV-resistant molecular clones and most NNRTI-resistant clinical HIV-1 isolates. It also has an excellent pharmacokinetic profile, warranting further clinical development for the treatment of HIV infection in both NNRTI-naive and -experienced patients.[10]

In order to further confirm the importance of the five-membered heterocycle, a novel series of 1,2,3-thiadiazole thioacetanilide (TTA) derivatives was designed and synthesized based on the general principle of bioisosterism in medicinal chemistry.[11,12] In the TTA analogues, the 1,2,3-thiadiazole ring was substituted for the triazole or tetrazole moiety in the corresponding lead compounds (Fig. 2), the other fragments which were considered to be necessary for conserving anti-HIV-1 activity, such as the 'S–CH_2–CO–NH' linker and the 2-substituted anilides, were left unchanged. Herein, a new approach for the synthesis of novel TTAs (**7a–d**), as well as the evaluation of their inhibitory effects on HIV replication in MT-4 cell culture, is reported.

The synthesis of the 1,2,3-thiadiazole thioacetanilides **7** was straightforward and is depicted in Scheme 1. 2-Halo-1-(substituted phenyl)ethanones (**2**), synthesized by direct halogenation of acetophenone or by Friedel–Crafts reaction of substituted benzene with 2-chloroacetyl chloride, were reacted with ethyl

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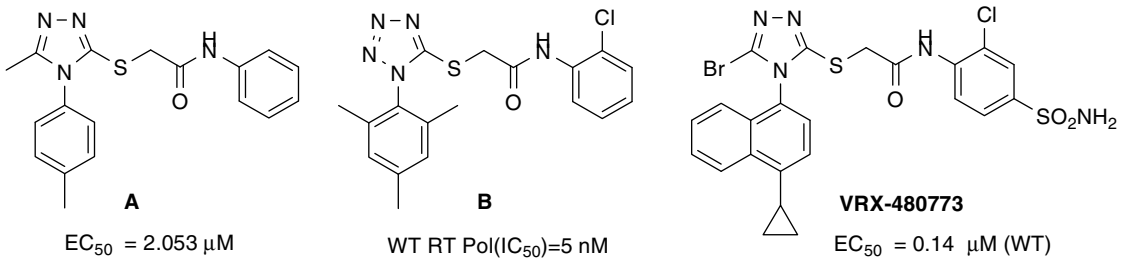


Figure 1. Sulfanyltriazole- and sulfanyltetrazole-type NNRTIs.

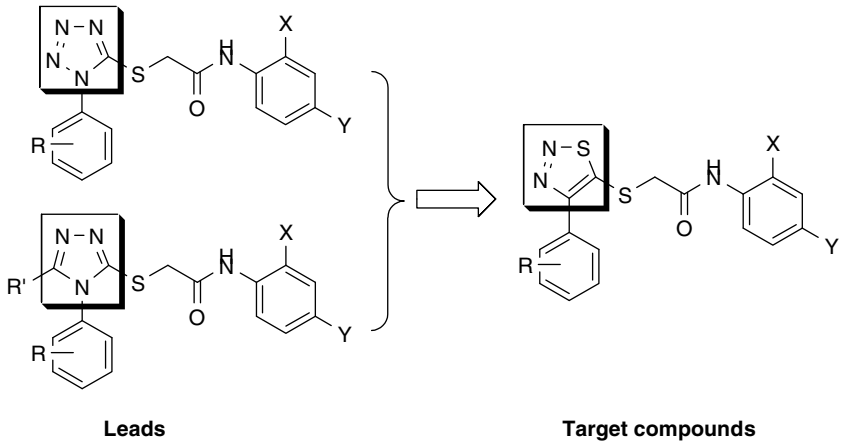
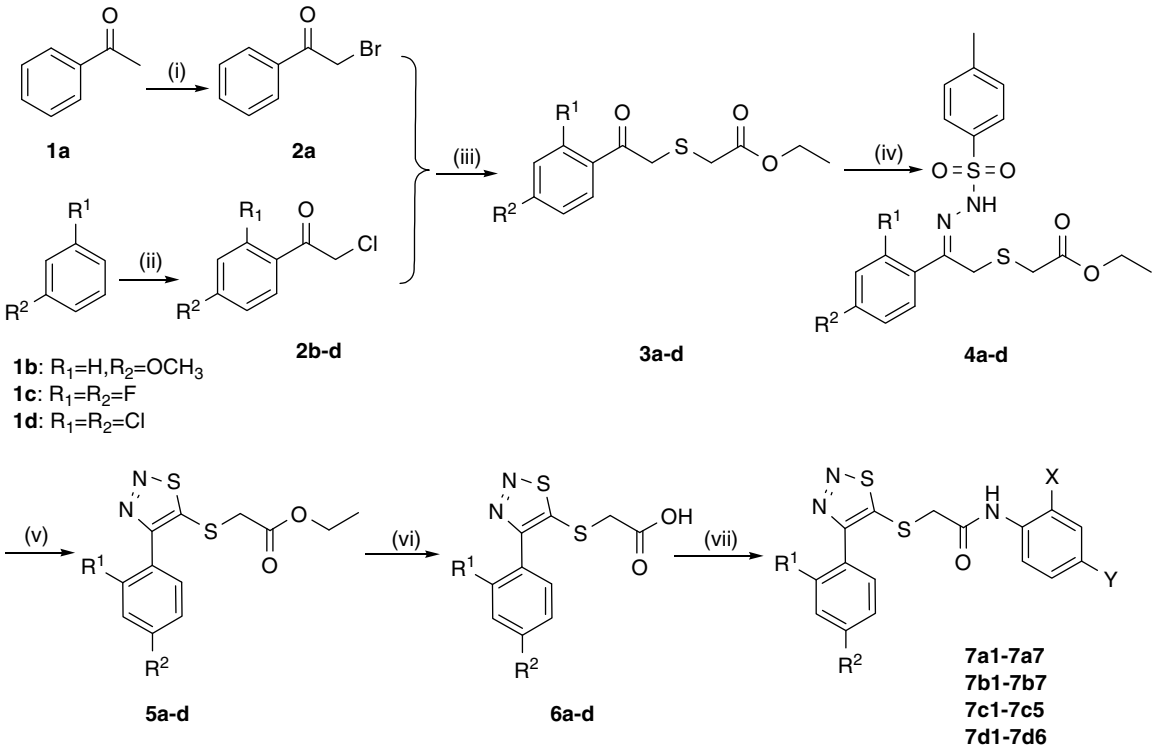


Figure 2. The newly designed 1,2,3-thiadiazole thioacetanilide (TTA) scaffold.



Scheme 1. Reagents and conditions: (i) Br_2, $AlCl_3$; (ii) $AlCl_3$, $ClCH_2COCl$, DCM; (iii) Na_2CO_3, $HSCH_2CO_2Et$, EtOH, rt; (iv) *p*-tosyl hydrazine, toluene, reflux; (v) excess $SOCl_2$; (vi) KOH–EtOH–H_2O, 50–70 °C; (vii) PCl_5, aniline, DCM, rt, 1–2 h.

2-mercaptoacetate in EtOH at ambient temperature for several hours to obtain the ethyl 2-(1-phenyl ethanone-2-thio)acetates (**3**). Condensation of an appropriately substituted **3** with *p*-tosyl hydrazine in refluxing toluene for 5–8 h, followed by recrystallisation from EtOH or by column chromatography on silica gel afforded the corresponding ethyl 2-(1-phenylethanone-2-thio)acetate

hydrazones (**4**), which are the key intermediates for the preparation of the substituted 1,2,3-thiadiazole heterocycles **5**. The ring closure reaction of compounds **4** was carried out via the reaction with thionyl chloride according to the method reported by Hurd and Mori.[13–15] The thioacetic acid derivatives **6** were produced by saponification of compounds **5** in KOH–EtOH–H_2O solution. The final 1,2,3-thiadiazole thioacetanilides **7** were synthesized by reaction of **6** with substituted anilines in the presence of 1.1 equiv mole of PCl_5. Both analytical and spectral data of all the newly synthesized compounds are in full agreement with the proposed structures.[16]

The mechanism of the Hurd–Mori ring closure reaction has been investigated and discussed in detail.[13,17] In brief, an intermediate thiadiazoline-1-one **II** is formed by condensation of **I** with the excess thionyl chloride, which readily aromatizes to form the 1,2,3-thiadiazoles **IV,** possibly through a Pummerer-typed rearrangement[18] in concomitance with the cleavage of the $PhSO_2-$ group through the transition state **III** (Fig. 3).

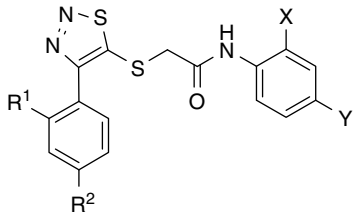
The preliminary activity and cytotoxicity of the newly designed and synthesized 1,2,3-thiadiazole thioacetanilides were tested in MT-4 cells for inhibition of HIV-1 (strain IIIB) and HIV-2 (strain ROD) by the MTT method.[19,20] The compounds nevirapine (NVP), delaviridine (DLV), efavirenz (EFV) and zidovudine (azidothymidine, AZT) were used as the reference drugs. The sulfanyltriazole **VRX-480773** was used as reference compound for comparative purposes. The experimental results indicated that most of the compounds exhibited potent inhibitory activity against HIV-1 (Table 1) and none of the compounds was active against HIV-2.

As shown in Table 1, most of the compounds inhibited HIV-1 replication in the lower micromolar concentration range. Among them, analogues of the **7d** series showed an EC_{50} value <0.2 μM, lower than the reference drugs of NVP and DLV. The most potent HIV-1 inhibitors were **7d2** (EC_{50} = 0.059 μM, CC_{50} > 283.25 μM, SI > 4883), **7d6** (EC_{50} = 0.099 μM, CC_{50} > 228.41 μM, SI > 2298), and **7d1** (EC_{50} = 0.118 μM, CC_{50} = 111.43 μM, SI = 943). The EC_{50} values of these two compounds were lower than that of the lead compound **VRX-480773**, which indicates that the 1,2,3-thiadiazole is an acceptable isosteric replacement for the triazole/tetrazole in the lead compounds.

In all series (**7a–d**), we observed that *p*-methoxy substitution on the phenyl of the thiadiazole ring increased the antiviral activity compared to the unsubstituted phenyl series (**7a**). The 2,4-dihalophenyl substituted compounds (**7c** and **7d**) were also found to be essential for keeping the potent activities. Interestingly, replacing the 2,4-difluoro (**7c** series) by 2,4-dichloro phenyl group (**7d** series) led to even superior anti-HIV-1 activities. No substantial differences in antiviral activity against HIV-1 were observed between the **7b** and **7c** series. From the structure–activity relationship (SAR) results, we find that the antiviral potency of the 1,2,3-thiadiazole thioacetanilides is closely related to the electronic or spatial characteristics of the aryl linked to the 1,2,3-thiadiazole core.

Substitution at the phenyl ring of the anilide moiety revealed that nitro and halogen at the *ortho* position were the preferred substituents, but the introduction of a fluorine atom in this position caused a substantial decrease in potency. However, substituents at the *para* position of the anilide almost did not influence the antiviral potency in the same series.

Table 1
Anti-HIV activity in MT-4 cells of 1,2,3-thiadiazole thioacetanilides (**7a–d**)



Code	R^1	R^2	X	Y	HIV-1(III_B)		
					EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]
7a1	H	H	Cl	H	26.11 ± 5.28	>345.4	>13
7a2	H	H	NO_2	H	⩾54.23	⩾246.72	NA[d]
7a3	H	H	F	H	>361.8	>361.8	NA
7a4	H	H	Br	H	22.42 ± 0.27	>307.64	>14
7a5	H	H	Br	CH_3	13.73 ± 3.83	>297.37	>22
7a6	H	H	Br	$CO_2C_2H_5$	5.21 ± 0.38	⩾232.03	⩾45
7a7	H	H	Br	CO_2CH_3	5.10 ± 1.34	>269.19	>53
7b1	H	OMe	Cl	H	2.63 ± 0.97	>318.97	>121
7b2	H	OMe	NO_2	H	0.99 ± 0.17	⩾280.78	⩾283
7b3	H	OMe	F	H	>332.94	>332.94	NA
7b4	H	OMe	Br	H	3.19 ± 0.07	>286.46	>90
7b5	H	OMe	Br	CH_3	5.06 ± 0.84	249.24 ± 11.95	49
7b6	H	OMe	Br	$CO_2C_2H_5$	3.01 ± 0.33	>245.86	>82
7b7	H	OMe	Br	CO_2CH_3	2.00 ± 1.25	195.40 ± 7.38	97
7c1	F	F	Cl	H	1.23 ± 0.10	178.08 ± 38.63	146
7c2	F	F	NO_2	H	4.14 ± 1.08	164.62 ± 8.86	40
7c3	F	F	F	H	5.45 ± 1.36	147.88 ± 26.90	27
7c4	F	F	Br	H	2.26 ± 0.16	144.47 ± 9.29	143
7c5	F	F	Br	CH_3	1.45 ± 0.22	>273.92	>189
7d1	Cl	Cl	Cl	H	0.118 ± 0.03	111.43 ± 93.95	943
7d2	Cl	Cl	NO_2	H	0.059 ± 0.02	>283.25	>4883
7d3	Cl	Cl	F	H	0.135 ± 0.13	19.53 ± 15.62	145
7d4	Cl	Cl	Br	H	0.149 ± 0.01	⩾146.88	⩾981
7d5	Cl	Cl	Br	CH_3	0.204 ± 0.02	>255.50	>1265
7d6	Cl	Cl	Br	$CO_2C_2H_5$	0.099 ± 0.01	>228.41	>2298
NVP					0.208	>15.02	>72
DLV					0.320	>3.827	>12
EFV					0.00440	>6.336	>1434
AZT					0.0151	>93.55	>6192
VRX-480773[e]					0.14	ND[f]	ND

[a] EC_{50}: concentration required to protect the cell against viral cytopathogenicity by 50% in MT-4 cells.
[b] CC_{50}: concentration that reduces the MT-4 cell viability by 50%.
[c] Selectivity index: ratio CC_{50}/EC_{50}, a higher SI means a more selective compound.
[d] NA: not active.
[e] Ref. 10.
[f] ND: not determined. The symbol '>' in the CC_{50} column is 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.

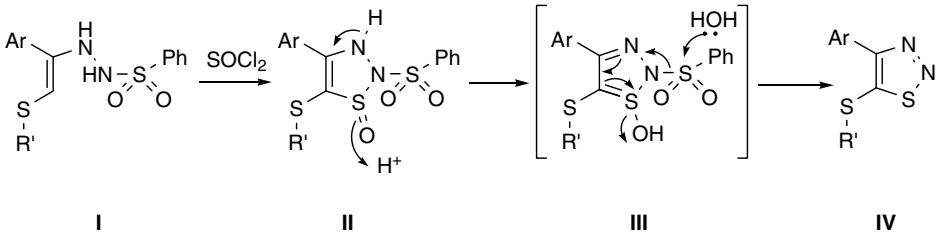


Figure 3. The mechanism of the Hurd–Mori reaction.

From the SAR studies, we found that the SAR features of the 4-substituted phenyl and anilide moieties of the TTAs were highly consistent with the previously observed sulfanyltriazole/tetrazole-type NNRTIs.[5–9] In addition, the TTA analogues proved active against HIV-1, but not HIV-2. Taking together these data we can assume that the novel TTAs most probably act as genuine NNRTIs. Experiments for inhibition of HIV-1 RT, activity against NNRTI-resistant strains and docking studies are in progress, and further results will be reported in due course.

In summary, the bioassay results show that our 'bioisosterism' based approach has led to the discovery of novel TTA-based anti-HIV agents. Some derivatives proved to be highly effective in inhibiting HIV-1 replication at nanomolar concentrations. Among them, compound **7d2** was identified as the most promising candidate (EC_{50} = 0.059 ± 0.02 μM, SI > 4883). Additional structural modifications of the TTA derivatives may further increase their potency as anti-HIV-1 agents.

Acknowledgments

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16. Selected data for compound **7d1**: ^{1}H NMR (600 MHz, $CDCl_3$, ppm) δ: 8.53 (s, 1H, NH), 8.27 (d, 1H, J = 8.4 Hz, PhH), 7.57 (d, 1H, J = 2.4 Hz, PhH), 7.44–7.10 (m, 5H, PhH), 3.84 (s, 2H, S-CH_2); ^{13}C NMR (150 MHz, $CDCl_3$, ppm) δ: 163.73 (C═O), 156.84 (thiadiazole), 148.28 (thiadiazole), 136.78, 135.12, 133.44, 132.80, 130.12, 129.19, 127.91, 127.51, 127.45, 125.67, 123.23, 121.53, 41.64 (S-CH_2); IR (KBr, cm^{-1}): 3355 (υ_{NH}), 1698 ($\upsilon_{C=O}$), 1592 ($\upsilon_{C=N}$), 1528 (δ_{NH}). EI-MS: m/z 430.2 (M^+), 432.2 (M+2), 434.2 (M+4). $C_{16}H_{10}Cl_3N_3OS_2$ (M_W: 430.76).
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