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Discovery of triazolinone non-nucleoside inhibitors of HIV reverse transcriptase

Zachary K. Sweeney *, Sahaja Acharya, Andrew Briggs, James P. Dunn, Todd R. Elworthy, Jennifer Fretland, Anthony M. Giannetti, Gabrielle Heilek, Yu Li, Ann C. Kaiser, Michael Martin, Y. David Saito, Mark Smith, Judy M. Suh, Steven Swallow, Jeffrey Wu, Julie Q. Hang, Amy S. Zhou, Klaus Klumpp

Roche Palo Alto LLC, 3431 Hillview Avenue, Palo Alto, CA 94304, USA

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ABSTRACT

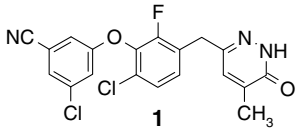
Novel non-nucleoside inhibitors of HIV-RT that contain pyridazinone isosteres were prepared, and a series of triazolinones were found to be potent inhibitors of HIV replication. These compounds were active against several NNRTI-resistant virus strains. Pharmacokinetic studies indicated that inhibitor **7e** has good bioavailability in rats. Several fragments of inhibitor **7c** were prepared, and the binding of these compounds to HIV-RT was analyzed by surface plasmon resonance spectroscopy.

The reverse transcriptase enzyme of the human immunodeficiency virus (HIV-RT) transcribes the RNA viral genome into DNA. This essential step in the viral replication cycle can be inhibited by a class of drugs known as non-nucleoside reverse transcriptase inhibitors (NNRTIs).[1] There are four marketed NNRTIs for the treatment of HIV infection, and several compounds that inhibit the replication of commonly observed NNRTI-resistant viruses are in development.[2,3]

In a previous letter,[4] we described the discovery of a series of pyridazinones that are potent inhibitors of viral replication in vitro. Pyridazinone **1**, was determined to have excellent activity against the wild-type virus and several commonly observed resistant viruses (Table 1). However, low aqueous solubility for a number of these inhibitors appeared to limit oral bioavailability at higher doses. In order to further optimize the potency and bioavailability of this series of NNRTIs, we focused on modification of the pyridazinone functionality. In this letter, we describe diaryl ether NNRTIs that contain modified pyridazinones and heterocyclic pyridazinone isosteres. This effort resulted in the discovery of a series of triazolinone inhibitors that have good oral bioavailability and excellent activity against common NNRTI-resistant viruses.

Crystal structures of inhibitors similar to **1** bound to HIV-RT demonstrated that the pyridazinones interact with the protein backbone through a pair of hydrogen bonds between the amide of the K103 amino acid and the N-NH acceptor–donor motif of the pyridazinone. Inspection of the small molecule crystal structures of the inhibitors themselves revealed that in the solid state the pyridazinones were associated through extensive aromatic stacking interactions. Therefore, our design strategy was to retain the N-NH binding motif while introducing groups that might perturb intermolecular stacking interactions and improve the solubility of the inhibitors.

Table 1
Activity of **1** against wild-type and mutant viruses[a]



1

WT	G190A	K103N/L100I	K103N/Y181C
0.001	0.001	0.004	0.019

[a] EC_{50} in μM versus the wild-type virus or the indicated mutant.

A simple scaffold that did not contain a fluorinated core ring was initially used to investigate the activity of the new inhibitors. Compounds were assessed only in terms of their ability to inhibit the polymerase activity of wild-type reverse transcriptase, as our experience with the pyridazinone compounds had suggested that potency against NNRTI-resistant viruses was likely to be highly dependent upon the substitution pattern of the phenyl rings. Selected NNRTIs prepared in this initial series are shown in Table 2, along with the corresponding activity in the polymerase enzyme assay.[5]

* Corresponding author. Tel.: +1 650 855 6376; fax: +1 650 852 1311.
E-mail address: zachary.sweeney@roche.com (Z.K. Sweeney).

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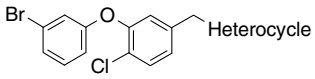
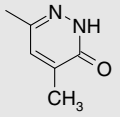
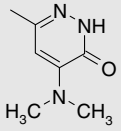
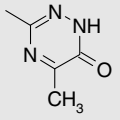
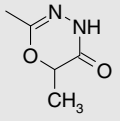
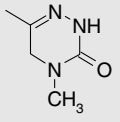
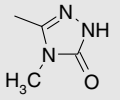
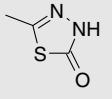
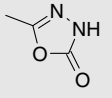
Table 2
Potency of a series of NNRTIs



Compound	Heterocycle	IC_{50}
2a		0.13
3a		0.23
4a		0.33
5a		0.05
6a		0.15
7a		0.69
8a		0.53
9a		5.80

IC_{50} in μM versus the wild-type virus.

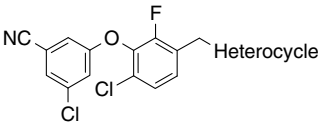
Almost all of the new compounds retained good potency in the enzyme assay. Substitution of the pyridazinone methyl group with the considerably larger dimethylamino group resulted in only a moderate loss in activity. Introduction of an additional heteroatom adjacent to the methylene bridge was also accommodated (i.e., triazinone **4a**). The non-aromatic oxadiazine **5a** and 1,2,4-triazine **6a** were equipotent to pyridazinone **2a** in this assay. Finally, good inhibition of polymerase activity was observed with compounds containing 5-membered heterocycles. For this series of inhibitors, triazolinone **7a** and thiadiazinone **8a** were somewhat more potent than oxadiazinone **9a**.

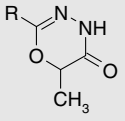
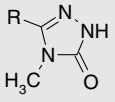
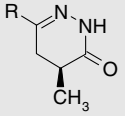
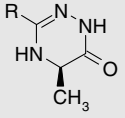
In order to further investigate compounds that had promising activity in the initial screen, inhibitors that contained more highly substituted biaryl ethers were prepared. These efforts focused on the evaluation of NNRTIs that feature a fluorinated central phenyl ring and a 3-chloro, 5-cyano substituted terminal phenyl ring so that the compounds could be easily compared to lead compound **1**. The dihydropyridazinone **10** and the dihydrotriazinone **11** were designed to project the methyl group out of the plane of the heterocycle, as it was thought that a similar orientation of the methyl group of oxadiazinone **5a** might be responsible for the potency of this inhibitor. Selected examples of this group of compounds are shown in Table 3. These NNRTIs again had excellent potency in the polymerase assay. The nearly identical activity of these diverse compounds suggests that the strength of the hydrogen bonding interactions of the heterocycle with the protein backbone do not strongly influence the thermodynamics of the protein–inhibitor interaction. It was later demonstrated that these compounds derive much of their potency from hydrophobic interactions of the biaryl ether (vide infra). However, excision of the methyl group from the heterocyclic ring of these inhibitors did result in a loss of activity.

The potency of the new set of inhibitors against several NNRTI-resistant viruses was good, and the EC_{50} of **5b**, **7b**, and **11** against the K103N/Y181C mutant virus was less than 50 nM.[6] Unfortunately, compound **5b** was determined to be a strong inhibitor of CYP3A4 (IC_{50} = 0.27 μM). Strong CYP inhibition was also observed with other 1,3,4-oxadiazin-5-ones, and this group of compounds was not investigated further. Dihydropyridazinone **10** was less potent than the other inhibitors in the cellular assay. Compound **11** and similar triazinones were rapidly metabolized in the presence of rat microsomal protein (Cl = 130 μg/mL/mg), and the reduced stability of compounds of this type was confirmed through in vivo studies.

Triazolinone **7b** showed good potency in the cell-based assay and was only slowly metabolized in the presence of human liver microsomes (Cl = 5 μg/mL/min). A set of triazolinones with

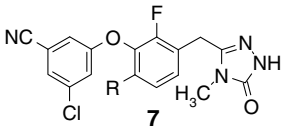
Table 3
Activity of inhibitors **5b**, **7b**, **10**, and **11**[a]



Compound	Heterocycle	WT IC_{50}	WT EC_{50}	K103N/Y181C EC_{50}
5b		0.013	0.005	0.034
7b		0.011	0.008	0.040
10		0.017	0.032	na
11		0.011	0.008	0.072

[a] IC_{50} or EC_{50} in μM versus the wild-type virus and the K103N/Y181C mutant virus.

Table 4
Activity of triazolinone inhibitors **7**[a]



Compound	R	WT	K103N/Y181C
7b	Cl	0.011	0.039
7c	Br	0.002	0.009
7e	CH_3	0.002	0.026
7f	Et	0.002	0.004
7g	c-Pro	0.002	0.011

[a] EC_{50} in μM versus the wild-type virus and the K103N/Y181C mutant virus.

different substituents on the central phenyl ring was prepared in an effort to further improve the potency of these compounds against NNRTI-resistant viruses (Table 4). From the group of triazolinones prepared, inhibitor **7c** was selected for further characterization.

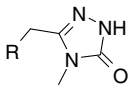
As indicated by the data in Table 5, **7c** was able to effectively reduce the replication of viruses containing mutations at positions 103, 181, 100, and 190 of the reverse transcriptase (Table 5). Surface plasmon resonance (SPR) experiments[7] showed that **7c** bound very tightly to an immobilized wild-type reverse transcriptase enzyme, although binding was readily reversible. Under these conditions, the dissociation constant (K_d) was calculated to be 4×10^{-9} M^{-1}. Finally, **7e** did not strongly inhibit the activity of major cytochrome p450 enzymes (e.g., CYP3A4 IC_{50} = 12 μM).[8]

The solubility of the triazolinone inhibitors was significantly improved relative to the analogous pyridazinone compounds. For example, although the solubility of compound **7c** (solubility in pH 6.5 buffer = 2.4 μg/mL) is low, this inhibitor is more than 10-fold more soluble than the pyridazinone lead **1**.[9] The permeability of **7c** as measured in CACO-2 experiments was high (P_{app} = 15.1 cm^2/s), and no indication of transporter-mediated efflux was observed. Administration of an aqueous suspension of this inhibitor to dogs (0.5 mg/kg iv, 2 mg/kg po) demonstrated that the **7c** was metabolically stable and well absorbed (Cl = 1.5 mL/min/kg, C_{max} = 1.3 μM, $AUC_{0-\infty}$ = 38.4 μM h, F% = 74).

Inhibitor deconstruction and fragment screening: Fragment screening has emerged as a useful method for the discovery of new templates for medicinal chemistry lead optimization programs.[10] However, there have been few reports of the successful application of fragment screening for the discovery of inhibitors that bind to induced pockets such as the HIV-RT NNRTI binding site. In order to gain insight into the size of fragments that would have been required to identify triazolinones as templates for further optimization, we synthesized various fragments of **7c** and studied their binding to immobilized HIV-RT using surface Plasmon resonance (SPR).[11] These smaller compounds were also assayed as inhibitors of the polymerase activity of HIV-RT. The K_d determined in the SPR study and the IC_{50} calculated from the polymerase assay are listed in Table 6.

In general, there was good agreement between the inhibitory IC_{50} derived from the functional polymerase assay and the binding constant extracted from the SPR experiments. The smallest fragments did not show any binding within the limits of detection of the SPR assay (~2 mM). Very weak binding was observed with halogenated benzyl triazolinone compounds **7j** and **7k** in the SPR assay, although this interaction still could not be detected in the enzymatic assay. Only larger compounds **7l** and **7m** showed micromolar inhibition of the polymerase activity. These molecules have more heavy atoms than the small compounds commonly included in fragment screening libraries.

Table 6
Activity of fragments **8** and inhibitor **7c**



Compound	R	WT IC_{50}	K_d
7h	H	>100	NB
7i	Ph	>100	NB
7j	4-Br-Ph	>100	388
7k	2-F,4-Br-Ph	>10	102
7l	2-F,3-OMe,-4-Br-Ph	34	4
7m	3-PhO-Ph	18	3
7c	See Table 4	0.005	0.004

The results from these experiments confirm the utility of SPR fragment screening for lead discovery. Fragment **7j**, which has a ligand efficiency (LE) of 0.3 kcal/mol/non-hydrogen atom, could have been identified as a useful starting point for the design of more potent triazolinone NNRTIs in a fragment screening approach to lead identification. It should be noted, however, that this LE value had previously been suggested to be a minimum value for a useful fragment lead.[12] Figure 1 plots the free energy of binding (ΔG) calculated from the SPR binding data versus the number of heavy atoms for compounds **7j**–**m** and **7c**. As has been observed in earlier work, there is a roughly linear correlation between binding energy and mass.[13] The slope of the plot indicates that each heavy atom contributes, on average, 0.59 kcal/mol to the binding energy of the molecule. This number is substantially greater than the slopes observed in most earlier deconstruction analyses for compounds that have between 15 and 25 heavy atoms, and might be explained by the hydrophobic nature of the NNRTI binding site. It is possible that a molecule such as **7c**, which has an excellent LE (0.43), may have been discovered starting from a fragment hit with a substantially worse binding efficiency. Such a finding may encourage optimization of even modestly efficient leads when the compound binding site is already known to be highly druggable.

Table 5
Activity of **7c** against a set of NNRTI-resistant viruses[a]

Virus	WT	Y181C	G190A	L100I/K103N
EC_{50}	0.002	0.005	0.001	0.002

[a] EC_{50} in μM against the indicated virus.

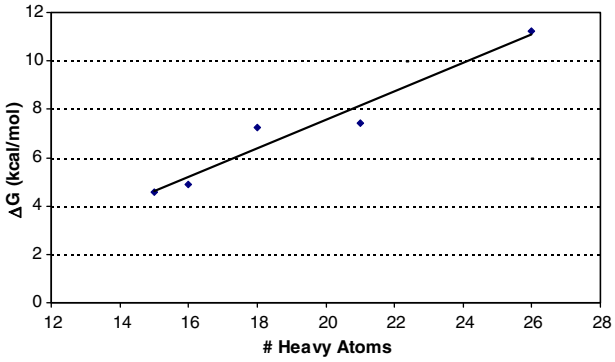
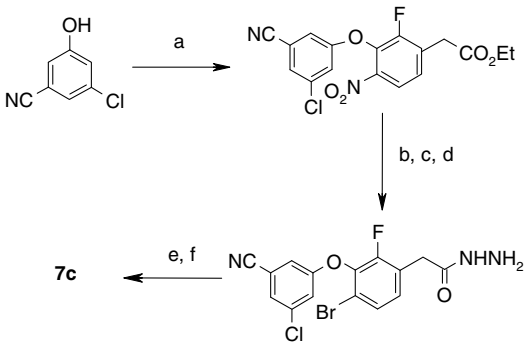


Figure 1. ΔG binding versus number of heavy atoms for **7j**–**m** and **7c**.



Scheme 1. Reagents: (a) KO^{t}Bu, ethyl (2, 3-difluoro-4-nitrophenyl)acetate, THF, 90%; (b) Fe, NH_4Cl, EtOH, H_2O, 91%; (c) ^{t}BuONO, $CuBr_2$, LiBr, CH_3CN, 50%; (d) N_2H_4, EtOH; (e) $OCNCH_3$, THF; (f) KO^{t}Bu, ^{t}BuOH, 74% for three steps.

The synthesis of inhibitor **7c** is outlined in Scheme 1.[14] An aromatic substitution reaction of 3-cyano, 5-chlorophenol with a difluoronitrophenyl acetate provided the diaryl ether in excellent yield. Reduction of the nitro group with iron, followed by a Sandmeyer reaction gave the chlorinated product. Sequential reaction of the ester with hydrazine and methyl isocyanate provided a semicarbazide that could be dehydrated with a catalytic amount of KO^{t}Bu in ^{t}BuOH to form the triazolinone NNRTI.

In summary, a number of non-nucleoside inhibitors of HIV reverse transcriptase were prepared. Evaluation of the potency and physical properties of these compounds led to the identification of a series of triazolinones that strongly inhibited wild-type and NNRTI-resistant viruses in vitro. These compounds had significantly improved physical properties relative to their pyridazinone analogs, and inhibitor **7c** was determined to have excellent bioavailability in animal studies. A deconstruction exercise was used to determine the suitability of fragment-based approaches for lead identification for the NNRTI binding site.

Acknowledgment

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5. Enzymatic assay: IC_{50} values were obtained from a scintillation counting assay using poly rA primer and tritiated dTTP.
6. Replication assay: EC_{50} values were obtained following incubation of compound with infected MT4 cells in the presence of 10% fetal bovine serum. The assay assesses the reduction in the cytopathic effect of HIV-1 on MT4 cells in the presence of HIV-RT inhibitors. Each value represents the average of at least two independent assays.
7. Surface plasmon resonance experiments were performed using HIV-RT protein immobilized to the CM5 sensor chip via an amide-coupling reaction. The binding experiments were performed using Hepes buffer (50 mM Hepes (pH 8.0), 150 mM NaCl, 10 mM $MgCl_2$, 0.01% Tween 20, 5% DMSO) as the running buffer. For characterization of other NNRTIs using SPR see: Geitmann, M.; Danielson, U. H. *Bioorg. Med. Chem.* **2007**, *15*, 7344.
8. CYP3A4 inhibition was assessed using 7-benzyloxy-4-(trifluoromethyl)-coumarin as a fluorogenic substrate.
9. Interestingly, the differences in solubility between these inhibitors cannot simply be attributed to differences in intermolecular interactions in the solid state. The melting temperature of crystals of **1** (181–182 °C) is nearly identical to that of **7c** (185–186 °C).
10. Abad-Zapatero, C. *Expert Opin. Drug Disc.* **2007**, *2*, 469.
11. For an early example of such a "inhibitor deconstruction" exercise see: Babaoglu, K.; Shoichet, B. K. *Nat. Chem. Biol.* **2006**, *2*, 720.
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14. For complete details of the synthesis of inhibitor **7c** and other NNRTIs described in this communication see: Saito, Y. D.; Smith, M.; Sweeney, Z. K. US 2007/078128 and Dunn, J. P.; Elworthy, T. R.; Stefanidis, D.; Sweeney, Z. K. WO 2006/010545.