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Original article

Synthesis and anti-HIV activity evaluation of 2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)-*N*-acetamides as novel non-nucleoside HIV-1 reverse transcriptase inhibitors

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ABSTRACT

A series of 2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (TTA) derivatives were synthesized and evaluated as potent inhibitors of HIV-1. Among the newly disclosed TTAs, compounds **7f**, **7g** and **7c** were the most potent inhibitors of HIV-1 replication of the series ($EC_{50} = 0.17 \pm 0.02$, 0.36 ± 0.19 and 0.39 ± 0.05 μM, respectively), coupled with a reasonable selectivity index ($SI > 1452$, >845, and >774, respectively). They possess improved or similar HIV-1 inhibitory activity compared with NVP ($EC_{50} = 0.208$ μM) and DLV ($EC_{50} = 0.320$ μM). The preliminary structure–activity relationships among the newly synthesized congeners are discussed briefly and rationalized by docking studies.

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 proved to be an important component of cocktail therapy [1–4]. However, the long-term usage of NNRTIs in HIV/AIDS patients may eventually lead to the development of virus-drug resistance. Therefore, it is imperative to look for novel NNRTIs with potent and broad spectrum anti-HIV mutant activity that are also safe and have excellent pharmacokinetic profiles [5].

Among the representatives of the NNRTIs, sulfanyltriazoles (**I**) and sulfanyltetrazoles (**II**) (Fig. 1) have interesting structures and offer various opportunities on the skeleton of sulfanylazoles as lead compounds [6–11]. Initially, we developed a series of 2-(4-(2,4-dichlorophenyl)-1,2,3-thiadiazol-5-ylthio)-*N*-acetamide (TTA) analogues (**III**, Fig. 1), which exhibited significant anti-HIV-1 activities [12,13]. Inspired by these promising results and in continuation of our work on the search of novel NNRTIs [14,15], we thought it worthwhile to synthesize new compounds of TTAs having 4-(2-naphthoyl) moiety attached to 1,2,3-thiadiazole, with the aim to strengthen the π–π stacking interaction between the inhibitors and aromatic residues (such as Tyr188 or Tyr181) of RT and to obtain new biologically active compounds (Fig. 2).

Molecular modeling studies of the sulfanyltriazole/tetrazoles family [6,10,11,13] revealed that the *N*-substituted anilide phenyl ring extends from the NNRTI binding pocket to the protein/solvent interface, which presents an attractive site (a tolerant region) for introducing structurally diverse moieties to generate novel molecules with reasonable anti-HIV activity (Fig. 2).

These backgrounds prompted us to further explore novel 4-(2-naphthoyl) TTAs bearing substituted anilide phenyl ring or heterocycles linked with the amide. In this paper, a series of novel TTA analogues were synthesized and evaluated for anti-HIV activity in MT-4 cell culture.

2. Results and discussion

2.1. Chemistry

A modified synthetic route of 1,2,3-thiadiazole thioacetanilides which requires the salt of 5-thiol-1,2,3-thiadiazole as one of the

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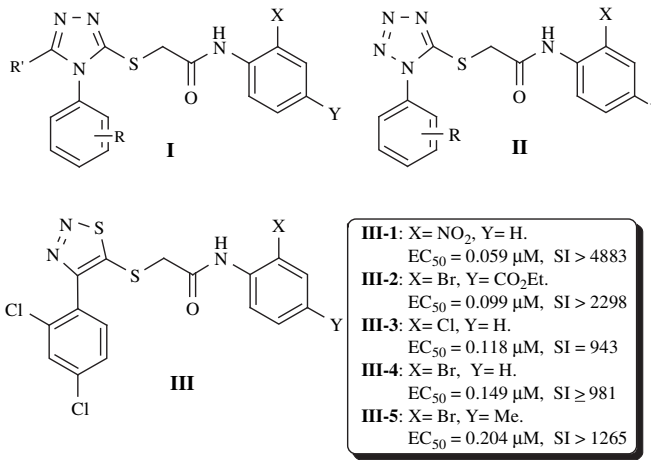


Fig. 1. Sulfanylazoles based NNRTIs.

key intermediates is developed as shown in Scheme 1. In this approach, the commercially available 1-(naphthalen-2-yl)ethanone (**1**) was utilized as starting material. 2-Bromo-1-(naphthalen-2-yl)ethanone (**2**), synthesized by direct bromination of **1**, was reacted with methyl 3-mercaptopropanoate in EtOH at ambient temperature for several hours to obtain the methyl 3-(1-(naphthalen-2-yl)ethanone)propanoate (**3**). The compound **4** was synthesized from semicarbazide by reaction with the compound **3**. Ring-closure reaction of **4** with $SOCl_2$ was carried out according to the similar methods reported in the literatures [16–18], producing the methyl 3-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)propanoate (**5**), which was purified by flash chromatography. 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 the C_5–*S* group [19]. The final 1,2,3-thiadiazole thioacetanilides **7** were synthesized by the reaction of **6** with various 2-chloro-*N*-(substituted aromatic group)acetamides in high yields. All the synthesized compounds **7a–r** were characterized by NMR, MS and IR. Both analytical and spectral data of all the compounds are in full agreement with the proposed structures. Moreover, comparison of the spectroscopic data of the new compounds with those of the previously reported analogues further confirmed the above structures [12].

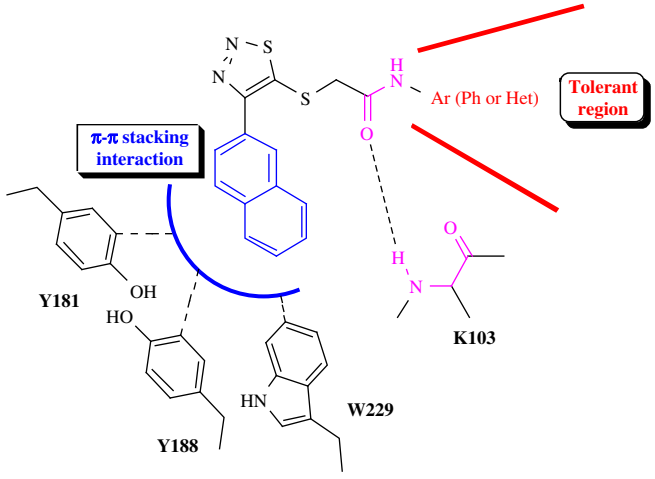


Fig. 2. Structure and proposed binding mode for newly designed TTAs to NNIBP.

2.2. Anti-HIV evaluation

All designed compounds were evaluated for cytotoxicity and anti-HIV activity in MT-4 cells, in comparison with nevirapine (NVP), delavirdine (DLV), efavirenz (EFV) and zidovudine (azidothymidine, AZT) used as reference drugs. The results, expressed as CC_{50}, EC_{50} and SI, are summarized in Table 1.

As can be seen from Table 1, nearly half of the test compounds inhibited HIV-1 replication at the lower micromolar concentration range. It is worth noting that a strikingly low cytotoxicity of these new TTAs was observed. In fact, the majority of them were non-cytotoxic for MT-4 cells at doses as high as 253 μM. Among all the newly disclosed TTAs, compounds **7f**, **7g** and **7c** were the most potent inhibitors of HIV-1 replication of the series ($EC_{50} = 0.17 \pm 0.02$, 0.36 ± 0.19 and 0.39 ± 0.05 μM, respectively), coupled with the highest selectivity index (SI > 1452, >845, and >774, respectively). They possess similar HIV-1 inhibitory activity compared with NVP ($EC_{50} = 0.208$ μM) and DLV ($EC_{50} = 0.320$ μM). Some other compounds, **7b, 7d**, **7e**, **7i**, and **7r**, also showed reasonable anti-HIV-1 potency ($EC_{50} < 4.5$ μM) and moderate selectivity indices.

Besides, the results shown in Table 1 revealed some important SAR information on the role of different substitutions in the aromatic ring linked with amide. In the case of 2-monosubstituted analogues (**7a**, **7b**, **7d**, **7g** and **7i**), a clear order of 2-substition for anti-HIV activity was observed by direct comparison: $NO_2 > Br > Cl > Me > F$. This conclusion is in agreement with the previous SAR studies grossly [12].

Furthermore, additional substitution with methyl at the *para*-position of anilide phenyl ring in compounds **7d** and **7g** led to derivatives **7e** and **7h** with reduced or deprived antiviral potency, respectively, suggesting that the nature (probably hydrophobicity) of methyl did not accommodate the chemical environment in this region of RT. In contrast, the increased activity against HIV-1 for **7c** (*versus* **7b**) and **7f** (*versus* **7d**) might suggest the detrimental effect of introducing a polar atom or moiety in the anilide phenyl ring to enhance antiviral activity. The above findings are also in agreement with the results of earlier SAR studies [12].

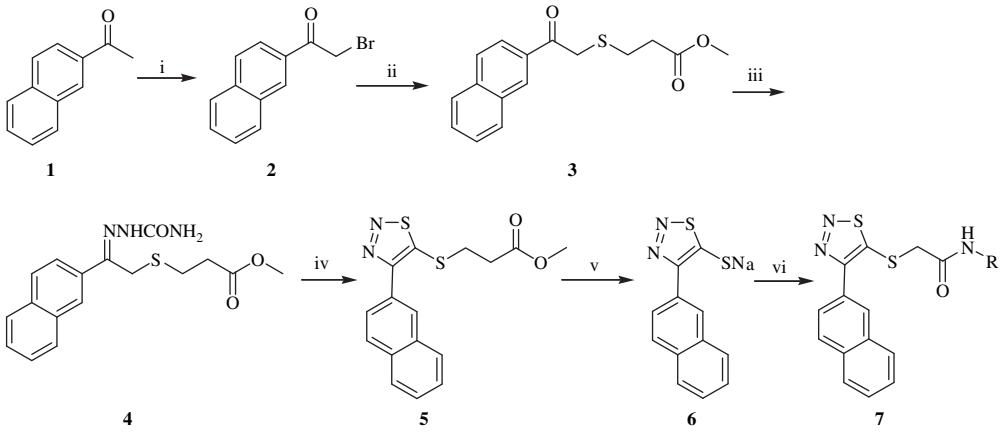
Moreover, it is worth noting that only compound **7r** demonstrated moderate antiviral activity ($EC_{50} = 4.42 \pm 0.09$ μM) among the *N*-substituted heterocycle derivatives **7o**–**r**, indicating that this region was structurally sensitive to modifications with the diverse heterocyclic substitutions.

All the compounds were evaluated for their, but did not show any, activity against HIV-2 (ROD) in MT-4 cells. These findings showed that this new series of TTAs was specific for HIV-1 and belonged to typical NNRTIs.

2.3. Molecular modeling analysis

In order to investigate the binding mode of our newly synthesized compounds and rationalize the SAR data, a molecular modeling of compound **7f** docked into the NNRTIs binding pocket (NNIBP) of HIV-1 RT was performed by means of Autodock Vina [http://vina.scripps.edu]. X-ray crystal structure of HIV-1 RT with benzophenone was taken from PDB (3DLG) and used for docking studies for the high degree of similarity between sulfanyltriazole/tetrazole leads and benzophenones [13]. Default parameters were used as described in the AutoDock manual unless otherwise specified. The theoretical binding mode of **7f** to the NNIBP is shown in Fig. 3.

Our studies suggest that this class of compounds shares the similar binding mode with sulfanyltriazoles [6] and sulfanyltetrazoles-based NNRTIs [10,11]. As illustrated in Fig. 3, the naphthalene



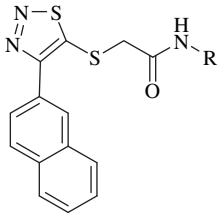
Scheme 1. Reagents and conditions: (i) Br_2/AcOH; (ii) $HSCH_2CH_2COOCH_3$, TEA, DCM; (iii) $NH_2NHCONH_2 \cdot HCl$, AcONa, EtOH; (iv) $SOCl_2$; (v) CH_3ONa/CH_3OH; (vi) $ClCH_2CONHR$, EtOH.

ring of **7f** 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 one phenyl ring is parallel to Tyr188 side chain and is orthogonal to the indole ring of Trp229, giving rise to a positive π-stacking interaction and CH–π interaction, respectively. The inhibitor's amide carbonyl forms a key hydrogen bond with the backbone N–H of Lys103 (not shown). The anilide moiety of **7f** is close to Pro236, and the 4-acetyl points toward the solvent exposed region. Thus, introduction of hydrophilic groups or heteroatoms in the anilide moiety led a dramatic increase in potency, which can explain the SAR conclusion at this region. In summary, the results of the AutoDocking analysis seem to support our proposed binding mode for newly designed TTAs to NNIBP (Fig. 2). Further optimization of TTA analogues will consider these aspects in further design attempts.

3. Conclusions

A new series of 2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio) acetamide (TTA) derivatives was synthesized and evaluated as potent HIV-1 inhibitors. Among them, the most potent HIV-1 inhibitors were **7f** ($EC_{50} = 0.17$ μM), **7g** ($EC_{50} = 0.36$ μM) and **7c**

Table 1
Anti-HIV activities, cytotoxicities and selectivity indices of 2-(4-(naphthalen-2-yl)-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	>257.92	>257.92	≥257.92	< or ×1	< or ×1
7b	2-Chlorophenyl	**2.91 ± 0.39**	>229.04	229.04 ± 13.50	**79**	<1
7c	2-Chloropyridin-3-yl	**0.39 ± 0.05**	>302.72	>302.72	**>774**	×1
7d	2-Bromophenyl	**1.38 ± 0.20**	>211.88	≥211.88	**≥154**	< or ×1
7e	2-Bromo-4-methylphenyl	**2.72 ± 0.28**	>265.73	>265.73	**>98**	×1
7f	4-Acetyl-2-bromophenyl	**0.17 ± 0.02**	>250.79	>250.79	**>1452**	×1
7g	2-Nitrophenyl	**0.36 ± 0.19**	>295.87	>295.87	**>845**	×1
7h	4-Methyl-2-nitrophenyl	>182.36	>182.36	182.36 ± 3.78	<1	<1
7i	*o*-Tolyl	**4.24**	>257.98	**≥257.98**	**≥61**	< or ×1
7j	Phenyl	≥24.45	>248.09	248.09 ± 13.09	≤10	<1
7k	*p*-Tolyl	>319.28	>319.28	>319.28	×1	×1
7l	4-Chlorophenyl	>125.70	>125.70	125.70 ± 17.62	<1	<1
7m	2,3-Dimethylphenyl	>308.23	>308.23	>308.23	×1	×1
7n	2,6-Dimethylphenyl	>308.23	>308.23	>308.23	×1	×1
7o	Pyridin-2-yl	>253.92	>253.92	253.92 ± 7.42	<1	<1
7p	Thiazol-2-yl	>239.35	>239.35	239.35 ± 16.18	<1	<1
7q	5-Methylbenzo[d]thiazol-2-yl	>163.14	>163.14	163.14 ± 7.91	<1	<1
7r	3-Methyl acetate-thiophen-2-yl	**4.42 ± 0.09**	>283.09	**>283.09**	**64**	×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 ≥1 or <1.
[d] The antiviral properties of these compounds were previously described [12].

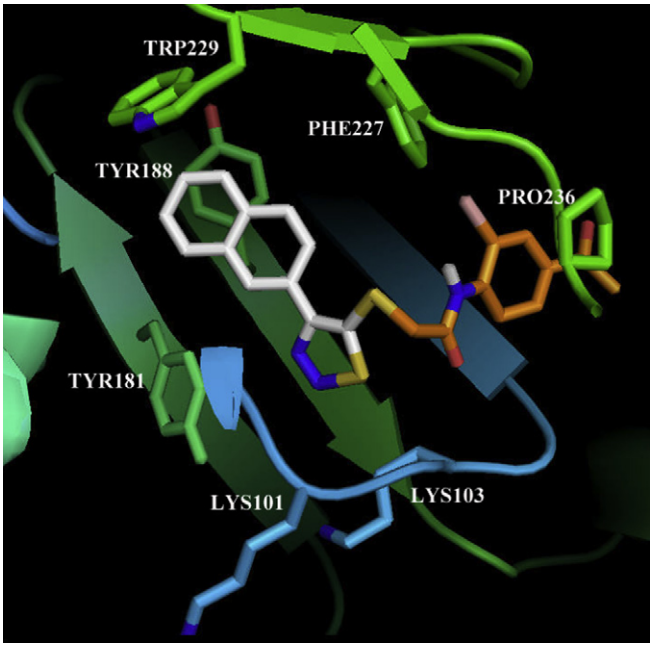


Fig. 3. Molecular model of **7f** in the allosteric site of HIV-1 RT (PDB code: 3DLG). The docking result of **7f** is showed by PyMOL.

($EC_{50} = 0.39$ μM), which possess similar HIV-1 inhibitory activity compared with NVP ($EC_{50} = 0.208$ μM) and DLV ($EC_{50} = 0.320$ μM). The preliminary structure–activity relationships among the newly disclosed congeners are discussed. Molecular modeling studies were employed to understand the interactions between these inhibitors and the RT, and to guide further SAR studies.

4. Experimental section

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 Bruker 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 an 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-(naphthalen-2-yl)-1,2,3-thiadiazole-5-thiolate (*6*)

To a solution of 1-(naphthalen-2-yl)ethanone (**1**, 17.0 g, 0.1 mol) in glacial acetic acid (200 ml) was added at room temperature a solution of Br_2 (16.0 g, 0.1 mol) in glacial acetic acid (30 ml) over a period of 30 min. The reaction mixture was stirred at room temperature for 6 h (monitored by TLC). Then the mixture was poured into ice water (100 g), extracted with dichloromethane (3 × 50 ml). The combined organic phase was washed with cold water (3 × 150 ml), 10% $NaHCO_3$ solution (3 × 50 ml), successively, and then dried over anhydrous Na_2SO_4. The drying agent was filtered off to give the dichloromethane solution (about 150 ml) of 2-bromo-1-(naphthalen-2-yl)ethanone (**2**), which was used without any further concentration or purification.

Methyl 3-mercaptopropanoate (11.4 ml, 0.1 mol) was added dropwise to the dichloromethane (DCM) solution of 2-bromo-1-(naphthalen-2-yl)ethanone (**2**, about 0.1 mol) obtained in the above step in ice bath. When the addition was finished with further stirring for 10 min, 0.1 mol of triethylamine (TEA) in 20 ml dichloromethane was added to the mixture, which was then stirred at room temperature overnight (checked by TLC). The mixture was diluted with CH_2Cl_2 (100 ml), washed with 0.1 N HCl solution, aqueous saturated $NaHCO_3$, water and brine, successively. The organic layer was dried with 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, then filtered while hot to remove precipitated sodium chloride. The filtrate was then mixed with compound **3** (30 g, 0.1 mol), and the resulting mixture was heated to reflux. Subsequently, a few drops of concentrated hydrochloric acid were added and heated under reflux with continuous removal of the generated water was continued overnight. Finally, the solution 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: 75.1%, m.p.162–164 °C. MS (ESI): *m*/*z* 346.3 (M + 1).

10 ml of thionyl chloride was added to the cooled suspension 0 °C of hydrazone **4** (3.5 g, 0.01 mol) in 10 ml of dry dichloromethane with vigorous stirring and cooling. The mixture was allowed to warm up to room temperature with continuous stirring. Monitoring of reaction progress with TLC showed that reaction was completed in 12 h at which time the solvent was removed under vacuum. The residue was diluted with ethyl acetate (50 ml), 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, yield: 71.5%, MS (ESI): *m*/*z* 331.3 (M + 1).

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-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)propanoate (**5**) (3.3 g, 0.01 mol) in 100 ml of methanol. After about 1 h, the reaction solution was evaporated *in vacuo* to about 10 ml. 50 ml of dry methylene chloride was added, causing precipitation of a red solid. This solid was collected, washed with methylene chloride, giving the desired product sodium 4-(naphthalen-2-yl)-1,2,3-thiadiazole-5-thiolate (**6**), yield: 92.4%, m.p.138–140 °C.

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

To the solution of sodium 4-(naphthalen-2-yl)-1,2,3-thiadiazole-5-thiolate (**6**) (1.05 mmol, 0.28 g) in ethanol (30 ml) were 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 recrystallisation using ethanol to yield the title compounds (**7a–r**).

4.1.3. N-(2-Fluorophenyl)-2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (*7a*)

Light yellow needle crystals, yield: 58.9%. mp: 163–165 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 11.07 (br s, 1H, NH), 8.41–7.55 (m, 7H, naphthalene–H), 8.19 (t, 1H, Ph′H), 7.20 (m, 3H, Ph′H), 3.91 (s, 2H, S–CH_2).

IR (KBr, cm^{-1}): 3247 (υ_{NH}), 1658 ($\upsilon_{C=O}$), 1541, 1492, 1457 ($\upsilon_{N=N}$), 1256, 1199, 756 (υ_{C-S}). MS (ESI): *m/z* 396.2 (M + 1). $C_{20}H_{14}FN_3OS_2$ (395.06).

4.1.4. N-(2-Chlorophenyl)-2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (**7b**)

White needle crystals, yield: 84.7%. mp: 179–180 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.58 (br s, 1H, NH), 8.51–7.57 (m, 7H, naphthalene–H), 7.41 (d, 1H, J = 2.4 Hz, Ph′H), 7.31 (d, 1H, J = 7.8 Hz, Ph′H), 7.25 (t, 1H, Ph′H), 7.07 (t, 1H, Ph′H), 3.92 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3296 (υ_{NH}), 1685 ($\upsilon_{C=O}$), 1594, 1525, 1438 ($\upsilon_{N=N}$), 1233, 744 (υ_{C-S}). MS (ESI): *m/z* 412.3 (M + 1), 414.3 (M + 3). $C_{20}H_{14}ClN_3OS_2$ (411.03).

4.1.5. N-(2-Chloropyridin-3-yl)-2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (**7c**)

Light yellow needle crystals, yield: 81.7%. mp: 161–163 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.60 (d, 1H, J = 8.4 Hz, pyridine–H), 8.53 (s, 1H, NH), 8.43–7.56 (m, 7H, naphthalene–H), 8.18 (dd, 1H, J = 1.8 Hz, J = 4.2 Hz, pyridine–H), 7.27 (m, 1H, pyridine–H), 3.93 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3439 (υ_{NH}), 1683 ($\upsilon_{C=O}$), 1520, 1453 ($\upsilon_{N=N}$), 1394, 748 (υ_{C-S}). MS (ESI): *m/z* 413.4 (M + 1), 415.3 (M + 3). $C_{19}H_{13}ClN_4OS_2$ (412.02).

4.1.6. N-(2-Bromophenyl)-2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (**7d**)

White needle crystals, yield: 69.8%. mp: 164–166 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.62 (br s, 1H, NH), 8.24 (d, 1H, J = 7.8 Hz, Ph′H), 8.41–7.55 (m, 7H, naphthalene–H), 7.47 (d, 1H, J = 7.8 Hz, Ph′H), 7.32 (dd, 1H, Ph′H), 7.00 (dt, 1H, Ph′H), 3.95 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3214 (υ_{NH}), 1653 ($\upsilon_{C=O}$), 1533, 1435 ($\upsilon_{N=N}$), 1227, 748 (υ_{C-S}). MS (ESI): *m/z* 456.3 (M + 1). $C_{20}H_{14}BrN_3OS_2$ (454.98).

4.1.7. N-(2-Bromo-4-methylphenyl)-2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (**7e**)

White needle crystals, yield: 70.8%. mp: 143–145 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.52 (br s, 1H, NH), 8.44–7.55 (m, 7H, naphthalene–H), 7.84 (d, 1H, J = 8.4 Hz, Ph′H), 7.28 (s, 1H, Ph′H), 7.10 (dd, 1H, Ph′H), 3.92 (s, 2H, S–CH_2), 2.28 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3234 (υ_{NH}), 3042, 2967, 2916, 1659 ($\upsilon_{C=O}$), 1526, 1444 ($\upsilon_{N=N}$), 814, 747 (υ_{C-S}). MS (ESI): *m/z* 470.2 (M + 1), 472.2 (M + 3). $C_{21}H_{16}BrN_3OS_2$ (468.99).

4.1.8. N-(4-Acetyl-2-bromophenyl)-2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (**7f**)

White crystals, yield: 85.7%. mp: 172–174 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.77 (s, 1H, NH), 8.54–7.55 (m, 7H, naphthalene–H), 8.40 (d, 1H, J = 8.4 Hz, Ph′H), 8.07 (d, 1H, J = 2.4 Hz, Ph′H), 7.83 (dd, 1H, J = 2.4 Hz, J = 8.4 Hz, Ph′H), 3.89 (s, 2H, S–CH_2), 2.55 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3233 (υ_{NH}), 1675 ($\upsilon_{C=O}$), 1595 ($\upsilon_{C=O}$), 1527, 1386 ($\upsilon_{N=N}$), 1266, 748 (υ_{C-S}). MS (ESI): *m/z* 498.2 (M + 1). $C_{22}H_{16}BrN_3O_2S_2$ (496.99).

4.1.9. 2-(4-(Naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)-N-(2-nitrophenyl)acetamide (**7g**)

Light yellow needle crystals, yield: 67.4%. mp: 151–153 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 11.12 (br s, 1H, NH), 8.65 (d, 1H, J = 8.4 Hz, Ph′H), 8.44–7.53 (m, 7H, naphthalene–H), 8.32 (dd, 1H, J = 1.8 Hz, J = 8.4 Hz, Ph′H), 7.19 (m, 2H, Ph′H), 3.89 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3339 (υ_{NH}), 1699 ($\upsilon_{C=O}$), 1588 ($\upsilon_{as\ NO_2}$), 1437 ($\upsilon_{N=N}$), 1339 ($\upsilon_{s\ NO_2}$), 1271, 1148, 740 (υ_{C-S}). MS (ESI): *m/z* 423.3 (M + 1). $C_{20}H_{14}N_4O_3S_2$ (422.05).

4.1.10. N-(4-Methyl-2-nitrophenyl)-2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (**7h**)

Light yellow needle crystals, yield: 84.3%. mp: 159–161 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 10.96 (br s, 1H, NH), 8.48 (d, 1H, J = 8.4 Hz, Ph′H), 8.43–7.52 (m, 7H, naphthalene–H), 8.07 (dd, 1H, J = 1.8 Hz, J = 8.4 Hz, Ph′H), 7.38 (m, 1H, Ph′H), 3.90 (s, 2H, S–CH_2), 2.36 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3313 (υ_{NH}), 1696 ($\upsilon_{C=O}$), 1516 ($\upsilon_{as\ NO_2}$), 1446 ($\upsilon_{N=N}$), 1340 ($\upsilon_{s\ NO_2}$), 1278, 825, 751 (υ_{C-S}). MS (ESI): *m/z* 437.3 (M + 1). $C_{21}H_{16}N_4O_3S_2$ (436.07).

4.1.11. 2-(4-(Naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)-N-o-tolylacetamide (**7i**)

White needle crystals, yield: 80.7%. mp: 138–140 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.72 (br s, 1H, NH), 8.40–7.56 (m, 7H, naphthalene–H), 7.71 (d, 1H, J = 8.4 Hz, Ph′H), 7.21 (t, 1H, Ph′H), 7.18 (m, 2H, Ph′H), 3.94 (s, 2H, S–CH_2), 2.01 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3213 (υ_{NH}), 3051, 2969, 2912, 2855, 1649 ($\upsilon_{C=O}$), 1539, 1457 ($\upsilon_{N=N}$), 1228, 748 (υ_{C-S}). MS (ESI): *m/z* 392.2 (M + 1). $C_{21}H_{17}N_3OS_2$ (391.08).

4.1.12. 2-(4-(Naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)-N-phenylacetamide (**7j**)

White needle crystals, yield: 76.1%. mp: 171–173 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 10.24 (br s, 1H, NH), 8.41–7.56 (m, 7H, naphthalene–H), 7.43 (d, 2H, J = 7.8 Hz, Ph′H), 7.30 (dt, 2H, Ph′H), 7.11 (dt, 1H, Ph′H), 3.86 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3285 (υ_{NH}), 1653 ($\upsilon_{C=O}$), 1530, 1445 ($\upsilon_{N=N}$), 756, 749 (υ_{C-S}). MS (ESI): *m/z* 378.3 (M + 1). $C_{20}H_{15}N_3OS_2$ (377.07).

4.1.13. 2-(4-(Naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)-N-p-tolylacetamide (**7k**)

White needle crystals, yield: 78.4%. mp: 163–165 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 10.27 (s, 1H, NH), 8.37 (s, 1H, naphthalene–1′-H), 8.17 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.4 Hz, naphthalene–4′-H), 8.01 (dd, 1H, J = 8.4 Hz, naphthalene–3′-H), 7.91–7.86 (m, 2H, naphthalene–5′, 8′-H), 7.57 (m, 2H, naphthalene–6′, 7′-H), 7.18 (d, 2H, J = 8.4 Hz, Ph′H), 7.06 (d, 2H, J = 8.4 Hz, Ph′H), 3.86 (s, 2H, S–CH_2), 2.29 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3244 (υ_{NH}), 3052, 2915, 1651 ($\upsilon_{C=O}$), 1541, 1405 ($\upsilon_{N=N}$), 1227, 813, 751 (υ_{C-S}). MS (ESI): *m/z* 391.9 (M + 1). $C_{21}H_{17}N_3OS_2$ (391.08).

4.1.14. N-(4-Chlorophenyl)-2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (**7l**)

White crystals, yield: 69.0%. mp: 147–149 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 10.33 (s, 1H, NH), 8.39 (s, 1H, naphthalene–1′-H), 8.16 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.4 Hz, naphthalene–4′-H), 8.05 (dd, 1H, J = 8.4 Hz, naphthalene–3′-H), 7.94–7.81 (m, 2H, naphthalene–5′, 8′-H), 7.59 (m, 2H, naphthalene–6′, 7′-H), 7.20 (d, 2H, J = 9 Hz, Ph′H), 7.15 (d, 2H, J = 9 Hz, Ph′H), 3.86 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3241 (υ_{NH}), 1652 ($\upsilon_{C=O}$), 1595, 1539, 1491 ($\upsilon_{N=N}$), 827, 746 (υ_{C-S}). MS (ESI): *m/z* 412.3 (M + 1), 414.3 (M + 3). $C_{20}H_{14}ClN_3OS_2$ (411.03).

4.1.15. N-(2,3-Dimethylphenyl)-2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (**7m**)

White needle crystals, yield: 68.7%. mp: 171–173 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.69 (br s, 1H, NH), 8.39–7.55 (m, 7H, naphthalene–H), 7.35 (d, 1H, J = 7.8 Hz, Ph′H), 7.07 (t, 1H, Ph′H), 7.00 (d, 1H, J = 7.8 Hz, Ph′H), 3.92 (s, 2H, S–CH_2), 2.22 (s, 3H, CH_3), 1.93 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3246 (υ_{NH}), 3050, 2966, 2915, 2852, 1652 ($\upsilon_{C=O}$), 1538, 1472 ($\upsilon_{N=N}$), 777, 745 (υ_{C-S}). MS (ESI): *m/z* 406.4 (M + 1). $C_{22}H_{19}N_3OS_2$ (405.1).

4.1.16. N-(2,6-Dimethylphenyl)-2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (**7n**)

White needle crystals, yield: 74.2%. mp: 188–190 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.38 (s, 1H, naphthalene–1′-H), 8.17 (dd, 1H, J_1 = 1.8 Hz, J_2 = 8.4 Hz, naphthalene–4′-H), 8.07 (dd, 1H, J = 8.4 Hz, naphthalene–3′-H), 7.95–7.90 (m, 2H, naphthalene–5′, 8′-H), 7.57 (m, 2H, naphthalene–6′, 7′-H), 7.50 (br s, 1H, NH), 7.09 (t, 1H, Ph′H), 7.03 (m, 2H, Ph′H), 3.97 (s, 2H, S–CH_2), 2.06 (s, 6H, CH_3). IR (KBr, cm^{-1}): 3235 (υ_{NH}), 3034,

2960, 2922, 2852, 1655 ($\upsilon_{C=O}$), 1533, 1474, 1440 ($\upsilon_{N=N}$), 1224, 760, 747 (υ_{C-S}). MS (ESI): *m/z* 406.4 (M + 1). $C_{22}H_{19}N_3OS_2$ (405.1).

4.1.17. 2-(4-(Naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)-N-(pyridin-2-yl)acetamide (**7o**)

White solids, yield: 59.7%. mp: 186–188 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.76 (s, 1H, NH), 8.42–7.54 (m, 7H, naphthalene–H), 8.20 (d, 1H, $J = 4.2$ Hz, pyridine–H), 8.13 (d, 1H, $J = 8.4$ Hz, pyridine–H), 7.73 (m, 1H, pyridine–H), 7.06 (dd, 1H, $J = 4.8$ Hz, $J = 7.2$ Hz, pyridine–H), 3.87 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3215 (υ_{NH}), 1683 ($\upsilon_{C=O}$), 1585, 1539, 1445 ($\upsilon_{N=N}$), 1314, 1163, 785, 747 (υ_{C-S}). MS (ESI): *m/z* 351.3 (M–N_2 + 1), 379.3 (M + 1), 401.3 (M + Na). $C_{19}H_{14}N_4OS_2$ (378.06).

4.1.18. 2-(4-(Naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)-N-(thiazol-2-yl)acetamide (**7p**)

White powder, yield: 35.8%. mp: 219–221 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 12.56 (br s, 1H, NH), 8.41–7.61 (m, 7H, naphthalene–H), 7.51 (d, 1H, $J = 3.6$ Hz, thiazole–H), 7.27 (d, 1H, $J = 3.6$ Hz, thiazole–H), 4.33 (s, 2H, S–CH_2). IR (KBr, cm^{-1}): 3193 (υ_{NH}), 1687 ($\upsilon_{C=O}$), 1589, 1403 ($\upsilon_{N=N}$), 1325, 1229, 1172, 1162, 969, 750 (υ_{C-S}). MS (ESI): *m/z* 385.2 (M + 1), 407.3 (M + Na). $C_{17}H_{12}N_4OS_3$ (384.02).

4.1.19. N-(5-Methylbenzo[d]thiazol-2-yl)-2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamide (**7q**)

White needle crystals, yield: 58.7%. mp: 196–198 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 12.71 (br s, 1H, NH), 8.42–7.59 (m, 7H, naphthalene–H), 7.77 (s, 1H, Ph′H), 7.65 (d, 1H, $J = 8.4$ Hz, Ph′H), 7.27 (d, 1H, $J = 8.4$ Hz, Ph′H), 4.36 (s, 2H, S–CH_2), 2.50 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3207 (υ_{NH}), 2997, 2919, 2854, 1698 ($\upsilon_{C=O}$), 1608, 1553, 1463 ($\upsilon_{N=N}$), 816, 748 (υ_{C-S}). MS (ESI): *m/z* 449.3 (M + 1). $C_{22}H_{16}N_4OS_3$ (448.05).

4.1.20. Methyl 2-(2-(4-(naphthalen-2-yl)-1,2,3-thiadiazol-5-ylthio)acetamido)thiophene-3-carboxylate (**7r**)

White solids, yield: 74.5%. mp: 109–111 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 10.94 (br s, 1H, NH), 8.46–7.54 (m, 7H, naphthalene–H), 8.02 (d, 1H, $J = 6.0$ Hz, thiophene–H), 7.46 (d, 1H, $J = 6.0$ Hz, thiophene–H), 3.87 (s, 2H, S–CH_2), 3.80 (s, 3H, OCH_3). IR (KBr, cm^{-1}): 3291 (υ_{NH}), 1673 ($\upsilon_{C=O}$), 1576 ($\upsilon_{C=O}$), 1445 ($\upsilon_{N=N}$), 1281, 1249, 779, 749 (υ_{C-S}). MS (ESI): *m/z* 442.3 (M + 1). $C_{20}H_{15}N_3O_3S_3$ (441.03).

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 [20,21]. 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 5-fold 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) [22] or HIV-2 (ROD) [23] 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 [24] 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 coloured 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 three 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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