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Novel 1,2,3-thiadiazole derivatives as HIV-1 NNRTIs with improved potency: Synthesis and preliminary SAR studies

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ABSTRACT

A novel synthetic route and anti-HIV activity evaluation of a new series of 2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)acetamide (TTA) derivatives are described. Bioactivity assay indicated that most of the title compounds showed good activities against HIV-1. In particular, compound **7c** displayed the most potent anti-HIV-1 activity (EC_{50} = 36.4 nM), inhibiting HIV-1 replication in MT-4 cells more effectively than NVP (by sevenfold) and DLV (by eightfold). The preliminary structure–activity relationships (SAR) of the newly synthesized congeners are discussed, and molecular modeling of compound **7c** in complex with HIV-1 RT is described, allowing rationalization of some SAR conclusions.

1. Introduction

The reverse transcriptase (RT) of the human immunodeficiency virus type 1 (HIV-1) is a key target for inhibition of HIV-1 replication, the RT can be inhibited by two classes of drug belonging either to the nucleoside reverse transcriptase inhibitors (NRTIs) or to the non-nucleoside reverse transcriptase inhibitors (NNRTIs). NNRTIs have become key components in the combination regiments of cocktail therapy.[1–4]

However, a number of problems still remain with these agents, in particular, the therapeutic potential of this class of drugs has been compromised by the rapid development of resistance, but their encouraging usage in combination therapy has rekindled interest in the search for novel and potent NNRTIs.[1–4]

Among the representatives of the NNRTIs, triazole/tetrazole thioacetanilides derivatives (**L1** and **L2**, Fig. 1) were reported as potent HIV-1 inhibitors, with low nanomolar intrinsic activity against the RT and submicromolar antiviral activity in HIV-infected cells.[5–12] Recently, we developed a series of 1,2,3-thiadiazole thioacetanilides (TTAs) (**L3**–**L7**, Fig. 2), which exhibited significant anti-HIV-1 activities.[13] Inspired by these promising results and in continuation of our work on the research of novel NNRTIs,[13–15] we thought it is worthwhile to synthesize new compounds of TTAs based on the previous SAR analysis, with the aim to improve the interaction between the inhibitors and RT and to obtain new biologically active molecules.

The previous SAR and molecular modeling of azole thioacetanilides indicated that increasing volume of the substituted phenyl linked with azole was beneficial for strengthening π–π stacking interaction between inhibitor and Tyr188 or Tyr181 of RT.[12,13] This background prompted us to further explore novel TTAs bearing a larger 2,4-dibromo substituted phenyl attached to the 1,2,3-thiadiazole ring. Additionally, in view of the fact that the strength of halogen bonding,[16,17] one of the important interactions in biological molecules, decreases in the order Br > Cl, it is likely that the 2,4-dibromo derivatives could form potentially enhanced halogen bond with carbonyl oxygen in the main chain of residues nearby, and lastly increasing the overall RT-NNRTI binding affinity.

On the other hand, it has been demonstrated that the N-substituted phenyl moiety sits at the protein/solvent interface near a region of the protein known to be flexible,[12,13] it might tolerate substitution by structurally diverse groups. Therefore, in several novel molecules, the anilide phenyl ring was also replaced by several substituted heterocycles to further investigate the SARs in this region.

Herein we reported a facile synthetic pathway and biological evaluation of a series of 2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-phenylacetamide derivatives (Fig. 3) as novel NNRTIs. It is a further object of this paper to develop our under-

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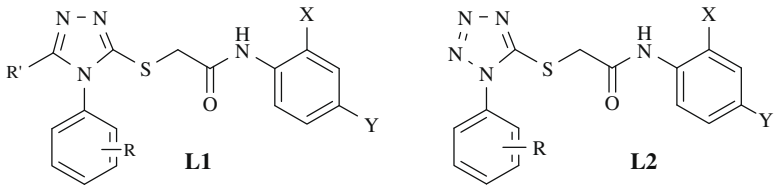
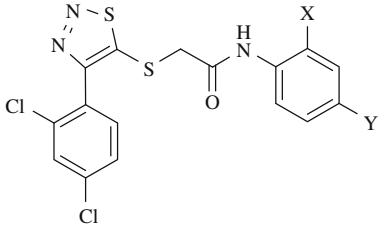


Figure 1. Triazole/tetrazole thioacetanilide based NNRTIs.



L3: X= NO_2, Y= H. EC_{50} = 0.059 μM, SI > 4883
L4: X= Br, Y= CO_2Et. EC_{50} = 0.099 μM, SI > 2298
L5: X= Cl, Y= H. EC_{50} = 0.118 μM, SI = 943
L6: X= Br, Y= H. EC_{50} = 0.149 μM, SI ≥981
L7: X= Br, Y= Me. EC_{50} = 0.204 μM, SI > 1265

Figure 2. 1,2,3-Thiadiazole based NNRTIs.

standing of the SAR among TTAs and to afford information to the ongoing modification of this scaffold.

2. Results and discussion

2.1. Chemistry

We described a convenient and original synthetic route for title compounds which requires the salt of 5-thiol-1,2,3-thiadiazole as the key intermediate. In this approach, 2-chloro-1-(2,4-dibromophenyl)ethanone (**2**), synthesized by Friedel–Crafts reaction of 1,3-dibromobenzene (**1**) with 2-chloroacetyl chloride, were reacted with 3-mercaptopropanoate in EtOH at ambient temperature for several hours to obtain the methyl 3-(1-(2,4-dibromophenyl)ethanone)propanoate (**3**) which was not purified and used for the next step directly. The compound **4** was synthesized from semicarbazide by reaction with the compound **3**. Ring-closure of compound **4** with thionyl chloride according to the method reported by Hurd and Mori,[18–20] and purification by flash chromatography produced the thiadiazole derivative **5**. Treatment of compound **5** with sodium methoxide afforded the desired sodium salt of 1,2,3-thiadiazole-5-thiolate **6** as a result of the β-elimination of the propyl ester group at C_5–S group.[21] The final 1,2,3-thiadiazole thioacetanilides **7** were synthesized by reaction of **6** with various 2-chloro-*N*-(substituted aromatic group)acetamides in high yields. These reactions are summarized in Scheme 1. The structures of all the synthesized title compounds were confirmed on the basis of spectral and analytical data.

It must be pointed out that we once reported a synthetic method for TTAs in our previous literature which requires the 1,2,3-thiadiazole-5-thioacetic acid (**9**), produced by hydrolysis of compounds **8** in KOH–EtOH–H_2O solution, as the key intermediates (Scheme 2).[13] In the last step, the title compounds **10** were obtained by acylation reaction of **9** with substituted anilines in the presence of 1.1 equiv mol of PCl_5. However, this route did not give us satisfactory results due to the low overall yield and complicated procedure in these two reaction steps. Consequently, a modified method was disclosed herein, which not only improved overall yield, but also provide possibility of further developing TTA derivatives with mild reaction condition and simple operation.

2.2. Anti-HIV cell-based assays and discussions

The newly synthesized TTA derivatives (**7a–r**) were evaluated for their anti-HIV activity and cytotoxicity in MT-4 cells infected with wild-type HIV-1 strain IIIB and HIV-2 strain ROD in comparison with nevirapine (NVP), delaviridine (DLV), efavirenz (EFV) and zidovudine (azidothymidine, AZT), which were used as reference drugs. The results, expressed as CC_{50} (50% cytotoxic concentration), EC_{50} (50% HIV-1 replication inhibitory concentration) and SI (selectivity index given by the CC_{50}/EC_{50} ratio), are summarized in Table 1.

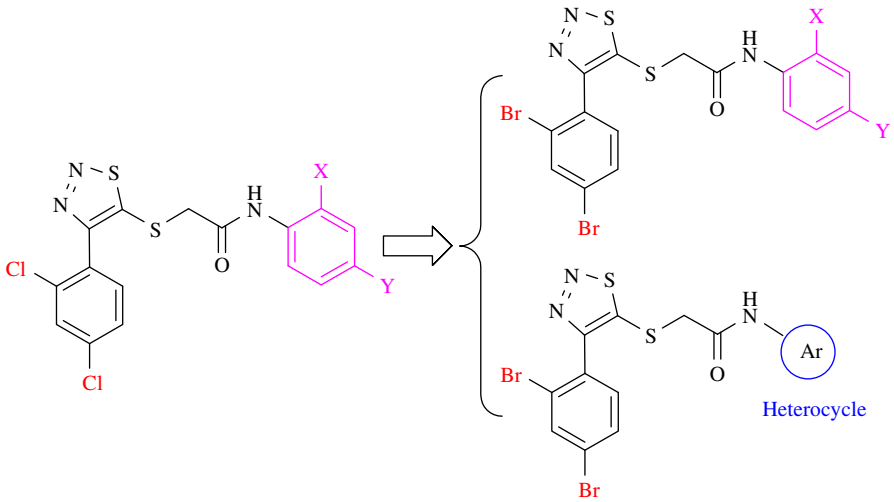
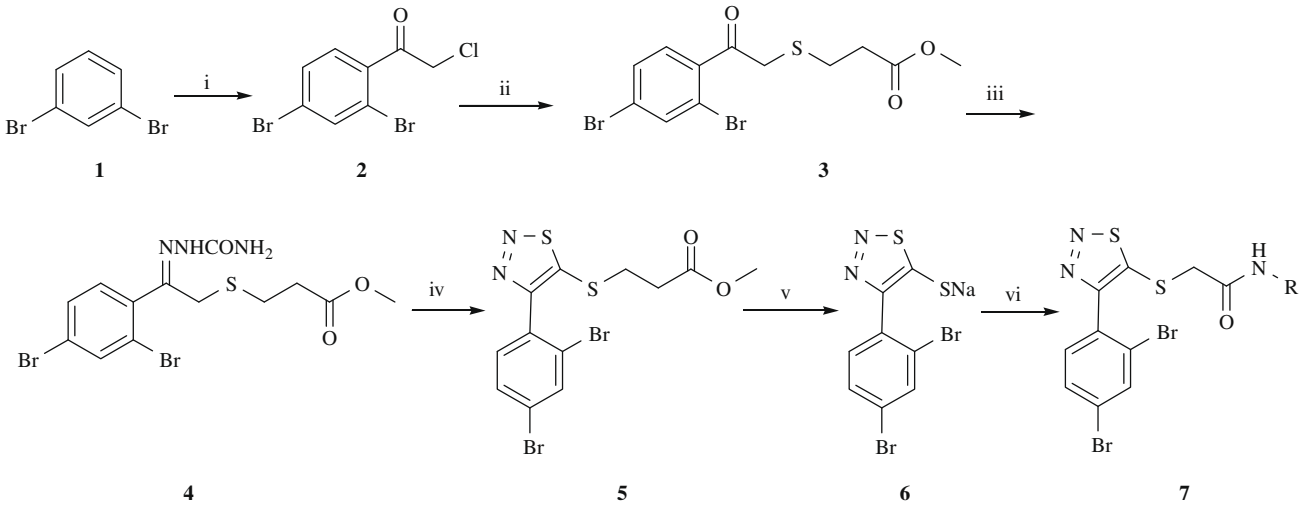
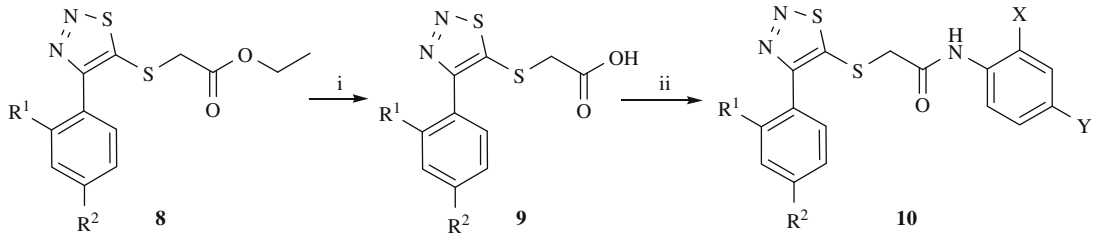


Figure 3. The design of novel 1,2,3-thiadiazole derivatives.



Scheme 1. Reagents: (i) $ClCOCH_2Cl/AlCl_3$; (ii) $HSCH_2CH_2COOCH_3$, Na_2CO_3, EtOH; (iii) $NH_2NHCONH_2{\cdot}HCl$, AcONa, EtOH; (iv) $SOCl_2$; (v) CH_3ONa/CH_3OH; (vi) $ClCH_2CONHR$, EtOH.



Scheme 2. Reagents and conditions: (i) KOH, EtOH–H_2O, 50–70 °C; (ii) PCl_5, aniline, DCM, rt, 1–2 h, Ref. 13.

The experimental results indicated that the majority of the tested TTA derivatives were found to be active against HIV-1 in the range of 0.0364–4.53 μM and none of the compounds was active against HIV-2. In particular, 10 compounds showed anti-HIV-1 activities at submicromolar concentrations. Interestingly, the cytotoxicity of TTAs was generally low. Owing to the above reasons, their SIs were in many cases similar to that of the reference drug. Compound **7c** was the most active derivative of this series, with EC_{50} = 36.4nM, CC_{50} > 240.1 μM, SI > 6460. **7c** was about sevenfold more active than NVP and eightfold more active than DLV.

In the N-substituted phenyl acetamide derivatives, as expected, we observed that 2,4-dibromo substitution on the phenyl of the thiadiazole ring increased the antiviral activity compared to the 2,4-dichloro substituted phenyl series.[12] Based on previous SAR studies on the phenyl-thiadiazole, we found that the antiviral potency of TTAs decreases in the order 2,4-dibromo > 2,4-dichloro > 2,4-difluorine.

Table 1 also reveals the potency order of the *ortho* substitution at the phenyl ring of the anilide moiety: NO_2 (**7g**, EC_{50} = 0.040 μM) > Cl (**7b**, EC_{50} = 0.083 μM) > Br (**7d**, EC_{50} = 0.12 μM) > F (**7a**, EC_{50} = 0.14 μM) > Me (**7i**, EC_{50} = 0.52 μM) > H (**7j**, EC_{50} = 0.54 μM), indicating that electron withdrawing groups, such as nitro and halogen, were the preferred substituents compared with electron-donating group methyl or hydrogen atom. Thus, the activity data of the TTAs are affected by the electronic nature or the steric demand of the *ortho* substitution. In addition, a decrease of activity was observed in the *para* mono-substituted isomer of TTAs with the *ortho* single substitution at the anilide moiety (**7k** vs **7i**; **7l** vs **7b**).

In the case of compounds **7d** and **7g**, introduction of methyl group on the *para* position at the phenyl ring of the anilide moiety led to **7e** and **7h** with decreased activity, whereas introduction of 4-acetyl in **7d** led to **7f** with slightly improved activity, indicating that the nature of the substituent at the *para* position influenced the anti-HIV-1 activity remarkably.

Significantly increased cytotoxicities were observed in **7j**, **7k** and **7l** characterized by the absence of the *ortho* substitution at the phenyl ring of the anilide moiety. The result indicates that the *ortho* substitutions played a determinant role in their cytotoxicity.

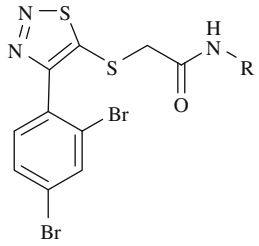
In the N-substituted heterocycle acetamide derivatives, pyridine derivative **7c** showed an improved anti-HIV-1 profile superior to that of **7b**, with a higher selectivity index. Moreover, pyridine derivative **7o**, thiazole derivative **7p** and thiophene derivative **7r** demonstrated reasonable antiviral activity. These results confirmed the idea that the introduction of structurally diverse heterocycles in this region could be a valid strategy to get novel molecules with increased or appreciable antiviral potency.

In addition, the finding that none of the title compounds was found effective against HIV-2 (Table 1) showed that this new series of TTAs was specific for HIV-1 and belonged to typical NNRTIs.

2.3. Molecular modeling analysis

Docking calculations were also performed by means of Autodock Vina to investigate the binding mode of compound **7c** into the NNRTIs binding pocket (NNIBP) of HIV-1 RT and to rationalize some SARs. Three-dimensional coordinates of the HIV-1 RT/GW564511 (benzophenone based NNRTI) complex (Brookhaven Protein Data Bank entry 3DLG) were used as the input structure because of the high degree of similarity between sulfanyltriazole/tetrazole leads and benzophenones.[11] Default parameters were used as described in the AutoDock manual unless otherwise specified. The theoretical binding mode of **7c** to the NNIBP is shown in Figure 4.

Table 1
Anti-HIV activities, cytotoxicities and selectivity indices of 2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)acetamide derivatives (**7a**–**r**)



No.	R	EC_{50}[a] (μM)		CC_{50}[b] (μM)	SI[c]	
		HIV-1 III_B	HIV-2 ROD		HIV-1 III_B	HIV-2 ROD
7a	2-Fluorophenyl	0.14 ± 0.01	>27.82	⩾27.82	>195	<or×1
7b	2-Chlorophenyl	0.083 ± 0.015	⩾221.30	>240.54	>2904	>or×1
7c	2-Chloropyridin-3-yl	0.0364 ± 0.0038	>240.08	>240.08	>6460	×1
7d	2-Bromophenyl	0.12 ± 0.01	171.78 ± 52.90	>221.59	>1786	>1
7e	2-Bromo-4-methylphenyl	0.22 ± 0.05	>216.21	>216.21	>958	×1
7f	4-Acetyl-2-bromophenyl	0.058 ± 0.026	>206.22	>206.22	>3546	×1
7g	2-Nitrophenyl	0.040	>235.76	>235.76	>5841	×1
7h	4-Methyl-2-nitrophenyl	0.079 ± 0.017	>229.68	>229.68	>2934	×1
7i	*o*-Tolyl	0.52 ± 0.18	>201.87	201.87 ± 35.85	381	<1
7j	Phenyl	0.54 ± 0.02	>24.83	24.83 ± 1.26	47	<1
7k	*p*-Tolyl	1.52 ± 0.96	>24.04	24.04 ± 0.28	16	<1
7l	4-Chlorophenyl	2.19 ± 0.60	>23.34	23.34 ± 0.69	11	<1
7m	2,3-Dimethylphenyl	1.44 ± 0.27	>240.95	240.95 ± 2.98	166	<1
7n	2,6-Dimethylphenyl	>198.73	>198.73	⩾198.73	<or×1	<or×1
7o	Pyridin-2-yl	1.17 ± 0.51	>42.43	42.43 ± 9.26	36	<1
7p	Thiazol-2-yl	4.53 ± 0.33	>87.76	87.76 ± 43.37	19	<1
7q	5-Methylbenzo[*d*]thiazol-2-yl	>23.55	>23.55	23.55 ± 1.42	<1	<1
7r	3-Methyl acetate-thiophen-2-yl	4.01 ± 1.55	>227.57	>227.57	>57	×1
NVP[d]		0.208		>15.02	>72	
DLV[d]		0.320		>3.827	>12	
EFV[d]		0.00440		>6.336	>1434	
AZT[d]		0.0151		>93.55	>6192	

[a] EC_{50}: 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}: 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}). The SI values: ×1 stand for ⩾1or<1.
[d] The antiviral properties of these compounds were previously described, Ref. 13.

Results showed that the 2,4-dibromophenyl ring of **7c** fits into the aromatic-rich binding pocket, surrounded by the aromatic side chains of Tyr188, Phe227 and Trp229. Detailed analysis of the binding mode shows that the phenyl ring is parallel to Tyr188 side chain, giving rise to a positive π-stacking interaction, and an interesting but indefinite interaction in which the 4-bromo of the phenyl points directly at, and perpendicular to, the highly conserved residue W229. The highly conserved amino acid residues in the NNIBP are essential for viral replication, and improved binding at these amino acids could result in the development of novel NNRTIs not sensitive to the common RT mutations.[22] Therefore, the activity was strongly affected by the chemical features of halogen atoms

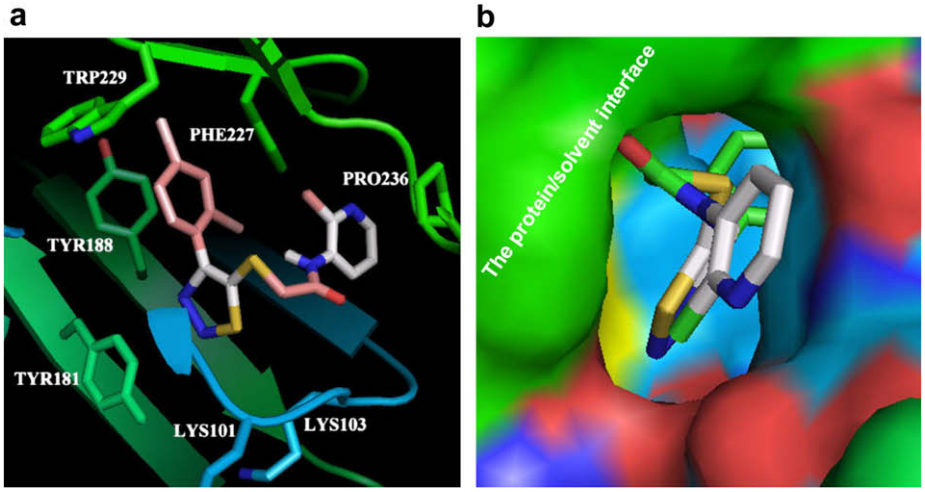


Figure 4. Model of **7c** docked into the RT non-nucleoside binding site (PDB code: 3DLG) using Autodock Vina [http://vina.scripps.edu]. The docking result of **7c** is showed by PyMOL. (a) Overview; (b) View is looking inward along the enzyme–solvent interface under Pro236, showing the solvent exposed lower surface of the 2-chloropyridin-3-yl group in **7c**.

in the 2,4-bisubstituted phenyl ring linked to thiadiazole and we can presume that replacing the 2,4-dibromo by 2,4-diiodine phenyl group may lead to even superior anti-HIV-1 activities.

The inhibitor's amide carbonyl forms a key hydrogen bond with the backbone N–H of Lys103 (not shown). In compound **7n**, it is likely that the steric factor of 2,6-dimethyl diminishes this hydrogen bond, yielding an overall negative effect on the activity.

The 2-chloropyridin-3-yl group of **7c** is close to the flexile Pro236 'hairpin loop' of the protein/solvent interface, and the nitrogen atom in the pyridine ring points toward the solvent exposed region (Fig. 4b), suggesting that this plasticity region could accommodate substantial modifications of the inhibitor molecule. The hydrophilic groups (atoms) has been proved to be the preferred substituents, which can explain the SAR conclusion of this region.

Overall, our studies suggest that this class of compounds share the similar bound conformation with sulfanyltriazoles[5] and sulfanyltetrazoles-based NNRTIs.[9,10] As illustrated in Fig. 4a, the compound **7c** binds in a 'kinked' conformation which involves a rotation about the 'S–CH_2–CO–NH' dihedral angle from 180° in the fully extended free-state conformation to almost 0° in the bound state. The thiadiazole is orthogonal to the 2,4-dibromophenyl ring, which orients the pharmacophores into the proper geometry for binding. This brings the two substituted aryl rings into close proximity and results in the amide N–H pointing toward the sulfur atom (probably forming intramolecular hydrogen bond).

In summary, the results of the AutoDocking analysis supported our newly designed and synthesized compounds. Further optimization of TTA analogues will consider these aspects in further design attempts.

3. Conclusions

In this article, we have described a improved synthetic route with easy operation and higher yield and anti-HIV-1 activity evaluation of a new series of 2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio) acetamides. The bioassay results revealed that the majority of the title compounds exhibited potent HIV-1 inhibitory activity. In particular, the *N*-(2-chloropyridin-3-yl) analogue **7c** turned out to be the most potent with an EC_{50} value of 36.4 nM, being about sevenfold more active than the reference compounds NVP and DLV. Based upon the preliminary SAR studies of these new 2,4-dibromophenyl substituted TTAs, some structural requirements for high potency against HIV-1 were identified and rationalized by the molecular modeling studies. Further synthesis of new TTA derivatives and quantitative SAR (QSAR) studies are currently performed in our laboratories.

4. Experimental

4.1. Chemistry

All melting points were determined on a micromelting point apparatus and are uncorrected. Infrared spectra (IR) were recorded with a Nexus 470FT-IR Spectrometer. ^{1}H NMR spectra were obtained on a Brucker Avance-600 NMR-spectrometer in the indicated solvents. Chemical shifts are expressed in δ units and TMS as internal reference. Mass spectra were taken on a LC Autosampler Device: Standard G1313A instrument. TLC was performed on Silica Gel GF254 for TLC (Merck) and spots were visualized by iodine vapors or by irradiation with UV light (254 nm). Flash column chromatography was performed on column packed with Silica Gel 60 (230–400 mesh). Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of the reaction solutions involved the use of rotary evaporator at reduced pressure.

4.1.1. General procedure for the synthesis of sodium 4-(2,4-dibromophenyl)-1,2,3-thiadiazole-5-thiolate (6)

To a vigorously stirred mixture of the commercially available 1,3-dibromobenzene (**1**, 23.6 g, 0.1 mol) and anhydrous aluminum chloride (14.6 g, 0.11 mol) at room temperature was added chloroacetyl chloride (13 g, 0.1 mol) dropwise. The reaction mixture was stirred at 60–65 °C for 10 h (monitored by TLC). After cooling, the mixture was then diluted with CH_2Cl_2 (40 mL). The separated organic phase was washed successively with water, aqueous saturated $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford crude product 2-chloro-1-(2,4-dibromophenyl)ethanone (**2**) as a light yellow oil (26.7 g, yield: 85.4%), which was used in the next step without any further purification.

2-Chloro-1-(2,4-dibromophenyl)ethanone (2, 0.1 mol) was added to a mixture of methyl 3-mercaptopropanoate (11.4 mL, 0.1 mol) and Na_2CO_3 (5.3 g, 0.05) in 100 mL EtOH. The reaction mixture was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure. The residue was then diluted with CH_2Cl_2 (100 mL), washed successively with aqueous 0.1 M HCl solution, aqueous saturated $NaHCO_3$, water and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford crude product 3 as a light yellow oil.

A mixture of semicarbazide hydrochloride (12.2 g, 0.12 mol) and sodium acetate (8.2 g, 0.1 mol) was dissolved in absolute ethanol (50 mL). The mixture was heated for 60 min under reflux, and then filtered while hot to remove precipitated sodium chloride. The filtrate was then mixed with compound **3** (39.6 g, 0.1 mol), and the resulting mixtures were heated to reflux, a few drops of concentrated hydrochloric acid were added and heating under reflux with continuous removal of the generated water was continued overnight. Finally, the solvent was cooled and filtered. The obtained solid was washed successively with water and diethyl ether and dried to give compound **4** as white solid, yield: 74.7%, mp 186–188 °C.

A 4.5 g (0.01 mol) portion of semicarbazone (**4**) was diluted with 10 mL of dry methylene chloride and this was added, fairly rapidly, using a dropping funnel, to 10 mL of thionyl chloride with rapid stirring which was continued for 6 h after addition was completed. The reaction mixture was then evaporated under reduced pressure and two portions of methylene chloride were added and removed under reduced pressure. The residue was dissolved in ethyl acetate, filtered and the filtrate was evaporated to a residue which was chromatographed on silica gel using ethyl acetate:petroleum ether (1:4). Fractions were collected and evaporated, giving the desired compound (**5**) as brilliant yellow oil (80.7%).

A solution of sodium methoxide (0.54 g, 0.01 mol) in 15 mL of methanol was added to a solution of methyl 3-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)propanoate (**5**) (4.4 g, 0.01 mol) in 100 mL of methanol. After about 1 h, the reaction solution was evaporated in vacuo to about 10 mL. Dry methylene chloride (50 mL) was added, causing precipitation of a white solid. This solid was collected, wash with methylene chloride, giving the desired product **6** as white powder (90.4%), mp: 98–100 °C.

4.1.2. General procedure for the synthesis of 2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio) acetamide (7a–r)

To the solution of sodium 4-(2,4-dibromophenyl)-1,2,3-thiadiazole-5-thiolate (**6**) (1.05 mmol, 0.4 g) in ethanol (30 mL) was added 2-chloro-*N*-(substituted aromatic group)acetamides (1.0 mmol). The reaction mixture was stirred at room temperature overnight. Upon completion of the reaction, the solvent was evaporated, leaving a residue which was treated with methylene chloride (30 mL) and washed with water (3 × 30 mL). The organic layer was dried over anhydrous sodium sulfate, the drying agent was filtered off, and the solvent was removed in vacuo. The residue was purified by recrystallization using ethanol to yield the title compounds (**7a–r**).

4.1.2.1. 2-(4-(2,4-Dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-(2-fluorophenyl)acetamide (7a). White needle crystals, yield: 49.8%. Mp: 121–123 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.21 (t, 1H, Ph'H), 8.10 (br s, 1H, NH), 7.92 (d, 1H, J_1 = 1.8 Hz, PhH), 7.57 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.4 Hz, PhH), 7.32 (d, 1H, J_2 = 8.4 Hz, PhH), 7.15 (m, 3H, Ph'H), 3.81 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3271 (ν_{NH}), 1684 ($\nu_{C=O}$), 1624, 1548, 1489, 1456 ($\nu_{N=N}$), 744 (ν_{C-S}). MS (ESI): *m*/*z* 502.1 (M+1), 504.1 (M+3), 506.1 (M+5). $C_{16}H_{10}Br_2FN_3OS_2$ (500.86).

4.1.2.2. *N*-(2-Chlorophenyl)-2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)acetamide (7b). Light yellow cubic crystals, yield: 59.4%. Mp: 161–163 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.52 (s, 1H, NH), 8.28 (d, 1H, J = 8.4 Hz, PhH), 7.90 (d, 1H, J = 1.2 Hz, Ph'H), 7.54 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.2 Hz, PhH), 7.37 (d, 1H, J = 1.2 Hz, PhH), 7.29 (m, 2H, Ph'H), 7.10 (t, 1H, Ph'H), 3.83 (s, 2H, S–CH_2). ^{13}C NMR ($CDCl_3$, ppm) δ: 163.7, 158.4, 147.9, 135.8, 133.5, 133.0, 131.0, 129.9, 129.2, 127.9, 125.7, 124.8, 124.7, 123.2, 121.5, 41.6. IR (KBr, cm^{-1}): 3365 (ν_{NH}), 1699 ($\nu_{C=O}$), 1592, 1528, 1440 ($\nu_{N=N}$), 1306, 1217, 750 (ν_{C-S}). MS (ESI): *m*/*z* 518.1 (M+1), 520.1 (M+3), 522.1 (M+5). $C_{16}H_{10}Br_2ClN_3OS_2$ (516.83).

4.1.2.3. *N*-(2-Chloropyridin-3-yl)-2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)acetamide (7c). White needle crystals, yield: 75.8%. Mp: 161–163 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.65 (d, 1H, J = 7.8 Hz, pyridine-H), 8.52 (br s, 1H, NH), 8.17 (dd, 1H, J = 1.2 Hz, J = 4.8 Hz, pyridine-H), 7.92 (d, 1H, J_1 = 1.8 Hz, PhH), 7.58 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.4 Hz, PhH), 7.32 (d, 1H, J_2 = 8.4 Hz, PhH), 7.28 (m, 1H, pyridine-H), 3.84 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3305 (ν_{NH}), 1680 ($\nu_{C=O}$), 1523, 1454 ($\nu_{N=N}$), 1392, 1212, 741(ν_{C-S}). MS (ESI): *m*/*z* 519.1 (M+1), 521.1 (M+3). $C_{15}H_9Br_2ClN_4OS_2$ (517.83).

4.1.2.4. *N*-(2-Bromophenyl)-2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)acetamide (7d). White cubic crystals, yield: 75.8%. Mp: 147–149 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.54 (s, 1H, NH), 8.25 (d, 1H, J = 7.8 Hz, Ph'H), 7.91 (d, 1H, J_1 = 1.8 Hz, PhH), 7.55 (m, 1H, PhH), 7.54 (m, 1H, Ph'H), 7.36 (m, 1H, Ph'H), 7.30 (m, 1H, PhH), 7.03 (t, 1H, Ph'H), 3.83 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3351 (ν_{NH}), 1690 ($\nu_{C=O}$), 1527, 1436 ($\nu_{N=N}$), 1303, 1217, 748 (ν_{C-S}). MS (ESI): *m*/*z* 562.1(M+1), 564.0 (M+3), 566.0 (M+5). $C_{16}H_{10}Br_3N_3OS_2$ (560.78).

4.1.2.5. *N*-(2-Bromo-4-methylphenyl)-2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)acetamide (7e). White solid, yield: 84.5%. Mp: 169–171 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.45 (s, 1H, NH), 8.10 (d, 1H, J = 8.4 Hz, Ph'H), 7.91 (d, 1H, J_1 = 1.8 Hz, PhH), 7.54 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.4 Hz, PhH), 7.36 (s, 1H, Ph'H), 7.32 (d, 1H, J = 8.4 Hz, PhH), 7.14 (s,1H, J = 8.4 Hz, Ph'H), 3.51 (s, 2H, S–CH_2), 2.32 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3275 (ν_{NH}), 3039, 2951, 2910, 1681 ($\nu_{C=O}$), 1529, 1488 ($\nu_{N=N}$), 1226, 1184, 740 (ν_{C-S}). MS (ESI): *m*/*z* 576.1 (M+1), 578.1 (M+3). $C_{17}H_{12}Br_3N_3OS_2$ (574.8).

4.1.2.6. *N*-(4-Acetyl-2-bromophenyl)-2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)acetamide (7f). White needle crystals, yield: 74.1%. Mp: 175–177 °C (decompose). ^{1}H NMR ($CDCl_3$, ppm) δ: 8.74 (s, 1H, NH), 8.44 (d, 1H, J = 8.4 Hz, Ph'H), 8.17 (s, 1H, Ph'H), 7.92 (d,1H, J_1 = 1.2 Hz, PhH), 7.90 (d, 1H, J = 8.4 Hz, Ph'H), 7.54 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, PhH), 7.31 (d, 1H, J_2 = 8.4 Hz, PhH), 3.84 (s, 2H, S–CH_2), 2.59 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3237 (ν_{NH}), 1681($\nu_{C=O}$), 1668 ($\nu_{C=O}$), 1527, 1391 ($\nu_{N=N}$), 1260, 1229, 736 (ν_{C-S}). MS (ESI): *m*/*z* 604.0 (M+1), 606.0 (M+3), 608.0 (M+5). $C_{18}H_{12}Br_3N_3O_2S_2$ (602.79).

4.1.2.7. 2-(4-(2,4-Dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-(2-nitrophenyl)acetamide (7g). Yellow needle crystals, yield: 74.1%. Mp: 156–158 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 11.10 (s, 1H, NH), 8.71 (d, 1H, J = 8.4 Hz, Ph'H), 8.24 (dd, 1H, J = 1.2 Hz, J = 8.4 Hz, Ph'H), 7.89 (d, 1H, J_1 = 1.2 Hz, PhH), 7.70 (t, 3H, Ph'H), 7.54 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, PhH), 7.34 (d, 1H, J_2 = 8.4 Hz, PhH), 7.27 (m, 2H, Ph'H), 3.85 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3298 (ν_{NH}), 1691 ($\nu_{C=O}$), 1500 ($\nu_{as\ NO_2}$), 1431 ($\nu_{N=N}$), 1338 ($\nu_{s\ NO_2}$), 1277, 1221, 742 (ν_{C-S}). MS (ESI): *m*/*z* 529.2 (M+1), 531.1 (M+3), 533.1 (M+5). $C_{16}H_{10}Br_2N_4O_3S_2$ (527.86).

4.1.2.8. 2-(4-(2,4-Dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-(4-methyl-2-nitrophenyl)acetamide (7h). Light yellow needle crystals, yield: 84.7%. Mp: 186–188 °C (decompose). ^{1}H NMR ($CDCl_3$, ppm) δ: 10.99 (s, 1H, NH), 8.56 (d, 1H, J = 8.4 Hz, Ph'H), 8.04 (m, 1H, J = 1.8 Hz, Ph'H), 7.89 (d, 1H, J_1 = 1.8 Hz, PhH), 7.55 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.4 Hz, PhH), 7.49 (dd, 1H, J = 1.8 Hz, J = 8.4 Hz, Ph'H), 7.34 (d, 1H, J_2 = 8.4 Hz, PhH), 3.83 (s, 2H, S–CH_2), 2.42 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3304 (ν_{NH}), 2914, 1690 ($\nu_{C=O}$), 1514 ($\nu_{as\ NO_2}$), 1445 ($\nu_{N=N}$), 1336 ($\nu_{s\ NO_2}$), 1277, 1220, 739 (ν_{C-S}). MS (ESI): *m*/*z* 543.2 (M+1), 545.1 (M+3). $C_{17}H_{12}Br_2N_4O_3S_2$ (541.87).

4.1.2.9. 2-(4-(2,4-Dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-*o*-tolylacetamide (7i). White lamella crystals, yield: 70.8%. Mp: 121–123 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 7.92 (s, 1H, J_1 = 1.2 Hz, PhH), 7.87 (br s, 1H, NH), 7.72 (d, 1H, J = 8.4 Hz, Ph'H), 7.56 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, PhH), 7.31 (d, 1H, J_2 = 8.4 Hz, PhH), 7.23 (t, 1H, Ph'H), 7.19 (m, 1H, Ph'H), 7.12 (t, 1H, Ph'H), 3.84 (s, 2H, S–CH_2), 2.13 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3242 (ν_{NH}), 3026, 2980, 2936, 2851, 1640 ($\nu_{C=O}$), 1532, 1490 ($\nu_{N=N}$), 1216, 751 (ν_{C-S}). MS (ESI): *m*/*z* 498.1(M+1), 500.1 (M+3). $C_{17}H_{13}Br_2N_3OS_2$ (496.89).

4.1.2.10. 2-(4-(2,4-Dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-phenylacetamide (7j). White solid, yield: 78.4%. Mp: 119–121 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 7.92 (d, 1H, J_1 = 1.2 Hz, PhH), 10.37 (br s, 1H, NH), 7.57 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, PhH), 7.41 (d, 1H, J_2 = 8.4 Hz, PhH), 7.32 (m, 4H, Ph'H), 7.17 (dt, 1H, Ph'H), 3.78 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3274 (ν_{NH}), 1676 ($\nu_{C=O}$), 1605, 1557, 1444 ($\nu_{N=N}$), 1220, 746 (ν_{C-S}). MS (ESI): *m*/*z* 484.2 (M+1), 486.1 (M+3). $C_{16}H_{11}Br_2N_3OS_2$ (482.87).

4.1.2.11. 2-(4-(2,4-Dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-*p*-tolylacetamide (7k). White crystals, yield: 80.7%. Mp: 155–157 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 7.92 (d, 1H, J_1 = 1.8 Hz, PhH), 7.86 (br s, 1H, NH), 7.57 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.4 Hz, PhH), 7.32 (d, 1H, J_2 = 8.4 Hz, PhH), 7.28 (d, 2H, J = 8.4 Hz, Ph'H), 7.13 (d, 2H, J = 8.4 Hz, Ph'H), 3.77 (s, 2H, S–CH_2), 2.33 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3249 (ν_{NH}), 3033, 2921, 2854, 1646 ($\nu_{C=O}$), 1541, 1421 ($\nu_{N=N}$), 1374, 1232, 815, 738 (ν_{C-S}). MS (ESI): *m*/*z* 498.1 (M+1), 500.1 (M+3). $C_{17}H_{13}Br_2N_3OS_2$ (496.89).

4.1.2.12. *N*-(4-Chlorophenyl)-2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)acetamide (7l). Light yellow solid, yield: 58.7%. Mp: 122–124 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 7.92 (d, 1H, J_1 = 1.2 Hz, PhH), 7.91 (br s, 1H, NH), 7.58 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, PhH), 7.36 (d, 2H, J = 9 Hz, Ph'H), 7.33 (d, 1H, J_2 = 8.4 Hz, PhH), 7.30 (d, 2H, J = 9 Hz, Ph'H), 3.77 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3307 (ν_{NH}), 1672 ($\nu_{C=O}$), 1541, 1492 ($\nu_{N=N}$), 1400, 1217, 823, 740 (ν_{C-S}). MS (ESI): *m*/*z* 518.1 (M+1), 520.1 (M+3). $C_{16}H_{10}Br_2ClN_3OS_2$ (516.83).

4.1.2.13. 2-(4-(2,4-Dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-(2,3-dimethylphenyl)acetamide (7m). White needle crystals, yield: 80.7%. Mp: 120–122 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 7.93 (s, 1H, J_1 = 1.2 Hz, PhH), 7.86 (s, 1H, NH), 7.56 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, PhH), 7.38 (d, 1H, J = 7.8 Hz, Ph'H), 7.31 (d, 1H, J_2 = 8.4 Hz, PhH), 7.11 (t, 1H, Ph'H), 7.05 (d, 1H, J = 7.8 Hz, Ph'H), 3.84 (s, 2H, S–CH_2), 2.29 (s, 3H, CH_3), 2.00 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3292 (ν_{NH}), 3058, 2961, 2920, 2854, 1680 ($\nu_{C=O}$), 1542, 1469 ($\nu_{N=N}$), 1216, 773, 741 (ν_{C-S}). MS (ESI): *m*/*z* 512.2 (M+1), 514.2 (M+3). $C_{18}H_{15}Br_2N_3OS_2$ (510.9).

4.1.2.14. 2-(4-(2,4-Dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-(2,6-dimethylphenyl)acetamide (7n). White lamella crystals, yield: 84.5%. Mp: 156–158 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 7.94 (d, 1H, J_1 = 1.2 Hz, PhH), 7.60 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, PhH), 7.55 (br s, 1H, NH), 7.33 (d, 1H, J_2 = 8.4 Hz, PhH), 7.13 (q, 1H, Ph'H), 7.06 (d, 1H, J = 7.8 Hz, Ph'H), 3.86 (s, 2H, S–CH_2), 2.10 (s, 6H, CH_3). IR (KBr, cm^{-1}): 3273 (ν_{NH}), 3020, 2962, 2924, 2851, 1645 ($\nu_{C=O}$), 1518, 1473, 1437 ($\nu_{N=N}$), 1214, 765, 740 (ν_{C-S}). MS (ESI): *m*/*z* 512.2(M+1), 514.1(M+3). $C_{18}H_{15}Br_2N_3OS_2$ (510.9).

4.1.2.15. 2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-(pyridin-2-yl)acetamide (7o). White needle crystals, yield: 58.7%. Mp: 128–130 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.82 (br s, 1H, NH), 8.27 (d, 1H, J = 4.8 Hz, pyridine-H), 8.15 (d, 1H, J = 8.4 Hz, pyridine-H), 7.91 (d, 1H, J_1 = 1.8 Hz, PhH), 7.76 (m,1H, pyridine-H), 7.56 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.4 Hz, PhH), 7.33 (d, 1H, J_2 = 8.4 Hz, PhH), 7.11 (m, 1H, pyridine-H), 3.81 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3195 (ν_{NH}), 1686 ($\nu_{C=O}$), 1586, 1535, 1438 ($\nu_{N=N}$), 1318, 789, 740 (ν_{C-S}). MS (ESI): *m*/*z* 585.1(M+1), 587.1(M+3). $C_{15}H_{10}Br_2N_4OS_2$ (483.87).

4.1.2.16. 2-(4-(2,4-Dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-(thiazol-2-yl)acetamide (7p). Yellow cubic crystals, yield: 54.2%. Mp: 217–219 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 12.48 (s, 1H, NH), 8.15 (d, 1H, J_1 = 1.2 Hz, PhH), 7.77 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, PhH), 7.52 (d, 1H, J_2 = 8.4 Hz, PhH), 7.51 (d, 1H, J = 3.6 Hz, thiazole-H), 7.28 (d,1H, J = 3.6 Hz, thiazole-H), 4.19 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3436 (ν_{NH}), 1684 ($\nu_{C=O}$), 1585, 1429 ($\nu_{N=N}$), 1330, 1227, 1176, 1163, 817, 740 (ν_{C-S}). MS (ESI): *m*/*z* 491.1(M+1), 493.1(M+3). $C_{13}H_8Br_2N_4OS_3$ (489.82).

4.1.2.17. 2-(4-(2,4-Dibromophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-(5-methylbenzo[*d*]thiazol-2-yl)acetamide (7q). Yellow solid, yield: 74.5%. Mp: 125–127 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 12.65 (s,1H, NH), 8.15 (d, 1H, J_1 = 1.8 Hz, PhH), 7.78 (s, H, Ph'H), 7.76 (m, 1H, PhH), 7.65 (d, 1H, J = 8.4 Hz, Ph'H), 7.52 (d, 1H, J_2 = 8.4 Hz, PhH), 7.26 (d, 1H, J = 8.4 Hz, Ph'H), 4.24 (s, 2H, S–CH_2), 2.50 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3435 (ν_{NH}), 2991, 2920, 2854, 1655 ($\nu_{C=O}$), 1608, 1550, 1435 ($\nu_{N=N}$), 815, 738 (ν_{C-S}). MS (ESI): *m*/*z* 555.0 (M+1), 557.1(M+3). $C_{18}H_{12}Br_2N_4OS_3$ (553.85).

4.1.2.18. Methyl 2-(2-(4-(2,4-dibromophenyl)-1,2,3-thiadiazol-5-ylthio)acetamido)thiophene-3-carboxylate (7r). Light yellow needle crystals, yield: 68.7%. Mp: 148–150 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 10.86 (s, 1H, NH), 8.05 (d, 1H, J = 5.4 Hz, thiophene-H), 7.90 (d, 1H, J_1 = 1.2 Hz, PhH), 7.55 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, PhH), 7.51 (d, 1H, J = 6.0 Hz, thiophene-H), 7.35 (d, 1H, J_2 = 8.4 Hz, PhH), 3.88 (s, 2H, S–CH_2), 3.81 (s, 3H, OCH_3). IR (KBr, cm^{-1}): 3275 (ν_{NH}), 1674 ($\nu_{C=O}$), 1571 ($\nu_{C=O}$), 1444 ($\nu_{N=N}$), 1282, 783, 736 (ν_{C-S}). MS (ESI): *m*/*z* 548.2 (M+1), 550.0 (M+3). $C_{16}H_{11}Br_2N_3O_3S_3$ (546.83).

4.2. Anti-HIV activity assays

Evaluation of the antiviral activity of the compounds against HIV-1 strain III_B and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described.[23,24] Stock solutions (10× final concentration) of test compounds were added in 25-μL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial fivefold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1(III_B)[25] or HIV-2 (ROD)[26] stock (50 μL) at 100–300 $CCID_{50}$ (cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells[27] were centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, and 50-μL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Multiscan Ascent Reader, Labsystems, Helsinki, Finland), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of tree wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD540) of the mock-infected control sample by 50%. The concentration achieving 50% protection from the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

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