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Tetrazole thioacetanilides: Potent non-nucleoside inhibitors of WT HIV reverse transcriptase and its K103N mutant

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Abstract—A series of aryltetrazolylacetanilides was synthesized and evaluated as HIV-1 non-nucleoside reverse transcriptase inhibitors on wild-type virus and on the clinically relevant K103N mutant strain. Extensive SAR investigation led to potent compounds, with nanomolar activity on K103N, and orally bioavailable in rats.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)[1] have found widespread use in HIV therapy in multidrug regimens and in the so-called highly active antiretroviral therapy (HAART), a combination of nucleoside HIV reverse transcriptase inhibitors (NRTIs), NNRTIs and protease inhibitors (PIs).[2] However, the main concern in the use of NNRTIs in the clinic still remains the rapid emergence of drug resistance, due to mutation of the HIV-RT enzyme.[3]

The most frequent HIV mutant strain observed in patients failing therapy with NNRTIs is K103N.[4] This mutation is observed in patients treated with each of the only three currently marketed NNRTI drugs: nevirapine, delavirdine and efavirenz,[4,5] thus creating cross-resistance and preventing therapeutic efficacy of any further treatment with available NNRTIs.

In this respect, the identification of novel NNRTIs, characterized by high potency on both the wild-type HIV-1 and the clinically relevant HIV-1 RT K103N mutant strain, is a constant goal for drug development.

From high-throughput screening (HTS) of our compound collection, two interesting compounds (**1**–**2**) were identified as potent low nanomolar inhibitors of HIV-1 RT polymerase, with submicromolar activity in a cell assay[6] and significant in vitro activity on the K103N mutant strain (Fig. 1).

The two lead compounds proved not to be orally bioavailable and were rapidly metabolized in rat plasma to the corresponding carboxylic acids.

This prompted us to undertake an extensive medicinal chemistry effort aimed at improving the pharmacokinet-

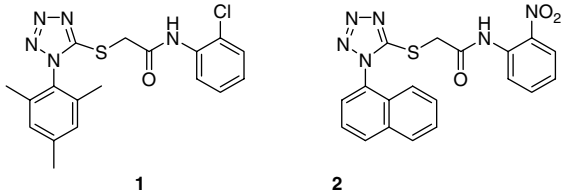

Figure 1. Lead tetrazole thioacetanilide inhibitors of HIV-RT, identified by HTS.

Keywords: HIV; Non-nucleoside reverse transcriptase inhibitors; Tetrazole thioacetanilides.
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ic properties together with the intrinsic activity on the K103N mutant.

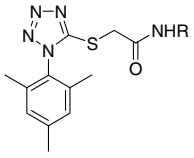
A SAR study at the anilide moiety of over 300 compounds (a selection displayed in Table 1) revealed that 2-substitution is the only allowed monosubstitution, nitro and halogen being the substituents of choice (compounds **1**–**5**). Monosubstitutions at the 3- or 4-positions are generally not well tolerated (compounds **6**–**7**), and 2,6-disubstitution completely abolishes activity (compound **8**). The preferred aromatic disubstitution pattern is 2,4 (compounds **9**–**11**), and, in this latter case, the preferred 2-substituents are halogens, as already seen for 2-monosubstitution, and also methyl (as in compound **11**). Introduction of a 2-methyl gained significant activity from inactive 4-substituted compounds (compare **11** with **7**), though to a lesser extent compared to chlorine (compare **10** with **7**).

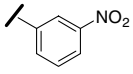
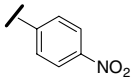
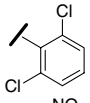
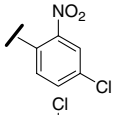
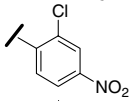
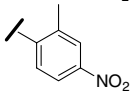
This observation proved to be extremely important when we discovered that anilides substituted in the 4-position with sulfonamide (Table 2, compound **12**), carboxamide, or aliphatic chains bearing a tertiary amine, were stable in rat plasma.

By introducing chlorine or methyl in the 2-position, we were able to obtain stability in rat plasma, conferred by the 4-substituent, and very high intrinsic potency on both the WT and K103N mutant enzymes and in spread cell based assays, conferred by the 2-substituent.

A simultaneous SAR study on the aryl linked to the tetrazole core (Table 3) provided a further opportunity to identify potent compounds. The pattern of aromatic substitution is more tolerant compared to the anilide, accepting most of the disubstitutions, except 3,4-disubstitution (compound **22**) and the preferred substituents are again halogens and methyl.

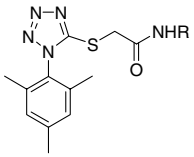
Table 1. SAR at anilide moiety

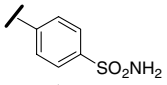
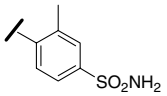
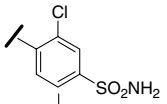
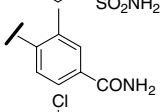
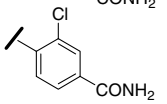
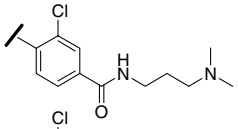
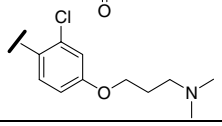


Compound	R	WT RT Pol IC_{50}[a] (nM)	K103N shift[b]	CIC_{95}[c] (nM) 10% FBS/50% NHS
3		3	3	11/90
4		7	5	100/810
5		31	3	312/1250
6		200	nd[d]	na[d]
7		na[d]	nd[d]	na[d]
8		na[d]	nd[d]	na[d]
9		4	3	31/400
10		22	5	78/1250
11		165	3	1250/na[d]

[a] IC_{50} values are means of at least two independent experiments.
[b] Ratio IC_{50} K103N RT Pol mutant/IC_{50} WT RT Pol.
[c] The two values of CIC_{95} refer to cell based assays in the presence of 10% FBS (fetal bovine serum) and 50% NHS (normal human serum). See Ref. 6.
[d] na: not active (>5 μM), nd: not determined.

Table 2. Anilide substitutions that confer stability in plasma



Compound	R	WT RT Pol IC_{50}[a] (nM)	K103N shift[b]	CIC_{95}[c] (nM) 10% FBS/50% NHS
12		1252	nd[d]	1250/na[d]
13		54	1	50/580
14		4	3	16/62
15		55	1	31/62
16		3	2	8/16
17		5	2	8/16
18		13	5	62/62

[a] IC_{50} values are means of at least two independent experiments.
[b] Ratio IC_{50} K103N RT Pol mutant/IC_{50} WT RT Pol.
[c] The two values of CIC_{95} refer to cell based assays in the presence of 10% FBS (fetal bovine serum) and 50% NHS (normal human serum). See Ref. 6.
[d] na: not active (>5 μM), nd: not determined.

More interestingly, the aryl SAR study gave compounds with low or no shift (K103N shift = 1) on the K103N mutant, which could be identified by appropriate substitutions on the aryl rings.

With the above SAR studies in hand, we envisaged the possibility to optimize the activity on both the wild-type RT enzyme and the K103N mutant strain by combining some of the most active anilides with some of the best aryls. The most relevant results are reported in Table 4.

We were delighted to see that by suitable combinations of the anilides and aryls it is possible to obtain very potent compounds on the WT RT, retaining the same low nanomolar enzymatic activity on the relevant mutant K103N.

The possibility to combine the best aryls with the anilides that confer the highest activity, but also the required metabolic stability, allowed also the optimization of the pharmacokinetic properties of the series (Table 5).

The 2-nitrophenylanilide **3** had the same pharmacokinetic profile as the lead compound **1**, characterized by total absence of oral exposure and extremely high values of plasma clearance, the latter being due, at least in part, to the already discussed hydrolytic metabolism.

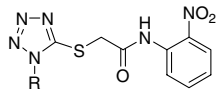
The substitution of the labile 2-nitroanilide with more stable anilides, such as in **13**–**14**, not only significantly decreased the plasma clearance, but produced orally bioavailable compounds.

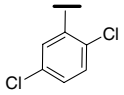
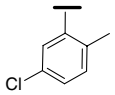
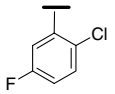
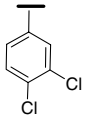
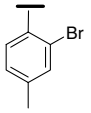
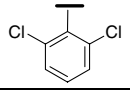
One of the best compounds tested was **29**, which exhibited acceptable oral bioavailability and plasma clearance.

In summary, an extensive SAR demonstrated that aryltetrazolyl thioacetanilides are potent inhibitors of HIV-1 RT, with low nanomolar intrinsic activity on the enzyme and submicromolar antiviral activity in HIV infected cells. With a suitable combination of substitution patterns both on the aryl linked to the tetrazole core and the anilide aryl, it is possible to identify compounds which maintain the same intrinsic activity on the wild-type HIV-1 enzyme and the clinically relevant K103N mutant.

The tetrazole derivatives were synthesized as outlined in Scheme 1, starting from the commercially available

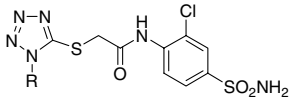
Table 3. SAR at aryl moiety

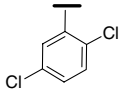


Compound	R	WT RT Pol IC_{50}[a] (nM)	K103N shift[b]	CIC_{95}[c] (nM) 10% FBS/50% NHS
19		4	1	31/200
20		23	1	78/1250
21		10	1	63/250
22		230	2	na[d]
23		3	3	8/62
24		12	1	78/125
25		18	1	63/250
26		3	3	8/31
27		4	1	15/62

[a] IC_{50} values are means of at least two independent experiments.
[b] Ratio IC_{50} K103N RT Pol mutant/IC_{50} WT RT Pol.
[c] The two values of CIC_{95} refer to cell based assays in the presence of 10% FBS (fetal bovine serum) and 50% NHS (normal human serum). See Ref. 6.
[d] na: not active (>5 μM).

Table 4. Effect of combination of aryl and anilide SAR on potency and resilience on K103 mutant



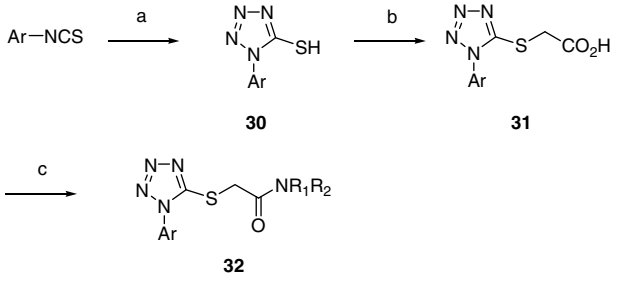
Compound	R	WT RT Pol IC_{50}[a] (nM)	K103N shift[b]	CIC_{95}[c] (nM) 10% FBS/50% NHS
28		9	1	62/250
29		3	1	16/125

[a] IC_{50} values are means of at least two independent experiments.
[b] Ratio IC_{50} K103N RT Pol mutant/IC_{50} WT RT Pol.
[c] The two values of CIC_{95} refer to cell based assays in the presence of 10% FBS (fetal bovine serum) and 50% NHS (normal human serum). See Ref. 6.

Table 5. Rat PK profiles[a]

Compound	*F* (%)	Clp (ml/min/kg)	$t_{1/2}$
3	0	272	
14	33	125	0.23
13	60	85	0.83
29	33	37	1.1

[a] Oral and iv dosing at 3 mg/kg. PO formulation: suspension in PEG400. iv formulation: solution in DMSO/PEG400/water 20:60:20.



Scheme 1. Reagents and conditions: (a) NaN_3, H_2O, reflux (70–95%); (b) chloroacetic acid, H_2O, 2 N NaOH, reflux (80–95%); (c) PCl_5, aniline, toluene/$CHCl_3$, rt 1–2 h then 70 °C 1–3 h. Purification by RP-HPLC.

isothiocyanates. Reaction with NaN_3 produced the corresponding tetrazole-thiols **30**, which were alkylated with chloroacetic acid in aqueous conditions. The final anilides were accessed by reaction of the thioacetic acids **31** with the required anilines in the presence of 1.2 equiv of PCl_5 by a slightly modified literature procedure.[7] All the final compounds have been purified by RP-HPLC.

Acknowledgments

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References and notes

1. De Clerck, E. *Farmaco* **1999**, *54*, 26 (rev).
2. Mocroft, A.; Vella, S.; Benfield, T. L.; Chiesi, A.; Miller, V.; Gargalianos, P.; d'Arminio-Monforte, A.; Yust, I.; Bruun, J. N.; Phillips, A. N.; Lundgren, J. D. *Lancet* **1998**, *352*, 1725.
3. Sorbera, L. A.; del Fresno, M.; Leeson, P. A.; Silvestre, J.; Rabasseda, X. *Drugs Future* **2002**, *27*, 331.
4. Bacheler, L. T. *Drug Resist. Updates* **1999**, *2*, 56.
5. (a) De Clerck, E. *Adv. Antiviral Drug Design* **2004**, *4*, 1 (rev); (b) Hsiou, Y.; Ding, J.; Das, K.; Clarks, A. D., Jr.; Boyer, P. L.; Lewi, P.; Janssen, P. A. J.; Kleim, J.-P.; Rősner, M.; Hughes, S. H.; Arnold, E. *J. Mol. Biol.* **2001**, *309*, 437.
6. Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V. V.; Schlabach, A. J.; Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lin, J.; Chen, I.; Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. A.; Huff, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096.
7. Rossi, S.; Pirola, O.; Selva, F. *Tetrahedron* **1968**, *24*, 6395.