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Discovery and optimization of pyridazinone non-nucleoside inhibitors of HIV-1 reverse transcriptase

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ABSTRACT

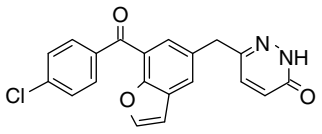
A series of benzyl pyridazinones were evaluated as HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs). Several members of this series showed good activity against the wild-type virus and NNRTI-resistant viruses. The binding of inhibitor **5a** to HIV-RT was analyzed by surface plasmon resonance spectroscopy. Pharmacokinetic studies of **5a** in rat and dog demonstrated that this compound has good oral bioavailability in animal species. The crystal structure of a complex between HIV-RT and inhibitor **4c** is also described.

Chemotherapy for the treatment of HIV infection often includes administration of an allosteric inhibitor of the viral reverse transcriptase (HIV-RT). These medicines, known collectively as non-nucleoside reverse transcriptase inhibitors (NNRTIs), bind to the transcriptase enzyme in a hydrophobic cavity adjacent to the polymerase catalytic site.[1,2] Association of an NNRTI with the reverse transcriptase is thought to inhibit chain elongation by altering the motions of the protein residues that interact with the nucleic acid chain.[3] Some NNRTIs have also been found to influence dimerization of the HIV-RT subunits,[4] HIV-RT RNase activity,[5–7] and the effectiveness of chain termination by deoxynucleoside drugs.[8]

Currently marketed NNRTIs include efavirenz, nevirapine, delavirdine, and etravirine. Efavirenz and nevirapine have good pharmacokinetic profiles and effectively inhibit replication of the wild-type virus,[9] but they are less effective against several commonly observed mutant viruses.[10] Etravirine shows improved potency against many NNRTI-resistant viruses,[11,12] but must be administered twice daily and is approved for use only in patients infected with HIV-1 strains resistant to an NNRTI and other antiretroviral agents. There is therefore a need for new NNRTIs that are active against virus strains resistant to current NNRTIs and have pharmacokinetic properties suitable for once-daily dosing.[13]

Our search for novel NNRTIs commenced with a high-throughput screen of the Roche compound library. From this screen, pyridazinone **1** was discovered to inhibit the activity of wild-type HIV-1 reverse transcriptase in an isolated enzyme assay (Table 1).[14] This compound was also found to be active against HIV replication in MT4 cells infected with wild-type HIV-1.[15] Subsequent studies

Table 1
Potency of lead compound **1**[a]



1

Assay	WT	K103N	Y181C
IC_{50}	0.40	0.26	0.36
EC_{50}	0.08	0.07	0.21

[a] IC_{50} and EC_{50} in μM.

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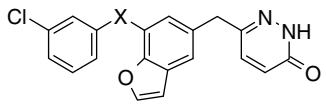
Table 2
SAR of phenyl-benzofuran linker[a]

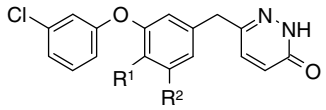


2

Compound	X	IC_{50}	CYP3A4 IC_{50}
2a	CO	8.2	0.6
2b	CH_2	3.9	0.18
2c	O	1.0	0.41

[a] IC_{50} (μM) versus the wild-type enzyme and for inhibition of recombinant CYP3A4 using BFC as a fluorogenic substrate.

Table 3
SAR of central phenyl ring substitution[a]



3

Compound	R^1	R^2	IC_{50}
3a	H	H	21
3b	H	Me	14
3c	Me	H	0.64
3d	Cl	H	0.10

[a] IC_{50} (μM) versus the wild type enzyme.

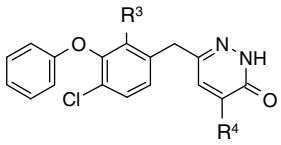
indicated that **1** inhibited the replication of the clinically relevant K103N and Y181C mutant viruses,[16] and this compound was chosen as a lead molecule for our discovery program.

Initial modifications focused on the influence of the linker between the terminal phenyl ring and the benzofuran portion of the inhibitors. As shown in Table 2, diaryl ether compounds proved to be somewhat more potent than their benzophenone analogs. However, the benzofuran lead compounds were found to be uniformly strong inhibitors of CYP3A4. A series of diaryl ethers that did not contain the benzofuran moiety were therefore prepared in an effort to discover NNRTIs that would not inhibit cytochrome p450 enzymes (Table 3). Excision of the furan hetereocycle (**3a**) resulted in a severe loss in activity, but analogs with R_1 = Cl or Me (**3c–d**) maintained good potency. These compounds were also relatively weak inhibitors of CYP3A4 (e.g., **3d**, CYP3A4 IC_{50} = 4.0 μM).

As we had identified a series of compounds that did not strongly interfere with CYP function, our attention turned to improving the potency of the pyridazinone inhibitors. Substitution of the pyridazinone ring revealed that addition of a methyl group in the 4-position could improve activity against the mutant viruses (Table 4 **4a** vs **4b**). Fluorination of the central ring also improved potency (**4c**). Compound **4d**, which contains both fluorine and methyl substitutions, was 25-fold more potent than precursor **4a** in testing against both the wild-type and mutant viruses.

Crystallographic studies of a complex of **4c** with the wild-type reverse transcriptase enzyme provided insight into the likely binding mode of this series of inhibitors (Fig. 1).[17–19] A bidentate hydrogen-bonding interaction between the pyridazinone ring and the K103 amide backbone anchors the compound to the NNRTI-binding pocket. The central ring of the inhibitor is positioned by the bridging methylene group to be nearly perpendicular to the plane of the pyridazinone ring, and the chlorine atom occupies a small

Table 4
Improvements in Potency[a]



Compound	R_3	R_4	WT	K103N	Y181C
4a	H	H	0.52	3.35	5.49
4b	H	Me	0.36	0.92	2.13
4c	F	H	0.10	0.43	0.5
4d	F	Me	0.02	0.05	0.21

[a] WT, K103N, and Y181C refer to IC_{50} values in μM versus the indicated enzyme.

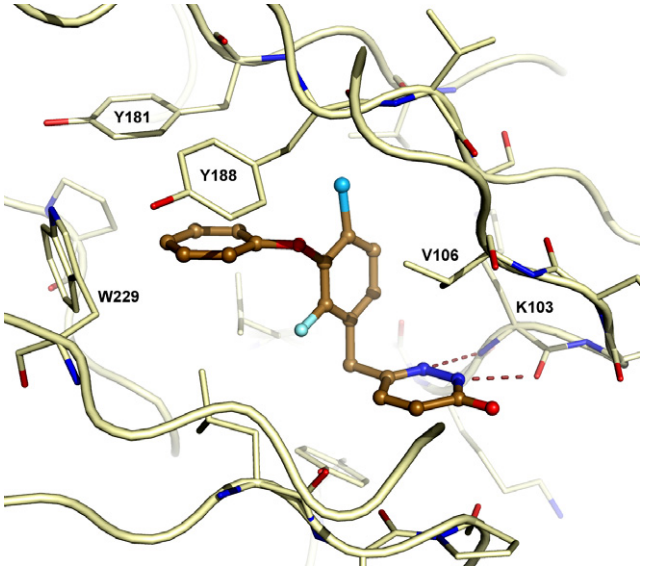


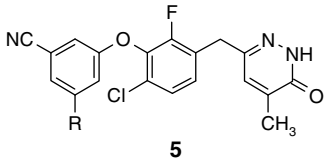
Figure 1. Inhibitor **4c** bound to wild-type HIV-RT. Only key protein residues are shown.

pocket defined by the side chains of V106 and V179. The terminal phenyl ring engages in hydrophobic interactions with Y188, Y181, and W229. The methyl group of **4d** appears to interact with P225, which is located on a flexible loop region.

In the course of our optimization program, it became apparent that appropriate substitution of the terminal phenyl ring improved the activity of the pyridazinone NNRTIs in the cellular assay. These modifications also strongly affected the potency of the inhibitors when they were tested against NNRTI-resistant mutant viruses. Analogs containing a 3,5-disubstituted aromatic ring had low nanomolar activity against the wild-type virus and a number of resistant mutant viruses (Table 5). These compounds maintained their potency in tests conducted in the presence of additional human serum.[20]

Inhibitor **5a** was selected for further characterization. Surface plasmon resonance experiments[21] showed that binding of this inhibitor to the wild-type reverse transcriptase and to the K103N and Y181C mutant viruses was rapid and reversible. For the wild-type enzyme, the dissociation constant (K_D) was calculated to be 8.4×10^{-9} M^{-1} ($k_{on} = 1.0 \times 10^5$ M^{-1} s^{-1}, $k_{off} = 8.4 \times 10^{-4}$ s^{-1}). None of the major cytochrome P450 enzyme isoforms were strongly inhibited by this compound (Table 6).[22] NNRTI **5a** was well absorbed when dosed orally in rats (5 mg/kg), with a C_{max} of 2.3 μM and an AUC of 22.4 μM h. Following intravenous

Table 5
Antiviral activity of pyridazinones **5a–c**



5

Compound	R_5	WT	WT + serum	K103N	Y181C	G190A	K103N/L100I	K103N/Y181C
Efavirenz	—	0.001	0.019	0.034	0.002	0.006	>0.50	0.083
5a	F	0.001	0.011	0.002	0.005	0.001	0.003	0.041
5b	OMe	0.002	0.015	nt	0.009	0.001	0.002	0.031
5c	CN	0.001	0.007	0.002	0.005	0.001	0.007	0.019

WT, K103N, Y181C, G190A, K103N/L100I, and K103N/Y81C refer to EC_{50} values in μM versus the wild-type virus and the 103, 181, 190, 103 + 100, and 103 + 181 mutant viruses, respectively. WT + serum refers to experiments performed in the presence of 40% human serum.

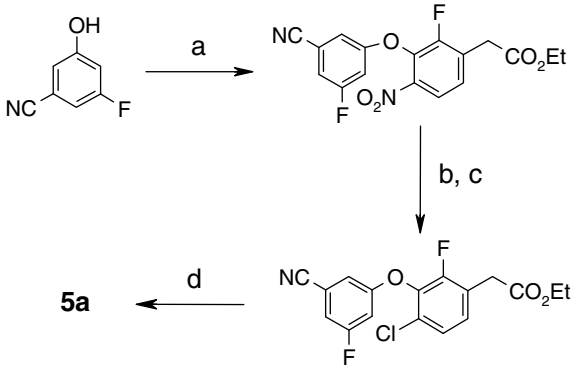
Table 6
CYP inhibition and pharmacokinetics for **5a** P450 inhibition (μM)

3A4	1A2	2D6	2C9	2C19
24	>50	>50	8	21
Pharmacokinetics		Rat		Dog
C_{max} (μ/mL)		2.3		1.47
AUC (μM h/mL)		22.4		8.8
$t_{1/2}$ (h)		3.8		6.4
Vd_{ss}		1.73		2.06
F%		61		34

administration (2.5 mg/kg), **5a** demonstrated a reasonable half-life (3.8 h), and the apparent bioavailability was determined to be 61%. Similar experiments in dogs showed that the compound had good stability and bioavailability in this species as well (Table 6).

The synthesis of inhibitor **5a** is outlined in Scheme 1.[23] Treatment of difluoronitrophenyl acetate with the potassium salt of 3-cyano, 5-fluorophenol provided the diaryl ether in excellent yield. Reduction of the nitro group with iron was followed by a Sandmeyer reaction to give the chlorinated intermediate. Addition of the sodium enolate of the aryl acetate to 3,6-dichloro-4-methylpyridazine formed the desired chloropyridazine adduct as a separable 2:1 mixture of regioisomers. Decarboxylation of the ester followed by hydrolysis of the chloropyridazine in refluxing acetic acid gave **5a**.

In summary, we have discovered a series of pyridazinones that display excellent potency against a wild-type HIV-RT and several NNRTI-resistant mutant viruses. These compounds have good bioavailability and low clearance in animals, and do not inhibit major CYP enzymes. Further optimization of these inhibitors is described in the following letter.



Scheme 1. Reagents and conditions: (a) KO*t*Bu, Ethyl (2, 3-difluoro-4-nitrophenyl)acetate, THF, 92%; (b) Fe, NH_4Cl, EtOH, H_2O, 93%; (c) ^tBuONO, $CuCl_2$, CH_3CN, 63%; (d) i—NaH, 3,6-dichloro-4-methylpyridazine, DMF; ii—LiOH, THF, H_2O, iii—NaOAc, HOAc, 30%.

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14. Enzymatic assay: IC_{50} values were obtained from a scintillation counting assay using poly-rA primer and tritiated dTTP.
15. 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.
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17. In addition to the marketed NNRTI delavirdine, there have been several recent examples of NNRTIs that bind to the K103 residue. For a very recent closely related series see: Tucker, T. J. et al *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2959.
18. This type of linked triaryl motif can also be found in the aminopyrimidine series of inhibitors that interact with the backbone of K101. See: Das, K.; Bauman, J. D.; Clark, A. D., Jr.; Frenkel, Y. V.; Lewi, P. J.; Shatkin, A. J.; Hughes, S. H.; Arnold, E. *Proc Natl. Acad. Sci. U.S.A.* **2008**, *105*, 1466.
19. Atomic coordinates the structure of **4c** bound to HIV-RT were deposited with the RCSB Protein Data Bank (PDB) under the Access Code 3DI6.
20. For these tests, the replication assay was conducted with additional 40% human serum.
21. 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 (10 mM Hepes (pH 8.0), 150 mM NaCl, 3 mM EDTA, 0.005% Tween 20, 1% DMSO) as the running buffer.
22. CYP inhibition experiments were determined using 7-benzyloxy-4-(trifluoromethyl)-coumarin as a fluorogenic substrate.
23. For a more detailed description of the synthesis of this compound and other pyridazinone inhibitors see: Dunn, J. P.; Dymock, B. W.; Mirzadegan, T.; Sjogren, E. B.; Swallow, S.; Sweeney, Z. K. WO 2004/085406; Kertesz, D. J.; Martin, M.; Palmer, W.S. US 2005/234236.