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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4444–4449

Tri-substituted triazoles as potent non-nucleoside inhibitors of the HIV-1 reverse transcriptase

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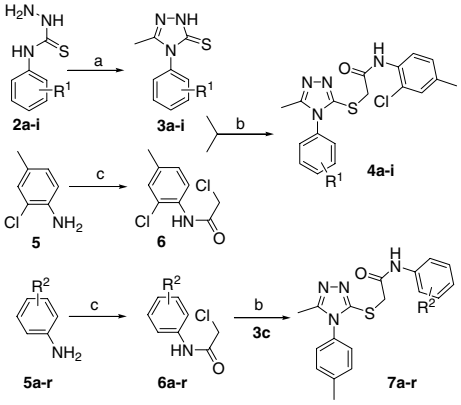
Received 5 May 2006; revised 9 June 2006; accepted 12 June 2006
Available online 27 June 2006

Abstract—A new series of 1,2,4-triazoles was synthesized and tested against several NNRTI-resistant HIV-1 isolates. Several of these compounds exhibited potent antiviral activities against efavirenz- and nevirapine-resistant viruses, containing K103N and/or Y181C mutations or Y188L mutation. Triazoles were first synthesized from commercially available substituted phenylthiosemicarbazides, then from isothiocyanates, and later by condensing the desired substituted anilines with thiosemicarbazones.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are key components of most current combination therapies used to fight HIV-1 infections.[1] Three molecules that belong to this class of compounds have been approved by the American Food and Drug Administration (FDA).[2] Nevirapine was approved in 1996 and continues to be used in various regimens. Efavirenz was approved in 1998 and is considered to be the current gold standard for NNRTIs. Delavirdine, approved in 1997, is not widely used, probably due to its lack of efficacy and its poor pharmacokinetic properties.[2] Because of the propensity of HIV to rapidly mutate, new agents with better activity profiles against mutant HIV-1 reverse transcriptases (RTs) are needed.[3]

We have discovered a new class of compounds that exhibit good antiviral efficacy against the wild type



Scheme 1. Reagents and conditions: (a) *N*,*N*-dimethylacetamide dimethylacetal, 100 °C; (b) K_2CO_3, DMF, rt; (c) chloroacetyl chloride, DIEA, CH_2Cl_2.

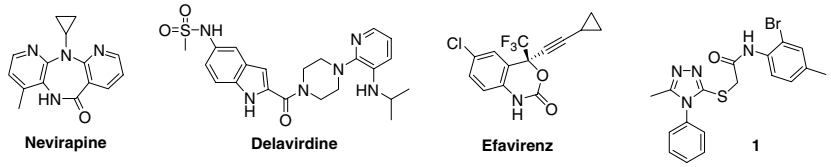


Figure 1. Currently approved NNRTIs nevirapine, delavirdine, and efavirenz, and new substituted triazole **1** from our high-throughput screening of 87,000 small molecules.

Keywords: Antiviral; HIV-1; NNRTI; Triazole; K103N; Y181C; Y188L.
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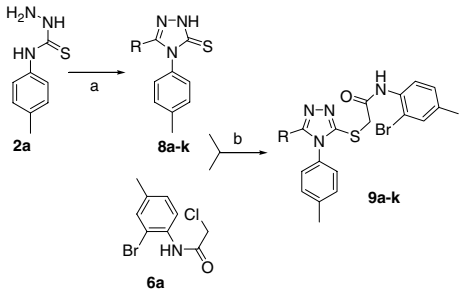
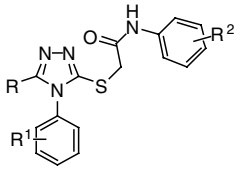
0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2006.06.048



Scheme 2. Reagents and conditions: (a) RCOOEt, EtOH, MeONa, reflux or CF_3COOH, reflux; (b) K_2CO_3, DMF, rt.

(WT) enzyme and against the double mutant K103N-Y181C that arises from the use of the three currently approved NNRTIs.[4,5] Compound **1** (Fig. 1) was discovered by screening a library of 87,000 compounds using a cell-based assay. This first compound showed moderate activity against viruses carrying WT or K103N-Y181C HIV-1 RT. Compound **1** was also active in an enzymatic assay against the purified WT HIV-1 RT.[6] A comprehensive literature search showed that SAR compounds could be accessed through a 3-step synthesis starting from various commercially available thiosemicarbazides.[7] Our syntheses first focused on several

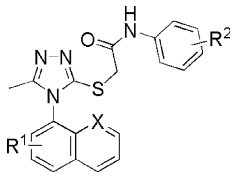
Table 1. Inhibition of HIV-1 (EC_{50}, μM)



Compound	R	R^1	R^2	WT	K103N-Y181C
1	Me	H	2-Br-4-Me	0.10	1.3
4a	Me	4-F	2-Cl-4-Me	2.3	> 10
4b	Me	4-CF_3	2-Cl-4-Me	0.019	0.23
4c	Me	4-Me	2-Cl-4-Me	0.0009	0.17
4d	Me	4-Et	2-Cl-4-Me	0.0002	0.06
4e	Me	3-Cl	2-Cl-4-Me	0.26	nd
4f	Me	3-CF_3	2-Cl-4-Me	> 0.6	nd
4g	Me	3-Me	2-Cl-4-Me	0.11	nd
4h	Me	2-Cl	2-Cl-4-Me	0.008	0.1
4i	Me	2,4-DiMe	2-Cl-4-Me	0.0001	0.06
7a	Me	4-Me	2-Br-4-Me	0.0004	0.1
7b	Me	4-Me	2-Cl	0.0007	0.15
7c	Me	4-Me	3-Cl	> 10	> 10
7d	Me	4-Me	4-Cl	0.40	> 10
7e	Me	4-Me	2-CF_3	0.007	> 10
7f	Me	4-Me	2-Ph	> 10	> 10
7g	Me	4-Me	2-SMe	0.0006	0.12
7h	Me	4-Me	2-Br	0.0004	0.076
7i	Me	4-Me	2-I	0.0005	0.22
7j	Me	4-Me	2,3-DiCl	0.0001	0.11
7k	Me	4-Me	2,4-DiCl	0.005	nd
7l	Me	4-Me	2,5-DiCl	0.10	nd
7m	Me	4-Me	2,3-DiMe	0.060	> 10
7n	Me	4-Me	2-Cl-4-COOMe	0.0004	0.12
7o	Me	4-Me	2-Cl-4-CN	0.003	0.9
7p	Me	4-Me	2-Br-4-*i*-Pr	0.14	> 10
7q	Me	4-Me	2-Br-4-OCF_3	0.058	> 10
7r	Me	4-Me	4-OPh	1.1	> 10
9a	H	4-Me	2-Br-4-Me	0.070	> 10
9b	Et	4-Me	2-Br-4-Me	0.0001	2.5
9c	*c*-Pr	4-Me	2-Br-4-Me	nd	> 10
9d	*i*-Pr	4-Me	2-Br-4-Me	nd	> 10
9e	OH	4-Me	2-Br-4-Me	nd	> 10
9f	CH_2OH	4-Me	2-Br-4-Me	nd	> 10
9g	CH_2NHMe	4-Me	2-Br-4-Me	nd	> 10
9h	CH_2SMe	4-Me	2-Br-4-Me	nd	> 10
9i	CH_2OMe	4-Me	2-Br-4-Me	nd	> 10
9j	CH_2NHMe	4-Me	2-Br-4-Me	nd	> 10
9k	CF_3	4-Me	2-Br-4-Me	0.0005	1.1
EFV				0.0004	0.011

Values are means of multiple experiments (nd, not determined); EFV, efavirenz.

Table 2. Inhibition of HIV-1 (EC_{50}, μM)



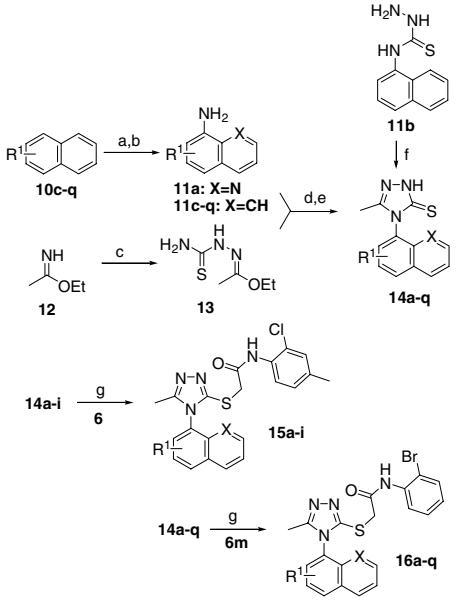
Compound	R^1	R^2	X	WT	K103N-Y181C	Y188L
1				0.10	1.3	> 10
15a	H	2-Cl-4-Me	N	0.008	nd	5.7
15b	H	2-Cl-4-Me	CH	0.004	0.054	0.59
15c	4-Me	2-Cl-4-Me	CH	0.001	0.016	0.10
15d	4-Cl	2-Cl-4-Me	CH	0.001	0.19	0.32
15e	4-OMe	2-Cl-4-Me	CH	0.001	nd	0.24
15f	2,4-DiMe	2-Cl-4-Me	CH	0.001	nd	0.090
15g	2-Me	2-Cl-4-Me	CH	0.001	0.060	0.20
15h	4-Et	2-Cl-4-Me	CH	0.001	0.032	0.11
15i	3,4-DiMe	2-Cl-4-Me	CH	0.001	nd	0.16
16a	H	2-Br	N	0.007	nd	4.2
16b	H	2-Br	CH	0.002	nd	0.72
16c	4-Me	2-Br	CH	0.0006	0.014	0.069
16d	4-Cl	2-Br	CH	0.0008	0.13	0.20
16e	4-OMe	2-Br	CH	0.0007	0.093	0.46
16f	2,4-DiMe	2-Br	CH	0.001	0.070	0.089
16g	2-Me	2-Br	CH	0.001	0.065	0.21
16h	4-Et	2-Br	CH	0.0005	0.022	0.058
16i	3,4-DiMe	2-Br	CH	0.0005	0.031	0.097
16j	4-$N(Me)_2$	2-Br	CH	0.0004	nd	0.24
16k	4,7-DiMe	2-Br	CH	0.0003	nd	0.012
16l	2,5-DiMe	2-Br	CH	0.0003	nd	0.33
16m	2,8-DiMe	2-Br	CH	0.001	nd	0.48
16n	4,6-DiMe	2-Br	CH	0.0005	nd	0.15
16o	4,6,7-TriMe	2-Br	CH	0.001	nd	0.11
16p	2,3,8-TriMe	2-Br	CH	0.003	nd	0.20
16q	2,3,5-TriMe	2-Br	CH	0.0008	nd	0.16
EFV				0.0004	0.011	0.073

Values are means of multiple experiments (nd, not determined); EFV, efavirenz.

substitutions at the 4-position of the triazole ring as well as various substituted anilines (Scheme 1).

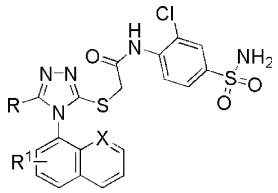
Ring cyclization of the desired thiosemicarbazides **2a–i** with dimethylacetamide dimethylacetal[8] led to the corresponding triazoles **3a–i** in good yields. Linkers **6a–r** were obtained by condensing anilines **5a–r** with chloroacetyl chloride in dichloromethane, with or without the presence of diisopropylethylamine. The two fragments were reacted together in dimethylformamide in the presence of potassium carbonate to give compounds **4a–i** and **7a–r** in excellent yield after precipitation (adding water to the reaction mixture induced precipitation of the pure product in most cases) or silica gel chromatography.

Various substitutions at the 5-position of the triazole were also explored through cyclization of a thiosemicarbazide with various esters or with trifluoroacetic acid[9] (Scheme 2). The resulting triazoles **8a–k** were condensed with the linkers **6a** following the same protocol as described earlier, to yield compounds **9a–k**. The antiviral activities of these series against wt and mutant HIV-1 were evaluated using a cell-based assay system employing VSV-G-



Scheme 3. Reagents and conditions: (a) HNO_3, 0 °C; (b) H_2, Pd–C or Raney-Ni, ethanol, rt; (c) thiosemicarbazide, DMF, rt; (d) DMF, reflux 3 h; (e) NaOH 1 N, 40 °C; (f) *N*,*N*-dimethylacetamide dimethylacetal, 100 °C; (g) K_2CO_3, DMF, rt.

Table 3. Inhibition of HIV-1 (EC_{50}, μM)



Compound	R	R^1	X	WT	Y188L
25a	Me	H	N	0.004	2.4
25b	Me	H	CH	0.0005	0.19
25c	Me	4-Me	CH	0.0002	0.020
25d	Me	4-Cl	CH	0.001	0.068
25e	Me	4-OMe	CH	0.0007	0.11
25f	Me	2,4-DiMe	CH	0.0001	0.004
25g	Me	4-$N(Me)_2$	CH	0.0005	0.088
25h	Me	4,7-DiMe	CH	0.0006	0.006
27a	Me	2-Me	N	0.001	0.39
27b	Me	2,5-DiMe	N	0.0006	0.015
27c	Me	2,5,7-TriMe	N	0.0007	0.011
27d	Me	6-OMe	N	0.0008	0.37
27e	Me	6-Br	N	0.0008	0.19
27f	Me	2-Me-6-Cl	N	0.002	0.10
30a	CF_3	H	CH	0.0005	0.049
30b	CF_3	4-Me	CH	0.0004	0.025
30c	CF_3	4-Cl	CH	0.004	0.041
30d	CF_3	4-OMe	CH	0.0004	0.014
30e	CF_3	4-$N(Me)_2$	CH	0.002	0.007
30f	CF_3	4,7-DiMe	CH	0.006	0.035
30g	CF_3	2,7-DiMe	CH	0.003	0.026
30h	CF_3	H	N	0.002	0.18
30i	CF_3	2-Me	N	0.0006	0.022
30j	CF_3	2,5,7-TriMe	N	0.001	0.004
EFV				0.0004	0.073

Values are means of multiple experiments; EFV, efavirenz.

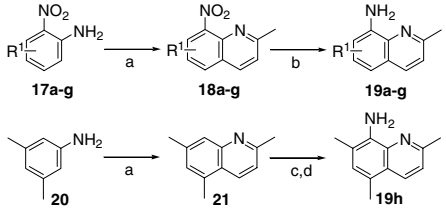
pseudotyped pNL4-3.Luc.R-E reporter virus and HeLa-JC53 cells[10] (Table 1). Compounds **4b–d** and **4h,i** showed a very strong inhibition of the wild type virus (sub-nanomolar range), along with an improved inhibition of the double mutant K103N-Y181C when compared to compound **1**. The presence of an ortho or para R^1 substituent improved activities against both viruses, with the biggest effect being observed when R^1 was in para position. We decided to select R^1 as a para methyl to study the SAR at R^2. Compounds **7a**, **b**, **g**, **j**, **k**, **m**, **r** showed the strongest HIV-1 inhibition, and shared the presence of an ortho halogen substitution on the aniline. A secondary substitution was accepted in most cases, as seen with compounds **7a**, **j**, **n**, **o**. Para substituents like methyl and methyl carboxylate showed similar good activity, while trifluoromethoxy and isopropyl exhibited a reduced activity. The range of substitutions allowed at the position 5 of our triazole proved to be narrow as seen in compounds **9a–k**; only methyl, ethyl, and trifluoromethyl showed good activity on the wild type virus, and methyl was the only substitution exhibiting sub-micromolar potency against K103N-Y181C mutant virus in this first series of compounds.

We later added another efavirenz-resistant mutant virus to our testing panel (Y188L), and we noticed that none of the compounds listed in Table 1 displayed any significant antiviral activity against this mutant ($EC_{50} > 2\mu M$); however, switching the *N*-4 substitution from a phenyl group to a naphthyl group, as shown with compound **15b**, gave us submicromolar potency against Y188L virus (Table 2). The need to explore more substituted aryls at the *N*-4 position of the triazole led us to design a more convergent and straightforward synthesis (Scheme 3). Instead of the classical scheme involving a thiosemicarbazide intermediate, we found that the thiosemicarbazone **13**[11] could be condensed and cyclized in two convenient steps in one pot with an aniline, an 8-aminoquinoline or a naphthylamine to give **14a–q**. Yields were only fair (50–60%), but the convenience of the reaction allowed us to synthesize many triazoles in a limited amount of time.

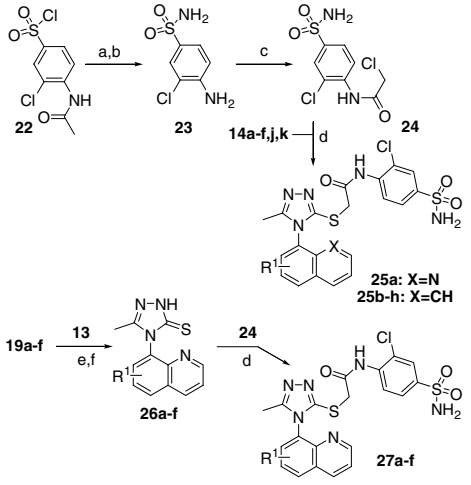
One of the hurdles in the synthesis of various substituted naphthalenes was the regioselectivity of the nitration. In some cases, we obtained two or more isomers that had to be separated and identified based on their NMR pattern. Reduction of the nitro compounds was accomplished using either Raney-nickel or palladium on carbon catalysts under 1–3 atm of hydrogen. A few substituted naphthylamines were commercially available and were condensed directly with the thiosemicarbazone **13**. The presence of two fused rings at *N*-4 dramatically

improved the antiviral activities against Y188L (Table 2). While *N*-4 phenyl-substituted triazole compounds were poorly active on Y188L mutants, most naphthalene compounds inhibited viral growth at submicromolar level, and compounds **16c**, **f**, **h**, **i**, **k** exhibited double-digit nanomolar activity on both mutant viruses. Since the Y188L mutant virus was more resistant to our series of compounds than the double mutant K103N-Y181C, we decided to test our newer compounds only against WT and Y188L viruses (see Table 3).

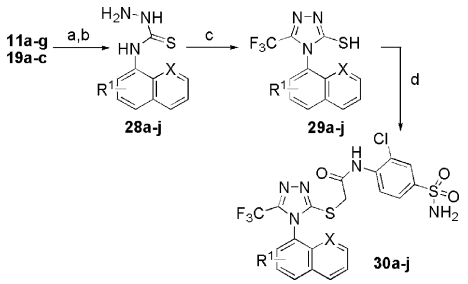
The trend observed with R^1 substituents in the previous set of compounds proved to be applicable to naphthalene substitutions, with the most active compounds having a para substitution. These results motivated us to expand our SAR to more bicyclic ring systems, and we turned toward the synthesis of various substituted quinolines (Scheme 4).[12] 2-Nitrosubstituted anilines were cyclized with acetaldehyde in the presence of hydrochloric acid to give nitroquinolines **18a–g**, which were subsequently reduced to give 8-aminoquinolines **19a–g**. Also, from 3,5-dimethylaniline **20**, we built the 2,5,7-trimethylquinoline **19h** following a slightly longer chemical route, involving a nitration step with potassium nitrite in sulfuric acid, followed by reduction with sodium dithionite. We also synthesized the linker **24** (Scheme 5) in 3 steps after noticing some similarities with another NNRTI scaffold published in a patent application.[13]



Scheme 4. Reagents and conditions: (a) H_2O, HCl, acetaldehyde, rt-70 °C; (b) H_2, Pd–C or Raney-Ni, ethanol, rt; (c) potassium nitrate, sulfuric acid, 0 °C to rt; (d) sodium dithionite, ethanol, H_2O, reflux.



Scheme 5. Reagents and conditions: (a) NH_3, THF; (b) HCl, EtOH/water; (c) chloroacetyl chloride, CH_2Cl_2; (d) K_2CO_3, DMF, rt; (e) DMF, reflux 3 h; (f) NaOH 1 N, 40 °C.



Scheme 6. Reagents and conditions: (a) $CSCl_2$; (b) hydrazine; (c) trifluoroacetic acid; (d) **24**, K_2CO_3, DMF, rt.

The linker was condensed with the previously obtained triazoles **14a–f,j,k** to yield compounds **25a–h**. This new linker brought several folds improvement in potency against the Y188L resistant mutant when compared to any other available R^2-substituted anilines. Quinolines **19a–f** were cyclized following a procedure described above to yield triazoles **26a–f**, and were condensed with linker **24** to give compounds **27a–f**. We also prepared a few 5-trifluoromethyl-substituted triazoles by condensing thiosemicarbazides **28a–j** with trifluoroacetic acid, followed by a ring cyclization, to yield compounds **30a–j** (Scheme 6).

Except for compounds **30b** and **30f**, the presence of the 5-trifluoromethyl replacing the 5-methyl-substitution on the triazole improved the antiviral activity against the Y188L mutant. Another observation was that when bearing the same R^1 substituents, naphthalene compounds were more active than the corresponding quinolines, as exemplified by the couples **25a–b**, **30a–h**, and to a lesser extent **25h–27b**. Compounds **25f,h**, and **30e**, **j** exhibited single-digit nanomolar activity against the Y188L mutant, with no cytotoxicity. This was a 10-fold improvement when compared to efavirenz tested side-by-side. More interestingly, in the case of compounds **30e** and **30j**, there was only a 4-fold loss of potency against Y188L as compared to WT. The loss of potency for efavirenz was about 180-fold. This improved activity profile against an efavirenz-resistant virus could prove extremely valuable, and warrants further studies on more efavirenz-resistant viruses.[14] A number of improved compounds from this triazole scaffold are currently being considered for clinical evaluation. The results of these studies will be presented at a later time.

Acknowledgments

Thanks to Robert Selliah and Haoyun An for some stimulating discussions, to our analytical group for numerous sample purifications and analyses, and to Heli Walker and Jae Hoon Shim for the construction of various mutant HIV-1 RTs.

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14. Results to be part of future publications.