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Scaffold hopping in the rational design of novel HIV-1 non-nucleoside reverse transcriptase inhibitors

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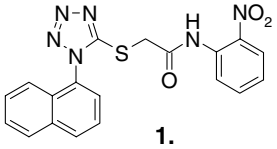
Abstract—High-throughput screening hit **1** was identified as a potent, broad-spectrum, non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1 replication. Analysis of the bound conformation of analogs of this inhibitor via molecular modeling and NMR contributed to the design of novel tertiary amide, carbamate, and thiocarbamate based NNRTIs.

In the more than 20 years since AIDS was discovered it has evolved into an unprecedented threat to global health. Worldwide, the pandemic has infected 70 million people, 25 million of whom have already succumbed to the disease. Additionally, there were 4.3 million new infections and 2.9 million deaths in 2006 alone.[1]

In industrialized nations, an aggressive educational program coupled with increasingly effective pharmaceutical intervention has diminished the number of new infections and increased the life span of infected individuals substantially. However, treatment failure as a result of in vivo selection of multi-drug resistant mutants remains a serious issue which must be continually addressed.

As part of our effort to develop improved NNRTIs, we were interested in evaluating a structurally distinct class of inhibitors with good anti-viral activity versus clinically relevant mutants and with DMPK properties consistent with once per day oral administration. To this end, a high-throughput screening campaign targeting the prevalent NNRTI resistant double mutant K103N/Y181C was initiated. The screen against this enzyme identified thio-tetrazole derivative **1** as a lead structure. We and others[2a–i] have revealed that these compounds and closely related triazole derivatives are extremely potent, broad-spectrum inhibitors of wild-type (WT) and mutant HIV-1 reverse transcriptases. These compounds however suffer from poor stability in the presence of human liver microsomes (HLM) which is predictive of rapid clearance in humans. In order to facilitate patient compliance, a demanding pharmacokinetic profile is absolutely required in a contemporary NNRTI; we were therefore challenged to address the metabolic weaknesses associated with these compounds.

Organic sulfur compounds are known to be prone to metabolic oxidation so we immediately considered the thio-ether linkage as a potential metabolic liability. However, SAR rapidly revealed the importance of the sulfur atom in this lead series. Simply substituting the sulfur with either oxygen (**2**), or carbon (**3**), led to 10- and 27-fold decreases in intrinsic wild-type potency, respectively (Table 1). It was interesting to note that no improvements in HLM stability were observed by these modifications. We therefore decided to continue the exploration of the thio-tetrazole series in order to



1.

Figure 1. Hit identified in high-throughput screening.

Keywords: NNRTI; Scaffold hopping; Tertiary amide; Tertiary carbamate; Thio-tetrazole; Tetrazole thioacetanilide.
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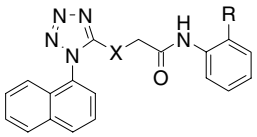
Table 1. SAR of naphthyl tetrazole linkers



Compounds	X	R	IC_{50} (nM)		$t_{1/2}$ HLM (min)
			WT	K103N/Y181C	
1	S	NO_2	<20	849	3
2	O	NO_2	200	11750	3
3	CH_2	NO_2	542	>10,000	7
4	S	Cl	20	1838	6

ascertain any structure–activity relationships while at the same time monitoring the effects of different substitutions on the metabolic stability. This exercise provided significant improvements in potency, particularly versus the enzyme containing the prevalent double mutant K103N/Y181C. It was very quickly discovered that the undesirable 2-nitro anilide could be replaced with a 2-Cl anilide as in **4** (Table 1) with only a 2-fold decrease in potency versus the double mutant. It was also revealed that the tetrazole *N*-naphthyl could be replaced by a 2-Cl phenyl derivative as in **5** (Table 2) resulting in only a 2-fold loss in WT activity compared to **1**. Furthermore, it was discovered that the potency could be significantly enhanced by the introduction of a substituent at the 4-position of this aromatic ring, *para* to the tetrazole.

The 4-methyl substituted analog **6** provided a 2-fold improvement in potency versus K103N/Y181C RT relative to **5**. Upon increasing the steric bulk of this substituent to cyclopropyl (**7**) or to *tert*-butyl (**8**), a further 8-fold gain versus the double mutant was realized and compounds having IC_{50} values less than 100 nM versus this enzyme were obtained. Interestingly, these improvements were not observed versus the WT enzyme. In fact, compounds **6**, **7**, and **8** were essentially equipotent versus WT.

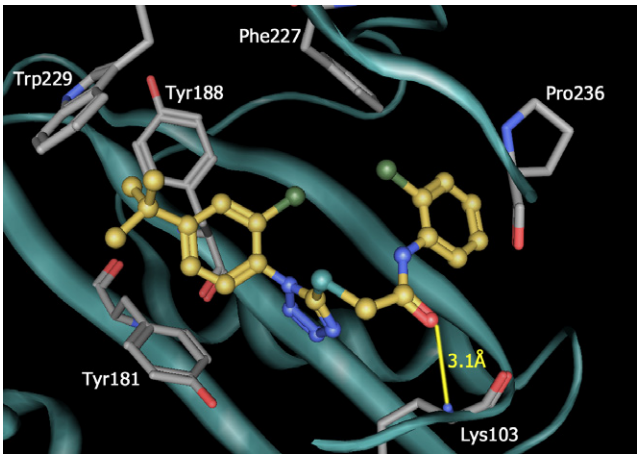
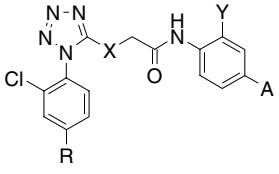


Figure 2. Model of **8** in the NNRTI binding pocket.

On the right-hand side of the molecule, further enhancements in potency could be attained by substituting the 2-Cl anilide at the 4-position. A wide array of functional groups were tolerated at this position, with sulfone (**9**), sulfonamide (**10**), and biphenyl derivatives **11** and **12** being optimal (Table 2). Replacement of the thio-ether by an all carbon linker in these more potent analogs provided **13** which was significantly less potent, losing 16-fold versus wild-type RT and nearly 80-fold versus K103N/Y181C. Some of the structural changes described above provided substantial gains in potency and moderate improvements in the HLM stability (i.e., compound **9**) however, these compounds still did not meet our target profile Figure 1.

Molecular modeling provided a complex structure of compound **8** bound in the WT NNRTI binding pocket (Fig. 2). Notable features include: (1) a key H-bond between the inhibitor's amide carbonyl and the backbone N–H of K103; (2) an interesting CH–π interaction in which the 4-substituent of the phenyl-tetrazole points directly at, and perpendicular to, the indole side chain of Trp229. Trp 229 is a highly conserved residue among all

Table 2. SAR of thio-tetrazole series



Compound	X	R	Y	A	IC_{50} (nM)		$t_{1/2}$ HLM (min)
					WT	K103N/Y181C	
5	S	H	NO_2	H	39	1995	ND
6	S	Me	Cl	H	9	809	2
7	S	*c*-Pr	Cl	H	13	103	ND
8	S	*t*-Bu	Cl	H	8	94	8
9	S	*t*-Bu	Cl	SO_2Me	3	27	45
10	S	*t*-Bu	Cl	SO_2NH_2	6	36	23
11	S	*t*-Bu	Cl	Ph-4-OCH_2CO_2H	7	23	7
12	S	*t*-Bu	Cl	Ph-4-CH_2CO_2H	8	29	23
13	CH_2	*t*-Bu	Cl	Ph-4-OCH_2CO_2H	115	1777	ND

known RT sequences[3] and has been implicated in template primer utilization (3) the 4-position of the anilide phenyl ring points directly toward a channel which is lined by Pro236 and leads to solvent; (4) the inhibitor binds in a 'kinked' conformation which involves a rotation about the S–CH_2–CO–N dihedral angle from 180° in the fully extended free-state conformation to almost 0° in the bound state. This brings the two substituted phenyl rings into close proximity and results in the amide N–H pointing toward the sulfur.

Interestingly, however, no discernable interaction was apparent for the thio-tetrazole motif which was vital for potency. These data suggested that the tetrazole portion of these inhibitors could simply be acting as a scaffold which orients the pharmacophores into the proper geometry for binding. Based on the above, we hypothesized that alternate, potentially more stable scaffolds could be designed.

Detailed examination of the bound conformation demonstrated the obvious *cis* orientation of the tetrazole appendages and that the *N*-2-chlorophenyl substituent rests essentially perpendicular to the plane of the tetrazole. We therefore anticipated that we could mimic this bound conformation with a variety of appropriately substituted tertiary amides, carbamates or thiocarbamates. The structural rationale for this idea is as follows: Tertiary amides such as **15** (Table 3) exist in either the *s-cis* or *s-trans* conformation. The *s-cis* conformation is preferred because of an unfavorable 1,5-steric interaction between the *N*-methyl substituent and the protons α to the carbonyl in the *s-trans* orientation. Furthermore, as in the bound conformation of the tetrazole derivatives, the aromatic ring is forced to be perpendicular to the plane of the amide in order to minimize a similar destabilizing steric interaction between the aromatic o-substituents and the protons α to the tertiary amide carbonyl.

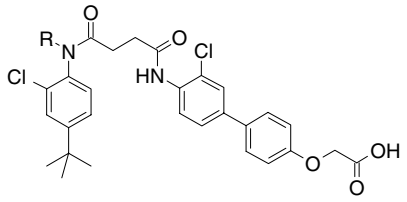
The scaffold hopping[5] hypothesis was evaluated when secondary amide **14**, a very weak inhibitor of K103N/Y181C which exists almost exclusively in the *s-trans* amide conformation, was converted to the tertiary amide **15**. By ^{1}H NMR in DMSO at room temperature, **15** exists as a 9:1 mixture of *s-cis*/*s-trans* amide rotamers. Gratifyingly, a 22-fold improvement in potency was observed relative to **14**. However, tertiary amide **15** was 14-fold less potent compared to its reference thio-tetrazole derivative **11** against K103N/Y181C and 96-fold less potent *versus* the WT enzyme. It is interesting to note that for the first time a compound was observed to be more potent against the double mutant K103N/Y181C than against WT reverse transcriptase. An X-ray crystal structure was obtained for **16** bound to the NNRTI binding pocket (data not shown) and indeed, it was found to bind as predicted with only minor shifts in binding mode relative to that of the thio-tetrazole inhibitors. In order to further evaluate the potential of the tertiary amide series, structure–activity relationship studies were initiated.

Initially, the tertiary amide N-substituent was examined. It was rapidly discovered that a small primary aliphatic group was required. The *N*-ethyl analog **16** (Table 3) was optimal with IC_{50}'s of 340 and 207 nM versus WT and K103/Y181C. This was roughly 2-fold better than *N*-methyl compound **15**. The *N*-allyl derivative **17** was equipotent to the *N*-ethyl compound **16** versus the double mutant, however, it was less potent against wild-type enzyme. The secondary aliphatic substituent *N*-cyclopropyl in **18** was not well tolerated, especially versus the wild-type RT. Trifluoroethyl analog **19** represents an attempt to subtly alter the electronic characteristics of **16**, while maintaining a similar steric volume. The compound demonstrated a decreased affinity, losing 3.5-fold affinity against both enzymes.

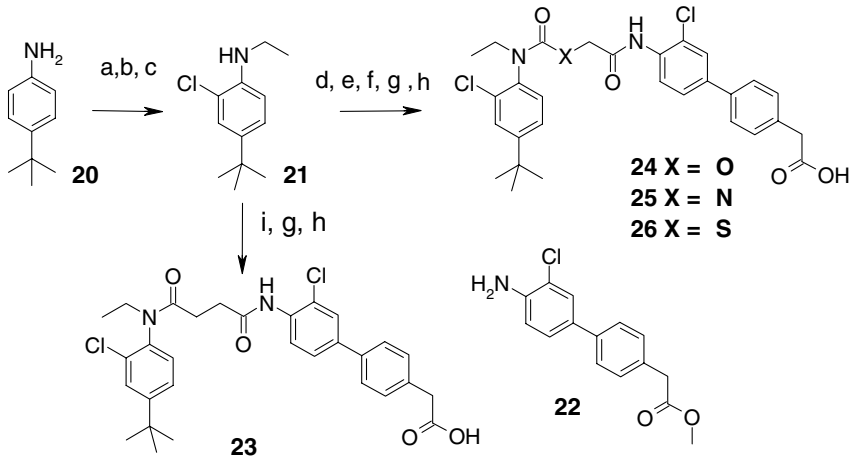
We next focused on the influence of altering the nature of the functional group of the alternate scaffold. The general synthetic route utilized to obtain the desired compounds was straightforward and is depicted in Scheme 1.[6] Chlorination of commercially available 4-*tert*-butyl aniline (**20**) was accomplished in acetonitrile using *N*-chlorosuccinimide. The 2-chloro aniline was then treated with acetyl chloride and the resulting amide was reduced using borane–dimethylsulfide complex to give the secondary aniline **21** in good yield. Compound **21** was treated with 3-carbomethoxypropionyl chloride to provide the amide intermediate. Alternatively, **21** was treated with phosgene to access the carbamoyl chloride which was in turn treated with the sodium salts of methyl glycolate, methyl thioglycolate or with glycine methyl ester hydrochloride to give the carbamate, thiocarbamate, and urea intermediates. Treatment of these intermediates with lithium hydroxide generated the corresponding acids. Finally, amide formation with aniline **22** in pyridine using phosphorus trichloride followed by treatment with sodium hydroxide in DMSO provided the desired inhibitors **23**, **24**, **25**, and **26**.

The amide **23** (Table 4) was prepared as a reference for this study and it is noteworthy that the small change in the biphenyl substitution from phenoxy-acetic acid (compound **19**) to the shorter acetic acid in compound **23** translated to 2.5-fold decrease in wild-type potency.

Table 3. SAR of tertiary amides

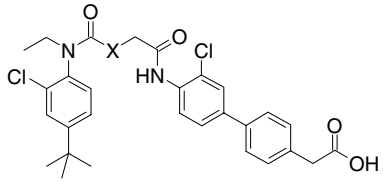


Compound	R	IC_{50} (nM)		$t_{1/2}$ HLM (min)
		WT	K103N/Y181C	
14	H	6060	4620	ND
15	Me	673	328	54
16	Et	340	207	6
17	Allyl	520	237	9
18	*c*-Pr	1922	506	15
19	CH_2CF_3	1117	718	ND



Scheme 1. Reagents and conditions: (a) *N*-chlorosuccinimide, CH_3CN; (b) acetyl chloride, pyridine; (c) BH_3–Me_2S, toluene, reflux; (d) phosgene, Et_3N, THF; (e) methyl glycolate, NaH, THF or methyl thioglycolate, NaH, THF or glycine methylester·HCl, Et_3N, THF; (f) LiOH, THF, MeOH; (g) **22**, PCl_3, pyridine; (h) NaOH, DMSO; (i) 3-carbomethoxypropionyl chloride, pyridine, THF.

Table 4. SAR of tertiary amides, carbamates, and thiocarbamates



Compound	X	IC_{50} (nM)		$t_{1/2}$ HLM (min)
		WT	K103N/Y181C	
23	C	829	305	3
24	O	109	98	9
25	N	1530	414	23
26	S	34	35	4

This decrease in affinity was not as pronounced versus the double mutant K103N/Y181C.

Carbamate **24** exists as a 3:1 mixture of *s-cis*/*s-trans* rotamers in DMSO (at room temperature) and is a potent inhibitor of both WT and K103/Y181C reverse transcriptases with IC_{50}'s of 109 and 98 nM, respectively. This represents an 8-fold improvement vs WT and a 3-fold improvement versus the double mutant relative to the amide **23**. The lower selectivity for the *s-cis* conformation in **24** can be explained by the smaller volume occupied by oxygen and its two lone pairs relative to CH_2 in **23**. The oxygen's smaller volume compared to that of CH_2 results in a decreased repulsive steric interaction with the *N*-ethyl substituent in the *s-trans* conformation and therefore has a weaker influence on overall molecular topography.

The urea **25** was not well tolerated and was 2-fold less potent than the corresponding amide **23**. Thiocarbamate **26**, on the other hand, is extremely potent showing equivalent IC_{50} values of 35 nM versus both WT and K103/Y181 enzymes. By ^1H NMR compound **26** exists in essentially only the *s-cis* conformation, an observation explained by the greater volume occupied by sulfur relative to both oxygen and CH_2. This inhibitor is essentially equipotent to the corresponding thio-tetrazole derivative **12**, versus the double mutant K103N/Y181C. However, against wild-type enzyme the compound is 4-fold less potent compared to **12**. This suggests that in the wild-type enzyme, the tetrazole is more than simply a scaffold and is likely involved in relatively weak associations with the side chains of Y181 and/or K103. It should be noted that none of these compounds resulted in a substantial improvement in the HLM stability.

In summary, a thorough understanding of the binding mode of the thio-tetrazole based inhibitors permitted the scaffold hop from tetrazoles to tertiary amides, carbamates, and thiocarbamates. Although these compounds did not provide the desired improvement in metabolic stability, they represent a novel,[4] potent class of NNRTIs with a broad spectrum of antiviral activity.

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