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Synthesis and biological evaluations of sulfanyltriazoles as novel HIV-1 non-nucleoside reverse transcriptase inhibitors

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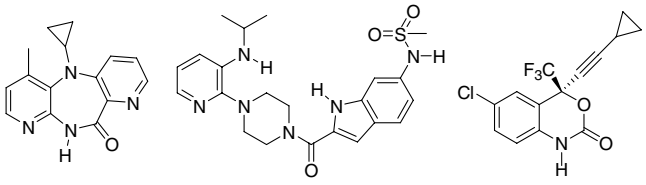
Abstract—A novel sulfanyltriazole was discovered as an HIV-1 non-nucleoside reverse transcriptase inhibitor via HTS using a cell-based assay. Chemical modifications and molecular modeling studies were carried out to establish its SAR and understand its interactions with the enzyme. These modifications led to the identification of sulfanyltriazoles with low nanomolar potency for inhibiting HIV-1 replication and promising activities against selected NNRTI resistant mutants. These novel and potent sulfanyltriazoles could serve as advanced leads for further optimization.

HIV-1 reverse transcriptase is a key enzyme in the HIV replication and has been a key target for developing anti-HIV drugs. Two types of reverse transcriptase inhibitors have been developed:[1,2] nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTI). Three NNRTIs, nevirapine (Viramune®),[3] delavirdine (Rescriptor®),[4] and efavirenz (Sustiva®)[5], have been approved by FDA for the treatment of HIV infection. However, significant resistance has been developed against the current NNRTIs and there is an urgent need to develop new anti-HIV agents that are effective against these resistant mutants.[6,7] The current efforts have been focused on developing inhibitors based on novel scaffolds and/or with new mechanism of action.[8,9] We have been using cell-based assays to identify HIV inhibitors in our studies,[10–12] which could potentially result in inhibitors which fall into both categories (see Fig. 1).

In our previous studies, we reported the discovery of novel oxindoles, quinolones, and pyrrolidinones as non-nucleoside reverse transcriptase inhibitors,[10–13] In this letter, we wish to report the identification of a novel series of sulfanyltriazoles as NNRTIs and our preliminary SAR studies on this scaffold. Through our HTS using the cell-based HIV replication assay, a sulfanyltriazole (**4a**) was identified as an inhibitor with low micromolar inhibitory activity. Sulfanyltriazoles have been reported to possess a number of biological activities, e.g. antibacterial and antifungal,[14] analgesic,[15] and anticonvulsant activities.[16] However, their anti-HIV activity has not been previously documented.[17] Compound **4a** has a simple, yet distinctively different chemical structure from the HIV inhibitors reported in the literature. To explore the SAR of this novel scaffold, we first prepared a series of analogs with varying amide moieties as illustrated in Scheme 1. Starting from



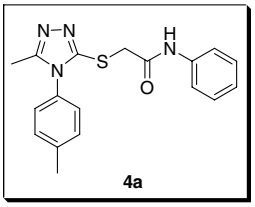


Figure 1.

Keywords: Sulfanyltriazoles; HIV-1 non-nucleoside reverse transcriptase inhibitor (NNRTI); SAR; Molecule modeling; HIV replication.
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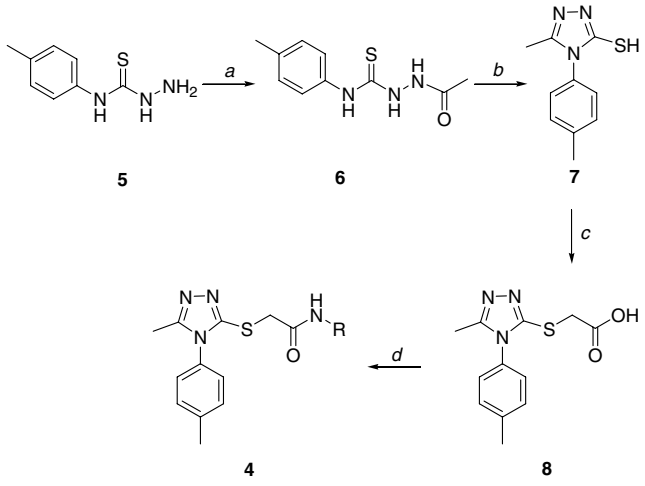


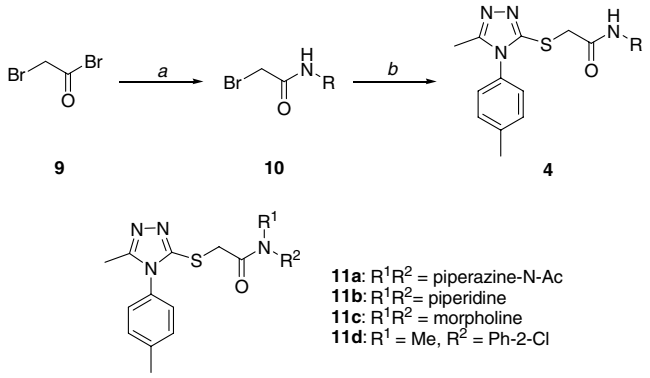
4a: R = Ph
4b: R = Ph-2-Me
4c: R = Ph-4-Me
4d: R = Ph-2-F
4e: R = Ph-2-Cl
4f: R = Ph-4-Cl
4g: R = Ph-2-Br
4h: R = Ph-3-Br
4i: R = Ph-4-Br
4j: R = Ph-2-CF_3
4k: R = Ph-3-CF_3
4l: R = Ph-2-CN
4m: R = Ph-3-CN
4n: R = Ph-4-CN
4o: R = Ph-3-OMe
4p: R = Ph-4-OMe
4q: R = Ph-2-CO_2Me
4r: R = Ph-3-CO_2Me
4s: R = Ph-4-CO_2Me
4t: R = Ph-2-NO_2
4u: R = Ph-3-NO_2
4v: R = Ph-4-NO_2
4w: R = Ph-2,3-Me_2
4x: R = Ph-2-Me-4-Cl
4y: R = Ph-2,4-Cl_2
4z: R = Ph-2,5-Cl_2
4aa: R = 2-naphthalyl
4ab: R = thiadiazole-3-Et
4ac: R = 2-Py
4ad: R = 4-Pyrimidyl
4ae: R = $(CH_2)_8$Me
4af: R = *t*Bu

Scheme 1. Synthesis of sulfanyltriazoles **4a**–**4af**. Reagents and conditions: (a) AcCl (1.1 equiv), Py (5.0 equiv), $CHCl_3$, 0–25 °C, 3 h, 90%; (b) 2.0 M NaOH (aqueous), reflux, 5 h, 80%; (c) $BrCH_2CO_2H$ (1.2 equiv), Et_3N (3.5 equiv), CH_2Cl_2, 25 °C, 5 h, 75%; (d) $ArNH_2$ (1.2 equiv), HATU (1.2 equiv), CH_2Cl_2, 25 °C, 1 h, 5–90%.

commercially available thiosemicarbazide **5**, the terminal amino group was first converted into 1-acetylthiosemicarbazide (**6**) by reacting with acetyl chloride in the presence of pyridine. Refluxing of **6** in sodium hydroxide solution led to the formation of triazolethiol **7** in excellent yield. Thiol **7** was then allowed to react with α-bromoacetic acid in the presence of triethylamine to give rise to the corresponding sulfanylacetic acid **8**. Under the HATU coupling conditions, acid **8** reacted with various anilines and amines to provide the desired analogs (**4a**–**4af**).

The HATU[18] coupling was generally smooth with amines and unhindered anilines. But the yields are low for the anilines with ortho substitutions, which were presumably a result of their unfavorable steric interactions with activated ester during the coupling. To circumvent this problem, analogs containing ortho substituted aniline moieties could be synthesized using an alternative route shown in Scheme 2. In this case, the hindered anilines were allowed to react with α-bromoacetyl bromide (**9**) to form the amide bond first. Subsequent displacement of the bromide with thiol **7** furnished the corresponding analogs in good yields. The same chemistry was employed to prepare a number of secondary amide analogs (**11a**–**11d**).

These sulfanyltriazoles were then tested for their inhibitory activity in the above-mentioned HIV replication assay, and their IC_{50}'s are shown in Table 1. These results



Scheme 2. Alternative synthesis of sulfanyltriazole **4**. Reagents and conditions: (a) $ArNH_2$ (1.2 equiv), Et_3N (5.0 equiv), CH_2Cl_2, 0–25 °C, 2 h, 77–89%; (b) **7** (1.2 equiv), Et_3N (2.0 equiv), CH_2Cl_2, 25 °C, 5 h, 73–85%.

suggest that substitution on the carboxamide phenyl ring has significant impact on antiviral activity. First, an electron-withdrawing group is favored at the ortho position for good antiviral activity. Analogs belonging to this group include **4d** (2-F, 68 nM), **4e** (2-Cl, 3 nM), and **4j** (2-CF_3, 72 nM), **4l** (2-CN, 170 nM), and **4t** (2-NO_2, 1 nM). It appears that a small, hydrophobic group is also preferred at this position (**4b**, **4d**, **4e**, **4j** vs **4l**, **4q**), with the exception of **4t**, which contains a relatively hydrophilic group. Among these, the ortho chloro (**4e**) and ortho nitro (**4t**) analogs are the most potent with single digit nanomolar potency. Consistent with this trend, analogs such as **4g** (2-Br), having relatively larger groups at the 2 position, did not show any inhibitory activity (see Scheme 3).

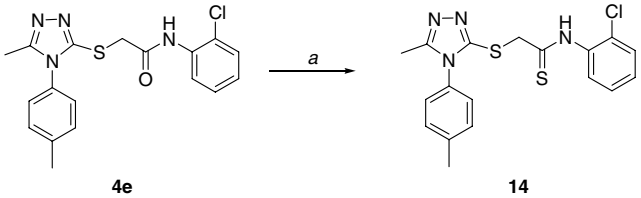
Analogs with single substitutions at the 3 position of the phenyl ring typically exhibited low or no inhibitory activity (**4h**, **4k**, **4m**, **4r**, and **4u**). The only exception is the methoxy analog **4o**, which exhibited an EC_{50} of 22 nM, suggesting that an electron-donating substituent might be favored at this position. Single substitutions at the 4 position of the phenyl ring had either minimum or negative impact on the inhibitory activity (**4c**, **4f**, **4i**, **4n**, **4p**, **4s**, and **4v**). Consistent with the observations for the single substitutions on the phenyl ring, the disubstituted analogs **4y** (2,4-Cl_2) and **4z** (2,5-Cl_2) exhibited EC_{50}s of 20 nM and 37 nM, respectively. The dimethyl analogs **4w** (2,3-Me_2) and **4x** (2-Me-4-Cl) also showed good inhibitory activity, and the 2-naphthalyl analog (**4aa**) also had a submicromolar EC_{50}. It appears that suitable substitutions on the phenyl ring could have a positive impact on the antiviral potency. The few heterocyclic (**4ab**–**4ad**), alkyl (**4ae** and **4af**), and tertiary amide (**11a**–**11d**) analogs were also examined, but none of them displayed any inhibitory activity, suggesting the importance of the phenyl moiety.

To explore the role of the other phenyl ring attached to the triazole moiety, analogs **12a**–**12g** were prepared using the same chemistry outlined in Scheme 1 (Fig. 2). Data clearly suggested that a phenyl ring (R in **12**) is favored over the methyl, ethyl, and benzyl replacements (**4e**, **12a**–**12d** vs **12e**–**12g**). Among all these

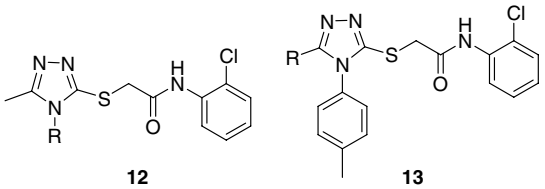
Table 1. Anti-HIV activities of sulfanyltriazoles[10]

Compound[a]	EC_{50} (μM)	IC_{50} (μM)
EFV	0.001	~10
NVP	0.050	>10
4a	2.053	>10
4b	0.112	>10
4c	>10	>10
4d	0.068	>10
4e	0.003	>10
4f	1.358	>10
4g	>10	>10
4h	>10	>10
4i	>10	>10
4j	0.072	>10
4k	2.869	>10
4l	0.170	>10
4m	>10	>10
4n	>10	>10
4o	0.022	>10
4p	1.837	>10
4q	0.614	>10
4r	>10	>10
4s	0.822	>10
4t	0.001	>10
4u	>10	>10
4v	>10	>10
4w	0.128	>10
4x	0.215	>10
4y	0.020	>10
4z	0.037	>10
4aa	0.412	>10
4ab	>10	>10
4ac	>10	>10
4ad	>10	>10
4ae	>10	>10
4af	>10	>10
11a	>10	>10
11b	>10	>10
11c	>10	>10
11d	>10	>10
12a	0.310	>10
12b	0.059	>10
12c	0.079	>10
12d	0.018	>10
12e	>10	>10
12f	>10	>10
12g	3.406	>10
13a	3.784	>10
13b	2.072	>10
13c	4.209	>10
13d	1.425	>10
13e	1.068	>10
14	>10	>10

[a] EFV, efavirenz; NVP, nevirapine.



Scheme 3. Synthesis of sulfanyltriazole **14**. Reagents and conditions: (a) Lawesson's reagent, toluene, 100 °C, 12 h, 70%.



12a: R = Ph
12b: R = Ph-2-OMe
12c: R = Ph-4-OMe
12d: R = Ph-2,3-$(OCH_2)_2$
12e: R = Me
12f: R = Et
12g: R = Bn

13a: R = Ph-4-Cl
13b: R = Ph-2-OMe
13c: R = 4-Py
13d: R = Bn
13e: R = CH_2OPh

Figure 2. Structures of sulfanyltriazoles **12** and **13**.

R groups examined, tolyl is the best for potency, and **4e** exhibited the highest anti-HIV activity. The 3-methyl group on the triazole was replaced with several larger substitutions (**13a**–**13e**) and none of them showed any antiviral activity, suggesting a relatively small hydrophobic pocket in the molecular target of these compounds in this region of the molecule (Fig. 3). The thioamide analog **14** exhibited no antiviral activity, demonstrating the importance of the carbonyl oxygen in the interaction with the target. It also suggests that the NH is not crucial to activity since a thioamide is a worse HB acceptor but a better HB donor than the corresponding amide.

A number of potent sulfanyltriazoles were selected to test against a few key NNRTI resistant mutants (Y181L, Y181C, K103N, and L100I), and the results are shown in Table 2. As the data demonstrated, the antiviral activities of all analogs are sensitive to these mutations in the NNRTI active site, which suggests

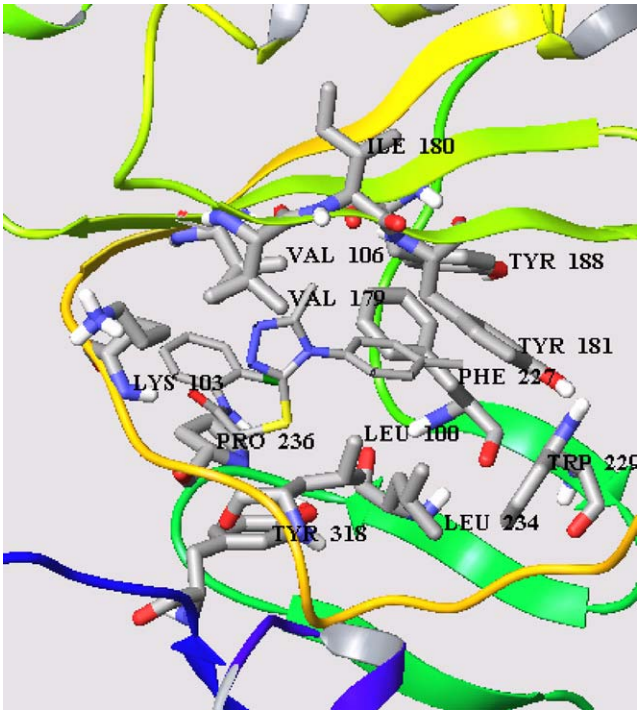


Figure 3. Docking of **4e** into the NNRT active site.[10]

Table 2. Antiviral activities of sulfanyltriazoles against HIV-1 NNRTI resistant mutants[11]

Compound	EC_{50} (μM) WT	EC_{50} (μM) Y188L	EC50 (μM) Y181C	EC_{50} (μM) K103N	EC_{50} (μM) L100I
EFV	0.001	0.313	0.001	0.019	0.013
NVP	0.050	>10	>10	5.053	0.164
4d	0.068	>10	4.028	3.273	1.376
4e	0.003	4.306	0.023	0.065	0.182[a]
4j	0.072	>10	1.377	1.511	0.488
4o	0.022	2.191	0.161	0.082	0.031
4t	0.001	3.595	0.016	0.006	0.024[a]
4y	0.020	>3.333	0.440	0.577	0.186
4z	0.037	>10	0.769	1.045	0.835
12b	0.059	>1.654	1.051	1.123	0.100
12c	0.079	>3.333	1.429	0.913	1.234
12d	0.018	>3.333	0.219	0.565	0.170

[a] Activity against K103N/L100I; for EFV: EC_{50} = 2526 nM.

that RT is the target of these sulfanyltriazoles. Several of these analogs (**4e**, **4o**, **4t**, **4y**, **12b**, and **12d**) exhibited good activity against L100I mutant with varying activities observed for the Y181C and K103N mutants. However, none of these analogs were effective in inhibiting the Y188L mutant virus. Among these analogs, **4e**, **4o**, **4t**, and **12d** exhibited good overall antiviral profile. In particular, the EC_{50}'s for **4o** (82 nM, ~4-fold shift from that of the WT virus) and **4t** (6 nM, 6-fold shift) against the K103N mutant are of interest considering that K103N is a key mutation associated with the current NNRTI resistance. Double mutants containing K103N are generally problematic for efavirenz. For example, the EC_{50} of efavirenz against K103/L100I is only 2.5 μM. On the other hand, sulfanyltriazoles **4e** and **4t** exhibited EC_{50}'s of 182 and 24 nM, respectively (Table 2), suggesting the potential of these sulfanyltriazoles to overcome the K103 related NNRTI resistant mutants.

Modeling studies were carried out to understand how **4e** interacts with the reverse transcriptase (Fig. 3). Our results suggest that the carbonyl oxygen forms a hydrogen bond with the K103 backbone NH, which is consistent with the good activity of these sulfanyltriazoles against the K103 mutants. The chlorophenyl moiety sits between P236 and V106, and points toward the solvent exposed region. The triazole moiety stays in the middle of the binding pocket, anchoring the three substituents on the ring into the optimal space for interactions with the enzyme. Finally the tolyl moiety fits into another important hydrophobic pocket, where many key resistant mutations take place, which include Y188L, Y181C, F227C, and L100I. Our studies suggest that this class of compounds shares the same pharmacophore with pyrrolidinone-[12] and benzophenone-based NNRTIs.[19]

In summary, a series of sulfanyltriazoles was discovered as novel NNRTIs, and their preliminary SAR has been established via chemical modifications. Molecular modeling studies were employed to understand the interactions between these inhibitors and the reverse transcriptase, and to guide the SAR studies. These studies led to the identification of inhibitors of single digit nM potency. Despite the fact that no optimization was directed toward the NNRTI resistant mutants, sulfanyltriazoles **4e**, **4o**, **4t**, and **12d** exhibited good activity against Y181C, K103N, and L100I, and promise great potential in overcoming these and other NNRTI resistant mutants. Future studies shall focus on optimizing the inhibitory activities against the NNRTI resistant mutants.

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