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Original article

Arylazolylthioacetanilide. Part 8☆: Design, synthesis and biological evaluation of Novel 2-(2-(2,4-Dichlorophenyl)-2*H*-1,2,4-triazol-3-ylthio)-*N*-arylacetamides As Potent HIV-1 inhibitors

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ABSTRACT

The development of novel HIV-1 NNRTIs offers the possibility of generating novel structures with increased potency. Based on the bioisosteric principle, a novel series of 2-(2-(2,4-dichlorophenyl)-2*H*-1,2,4-triazol-3-ylthio)-*N*-arylacetamide derivatives were designed, synthesized using a simple and efficient synthetic route, structurally confirmed by spectral analysis, evaluated for their anti-HIV activity in MT-4 cells and their inhibitory effect on HIV-1 RT. The results showed that some of the new compounds displayed low micromolar potency for inhibiting HIV-1 replication and promising activities against several selected resistant strains that confer resistance to current NNRTIs. However, all newly synthesized derivatives were not active against HIV-2 replication.

1. Introduction

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]. The introduction of highly active anti-retroviral therapy (HAART) has dramatically decreased the morbidity and mortality resulting from the infection with HIV. However, the AIDS prevalence remains one of the world's most serious health problems [2]. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) nowadays represent very potent anti-AIDS drugs that specifically target to HIV-1 reverse transcriptase (RT) [3]. However, to a great extent, the effectiveness of NNRTI drugs can be hampered by rapid emergence of drug-resistant viruses and severe side effect in the long-term usage. Therefore, the identification of novel NNRTIs, characterized by high potency against both the wild-type HIV-1 and the clinically relevant mutant strains of HIV-1, as well as improved pharmacokinetic profiles, is a constant goal for drug development [4].

Five-membered azoles heterocycles represent an important class of lead structures for novel antiviral drug development [5]. Arylazolylthioacetanilide derivatives have been of great interest for the development of novel NNRTIs, because of their high potency against HIV-1 wild-type and resistant strains, favorable bioavailability and low toxicity [6–12]. Several triazolylthioacetanilide NNRTIs, such as VRX-480733 [13] and RDEA806 (in phase IIa clinical trials) [14], have been or are currently being entered into clinical trials (Fig. 1).

Meanwhile, during the course of our investigations on potential NNRTIs, we embarked on in the chemical and biological studies of a number of novel arylazolylthioacetanilide derivatives [15–21]. Most of these compounds displayed potent anti-HIV properties in cell lines infected with either wild type or mutant HIV-1. In particular, 1,2,3-thiadiazole derivative **ZP7** displayed the most potent anti-HIV-1 activity (EC_{50} = 36.4 nM), inhibiting HIV-1 replication in MT-4 cells more effectively than nevirapine (NVP) (by sevenfold) and delavirdine (DLV) (by eightfold) [16]. Molecular modeling studies demonstrated that the five-membered heterocycle portion of these inhibitors could be acting as a scaffold which

☆ Parts 1-7 were reported in references 15-21.

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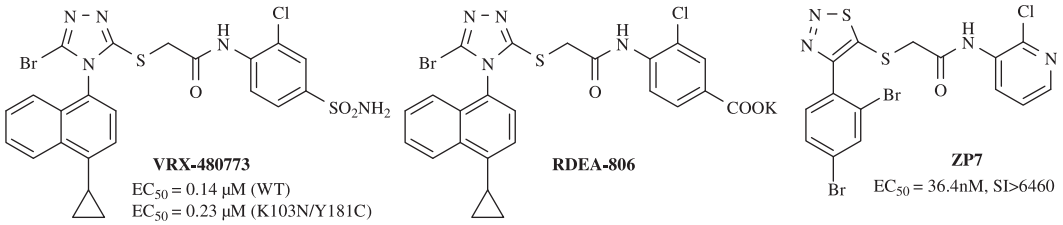


Fig. 1. Triazole/1,2,3-thiadiazole thioacetanilide-based NNRTIs.

orients the pharmacophores into the proper geometry for binding and key structural element to form hydrogen bond with K103 [6,12]. Therefore, there are remarkable differences in the electronic and conformational contribution of the heterocyclic groups to the binding of the inhibitors with the HIV-1 RT [12]. In the light of the above promising results and our continued interest in the study of antiviral activity of arylazolylthioacetanilide derivatives, a series of novel 1,2,4-triazole thioacetanilide derivatives were designed and synthesized based on the general principle of bioisosterism in medicinal chemistry [22]. In the newly synthesized analogues, novel 1,2,4-triazole ring (2-aryl) was substituted for the 1,2,4-triazole (4-aryl) or 1,2,3-thiadiazole moiety in the corresponding parent leads (see Fig. 2), the other fragments which were considered to be necessary for conserving anti-HIV-1 activity, such as the "$-SCH_2CONH-$" linker and the substituted arylamines, were maintained. The synthesis, the anti-HIV-1 activities evaluation of these novel derivatives against HIV-1 (IIIB), HIV-2 (ROD), and several key mutant HIV-1 strains as well as HIV RT inhibitory assay are presented in the following sections.

2. Results and discussion

2.1. Chemistry

The general synthetic route utilized to obtain the desired compounds 2-(2-(2,4-dichlorophenyl)-2*H*-1,2,4-triazol-3-ylthio)-*N*-arylacetamide was straightforward and is outlined in Scheme 1. The commercially available 1-(2,4-dichlorophenyl)hydrazine (**1**) was converted to the hydrazone (**2**) by reaction with 2-oxoacetic acid. The hydrazone was treated with triethylamine and diphenylphosphoryl azide in toluene to give 2-(2,4-dichlorophenyl)-2*H*-1,2,4-triazol-3(4H)-one (**3**). During the reaction of Curtius Rearrangement, nitrogen evolution began at 60–65 °C and occasionally became vigorous if temperature was not gradually risen [23]. Compound **3** was treated with phosphoryl trichloride under reflux condition to afford 5-chloro-1-(2,4-dichlorophenyl)-1*H*-1,2,4-triazole (**4**) The ethyl 2-(2-(2,4-dichlorophenyl)-2*H*-1,2,4-triazol-3-ylthio)acetate (**5**) was synthesized by reaction of **4** with ethyl 2-mercaptoacetate in high yield. The thioacetic acid derivative **6** was obtained by saponification of compound **5** in NaOH-DMSO-H_2O solution. The final triazole thioacetanilides **7** were synthesized by reaction of **6** with substituted arylamines 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.

2.2. Biological activity

2.2.1. Anti-HIV activities evaluation

The newly synthesized 1,2,4-triazolacetamide derivatives were evaluated for anti-HIV activity by determining their ability to inhibit the replication of HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cells in comparison with nevirapine (NVP), zidovudine (azidothymidine, AZT), dideoxycitidine (DDC) and dideoxyinosine (DDI) used as reference drugs. The cytotoxicity of the compound was determined in parallel. The results of inhibitory concentration (EC_{50}), cytotoxic concentration (CC_{50}), and SI (selectivity, given by the CC_{50}/EC_{50} ratio) values for different compounds are depicted in Table 1. Some active compounds were also evaluated against a number of clinically significant NNRTIs resistant strains, containing E138K, K103N, L100I, Y181C, Y188L and RES056 (K103N + Y181C), F227L + V106A double mutations, using AZT, NVP, efavirenz (EFV), delavirdine (DLV), and etravirine (TMC125) as standard drugs and the positive results are presented in Table 2.

The experimental results indicated that more than half of the tested 1,2,4-triazolacetamide derivatives were found to be active against HIV-1 and none of the compounds was active against HIV-2. In particular, six of these compounds exhibited moderate to good activities against HIV-1 strain IIIB with EC_{50} values in the range of

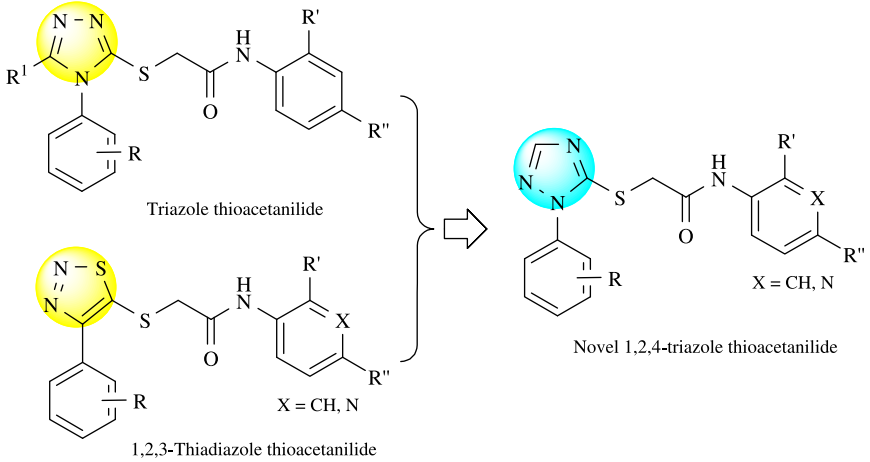
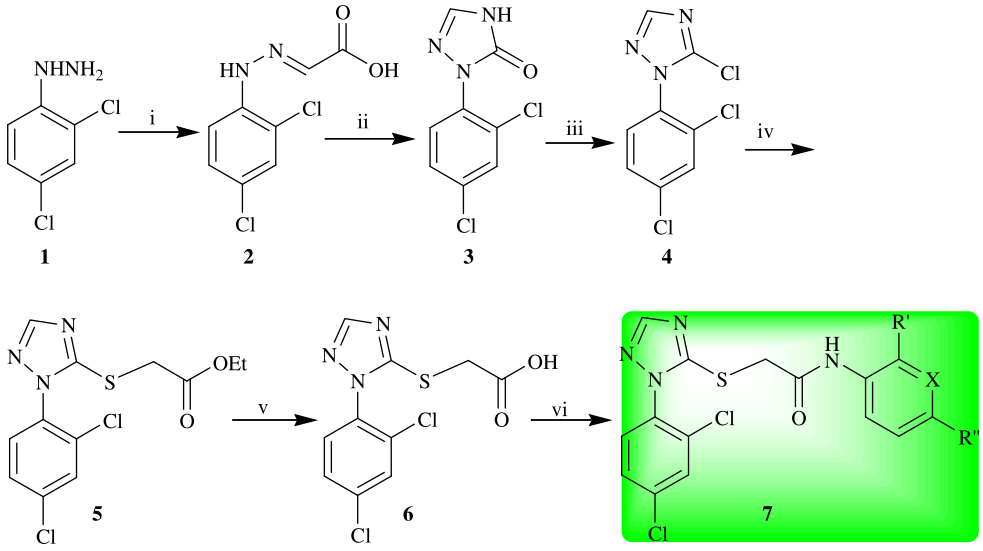
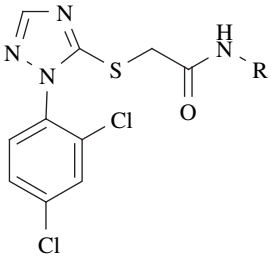


Fig. 2. The design of novel 1,2,4-triazole thioacetanilide based on bioisosterism principle.



Scheme 1. Reagents and conditions: (i) $HCOCO_2H$, H_2O; (ii) DPPA, TEA, Tol; (iii) $POCl_3$; (iv) NaH, $HSCH_2COOEt$, THF; (v) NaOH, H_2O/DMSO, HCl; (vi) PCl_5, aniline, THF, rt.

Table 1
Anti-HIV activities, cytotoxicities and selectivity indices of *N*-aryl-2-(2-(2,4-dichlorophenyl)-2*H*-1,2,4-triazol-3-ylthio)acetamide derivatives (**7a**–**o**).



No.	R	EC_{50}(μM)[a]		CC_{50} (μM)[b]	SI[c]	
		HIV-1	HIV-2		HIV-1	HIV-2
		III_B	ROD		III_B	ROD
7a	2-chlorophenyl	**6.21 ± 0.87**	>29.13	29.13 ± 3.36	**5**	<1
7b	2-fluorophenyl	>38.39	>38.39	38.36 ± 6.72	<1	<1
7c	2-bromophenyl	**4.85 ± 0.83**	>95.12	95.12 ± 54.87	**20**	<1
7d	2,4-dichlorophenyl	**34.36 ± 13.57**	>171.88	171.88 ± 9.93	**5**	<1
7e	2-chloropyridin-3-yl	**4.99 ± 0.92**	>98.46	98.46 ± 13.96	**20**	<1
7f	4-methyl-2-nitrophenyl	>285.20	>285.2	>285.20	X1	X1
7g	2-nitrophenyl	**2.78 ± 0.97**	>186.91	186.91 ± 9.62	**67**	<1
7h	o-tolyl	**32.42 ± 1.25**	>106.41	106.41 ± 8.90	**3**	<1
7i	4-acetyl-2-bromophenyl	>114.21	>114.21	114.21 ± 4.86	<1	<1
7j	2-bromo-4-methylphenyl	>184.25	>184.25	>184.25	X1	X1
7k	2-bromo-4-chlorophenyl	>188.90	>188.9	188.90 ± 2.78	<1	<1
7l	2-bromo-4-methoxy carbonylphenyl	**4.13 ± 0.41**	>59.34	59.34 ± 28.60	**14**	<1
7m	2-bromo-4-ethoxy carbonylphenyl	**4.68 ± 0.72**	>33.06	33.06 ± 2.58	**7**	<1
7n	4-methoxy carbonylphenyl	>26.27	>26.27	26.27 ± 8.74	<1	<1
7o	4-ethoxy carbonylphenyl	>22.82	>22.82	22.82 ± 4.36	<1	<1
ZP7[d]		0.0364 ± 0.0038	>240.08	>240.08	>6460	X1
NVP		0.19 ± 0.04	>15	>15	>80	
AZT		0.0076 ± 0.0014	0.0033 ± 0.0006	>88.26	>11587	>28348
DDC		0.75 ± 0.55	0.88 ± 0.53	>94	>127	>108
DDI		8.86 ± 2.89	16 ± 5.16	>211	>24	>13

Bold represents the values of active compounds.
[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cell against 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: X 1 stand for ≥1or<1.
[d] The antiviral properties of these compounds were previously described, Ref. [16].

Table 2
Anti-HIV mutants activities of selected 1,2,4-triazolacetamide derivatives.

Compd.	EC_{50}(μM)[a] (fold-resistance)[b]		
	E138K	K103N	L100I
7a	20.86 ± 0.87 (3.6)	16.05 ± 0.56 (2.8)	21.42 ± 1.31 (3.7)
7c	28.22 ± 15.65 (6.6)	8.36 ± 0.33 (1.9)	25.69 ± 20.17 (6.0)
7e	6.79 ± 0.31 (4.3)	10.49 ± 0.41 (2.8)	15.38 ± 3.11 (4.4)
7g	9.45 ± 4.57 (2.3)	7.42 ± 2.76 (1.8)	8.51 ± 2.85 (2.1)
7l	≥15.25 (≥4.3)	7.73 ± 1.67 (2.2)	6.95 ± 1.86 (2.0)
7m	>38.66 (>6.7)	9.54 ± 0.91 (1.6)	10.75 ± 1.72 (1.9)
AZT	0.02 ± 0.009 (1.4)	0.011 ± 0.0041 (0.71)	0.0049 ± 0.0015 (0.32)
NVP	0.18 ± 0.057 (1.2)	7.25 ± 0.64 (48.25)	1.92 ± 1.20 (12.75)
EFV	0.0055 ± 0.00078 (1.4)	0.108 ± 0.022 (28.3)	0.054 ± 0.025 (14.2)
DLV (Mesylate)	0.06 ± 0.023 (1.9)	1.72 ± 0.29 (52.8)	2.23 ± 0.33 (68.3)
TMC125	0.008 ± 0.002 (2.6)	0.00184 ± 0.00021 (0.62)	0.0046 ± 0.0016 (1.54)

[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cell against HIV-1-induced cytotoxicity, as determined by the MTT method.
[b] ratio of EC_{50} value against drug-resistant strain and EC_{50} of the wt HIV-1 IIIB ($EC_{50}^{\text{mutant strains}}/EC_{50}^{\text{wt}}$).

2.78–6.21 μM, which are much better than that of DDI. The most potent and selective compound was found to be **7g** having an EC_{50} value of 2.78 ± 0.97 μM against HIV-1(IIIB) with a selectivity index (SI) of 67.

Table 1 also reveals the potency order of the *ortho* substitution at the phenyl ring of the anilide moiety as following: NO_2 (**7g**, EC_{50} = 2.78 ± 0.97 μM) > Br (**7c**, EC_{50} = 4.85 ± 0.83 μM) > Cl (**7a**, EC_{50} = 6.21 ± 0.87 μM) > Me (**7h**, EC_{50} = 32.42 ± 1.25 μM) >F (**7b**, EC_{50} > 38.39 μM), indicating that the activities data of the 1,2,4-triazolacetamide derivatives are affected by the electronic nature or the steric demand of the *ortho* substitution. Significantly decreased potency was observed in **7n** and **7o** characterized by the absence of the *ortho* substitution at the phenyl ring of the anilide moiety. The result demonstrated that the *ortho* substitutions played a determinant role in keeping anti-HIV activity. This conclusion is grossly in agreement with the previous structure-activity relationship (SAR) studies in 1,2,3-thiadiazole series [15,17]

It is worth noting that, in the case of compound **7c**, introduction of methoxy carbonyl group and ethoxy carbonyl group on the *para* position at the phenyl ring of the anilide moiety led to **7l** and **7m** with the retained activity. Whereas introduction of 4-acetyl in **7c** led to **7i** with deprived activity, indicating that the nature of the substituent at the *para* position influenced the anti-HIV-1 activity remarkably. Furthermore, additional substitution with methyl and chlorine atom at the *para* position of anilide phenyl ring in compounds **7g** and **7a** led to derivatives **7f** and **7d** respectively, with strikingly reduced or deprived antiviral potency, suggesting that the nature (probably hydrophobicity) of methyl and chlorine atom did not accommodate the chemical environment in this region of RT.

In the 1,2,3-thiadiazole series, we have demonstrated that the introduction of pyridine moiety at the aromatic amine domain led to substantial improvement in potency and selectivity [16]. Interestingly, this beneficial effect was also observed in this closely related 1,2,4-triazolyl series (as shown in **7e** and **7a**). Thus again, 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.

Table 3
Inhibitory activity of selected 1,2,4-triazolacetamide derivatives against HIV-1 RT.

Compd.	IV-**7g**	IV-**7l**	IV-**7m**	NVP
IC_{50} (μM)[a]	>471.41	385.32	297.42	4.39

[a] 50% of the inhibitory concentration of selected 1,2,4-triazolacetamide derivatives required to inhibit biotin deoxyuridine triphosphate (biotin-dUTP) incorporation into the HIV-1 RT by 50%.

From the SAR studies, we found that the SAR features of the 1,2,4-triazolacetamides were highly consistent with the previously observed arylazolylthioacetanilide-typed NNRTIs [15–21].

Most NNRTIs are specific for HIV-1 RT and demonstrate minimal inhibition of HIV-2 RT. Therefore, based on the above SAR conclusions and the fact that all the derivatives did not show any activity against HIV-2 (ROD) in MT-4 cells, it can be concluded that this new series of 1,2,4-triazolacetamide derivatives was specific for HIV-1 and belonged to typical NNRTIs.

The six active compounds **7a**, **7c**, **7e**, **7g**, **7l** and **7m** were further tested for their inhibitory effects against the HIV-1 mutant strains (Table 2). To define the resistance profile of these compounds, both the absolute activity against the HIV-1 mutants (EC_{50} values) and the relative activity (fold-resistance) need to be considered. In this assay, the antiviral activities of the tested analogues are sensitive to these mutations in the NNRTI active site, which once again suggests that RT is the target of these 1,2,4-triazolacetamide derivatives. As the data demonstrated, the selected compounds retained in part their activities against the E138K, K103N and L100I mutant strains, with EC_{50} values at the low micromolar level and lower fold-resistance values, whereas they did not show activity against the HIV-1 Y181C, Y188L, F227L + V106A, and RES056 (K103N + Y181C) mutant strains. Especially, the **7g** showed the highest inhibitory activity against the HIV-1 K103N strain, which is the most frequent drug resistance mutation observed in patents failing therapy with NNRTIs [24].

2.2.2. HIV-1 RT inhibition assay

Since arylazolylthioacetanilide derivatives was previously confirmed to target at the HIV-1 RT, three selected title compounds (**7g**, **7l** and **7m**) were tested in enzyme assays against highly purified recombinant HIV-1 RT using poly(rC)-oligo(dG) as template primer. The results showed that the enzymatic inhibitory activity of these compounds is very weak, regardless of the structural pattern (Table 3). Thus, the possibility cannot be excluded that these new compounds might interfere with another target or act on reverse transcriptase in a different way other than the typical NNRTIs. Studies are in progress to determine the exact mechanism for the anti-HIV activity of these compounds.

3. Conclusion

In summary, our 'bioisosterism' approach has led to the discovery of novel 1,2,4-triazolacetamide-based anti-HIV agents. The preliminary SAR was discussed. Some derivatives proved to be effective in inhibiting HIV-1 replication at low micromolar concentrations. Among them, compound **7g** was identified as the most promising lead with favorable inhibitory activity against the

wild-type HIV-1 and the viral strain that carry the K103N mutation. The RT inhibitory potency was also assayed. All of them proved to be more weak in enzymatic assays than in cellular tests. Accordingly, it may be difficult to explain their antiviral activity only in terms of RT inhibition. This result may reflect the interference of the test compounds with viral replication steps other than reverse transcription. These studies provide more insight into the SARs of 1,2,4-triazolacetamide derivatives as HIV inhibitors, and should help in designing new arylazolylthioacetanilide derivatives, which could be useful for the development of antiviral agents.

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 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 (200–300 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.

4.2. General procedure for the synthesis of triazole thioacetanilides (**7a**–**o**)

4.2.1. General Procedure for the synthesis of 2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3- ylthio)acetic acid (**6**)

To a stirred solution of 4.9 g (27.7 mmol) of 1-(2,4-dichlorophenyl)hydrazine (**1**) in 30 mL of water was added 3 mL of concentrated hydrochloric acid followed by a solution of 4.5 g (30.5 mmol, 50%) of 2-oxoacetic acid in 5 mL of water. The precipitate that formed was collected by suction filtration and dried at reduced pressure to afford 2-(2-(2,4-dichlorophenyl)hydrazono) acetic acid (**2**) 4.8 g (75%) of a yellow solid, mp: 158 °C ^{1}H NMR (DMSO-d6, ppm) δ: 6.91–7.05 (m, 3H, Ph), 7.55 (s, 1H, N=CH), 10.5 (s, 1H, CO_2H), 11.9 (bs, 1H, NH). ESI-MS: *m*/*z* 233.1 (M + 1).

To a stirred suspension of 3.0 g (12.9 mmol) of **2** in 80 mL of dry toluene was added 1.8 mL (1.3 g, 12.9 mmol) of triethylamine and 2.8 mL (3.6 g, 12.9 mmol) of diphenylphosphoryl azide. This mixture was slowly heated to reflux temperature with stirring. Rapid gas evolution began at 60 °C and continued for 1 h. The clear orange solution was cooled and then poured into 100 mL of 10% aqueous potassium hydroxide. The basic extract was acidified with concentrated hydrochloric acid and the precipitated solid was recrystallized from ethyl acetate/heptane to afford 2-(2,4-dichlorophenyl)-2*H*-1,2,4-triazol-3(4*H*)-one (**3**) 2.55 g (86%) of a tan solid, mp: 189–190 °C. ^{1}H-NMR (DMSO-d6, ppm) δ: 12.1 (s, 1H, NH), 8.11 (s, 1H, triazole-H), 7.65 (d, 1H, J = 2.4 Hz, PhH), 7.47 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.4 Hz, PhH), 7.39 (d, 1H, J = 8.4 Hz, PhH). ^{13}C-NMR (DMSO-d6, ppm) δ: 153.35, 137.76, 134.68, 133.96, 132.81, 131.62, 130.46, 128.89. ESI-MS: *m*/*z* 230.4 (M + 1), 232.3 (M + 3).

The reaction mixture of **3** (0.23 g, 1 mmol) and 5 mL phosphoryl trichloride was stirred at reflux for 10 h (monitored by TLC). After cooling, the mixture was concentrated under reduced pressure to give crude residue, then diluted with EtOAc (40 mL). The separated organic phase was washed successively with water, aqueous saturated $NaHCO_3$ and brine, and then dried over Na_2SO_4, filtered and concentrated to afford crude product 5-chloro-1-(2,4-dichlorophenyl)-1*H*-1,2,4- triazole (**4**) as a light yellow solid (0.21 g, yield: 84.6%), which was used in the next step without any further purification. ^{1}H-NMR (DMSO-d6, ppm) δ: 8.38 (s, 1H, triazole-H), 8.04 (d, 1H, J_1 = 2.4 Hz, PhH), 7.84 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.4 Hz, PhH), 7.73 (d, 1H, J_2 = 8.4 Hz, PhH).

5-chloro-1-(2,4-dichlorophenyl)-1*H*-1,2,4-triazole (**4**) (0.5 g, 2 mmol) was added to a mixture of ethyl 2-mercaptoacetate (0.23 mL, 2.1 mmol) and NaH (0.084 g, 2.1 mmol) in 10 mL THF. The reaction mixture was stirred at 0 °C for 10 min and then refluxed for 8 h. The solvent was evaporated under reduced pressure and the residue was then diluted with CH_2Cl_2 (30 mL), washed successively with water (2 × 30 mL), aqueous saturated $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford crude product **5** as a light yellow oil, which was used in the next step without any further purification. ESI-MS: *m*/*z* 232.3 (M + 1), 234.4 (M + 3). $C_{12}H_{11}Cl_2N_3O_2S$ (Exact Mass: 330.99).

Ester **5** (0.4 g, 1.2 mmol) was dissolved in DMSO (1 mL) and aqueous 1 N NaOH (2.0 mL, 2.0 mmol) solution was added to the solution. The reaction mixture was stirred at room temperature for 1 h and acidified with aqueous 1 N HCl. The mixture was then diluted with EtOAc (100 mL) and successively washed with water and brine, dried with Na_2SO_4, filtered and concentrated under vacuum to give compound 2-(2-(2,4-dichlorophenyl)-2*H*-1,2,4-triazol-3-ylthio)acetic acid (**6**) as light yellow oil (0.35 g, 97% yield).

4.2.2. General Procedure for the synthesis of 2-(2-(2,4-dichlorophenyl)-2H-1,2,4- triazol-3-ylthio)acetamides (**7a**–**7o**)

PCl_5 (0.23 g, 1.1 mmol) was added to an ice-cold solution of compound **6** (0.30 g, 1 mmol) in ether (15 mL). The reaction mixture was stirred at room temperature for 2 h and then was concentrated under reduced pressure. The crude product was dissolved in THF (15 mL). The arylamine (1 mmol) and triethylamine (1.2 mmol) was added to the solution successively. The reaction mixture was stirred at room temperature for 2 h and then was then evaporated under reduced pressure. The residue was chromatographed on silica gel using ethyl acetate:petroleum ether (1:4). Pure fractions were collected and concentrated, giving the desired compound (**7a**–**7o**).

4.2.3. N-(2-chlorophenyl)-2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)acetamide (**7a**)

White needle crystals, yield: 45.7%. Mp: 118–121 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.76 (s, 1H, NH), 8.33 (d, 1H, J = 8.4 Hz, PhH), 8.08 (s, 1H, triazole-H), 7.60 (d, 1H, J = 2.4 Hz, PhH), 7.43–7.26 (m, 4H, PhH), 7.05 (dt, 1H, PhH), 4.03 (s, 2H, S–CH_2). ESI-MS: *m*/*z* 413.5 (M + 1), 415.4 (M + 3), 417.5 (M + 5), 435.4 (M + Na). $C_{16}H_{11}Cl_3N_4OS$ (411.97).

4.2.4. 2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)-N-(2-fluorophenyl)acetamide (**7b**)

White powder, yield: 50.4%. mp: 108–110 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 10.17 (s, 1H, NH), 8.34–8.31 (m, 1H, PhH), 8.11 (s, 1H, triazole-H), 7.61 (d, 1H, J = 2.4 Hz, PhH), 7.41 (dd, 1H, J_1 = 2.4 Hz, J_2 = 8.4 Hz, PhH), 7.37 (d, 1H, J = 8.4 Hz, PhH),7.14–7.05 (m, 3H, PhH), 3.96 (s, 2H, S–CH_2). IR (KBr): 3262 (NH), 1691 (C=O), 1624, 1557, 1496, 1456 (N=N), 758 (C–S). ESI-MS: *m*/*z* 397.1 (M + 1), 399.0 (M + 3). $C_{16}H_{11}Cl_2FN_4OS$ (396).

4.2.5. N-(2-bromophenyl)-2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)acetamide (**7c**)

White cubic crystals, yield: 35.2%. mp: 115–116 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.55 (s, 1H, NH), 8.26 (d, 1H, J = 8.4 Hz, PhH), 8.08 (s,

1H, triazole-H), 7.60 (d, 1H, $J = 2.4$ Hz, PhH), 7.60 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.4$ Hz, PhH), 7.54 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.4$ Hz, PhH), 7.42 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.4$ Hz, PhH), 7.37 (d, 1H, $J = 8.4$ Hz, PhH), 7.05 (dt, 1H, PhH), 4.04 (s, 2H, S–CH_2). IR (KBr): 3338 (NH), 1684 (C=O), 1591, 1527, 1496, 1436 (N=N), 1060, 744 (C–S). ESI-MS: *m/z* 457.0 (M + 1), 459.0 (M + 3). $C_{16}H_{11}BrCl_2N_4OS$ (455.92).

4.2.6. N-(2,4-dichlorophenyl)-2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)acetamide (7d)

White needle crystals, yield: 44.7%. mp: 115–118 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.90 (s, 1H, NH), 8.26 (d, 1H, $J = 9.6$ Hz, PhH), 8.07 (s, 1H, triazole-H), 7.43–7.37 (m, 4H, PhH), 7.25 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.4$ Hz, PhH), 4.04 (s, 2H, S–CH_2). IR (KBr): 3345 (NH), 1692 (C=O), 1580, 1518, 1496, 1413 (N=N), 1385, 1364, 1061, 821, 776 (C–S). ESI-MS: *m/z* 447.1 (M + 1), 449.1 (M + 3). $C_{16}H_{10}Cl_4N_4OS$ (445.93).

4.2.7. N-(2-chloropyridin-3-yl)-2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)acetamide (7e)

White needle crystals, yield: 65.4%. mp: 143–145 °C. ^{1}H NMR (DMSO, ppm) δ: 10.15 (s, 1H, NH), 8.68 (d, 1H, $J = 7.8$ Hz, pyridine-H), 8.28 (d, 1H, $J_1 = 1.8$ Hz, PhH), 8.20 (dd, 1H, $J = 1.2$ Hz, $J = 4.8$ Hz, pyridine-H), 8.01 (s, 1H, triazole-H), 7.61 (dd, 1H, $J_1 = 1.8$ Hz, $J_2 = 8.4$ Hz, PhH), 7.40 (d, 1H, $J_2 = 8.4$ Hz, PhH), 7.33–7.29 (m, 1H, pyridine-H), 4.28 (s, 2H, S–CH_2). IR (KBr): 3287 (NH), 1652 (C=O), 1582, 1544, 1520, 1493, 1453 (N=N), 1395, 1072, 809, 799, 785 (C–S). ESI-MS: *m/z* 414.1 (M + 1), 416.1 (M + 3), 418.3 (M + 3), 436.3 (M + Na^+), 438.2 (M + 2 + Na^+). $C_{15}H_{10}Cl_3N_5OS$ (412.97).

4.2.8. 2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)-N-(4-methyl-2-nitrophenyl)acetamide (7f)

Light yellow powder, yield: 37.4%. mp: 185–186 °C. ^{1}H-NMR ($CDCl_3$, ppm) δ: 11.07 (s, 1H, NH), 8.55 (d, 1H, $J = 8.4$ Hz, PhH), 8.01 (s, 1H, triazole-H), 7.97 (s, 1H, PhH), 7.61 (d, 1H, $J = 1.8$ Hz, PhH), 7.46–7.42 (m, 3H, PhH), 4.11 (s, 2H, S–CH_2), 2.40 (s, 3H, CH_3). MS (ESI): *m/z* 438.2 (M + 1), 540.1 (M + 3). $C_{17}H_{13}Cl_2N_5O_3S$ (437.01).

4.2.9. 2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)-N-(2-nitrophenyl)acetamide (7g)

Yellow needle crystals, yield: 53.8%. mp: 153–154 °C. ^{1}H-NMR ($CDCl_3$, ppm) δ: 11.19 (s, 1H, NH), 8.68 (d, 1H, $J = 8.4$ Hz, PhH), 8.18 (dd, 1H, $J = 1.8$ Hz, $J = 8.4$ Hz, PhH), 8.06 (s, 1H, triazole-H), 7.65 (t, 1H, PhH), 7.61 (d, 1H, $J_1 = 1.8$ Hz, PhH), 7.46–7.42 (m, 2H, PhH), 7.22 (m, 1H, PhH), 4.13 (s, 2H, S–CH_2). IR (KBr): 3295 (NH), 1685 (C=O), 1606, 1585, 1497 (NO_2), 1436 (N=N), 1362 (NO_2), 1337, 1279, 742 (C–S). MS (ESI): *m/z* 424.1 (M + 1), 426.3 (M + 3). $C_{16}H_{11}Cl_2N_5O_3S$ (423).

4.2.10. 2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)-N-o-tolylacetamide (7h)

White lamella crystals, yield: 50.7%. mp: 121–122 °C. ^{1}H-NMR ($CDCl_3$, ppm) δ: 9.42 (brs, 1H, NH), 8.06 (s, 1H, triazole-H), 7.91 (d, 1H, $J_1 = 7.8$ Hz, PhH), 7.61 (d, 1H, $J = 2.4$ Hz, Ph′H), 7.43 (dd, 1H, $J_1 = 7.8$ Hz, $J_2 = 2.4$ Hz, PhH), 7.37 (d, 1H, $J_2 = 2.4$ Hz, PhH), 7.22 (t, 1H, Ph′H), 7.18 (d, 1H, $J = 7.2$ Hz, Ph′H), 7.08 (t, 1H, Ph′H), 3.96 (s, 2H, S–CH_2), 2.27 (s, 3H, CH_3). IR (KBr): 3258 (NH), 1687 (C=O), 1591, 1549, 1494, 1458 (N=N), 1365, 1314, 999, 828, 771 (C–S). MS (ESI): *m/z* 393.1(M + 1), 395.1 (M + 3). $C_{17}H_{14}Cl_2N_4OS$ (392.03).

4.2.11. N-(4-acetyl-2-bromophenyl)-2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)acetamide (7i)

White needle crystals, yield: 55.4%. mp: 131–133 °C. ^{1}H-NMR ($CDCl_3$, ppm) δ: 9.27 (s, 1H, NH), 8.36 (d, 1H, $J = 8.4$ Hz, Ph′H), 8.24 (s, 1H, Ph′H), 8.09 (s, 1H, triazole–H), 7.98 (d, 1H, $J_1 = 1.2$ Hz, PhH), 7.91 (d, 1H, $J = 8.4$ Hz, Ph′H), 7.64 (dd, 1H, $J_1 = 1.2$ Hz, $J_2 = 8.4$ Hz, PhH), 7.45 (d, 1H, $J_2 = 8.4$ Hz, PhH), 3.85 (s, 2H, S–CH_2), 2.58 (s, 3H, CH_3). MS (ESI): *m/z* 199.1 (M + 1), 202.1 (M + 3), 204.1 (Mv+ 5). $C_{18}H_{13}BrCl_2N_4O_2S$ (497.93).

4.2.12. N-(2-bromo-4-methylphenyl)-2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)acetamide (7j)

White solid, yield: 48.6%. mp: 125–126 °C. ^{1}H-NMR ($CDCl_3$, ppm) δ: 9.55 (s, 1H, NH), 8.17 (d, 1H, $J = 8.4$ Hz, Ph′H), 8.04 (s, 1H, triazole–H), 7.88 (d, 1H, $J_1 = 1.8$ Hz, PhH), 7.59 (dd, 1H, $J_1 = 1.8$ Hz, $J_2 = 8.4$ Hz, PhH), 7.43 (s, 1H, Ph′H), 7.38 (d, 1H, $J = 8.4$ Hz, PhH), 7.20 (s, 1H, $J = 8.4$ Hz, Ph′H), 3.57 (s, 2H, S–CH_2), 2.39 (s, 3H, CH_3). MS (ESI): *m/z* 471.2 (M + 1), 473.2 (M + 3). $C_{17}H_{13}BrCl_2N_4OS$ (469.94).

4.2.13. N-(2-bromo-4-chlorophenyl)-2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)acetamide (7k)

White solid, yield: 49.8%. mp: 163–165 °C. ^{1}H-NMR ($CDCl_3$, ppm) δ: 9.67 (s, 1H, NH), 8.24 (d, 1H, $J = 8.4$ Hz, Ph′H), 8.08 (s, 1H, triazole-H), 7.61 (d, 1H, $J = 1.8$ Hz, PhH), 7.54 (d, 1H, $J = 2.4$ Hz, Ph′H), 7.42 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.4$ Hz, Ph′H), 7.38 (d, 1H, $J = 8.4$ Hz, PhH), 7.28 (dd, 1H, $J_1 = 1.8$ Hz, $J_2 = 8.4$ Hz, PhH), 4.02 (s, 2H, S–CH_2). MS (ESI): *m/z* 491.1 (M + 1), 493.1 (M + 3), 495.0 (M + 5). $C_{16}H_{10}BrCl_3N_4OS$ (489.88).

4.2.14. Methyl 3-bromo-4-(2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)acetamido) benzoate (7l)

White needle crystals, yield: 59.3%. mp: 164–166 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.84 (s, 1H, NH), 8.24 (d, 1H, $J = 8.4$ Hz, Ph′H), 8.23 (d, 1H, $J = 1.8$ Hz, Ph′H), 8.09 (s, 1H, triazole-H), 7.97 (dd, 1H, $J_1 = 1.8$ Hz, $J_2 = 8.4$ Hz, Ph′H), 7.61 (d, 1H, $J = 2.4$ Hz, PhH), 7.41 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.4$ Hz, PhH), 7.38 (d, 1H, $J = 8.4$ Hz, PhH), 4.05 (s, 2H, S–CH_2), 3.91 (s, 3H, OCH_3). MS (ESI): *m/z* 515.3 (M + 1), 517.4 (M + 3), 537.0 (M + Na^+). $C_{18}H_{13}BrCl_2N_4O_3S$ (513.93).

4.2.15. Ethyl 3-bromo-4-(2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)acetamido) benzoate (7m)

White needle crystals, yield: 67.1%. mp: 103–105 °C. ^{1}H-NMR ($CDCl_3$, ppm) δ: 9.82 (s, 1H, NH), 8.45 (d, 1H, $J = 8.4$ Hz, Ph′H), 8.23 (d, 1H, $J = 1.8$ Hz, Ph′H), 8.09 (s, 1H, triazole-H), 7.99 (dd, 1H, $J_1 = 1.8$ Hz, $J_2 = 8.4$ Hz, Ph′H), 7.61 (d, 1H, $J = 2.4$ Hz, PhH), 7.42 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.4$ Hz, PhH), 7.38 (d, 1H, $J = 8.4$ Hz, PhH), 4.36 (s, 2H, OCH_2), 4.05 (s, 2H, S–CH_2), 1.39 (s, 3H, CH_3). IR (KBr): 3228 (NH), 1720 (C=O), 1684, 1600, 1584, 1529, 1494, 1458 (N=N), 1365, 1285, 1271, 1131, 1106, 763 (C–S). MS (ESI): *m/z* 529.2 (M+1), 531.1 (M + 3), 533.2 (M + 5), 551.2 (M + Na^+), 551.2 (M+2 + Na^+). $C_{19}H_{15}BrCl_2N_4O_3S$ (527.94).

4.2.16. Methyl 4-(2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)acetamido)benzoate (7n)

White needle crystals, yield: 68.2%. mp: 153–154 °C. ^{1}H-NMR ($CDCl_3$, ppm) δ: 10.43 (s, 1H, NH), 8.16 (s, 1H, triazole-H), 8.01 (d, 2H, $J = 8.4$ Hz, Ph′H), 7.64 (d, 2H, $J = 8.4$ Hz, Ph′H), 7.61 (d, 1H, $J = 2.4$ Hz, PhH), 7.43 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.4$ Hz, PhH), 7.37 (d, 1H, $J = 8.4$ Hz, PhH), 3.90 (s, 2H, S–CH_2), 3.88 (s, 3H, OCH_3). MS (ESI): *m/z* 437.3 (M + 1), 439.3 (M + 3), 441.4 (M + 5), 559.3 (M + Na^+), 461.3 (M + 2 + Na^+). $C_{18}H_{14}Cl_2N_4O_3S$ (436.02).

4.2.17. Ethyl 4-(2-(2-(2,4-dichlorophenyl)-2H-1,2,4-triazol-3-ylthio)acetamido)benzoate (7o)

White needle crystals, yield: 71.4%. mp: 147–149 °C. ^{1}H-NMR ($CDCl_3$, ppm) δ: 10.43 (s, 1H, NH), 8.16 (s, 1H, triazole-H), 8.01 (d, 2H, $J = 8.4$ Hz, Ph′H), 7.63 (d, 2H, $J = 8.4$ Hz, Ph′H), 7.61 (d, 1H, $J = 2.4$ Hz, PhH), 7.42 (dd, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.4$ Hz, PhH), 7.37 (d, 1H, $J = 8.4$ Hz, PhH), 4.35 (s, 2H, OCH_2), 4.04 (s, 2H, S-CH_2), 1.41 (s, 3H,

CH_3). IR (KBr): 3319 (NH), 1700 (C═O), 1684, 1604, 1529, 1495, 1435, 1411 (N═N), 1369, 1317, 1290, 1169, 1156, 1117, 1001, 768 (C–S). MS (ESI): *m/z* 451.2 (M + 1), 453.2 (M + 3), 455.1 (M + 5), 473.3 (M + Na^+), 475.3 (M + 2 + Na^+). $C_{19}H_{16}Cl_2N_4O_3S$ (450.03).

4.3. Biological activity

4.3.1. In vitro anti-HIV assay

The methodology of the anti-HIV assay has been previously described [25,26]. 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(IIIB) [27] or HIV-2 (ROD) [28] stock (50 μL) at 100–300 CCID50 (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 [29] 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 50% effective concentration (EC_{50}) was defined as the compound concentration required inhibiting virus-induced syncytium formation by 50%.

4.3.2. HIV-1 RT inhibition assay

Inhibition of HIV-1 RT was developed using nucleotides linked to microtiter plate with colorimetric detection of incorporated biotin-dUTP into homopolymer template primers [30]. The incorporated quantities of the biotin-dUTP into the enzyme represented the activity of HIV-1 RT. IC_{50} values corresponded to the concentration of the 1,2,4-triazolacetamide derivatives required to inhibit biotin-dUTP incorporation into the HIV-1 RT by 50%.

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