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Thiotetrazole alkynylacetanilides as potent and bioavailable non-nucleoside inhibitors of the HIV-1 wild type and K103N/Y181C double mutant reverse transcriptases

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Abstract—A series of aryl thiotetrazolylacetanilides were synthesized and found to be potent inhibitors of the HIV-1 wild type and K103N/Y181C double mutant reverse transcriptases. The incorporation of an alkynyl fragment on the aniline provided inhibitors with excellent cellular activity and extensive SAR led to the identification of one inhibitor having good oral bioavailability in rats.

In 2006, 4.3 million people became newly infected with HIV, bringing the total number of people living with AIDS throughout the world to an unprecedented level of 39.5 million.[1] Although the introduction of highly active antiretroviral therapy (HAART) in 1996 led to a drastic reduction of AIDS related mortality, 2.9 million deaths were nonetheless recorded in the past year as a consequence of this disease. Despite the recent availability of simplified regimens, many patients still undergo treatment changes due to complex dosing schedules, metabolic complications or overwhelming side effects.[2] Moreover, treatment failure following appearance of drug resistance,[3] transmission of resistant viruses,[4] and superinfection[5] are serious concerns and new therapeutic drugs are therefore urgently needed.[6]

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are important components of the first line HAART regimens. However, selection of resistance mutations rapidly occur and cross resistance within the class is then generally observed.[7] Thus, the next generation NNRTI must be potent against mutant strains that confer resistance to current NNRTIs. Moreover, excellent pharmacokinetics should be targeted in order to achieve a once a day dosing.[8]

With these goals in mind, we initiated a high throughput screening (HTS) campaign aimed at identifying a potent and structurally novel NNRTI. Using an enzymatic assay,[9] we screened for inhibition against the clinically relevant K103N/Y181C HIV-1 double mutant reverse transcriptase (RT) since this mutation leads to broad resistance against all the available NNRTIs.[10] Following this exercise, we discovered that compound **1** is a potent inhibitor of the HIV-1 K103N/Y181C RT (Fig. 1). We subsequently found that compound **1** is also a low nanomolar inhibitor of the wild type (WT) RT.[11]

We recently reported the SAR study on the aryl tetrazole and the anilide moiety and demonstrated that the nitro group can be replaced by a chlorine atom with approximately twofold loss in activity against the double mutant.[12] Additionally, optimization of the aryl tetrazole led to the introduction of the 2-chloro-4-*tert*-butyl scaffold shown in compound **2**. This potent inhibitor, which has an IC_{50} value below 100 nM against

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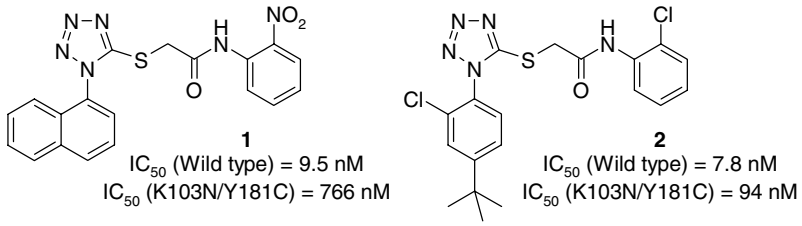


Figure 1. Hit and lead compounds identified following high throughput screening and hit to lead campaigns.

the double mutant RT, was used as a starting point for our NNRTI lead optimization program.

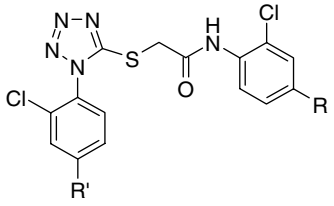
In the tetrazolyl series, we[12,13] and others[11] have demonstrated that the introduction of substituents at the *para* position of the anilide leads to substantial improvement in potency. Interestingly, this beneficial effect has also been observed in a closely related triazolyl series.[14] Being well aware of this fact and having secured the left and the right sides of the inhibitor, we embarked on finding a novel appendage that would allow modulation of the overall physico-chemical properties of the molecule while retaining excellent potency against the double mutant RT.

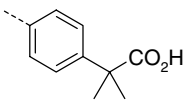
As shown in Table 1, the introduction of a carboxylic acid, a sulfonamide or a methylsulfone at the *para* position of the anilide gave inhibitors with low nanomolar potencies against the wild type and the K103N/Y181C double mutant RT (compounds **3**, **5**, and **7**). Although the presence of the bulky *tert*-butyl group ensured good potency, this fragment was found to be metabolically unstable and was therefore replaced by a cyclopropyl group.[15] While the impact of this change on the IC_{50} values is considerable in the case of the carboxylic acid **4**, the effect is insignificant in the case of the sulfonamide **6**, validating this modification.

The introduction of a phenyl acetic acid moiety (compound **8**) gave an acceptable IC_{50} value for the wild type and the double mutant RT. However, since it presents the disadvantage of increasing the molecular weight of the inhibitor, we hypothesized that this extraneous phenyl group could be replaced by a simpler linear fragment such as a saturated carbon chain. In the event, application of this hypothesis led to poor activity against the double mutant, as indicated by compound **9**. However, the assumption proved to be successful when an alkynyl moiety was introduced (compound **10**). Moreover, a threefold improvement in potency was observed by replacing the propiolic acid fragment by a homopropargylic alcohol group (compound **11**). Gratifyingly, no erosion of potency was observed when going to the cyclopropyl analogue **12**. In addition, the presence of the alcohol at the terminal position should allow the introduction of diverse functional groups.

A molecular model of compound **12** with the HIV-1 WT RT was generated in the allosteric site of the enzyme (Fig. 2). As illustrated, the tetrazole is orthogonal to the cyclopropyl-chlorophenyl, whereas the amide is in a *trans* conformation, bringing the two chlorine atoms of both aryl groups in close proximity. The carbonyl of the inhibitor is involved in a H-bond interaction with

Table 1. SAR on the right hand side anilide



Compound	R′	R	IC_{50}[a] (nM)[9]	
			WT	K103N/Y181C
3	*t*-Bu	CO_2H	5.0	66
4	*c*-Pr	CO_2H	16	151
5	*t*-Bu	SO_2NH_2	5.8	36
6	*c*-Pr	SO_2NH_2	5.0	45
7	*t*-Bu	SO_2Me	3.1	26
8	*t*-Bu		10	33
9	*t*-Bu	$CH_2CH_2CO_2H$	10	150
10	*t*-Bu	$C{\equiv}CCO_2H$	2.9	38
11	*t*-Bu	$C{\equiv}CCH_2CH_2OH$	5.5	12
12	*c*-Pr	$C{\equiv}CCH_2CH_2OH$	2.0	8.9

[a] IC_{50} values are means of at least two independent experiments.

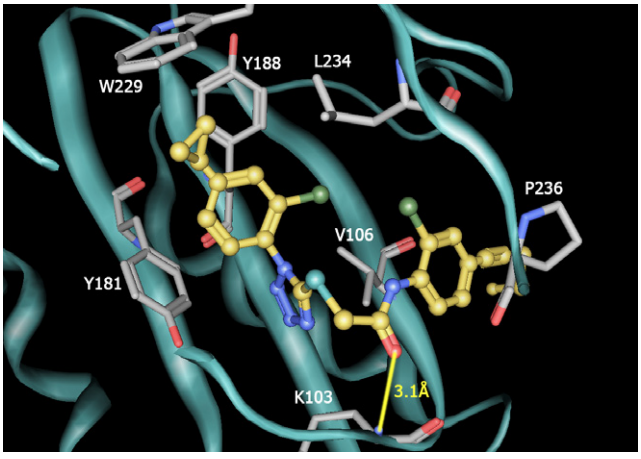


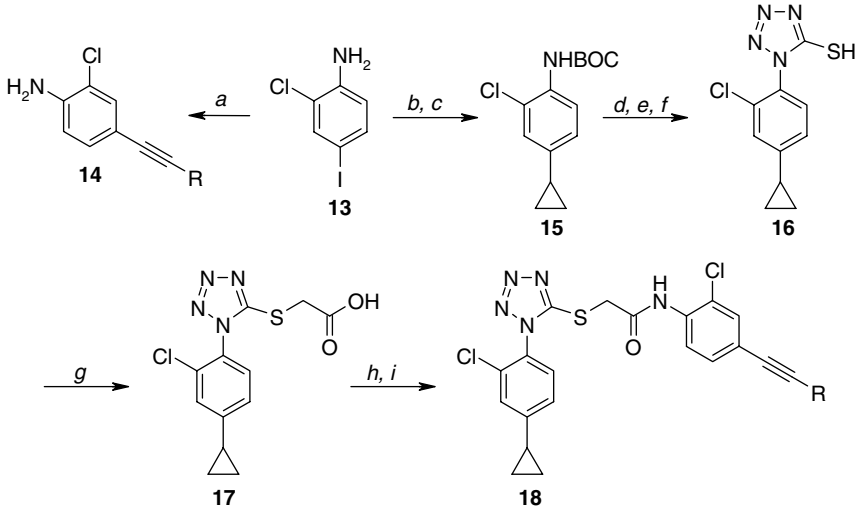
Figure 2. Molecular model of **12** in the allosteric site of HIV-1 WT RT.

the backbone NH of K103 and the left hand side aryl group binds in a hydrophobic pocket formed by residues W229, L234, Y188, and Y181. Detailed analysis shows that the cyclopropyl group sits over W229, while the left hand side phenyl is parallel to Y188. Interestingly, the alkynyl group extends between P236 and V106, and exposes its terminus to the solvent. Consequently, this feature should be useful in the modulation of the physico-chemical properties of the inhibitors.

The synthetic route that we designed to access the alkynyl inhibitors is expeditious and tolerates chemical diversity (Scheme 1). As illustrated, 2-chloro-4-iodoaniline **13** was submitted to a standard Sonogashira coupling reaction leading to alkynylanilines **14**. The same starting aniline **13** was protected as a *tert*-butyl carbamate employing conditions developed for electronically deactivated anilines.[16] The installation of the cyclopropyl unit was performed under Negishi conditions giving the BOC-protected 2-chloro-4-cyclopropyl aniline **15**. The amine was then liberated under acidic conditions and subsequently transformed into the corresponding arylthiotetrazole **16** in a two-step sequence. Alkylation with bromo acetic acid provided compound **17** which was activated as the corresponding acid chloride and coupled with anilines **14** to afford the desired inhibitors **18**.

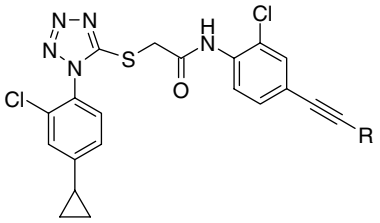
An extensive SAR study was then performed on the terminal position of the alkyne. As shown in Table 2, the enzymatic potency is given exclusively for the key double mutant RT since all of the prepared inhibitors showed IC_{50} values below 10 nM against the WT RT. The cellular activity was measured for both the WT and the K103N/Y181C double mutant RT viruses and the half-lives in rat liver microsomes (RLM) were also measured in order to select compounds for PK evaluation. As illustrated, the introduction of a terminal alkyne (compound **19**) led to mediocre potency against the double mutant, showing the necessity of having a substituted alkynyl group. Unfortunately, the propargylic alcohol **20** and the acid **21** suffered either from poor metabolic stability or unacceptable cellular activity. To our great pleasure, blocking the propargylic position with methyl groups led to enhanced stability and potency, as illustrated by compound **22**. Additionally, this scaffold was utilized as a handle for the introduction of basic and acidic groups, leading to potent inhibitors **23** and **24**, respectively. The dimethyl propargylic amine **25** gave equivalent potency as the alcohol **22** against the double mutant RT, but poorer half-life in rat liver microsomes. Fortunately, good metabolic stability was obtained by capping the amine with an acetic acid group (compound **26**) or an amino acetate (compound **27**). The homopropargyl alcohol **28** and the acid **29** gave very low half-lives in RLM. Happily, further elaboration leading to the aminoacetic acid **30** and the dimethyl propionic acid **31**, gave satisfactory potency and metabolic stability. Hoping to further improve on the activity of compound **31**, we transformed the acid into the corresponding acyl sulfonamide **32**, leading to a twofold improvement of the EC_{50} value for the double mutant RT. However, attempts to improve the metabolic stability of **31** by replacing the methyl groups with a cyclopropane (compound **33**) led to a reduction in the half-life in RLM. Furthermore, homologation of the acid (compound **34**) led to a minor improvement in cellular activity accompanied by a drastic reduction in metabolic stability.

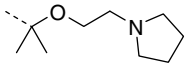
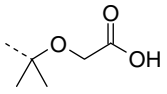
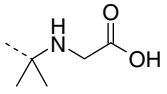
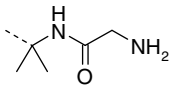
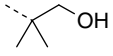
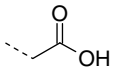
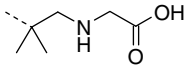
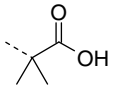
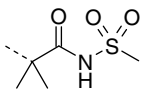
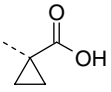
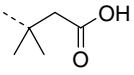
Compounds having good cellular activity against the WT and the double mutant RT in conjunction with adequate half-life in RLM were selected for pharmacokinetic evaluation in rats (Table 3). Based on these criteria, compounds **27**, **30**, **31**, and **32** were chosen for PK profiling. Unfortunately, low exposure was achieved following oral dosing with compounds **27**, **30**, and **32** at 5 mg/kg. However, to our delight, a C_{max} of 5.0 μM and



Scheme 1. Synthetic route to compound **18**. Reagents and conditions (a) R-C≡C-H (1.0 eq), CuI (0.1 eq), $Pd(PPh_3)_4$ (0.1 eq), Et_2NH (3 eq), THF, 70 °C, o.n., 75–90%; (b) NaHMDS (2.1 eq), BOC_2O (0.9 eq), THF, rt, o.n., 84%; (c) *c*-PrBr (3.0 eq), *n*-BuLi 2.5 M in hexanes (3.1 eq), $ZnBr_2$ (3.6 eq), $Pd(PPh_3)_4$ (0.1 eq), THF, reflux, 2 h, 72%; (d) HCl 4.0M in dioxane, rt, 5 h, 90%; (e) thiophosgene (1.1 eq), Et_3N (3.3 eq), *i*-PrOH, rt, 4 h; (f) NaN_3 (2.0 eq), acetonitrile, reflux, 48 h, 60% (2 steps); (g) $BrCH_2CO_2H$ (1.1 eq), Et_3N (2.5 eq), EtOH, rt, 2 h, 73%; (h) ClCOCOCl (1.1 eq), DMF (cat.), CH_2Cl_2, rt, 1.5 h; (i) aniline **14** (1.1 eq), pyridine (2.0 eq), CH_2Cl_2, rt, 4 h, 55–75% (2 steps).

Table 2. SAR on the alkynyl moiety



Compound	R	IC_{50}[a] (nM) K103N/ Y181C	EC_{50}[a] (nM)[17] WT	EC_{50}[a] (nM)[17] K103N/ Y181C	$t_{1/2}$[a] (min) RLM[18]
19	H	44	82	505	4
20	CH_2OH	20	5.4	31	7
21	CO_2H	47	66	355	20
22		17	2.4	17	84
23		80	3.4	22	15
24		23	6.2	29	29
25		38	2.7	25	31
26		30	1.9	29	70
27		7.9	1.1	8.1	109
28		17	8.2	51	9
29		32	11	139	19
30		23	1.6	13	79
31		28	7.1	58	90
32		19	4.2	27	82
33		43	12	54	30
34		26	5.8	36	21

[a] IC_{50}, EC_{50}, and $t_{1/2}$ values are means of at least two independent experiments.

Table 3. Rat PK profiles of best inhibitors[a]

Compound	iv (2 mg/kg) CL (mL/min kg)	iv (2 mg/kg) $t_{1/2}$ (h)	Oral (5 mg/kg) C_{max} (μM)	Oral (5 mg/kg) AUC (μM h)	Oral (5 mg/kg) *F* (%)
27	25.6	1.6	0.28	1.0	21
30	41.9	1.5	0.26	0.84	29
31	10.8	3.5	5.0	9.5	66
32[b]	17.5	0.72	0.63	1.7	22

[a] iv formulation: 70% PEG-400 (30% water). PO formulation: 0.5% methocel and 0.3% Tween 80.
[b] Sodium salt.

an AUC of 9.5 μM h were observed for inhibitor **31**. Following intravenous administration at 2 mg/kg, an acceptable clearance and half-life were obtained and an oral bioavailability of 66% was calculated for this inhibitor.

In summary, we have demonstrated that the structurally novel thiotetrazolyl acetanilides are potent non-nucleoside inhibitors of the HIV-1 wild type and K103N/Y181C double mutant reverse transcriptases.[19] Introduction of an alkynyl moiety at the *para* position of the anilide led to further improvement in potency and allowed the modulation of physico-chemical properties of the inhibitors. Extensive SAR studies led to the identification of potent and metabolically stable compounds. Following this exercise, rat PK evaluation of selected inhibitors was conducted and compound **31** was found to have the best PK profile.

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