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Structure-based bioisosterism design, synthesis and biological evaluation of novel 1,2,4-triazin-6-ylthioacetamides as potent HIV-1 NNRTIs

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ABSTRACT

The development of new HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) offers the possibility of generating novel chemical entities of increased potency. Previous investigations in our laboratory resulted in the discovery of several novel series of arylazolylthioacetanilides as potent NNRTIs. In this study, based on the structure-based bioisosterism strategy, novel 1,2,4-triazin-6-yl thioacetamide derivatives were designed, synthesized and evaluated for their anti-HIV activity in MT-4 cells. Among them, the most promising compound was **8b15** with double-digit nanomolar activity against wild-type HIV-1 (EC_{50} = 0.018 ± 0.007 μM) and moderate activity against the double mutant strain RES056 (EC_{50} = 3.3 ± 0.1 μM), which indicated that 1,2,4-triazin-6-yl thioacetamide can be used as a novel scaffold to develop a new class of potent NNRTIs active against both wild-type and drug-resistant HIV-1 strains. In addition, preliminary structure–activity relationship (SAR) and molecular modeling results are also briefly discussed, which provide some useful information for the further design of novel NNRTIs.

HIV-1 reverse transcriptase (RT) is an enzyme essential for the replication of the virus, being responsible for the reverse transcription of viral RNA into cDNA. As one of the main anti-HIV therapeutic targets, RT inhibitors are widely used in the clinic for treatment of AIDS patients.[1,2] Among RT inhibitors, non-nucleoside reverse transcriptase inhibitors (NNRTIs) with their unique antiviral potency and high selectivity, nowadays are a standard component of highly active antiretroviral therapy (HAART).[3] Nevertheless, the long-term use of NNRTIs in the clinical leads to the emergence of drug-resistant viruses and potentially severe side effects. Thus, there is a continued need for next-generation NNRTIs with better resistance profiles and improved safety and tolerability.[4]

Recently, arylazolylthioacetanilides were identified as new potential NNRTIs that displayed excellent activities against not only wild-type (WT) viruses but also a broader panel of NNRTI-resistant viruses, including K103 N and/or Y181C mutant strains, as well as good pharmacokinetic profiles.[5–11] Among them, 1,2,4-triazole derivatives VRX-480733[12] and RDEA806[13], were chosen as candidates for further studies. Especially, RDEA806 is now undergoing phase IIa clinical trials as a promising new drug candidate in Ardea Biosciences Company (Fig. 1).

Prompted by these promising results, many research groups are continuously trying to optimize the arylazolylthioacetanilide scaffold by substituting appropriate functional groups around the azole core.[5] Meanwhile, during the course of our continued effort toward the development of potent NNRTIs, we embarked on a chemical evolution through the replacement of the central core in bioactive lead molecules, a common and efficient 'follow-on'-based medicinal chemistry strategy to find proprietary and novel drug candidates.[14]

Replacement of the triazole ring by an array of other five-membered heterocycles led to the identification of additional series of arylazolylthioacetanilides that possessed potent anti-HIV activities in a cell-based replicon system.[15–23] Especially, 1,2,3-thiadiazole derivative **ZP7** (Fig. 1) exhibited the highest anti-HIV-1 activity (EC_{50} = 36 nM), inhibiting HIV-1 replication in MT-4 cells at sevenfold and eightfold higher efficiency than nevirapine (NVP) and delavirdine (DLV), 'respectively'.[16] Encouraged by these promising results, and in order to further explore the chemically diversed space and the SARs of arylazolylthioacetanilides, we employed the structure-based bioisosterism strategy, an excellent tool for lead optimization to produce the desired potency, selectivity, and the required ADME profiles for a marketable drug.[24]

This type of structure-based bioisosterism approach was based on the following considerations: molecular modeling studies

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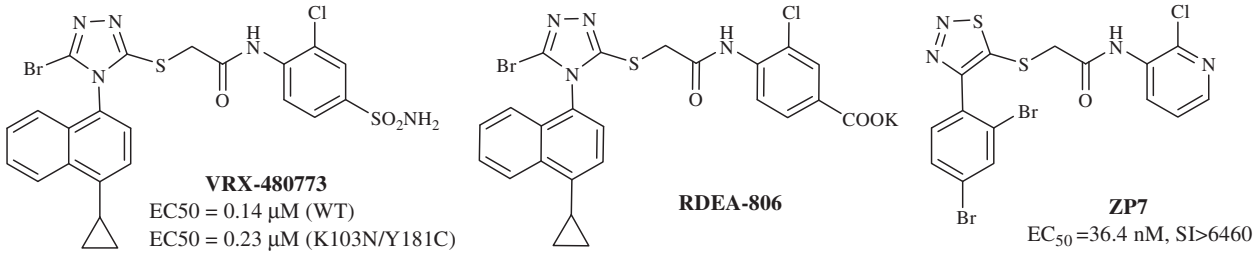


Figure 1. Azolylthioacetanilide-based NNRTIs.

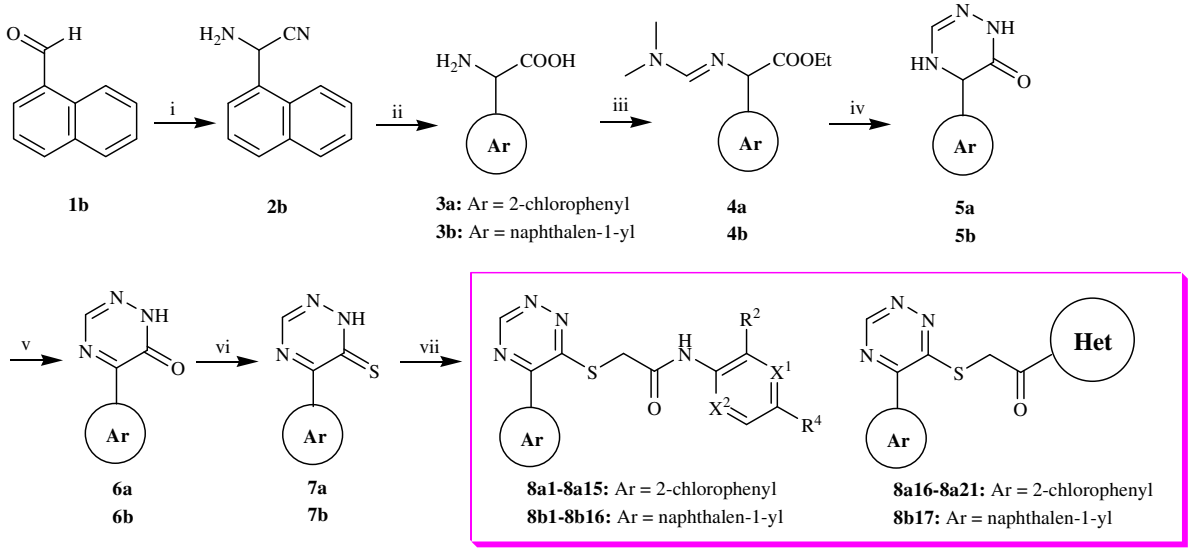
elucidated that the five-membered heterocycle portion of arylazolylthioacetanilides could be acting as a scaffold which orients the pharmacophores into the proper geometry for binding to the HIV-1 RT. It was also revealed that there are differences in the electronic and conformational contribution of the five-membered heterocyclic moiety to the binding of the inhibitors to RT.[16] Therefore, it is likely that other heterocycles with synthetic accessibility and drug-like properties are also acceptable isosteric replacements for the five-membered azoles moiety in the lead compounds. 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, might tolerate substitution by structurally diverse groups. Therefore, different types of modifications are worthy being carried out on the prototype compounds to further explore the structural features required for anti-HIV-1 activity in this region.

In continuation of our ongoing programs directed toward the development of novel anti-HIV agents, we now report design, a concise and practical synthetic route, and in vitro biological evaluation of novel 1,2,4-triazin-6-ylthioacetamides as potent HIV-1 NNRTIs. In addition, preliminary structure–activity relationship (SAR) and molecular modeling results of these new compounds are also discussed.

To achieve the synthesis of the target compounds 1,2,4-triazine thioacetanilides **8a** and **8b**, the steps outlined in Scheme 1 were adopted. For the 2-(5-(2-chlorophenyl)-1,2,4-triazin-6-ylthio)-*N*-arylacetamide (**8a**) series, by refluxing the commercially available 2-amino-2-(2-chlorophenyl)acetic acid (**3a**) in 5.5 equiv of dimethylformamide dimethyl acetal for 3 h, an essentially quantitative conversion to reasonably stable ethyl 2-(2-chlorophenyl)-2-((dimethylamino)methyleneamino) acetate (**4a**) was achieved.[25] Treatment of **4a** with excess hydrazine hydrate gave 5-(2-chlorophenyl)-4,5-dihydro-1,2,4-triazin-6(1*H*)-one (**5a**), which was treated with potassium permanganate in an acetone/acetic acid solution to give the desried oxidation product **6a** in satisfactory yields.[26] Compound **6a** was reacted with phosphorus pentasulfide in pyridine under reflux to afford 5-(2-chlorophenyl)-1,2,4-triazine-6(1*H*)-thione (**7a**). The final triazine thioacetanilides (**8a**) were synthesized by reaction of intermediates **7a** with suitable 2-chloro-*N*-aryl-substituted acetamides (or other alkyl halides) in good yields. 2-Chloro-*N*-phenyl acetamides (or other alkyl halides) were synthesized according to the reported literature.[27]

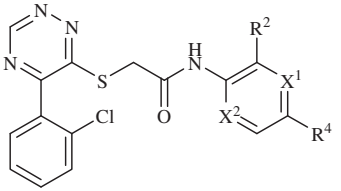
For the 2-(5-(naphthalene-1-yl)-1,2,4-triazin-6-ylthio)-*N*-arylacetamide (**8b**) series, treatment of 1-naphthaldehyde (**1b**) with aqueous ammonia followed by trimethylsilanecarbonitrile (TMSCN) afforded 2-amino-2-(naphthalen-1-yl) acetonitrile (**2b**), which was subjected to hydrolysis of the nitrile group in an acidic conditions to give 2-amino-2-(naphthalen-1-yl)acetic acid (**3b**).[28] The posterior synthetic steps were identical to those of the **8a** series. The purity of the synthesized compounds was monitored by TLC. All the MS, IR, ^{1}H NMR and ^{13}C NMR spectral data of the title compounds were in accordance with assumed structures.[29]

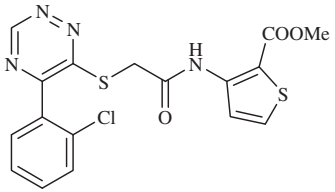
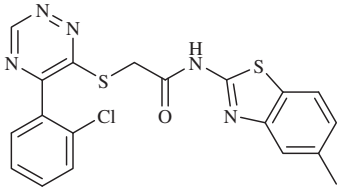
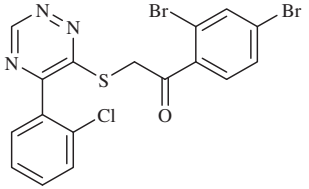
The newly synthesized 1,2,4-triazin-6-ylthioacetamide derivatives were evaluated for anti-HIV activities by determining their ability to inhibit the replication of the HIV-1 III_B strain[30], HIV-1 mutant strain RES056 (K103 N/Y181C double RT mutant), and HIV-2 ROD strain[31] in MT-4 cells[32] in comparison with reference drugs, that is nevirapine (NVP), zidovudine (azidothymidine,



Scheme 1. Reagents and conditions: (i) NH_3 aq, MeOH, 0 °C, 20 min, and then TMSCN, rt, 24 h, 70.0% over two steps; (ii) 6 N HCl, reflux, 76.8%; (iii) dimethylformamide dimethyl acetal; (iv) $N_2H_4{\cdot}H_2O$, EtOH; (v) Br_2, AcOH; (vi) P_2S_5, Pyridine; (vii) $ClCH_2CONHPh$ (other alkyl halides), Na_2CO_3 or NaOH, EtOH.

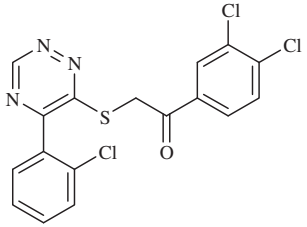
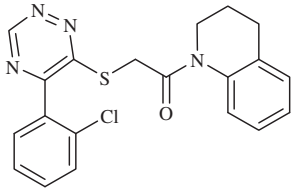
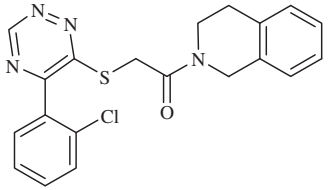
Table 1
Anti-HIV activity, cytotoxicity and selectivity indices of 2-(5-(2-chlorophenyl)-1,2,4-triazin-6-ylthio)-*N*-arylacetamide derivatives (**8a1–8a16**)



Code	X^1	X^2	R^2	R^4	EC_{50}(μM)[a]			CC_{50}[b] (μM)	SI[c]		
					HIV-1 III_B	RES056	HIV-2 ROD		HIV-1 III_B	RES056	HIV-2 ROD
8a1	CH	CH	H	H	0.42±0.056	>127	>127	127±42	296	<1	<1
8a2	CH	CH	Cl	H	0.82 ± 0.35	⩾55	>90	90 ± 37	1094	⩽2	<1
8a3	CH	CH	F	H	0.21 ± 0.045	>98	>98	98 ± 15	471	<1	<1
8a4	CH	CH	Br	H	0.092 ± 0.030	⩾55	>73	73 ± 35	792	⩽1	<1
8a5	CH	CH	Br	Me	0.178 ± 0.062	⩾42	>45	45 ± 16	253	⩽1	<1
8a6	CH	CH	NO_2	Me	0.048 ± 0.007	⩾22	>158	158 ± 52	3326	⩽1	<1
8a7	CH	CH	NO_2	H	0.0230 ± 0.015	⩾11	>63	63 ± 50	2155	⩽6	<1
8a8	CH	CH	Br	COMe	0.086 ± 0.017	>34	>35	34 ± 8.2	404	<1	<1
8a9	N	CH	Cl	H	0.074 ± 0.005	23 ± 4.5	>167	167 ± 8.0	2237	=7	<1
8a10	CH	CH	Cl	Cl	0.258 ± 0.094	>51	>51	51 ± 37	190	<1	<1
8a11	CH	CH	Me	H	0.297 ± 0.054	>142	>142	142 ± 18	465	<1	<1
8a12	CH	CH	Br	COOMe	0.190 ± 0.120	>59	>59	59 ± 40	311	<1	<1
8a13	CH	CH	Br	COOEt	0.236 ± 0.079	>69	>69	69 ± 45	302	<1	<1
8a14	CH	N	Br	Me	>31	-	>144	144 ± 6.1	<1		<1
8a15	CH	N	H	H	3.3 ± 1.6	>123	>123	172 ± 55	51	<1	<1
8a16					2.9 ± 1.2	>105	>105	105 ± 42	35	<1	<1
8a17					>32	—	>32	137 ± 26	<1	-	<1
8a18					>28	—	>28	28 ± 2.7	<1	-	<1

(*continued on next page*)

Table 1 (*continued*)

Code	X^1	X^2	R^2	R^4	EC_{50}(μM)[a]			CC_{50}[b] (μM)	SI[c]		
					HIV-1 III_B	RES056	HIV-2 ROD		HIV-1 III_B	RES056	HIV-2 ROD
8a19					6.2 ± 0.9	>33	>33	33 ± 2.6	5		<1
8a20					>35	—	>35	35 ± 0.98	<1	-	<1
8a21					0.731 ± 0.58	⩾43	>38	⩾38	⩾52	X1	<orX1
NVP					0.090 ± 0.030	11 ± 2.2	—	>15	>168	>1	
AZT					0.021 ± 0.024	0.009 ± 0.0001	0.004 ± 0.00112	249 ± 13	12221	29564	56907
DDC					1.0 ± 0.4	_	1.3 ± 0.6	>95	>93	_	>74
EFV					0.005 ± 0.0003	0.57 ± 0.063	—	>6.3	>1187	>11	—
DLV					0.032 ± 0.004	>36	—	>36	>1096	X1	—

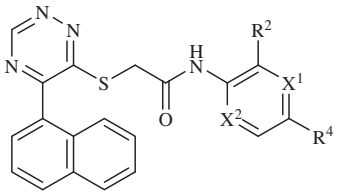
In bold are the values of active compounds.

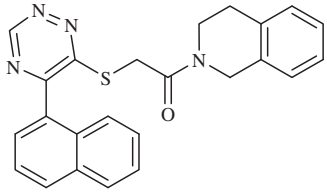
[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cells against HIV-1-induced cytopathicity, as determined by the MTT method.

[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: X1 stands for ⩾1 or <1.

Table 2
Anti-HIV activity, cytotoxicity and selectivity indices of 2-(5-(naphthalen-1-yl)-1,2,4-triazin-6-ylthio)-*N*-arylacetamide derivatives (**8b1–8b16**)



Code	X^1	X^2	R^2	R^4	EC_{50}[a] (μM)			CC_{50}[b] (μM)	SI[c]		
					HIV-1 III_B	RES056	HIV-2 ROD		HIV-1 III_B	RES056	HIV-2 ROD
8b1	CH	CH	H	H	1.53 ± 0.107	>154	>154	154 ± 63	101	<1	< 1
8b2	CH	CH	Cl	H	0.135 ± 0.059	>63	>63	63 ± 34	470	<1	<1
8b3	CH	CH	F	H	0.359 ± 0.077	>65	>65	65 ± 32	184	<1	<1
8b4	CH	CH	Br	H	0.173 ± 0.027	⩾43	>87	87 ± 48	503	⩽2	<1
8b5	CH	CH	Br	Me	0.195 ± 0.015	46 ± 0.9	>166	166 ± 59	852	4	<1
8b6	CH	CH	NO_2	Me	0.046 ± 0.005	5.2 ± 0.7	>147	147 ± 77	3167	28	<1
8b7	CH	CH	NO_2	H	0.043 ± 0.0	5.6 ± 0.07	>142	142 ± 23	3255	25	<1
8b8	CH	CH	Br	COMe	0.051 ± 0.002	4.3 ± 0.7	>133	133 ± 55	2681	31	<1
8b9	N	CH	Cl	H	0.059 ± 0.005	7.0 ± 0.2	>44	44 ± 9.2	751	6	<1
8b10	CH	CH	Cl	Cl	>254	–	>254	254 ± 11	<1	–	<1
8b11	CH	CH	Me	H	0.828 ± 0.388	>158	>158	158 ± 59	194	<1	<1
8b12	CH	CH	Br	COOMe	0.069 ± 0.01	25 ± 13	>173	173 ± 45	2561	7	<1
8b13	CH	CH	Br	COOEt	0.210 ± 0.038	>105	>105	105 ± 37	524	<1	<1
8b14	CH	CH	Cl	COOEt	0.205 ± 0.063	>150	>150	150 ± 12	729	<1	<1
8b15	CH	CH	Br	SO_2NH_2	0.018 ± 0.007	3.3 ± 0.07	>23	23 ± 0.3	1293	=7	<1
8b16	CH	CH	Cl	NO_2	>150	–	>150	150 ± 0.5	<1	–	<1
8b17					0.751 ± 0.339	>13	>13	13 ± 5.7	17	<1	<1
NVP					0.200 ± 0.105	>15	–	>15	>75	<1	–
AZT					0.006 ± 0.0005	–	–	⩾50	⩾8295	–	–
DDC					1.4 ± 0.05	–	–	>95	>68	–	–
EFV					0.007 ± 0.0008	–	–	>6.3	>964	–	–
DLV					0.035 ± 0.004	>36	–	>36	>1034	<1	–

In bold are the values of active compounds.
[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cells against HIV-1-induced cytopathicity, 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: X1 stands for ⩾1 or <1.

AZT), dideoxycytidine (DDC), delavirdine (DLV) and efavirenz (EFV). The cytotoxicity of the compounds was determined in parallel. The methodology of the anti-HIV assay has been previously described.[33,34] Inhibitory concentration (EC_{50}), cytotoxic concentration (CC_{50}), and selective index (SI, given by the CC_{50}/EC_{50} ratio) for different compounds are presented in Table 1 and Table 2.

As can be seen from Table 1 and Table 2, the experimental results indicated that the majority of the tested 1,2,4-triazin-6-ylthioacetamide derivatives were found to be active against HIV-1 in the range of 0.018–6.2 μM and none of the compounds was active against HIV-2. In fact, 28 compounds showed anti-HIV-1 activities at submicromolar concentrations. Especially, compound **8b15** was the most potent inhibitor against HIV-1(III_B) replication of these two series, with an EC_{50} = 18 nM, CC_{50} = 3.3 μM, SI = 1293. **8b15** was about seventy-eight times more active than DDC (EC_{50} = 1.4 ± 0.05 μM), eleven fold more active than NVP (EC_{50} = 0.200 ± 0.105 μM) and twofold more active than DLV (0.035 ± 0.005 μM), but was still inferior to EFV (EC_{50} = 6.6 ± 0.9 nM).

Some other compounds, **8a4**, **8a6–9**, **8b2**, **8b4–9** and **8b12–14** also exhibited moderate to good activities against HIV-1 strain III_B with EC_{50} values in the range of 0.030–0.195 μM, reaching the same order of magnitude as that of NVP (0.090–0.200 μM). These results indicated that the 1,2,4-triazine is an appropriate hopping scaffold instead of the five-membered heterocycles of the lead compounds.

Compared with the results shown in Table 1 and Table 2, substantial difference in antiviral activity against HIV-1(III_B) can be observed in the two series of compounds, with the following active sequence (Ar): naphthalen-1-yl > 2-chlorophenyl. Therefore, the aryl group linked to the 1,2,4-triazine core was further confirmed to be crucial for the antiviral activity, and this agrees with previous SAR findings in the arylazolylthioacetanilides series. Probably, the hydrophobicity of the aryl group helps to improve the binding affinity (π–π interaction) between the active binding site and the inhibitors, and thus enhances the biological activity.

Just as SAR of arylazolylthioacetanilides made in our previous studies, the electronic nature or the steric demand of the substituents at *ortho*-position of phenyl ring of the anilide moiety essentially influenced the anti-HIV-1 activity. Table 1 revealed the potency contribution of the *ortho* substitution at the phenyl ring of the anilide moiety in 2-chlorophenyl (Ar) series, according to the sequence: NO_2 (**8a7**, EC_{50} = 0.030 ± 0.015 μM) > Br (**8a4**, EC_{50} = 0.092 ± 0.30 μM) > F (**8a3**, EC_{50} = 0.21 ± 0.045 μM) > Me (**8a11**, EC_{50} = 0.297 ± 0.054 μM) > H (**8a1**, EC_{50} = 0.42 ± 0.056 μM) > Cl (**8a2**, EC_{50} = 0.82 ± 0.35 μM). Analogously, the antiviral potency of naphthalen-1-yl series was decreased in the following order: NO_2 (**8b7**, EC_{50} = 0.043 μM) > Cl (**8b2**, EC_{50} = 0.135 ± 0.059 μM) > Br (**8b4**, EC_{50} = 0.173 ± 0.027 μM) > F (**8b3**, EC_{50} = 0.359 ± 0.077 μM) > Me (**8b11**, EC_{50} = 0.828 ± 0.388 μM) > H (**8b1**, EC_{50} = 1.5 ± 0.1 μM).

Obviously, it should highlight that the 2-nitro substitution on the phenyl ring always furnished the most potent compounds of these two series. These SAR conclusions are also grossly in agreement with the previously reported results in the arylazolylthioacetanilide series.[15–23]

In addition, from the SAR point of view, the anti-HIV activity is also strongly dependent on the nature of the *para* position of the anilide moiety. For instance, when the methyl group is introduced to the *para* position of the anilide moiety, the bioactivity was decreased (**8a4**/**8a5**, **8a7**/**8a6**, **8b4**/**8b5**, **8b7**/**8b6**), which was likely due to their increased hydrophobicity in this region which would lead to unfavorable interactions with the reverse transcriptase as suggested by the molecular modeling studies. Whereas introduction of 4-acetyl or formic esters led to compounds with slightly improved or equative activity. Moreover, it is interesting to note in Table 2 that, compound **8b15**, with a sulfonamide group at the *para* position of the anilide moiety possessed outstanding inhibitory activity comparing to its counterparts. Practically, the sulfonamide is a common privileged group used in a lot of NNRTI scaffolds because its increased polarity can accommodate the chemical environment in this region of RT, leading to the possibility of increasing affinity by generating hydrogen bonding interactions.[5]

To further explore the salient features controlling the activity, the N-substituted heterocycle acetamide derivatives were also synthesized. Among them, pyridine derivatives **8a9** and **8b9** showed significantly improved anti-HIV-1 profiles superior to that of **8a2** and **8b2**, 'respectively'. Moreover, pyridine derivative **8a15** and thiazole derivative **8a16** demonstrated reasonable antiviral activity. These results confirmed the concept 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.

Moreover, in order to obtain comprehensive SAR indications and to identify more potent NNRTIs, the replacement of the N-substituted heterocycle acetamide moiety afforded phenylethanone derivatives (**8a18**, **8a19**) and 3,4-dihydro(iso)quinolin-2(1*H*)-yl ethanone derivatives (**8a20**, **8a21**, **8b17**). A brief investigation of the SARs revealed that the various substituting halogen atoms and subsitituting position on the phenyl ring of phenylethanone series markedly influenced the antiviral activity. It was also observed that, 3,4-dihydroisoquinolin-2(1*H*)-yl ethanone derivatives (**8a21**, **8b17**) remained their anti-HIV activity in the submicromolar concentration range.

Table 3
Inhibitory activity of compounds **8a6** and **8a7** against HIV-1 RT

Compd	**8a6**	**8a7**	NVP
IC_{50}[a] (μM)	18	6.5	2.7

[a] 50% inhibitory concentration of tested compounds required to inhibit biotin deoxyuridine triphosphate (biotin-dUTP) incorporation into the HIV-1 RT by 50%.

Lastly, the bioactivity results also showed that none of the title compounds was active against the HIV-2 strain, while five compounds (**8b6–8b9**, **8b15**) possessed potent activity against RES056 resistant mutant strain of HIV-1 in MT-4 cells with EC_{50} values in the low micromolar range (3.3–7.0 μM). Compound **8b15** possessed the highest activity against the RES056 mutant strain of HIV-1 with an EC_{50} value of 3.3 ± 0.075 μM. Some other three compounds (**8a9**, **8b5**, **8b12**) were also active at micromolar concentrations against the RES056 resistant mutant Figure 2.

With the aim to further confirm the drug target of 1,2,4-triazin-6-ylthioacetamide derivatives, the selected title compounds **8a6** and **8a7** were tested in enzymatic assays against highly purified recombinant HIV-1 RT using poly(rC)–oligo(dG) as template primer. Inhibition of HIV-1 RT has been performed using nucleotides linked to the microtiter plate with colorimetric detection of incorporated biotin-dUTP into homopolymer template primers.[35] 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-triazin-6-ylthioacetamide derivatives required to inhibit biotin-dUTP incorporation into the HIV-1 RT by 50%. As shown in Table 3, compounds **8a6** and **8a7** exhibited moderate inhibition of enzymatic activity with IC_{50} values of 18 μM and 6.5 μM, which were slightly higher than that of NVP (2.7 μM).

With the aim to understand the interactions between these inhibitors and the target, molecular modeling of compound **8b15** docked into the NNRTIs binding pocket (NNIBP) of HIV-1 RT was employed by means of Autodock Vina [http://www.vina.scripps.edu]. X-ray crystal structure of HIV-1 RT with benzophenone taken from PDB (3DLG) was used as the input structure for docking calculations because of the high degree of structural similarity between arylazolylthioacetanilides and benzophenones.[5] Default parameters were used as described in the Autodock Vina manual unless otherwise specified. The theoretical binding mode of **8b15** to the NNIBP is shown in Figure 3.

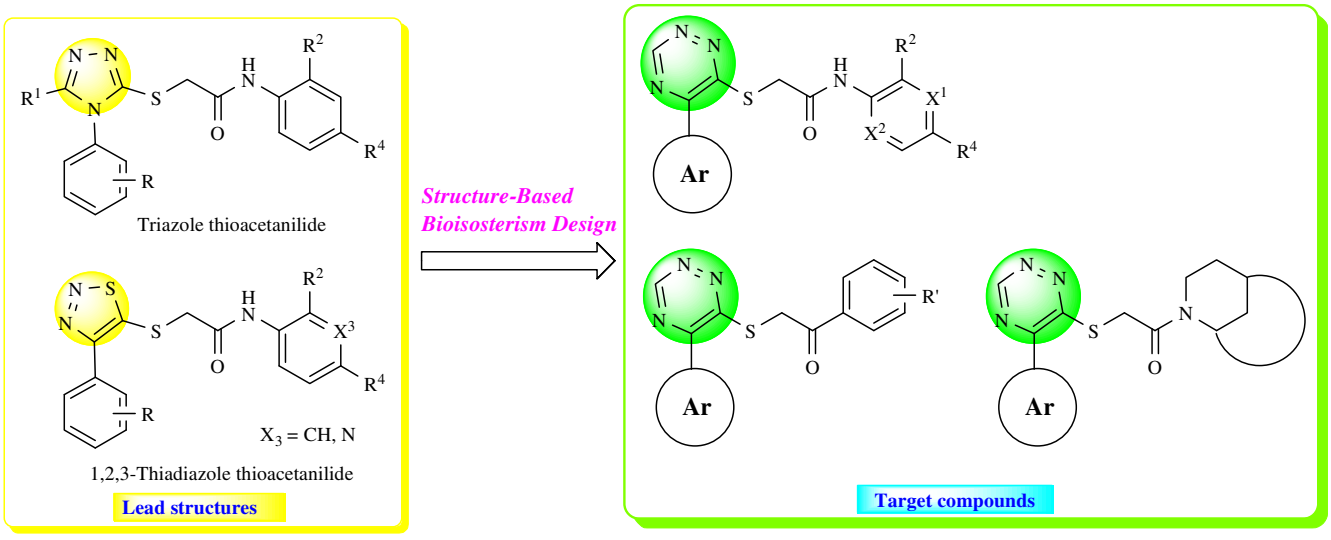
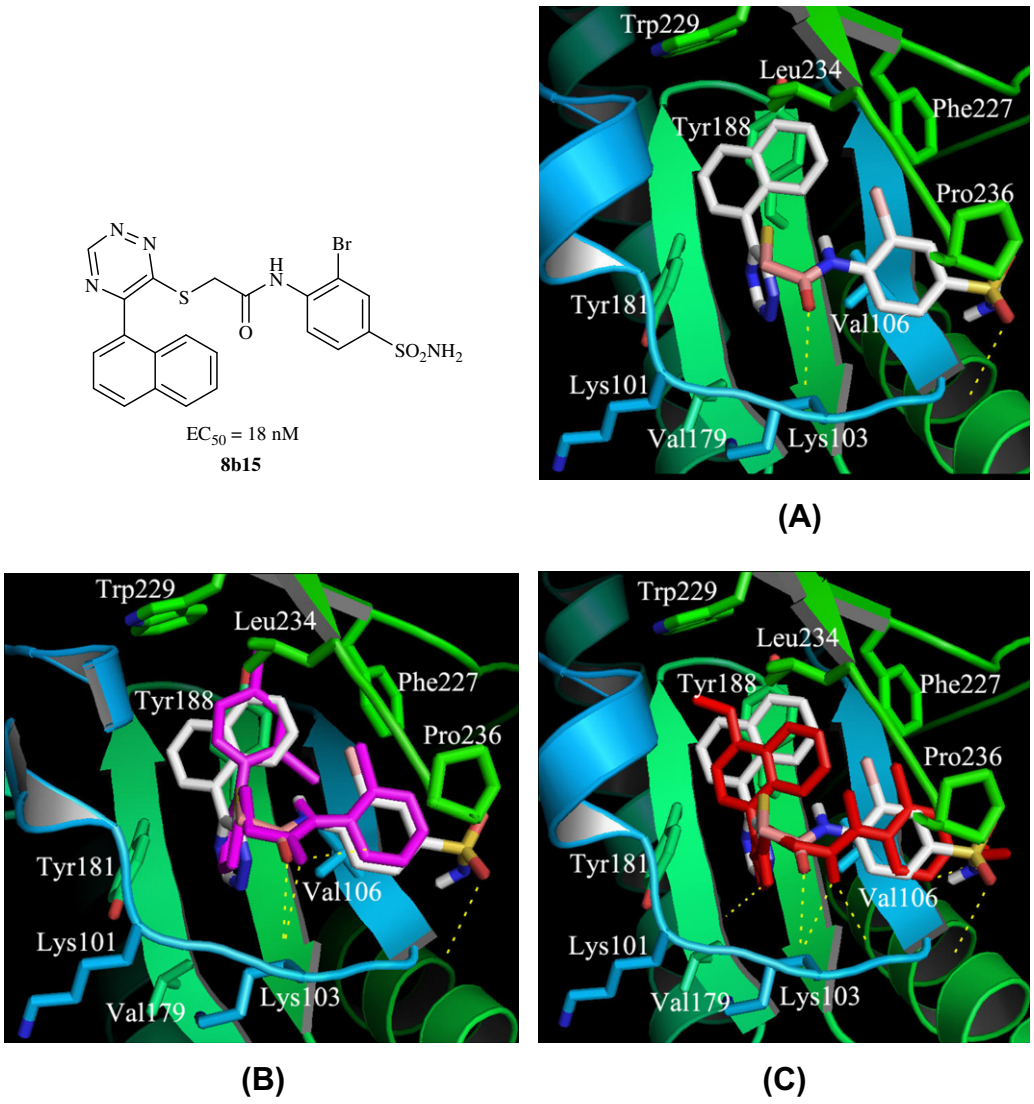


Figure 2. Structure-based bioisosterism replacement of azoles by 1,2,4-triazine.



EC_{50} = 18 nM
8b15

Figure 3. (A) Predicted binding mode and molecular docking of compound **8b15** into the allosteric site of HIV-1 RT (PDB code: 3DLG); (B) Superimposition of the docked conformations of **8b15** (white) and **ZP7** (purple) in the HIV-1 RT (PDB code: 3DLG); (C) Superimposition of the docked conformations of **8b15** (white) and **RDEA806** (red) in the HIV-1 RT (PDB code: 3DLG). The docking results are shown by PyMOL. Hydrogen bonds are indicated by dashed lines.

Results suggested that conpound **8b15** was able to bind into NNIBP of the RT in a similar manner to previous arylazolylthioacetanilide derivatives (Figures 3b, **c**).[17] As illustrated in Figure. 3**a**, the naphthalene ring of **8b15** fits into the aromatic-rich binding pocket, surrounded by the aromatic side chains of Tyr188, Phe227, and Trp229. Detailed analysis of the binding mode showed that one phenyl ring interacts favorably with the Tyr188 side chain, giving rise to a positive π–π stacking interaction. The inhibitor's amide carbonyl forms a key hydrogen bond with the backbone N-H of Lys103, which is important for the affinity between inhibitor and RT. The 2-bromo-4-sulfonamidephenyl moiety of **8b15** is close to Pro236, and the sulfonamide moiety points toward the solvent exposed region. Therefore, 4-substituent at the phenyl ring of the anilide moiety allows hydrophilic groups as the preferred substituents, which can explain why **8b15** stands out of other congeners.

In summary, the computational approach has helped in better understanding of inhibitor binding to the enzyme active site. Further structural optimization will consider these aspects in future design attempts.

In this article, based on the structure-based bioisosterism design, replacement of the five-membered heterocycle fragment in the previously reported lead structures with a 1,2,4-triazine group led to the discovery of a novel series of potent NNRTIs. SAR of the lead structure was further extended. Among the newly synthesized compounds, the most active compound was **8b15**, which contained the sulfonamide moiety. The HIV-1(III_B) inhibitory activity of **8b15** (EC_{50} = 0.018 ± 0.007 μM) was about seventy-eight times more active than DDC (EC_{50} = 1.40 ± 0.05 μM), eleven fold more active than NVP (EC_{50} = 0.200 ± 0.105 μM) and twofold more active than DLV (0.035 ± 0.005 μM). It also showed significant activity against the RES056 mutant strain of HIV-1 with an EC_{50} value in 3.3 ± 0.07 μM. A molecular modeling study on compound **8b15** was performed to gain insight into its binding mode with the allosteric site of HIV-1 RT, and to provide the basis for further structure-guided design of new NNRTIs related with an arylazolyl(azinyl)thioacetanilide scaffold.

In conclusion, the 1,2,4-triazin-6-ylthioacetamide derivatives are interesting NNRTIs that are endowed with potent antiviral activities. Ongoing studies involve extension of the illustrated SAR strategy to identify additional activity parameters in the arylazinylthioacetanilide scaffold to overcome viral resistance, which will be published due course of time.

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29. *Representative characterization data:* ***8a2***: white solid, yield: 71.4%. mp: 101–103 °C. ^{1}H-NMR (600 MHz, DMSO-d_6, ppm) δ: 9.90 (*s*, 1H, NH), 9.70 (s, 1H, CH═N), 7.75 (d, 1H, *J* = 7.8 Hz, PhH), 7.71 (d, 1H, *J* = 7.8 Hz), 7.66–7.62 (m, 2H), 7.58 (t, 1H, *J* = 7.2 Hz), 7.49 (d, 1H, *J* = 7.8 Hz), 7.33 (t, 1H, *J* = 7.8 Hz), 7.19 (t, 1H, *J* = 7.2 Hz, Ph'H), 4.38 (s, 2H, CH_2-S). ^{13}C NMR (150 MHz, DMSO-d_6, ppm): 166.5 (C═O), 161.8, 156.4, 154.4, 135.3, 133.0, 131.9, 131.0, 130.6, 130.2, 128.5, 128.2, 127.0, 126.6, 126.2, 35.2 (CH_2-S). IR (KBr, cm^{-1}): 3258 (υ_{NH}), 3066, 2929, 1689 ($\upsilon_{C=O}$), 1592, 1525, 1468, 1441, 1311, 1235, 1143, 762 (υ_{C-Cl}), 750 (υ_{C-S}). $C_{17}H_{12}Cl_2N_4OS$ (Exact Mass: 390.01). **8a4**: white solid, yield: 68.3%. mp: 99–102 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 9.83 (*s*, 1H, NH), 9.70 (s, 1H, CH═N), 7.71 (d, 1H, *J* = 8.4 Hz, PhH), 7.66–7.62 (m, 4H), 7.58 (t, 1H, *J* = 7.8 Hz), 7.37 (t, 1H, *J* = 7.8 Hz), 7.14 (t, 1H, *J* = 7.2 Hz, Ph'H), 4.36 (s, 2H, CH_2-S). ^{13}C NMR (150 MHz, DMSO-d_6, ppm): 166.5 (C═O), 161.7, 156.4, 154.3, 136.6, 133.4, 133.3, 133.0, 131.9, 131.0, 130.7, 128.8, 128.5, 127.7, 127.0, 117.9, 35.2 (CH_2-S). IR (KBr, cm^{-1}): 3336 (υ_{NH}), 3062, 1688 ($\upsilon_{C=O}$), 1594, 1526, 1437 ($\upsilon_{N=N}$), 1311, 1233, 1024, 756 (υ_{C-S}). MS (ESI): *m*/*z* 435.3 (M+1), 437.2 (M+3). $C_{17}H_{12}BrClN_4OS$ (Exact Mass: 433.96). **8b15**: grey solid, yield: 66.4%. mp: 197–199 °C. ^{1}H-NMR (400 MHz, DMSO-d_6, ppm) δ: 9.95 (s, 1H, SO_2NH_2), 9.75 (s, 1H, SO_2NH_2), 8.18 (*d*, 1H, *J* = 7.8 Hz, NH), 8.09 (d, 1H, *J* = 8.2 Hz, CH═N), 8.04 (d, 1H, *J* = 1.96 Hz, PhH), 7.90 (d, 1H, *J* = 8.5 Hz, PhH), 7.80–7.45 (m, 8H), 4.33 (s, 2H, CH_2-S). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 166.8 (C═O), 161.9, 157.6, 154.2, 142.0, 139.4, 133.7, 131.6, 131.4, 130.4, 130.0, 129.1, 127.8, 127.8, 127.2, 126.0, 125.9, 125.7, 125.0, 116.4, 35.2 (CH_2-S). MS (ESI): *m*/*z* 530.2 (M+1), 532.2 (M+3). $C_{21}H_{16}BrN_5O_3S_2$ (Exact Mass: 528.99).
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