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Discovery of piperidin-4-yl-aminopyrimidines as HIV-1 reverse transcriptase inhibitors. *N*-Benzyl derivatives with broad potency against resistant mutant viruses

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ABSTRACT

An analysis of the binding motifs of known HIV-1 non-nucleoside reverse transcriptase inhibitors has led to discovery of novel piperidine-linked aminopyrimidine derivatives with broad activity against wild-type as well as drug-resistant mutant viruses. Notably, the series retains potency against the K103 N/Y181C and Y188L mutants, among others. Thus, the *N*-benzyl compound **5k** has a particularly attractive profile. Synthesis and SAR are presented and discussed, as well as crystal structures relating to the binding motifs.

Treatment of HIV has become more efficacious in recent years in large part due to use of combination therapies employing sets of drugs with different mechanisms of action.[1] Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are valuable components of these treatment regimens. These drugs bind in an allosteric site near the catalytic region of the HIV-1 RT enzyme, thereby inducing changes in protein geometry which effectively disable the enzyme from carrying out its function.[2] However, mutation of the RT enzyme is facile and has resulted in viable viral strains resistant to first generation NNRTIs.[3] Thus, drugs such as Efavirenz and Nevirapine have been in clinical use for some time, and are quite effective against the wild-type virus found in treatment-naive patients. However, they have a low barrier to resistance and are less effective in treatment-experienced patients who carry viruses with NNRTI resistant mutations.[4]

Given the effectiveness of the initial NNRTI drugs, considerable effort has been applied towards finding new molecules which retain activity against the important mutant viruses.[5] Among the strains most often associated with resistance in the clinic are those with mutations at K103N and Y181C, found singly or in combination with other variants in the NNRTI binding site.[6] For example, K103N/Y181C (KY) is a frequently encountered double mutant.[7] We report here a new effort towards finding NNRTIs which are broadly active against resistant viruses as well as the wild-type virus.

Etravirine (TMC125) is a second generation NNRTI which has recently been approved for clinical use (Fig. 1).[8] It has good potency against wild-type (WT) and many resistant viruses, but its development was challenged by poor solubility.[9] The benzophenone compounds GW-678248 and GW-69564 have also been described as having broad potency against WT and the important mutants.[10] In addition, oral bioavailability of these compounds could be enhanced by conversion to *N*-acyl prodrugs, and GW-678248 was developed as such.[11] We considered combining the perceived advantages of Etravirine and the benzophenone series into a single molecule.

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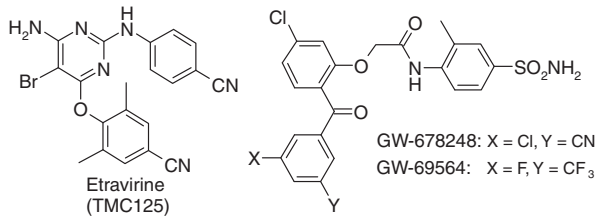


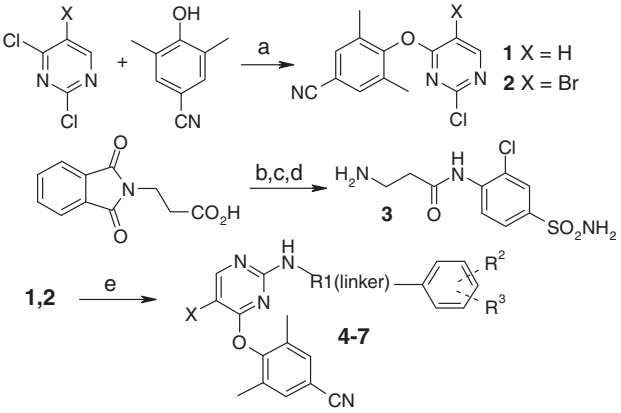
Figure 1. Structures of Etravirine and benzophenones.

A co-crystal structure of Etravirine bound in the NNRTI pocket was generated in our laboratories,[12] and was overlaid with the published structure of GW-69564, as seen in Figure 2. One evident difference in the way these compounds occupy the site is the 'horseshoe' conformation of Etravirine as compared to the largely 'extended' shape of GW-69564. Further differences in binding modes include the polar interactions with the protein backbone. Thus, Etravirine makes a hydrogen bond from the aminopyrimidine NH to the backbone carbonyl of lysine K101, while GW-69564 has a similar interaction between its amide carbonyl and the NH of the K103 amide. Also, the core ring of each drug sits differently in the central space so that the phenyl 'tail' rings enter the back pocket at different angles. Each tail ring then effects the important edge-to-face interaction with conserved tryptophan W229 from a different angle, and the protein back-pocket regions also show shifts for the residues of Y188, W229 and Y181. Lastly, GW-69564 interacts in a front pocket region not accessible to Etravirine. There the anilide ring can stack with P236 and the side-chain of V106. The 4-sulfonamide itself is positioned for possible hydrogen bonding with P236, and also appears to possess a vector to solvent.

Our plan was to prepare hybrid molecules using a back pocket substructure similar to that of Etravirine, combined with features from the benzophenones to give the additional polar and front pocket interactions. We conjectured this might give a drug with similar or superior potency and mutant profile to the parents, as well as possibly improved pharmacokinetic properties. The structure overlay in Figure 2 implies that blending the two molecules should be synthetically feasible. That is, the geometry looks promising for attaching a phenylsulfonamide head group to an aminopyrimidine core/tail unit *via* an appropriate linker unit. Thus, we set out to make the structures necessary to test our hypothesis.

We began with the preparation of alkyl-chain linked amides such as **4** (Scheme 1). Syntheses proceeded *via* tandem nucleophilic displacements on 2,4-dichloropyrimidines, the first being with 2,6-dimethyl-4-cyanophenol to give reagents **1** and **2**. Further treatment with amines such as **3** (prepared as shown from protected β-alanine) gave products as **4**.

Inhibitory activities of the products were measured against recombinant wild-type and mutant enzymes.[13] Bioassay results with **4** were disappointing (Table 1), but the concept remained of sufficient interest that a parallel synthesis effort was initiated to study other types of linkers. Intermediates **1** and **2** were then treated with a variety of commercially available amines, and the activities of the most interesting products (e.g., **5**–**7**) are also shown in Table 1. The *N*-benzyl piperidine **5b** was found to have the best activity profile, and a detailed SAR exploration of that scaffold was initiated. The *N*-phenyl piperidine **6** was less active but remained of interest for future work.



Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF; (b) $(COCl)_2$, DMF; (c) TEA, CH_2Cl_2, 4-aminosulfonyl-2-chloro-aniline; (d) H_2NNH_2, EtOH, 90 °C; (e) **3** or other amines, DIPEA, NMP, 100 °C.

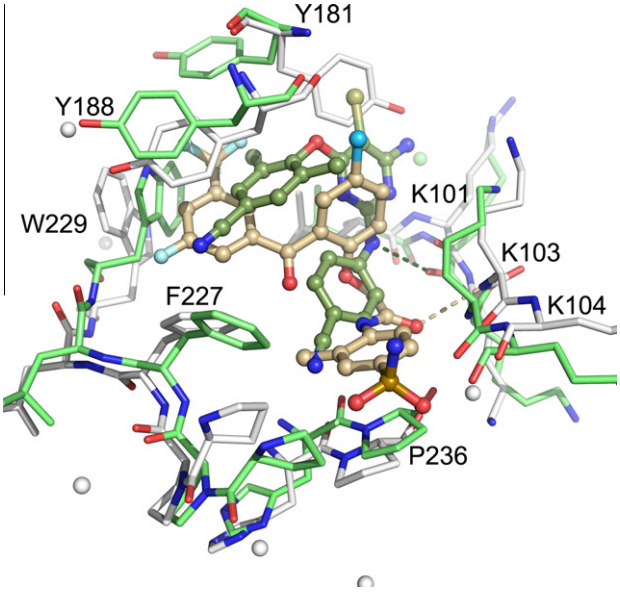
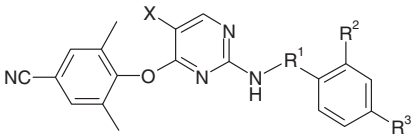
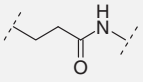
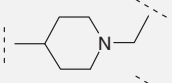
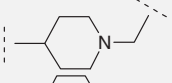
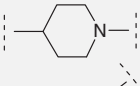
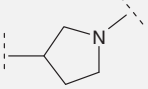


Figure 2. Overlay of crystal structures of Etravirine (green) and GW-69564 (tan) bound to wild type HIV-RT enzyme.

Table 1
Enzyme inhibitory potency of derivatives **4**–**7**



#	R^1	X	R^2	R^3	WT[a]	KY[a]	Y188L[a]
4		H	Cl	SO_2NH_2	900	>1K	>10K
5a		H	H	H	87	216	372
5b		Br	H	H	19	15	70
6		Br	H	H	192	112	334
7		Br	H	H	67	>1K	718

[a] IC_{50} in nM, heteropolymeric enzyme inhibition assay (see Ref. 13).

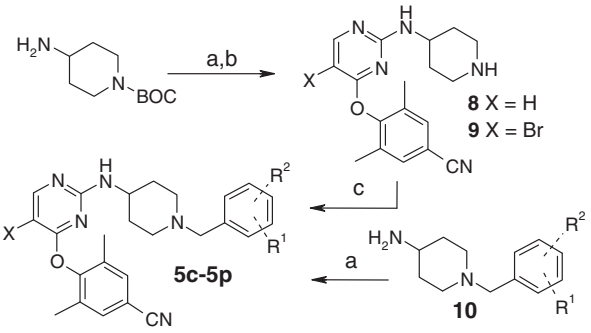
Further examples of *N*-benzyl piperidines were made in high yields *via* reactions of **1** and **2** with 4-amino-*N*-Boc-piperidine followed by deprotection, affording intermediates **8** and **9** (Scheme 2). Treatment of **8** and **9** with various benzyl bromides then gave products **5c**–**5p**. Alternatively, reactions of 4-(*N*-boc-amino)-piperidine with the benzyl bromides and deprotection gave amine reagents **10**, and further treatments with **1** and **2** then gave the same final products.[14]

Antiviral efficacy data[15] as well as enzyme data are presented in Table 2 for a selection of these compounds. Enzyme data for the chloride and nitrile-substituted *N*-benzyl analogs **5c**–**5g** implied a preference for a polar group in the 4-position. Such substituents turned out to be vital for antiviral potency, particularly against the KY mutant, as can be seen by comparing analogs **5h**–**5p** with unsubstituted **5a** and **5b**. Sulfonyl substituents were best in the 4-position, as among analogs **5h**–**5m**, and addition of a 2-chloride in combination with a 5′-bromide on the core ring led to the excellent antiviral profile noted for the 4-methylsulfone **5k**. Corresponding 4-sulfonamide **5m** had a similar profile, albeit with lower KY activity. Other combinations of substituents such as the nitrile **5n**, carboxamide **5o** and carboxylic acid **5p** were less potent, although acid **5p** was of note for its potential solubility advantage.

The best *N*-benzyl head-ring substitution patterns were similar to that of GW-69564, which we took as early evidence that the compounds were binding in accord with the design. However, the presence of the basic nitrogen in the piperidine linker group was difficult to rationalize since there was no salt bridging partner proximal to that amine in our docked models. The question was resolved when we obtained a crystal structure for **5h** bound to the wild type enzyme, and that structure showed a water molecule linking the piperidine nitrogen with the backbone NH of K103 (Fig. 3).[12] From this structure we surmised that the piperidine nitrogen is not formally protonated, as it would be difficult to understand the stabilization of a charged species in the lipophilic binding site environment. It is then possible that some of the enhanced potency seen with electron withdrawing R^3 groups in Table 2 results from the reduced basicity of the piperidine nitrogen, and thence a lower cost of binding the neutral form.

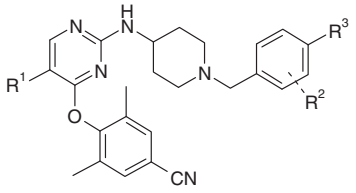
Further aspects of the modeling hypothesis were confirmed when the **5h** crystal structure was overlaid with the protein bound structures of Etravirine and GW-69564 as shown in Figure 4. Thus, in **5h** the edge-to-face interaction of the tail ring with W229 and the hydrogen bond between the aminopyrimidine NH and the K101 backbone carbonyl both emulate Etravirine, and these factors may contribute to the similarity in mutant profile for the two compounds. Likewise, the water-mediated H-bond of **5h** with K103 effectively parallels the interaction of the GW-69564 carbonyl with the same K103 NH, and occupancy of the lipophilic P236/V106 front pocket is similar as well. Finally, an interaction between the 4-sulfone oxygen of **5h** and V106 can be seen near the protein/solvent interface (Fig. 3). Therefore, the hybrid molecule **5h** appears to have achieved the main interactions hoped for in the design.

The best compounds from this study were the sulfone **5k** and the sulfonamide **5m**. It may be noted that antiviral activities for these compounds were more favorable than might have been predicted from their enzyme inhibitory potencies. These differences relate to template-specific variations between HIV-RT-RNA binding affinity and polymerase processivity, and have been observed in most NNRTI series.[16] An expanded set of antiviral data for **5k**



Scheme 2. Reagents and conditions: (a) **1** or **2**, DIPEA, NMP, 100 °C; (b) TFA, CH_2Cl_2; (c) benzyl bromides, K_2CO_3, DMF.

Table 2
Enzyme and antiviral potency of *N*-benzyl piperidines **5a**–**5p**



				Enzyme IC_{50} (nM)			Antiviral IC_{50} (nM)		
#	R^1	R^2	R^3	WT[a]	KY[a]	Y188L[a]	WT[b]	KY[b]	Y188L[b]
5a	H	H	H	87	216	372	3.3	>100	43.6
5b	Br	H	H	19	15	70	6.5	>100	9.1
5c	Br	H	3-Cl	241	229	—	—	—	—
5d	Br	H	4-Cl	152	129	793	—	—	—
5e	Br	H	2-CN	29	62	43	—	—	—
5f	Br	H	3-CN	23	27	37	—	—	—
5g	Br	H	4-CN	21	18	41	—	—	—
5h	H	H	4-SO_2CH_3	34	50	72	0.3	20.2	16.9
5i	Br	H	4-SO_2CH_3	28	14	28	5.2	35.7	<1.2
5j	H	2-Cl	4-SO_2CH_3	22	30	263	24.6	67.2	46.5
5k	Br	2-Cl	4-SO_2CH_3	45	28	216	6.1	7.3	2.8
5l	H	2-Cl	4-SO_2NH_2	84	25	105	4.4	21.4	1.4
5m	Br	2-Cl	4-SO_2NH_2	90	21	208	9.9	18.9	3.2
5n	Br	2-Cl	4-CN	146	159	—	17.6	67.4	11.6
5o	Br	2-Cl	4-$CONH_2$	131	69	519	41.5	52.7	30.2
5p	Br	2-Cl	4-CO_2H	32	28	86	3.9	74.2	—

[a] Enzyme inhibition, heteropolymeric assay.
[b] Cell based antiviral assay in the presence of 10% FBS (see Ref. 15).

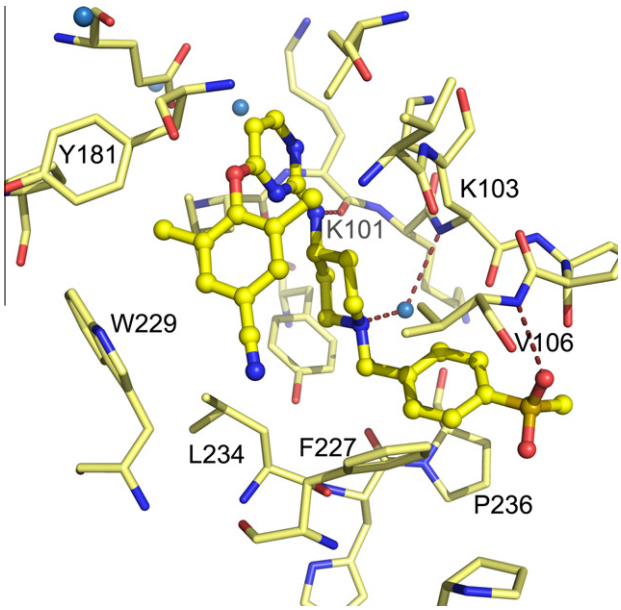


Figure 3. Crystal structure of *N*-benzyl piperidine **5h** showing water bridged H-bond interaction with K103 NH.

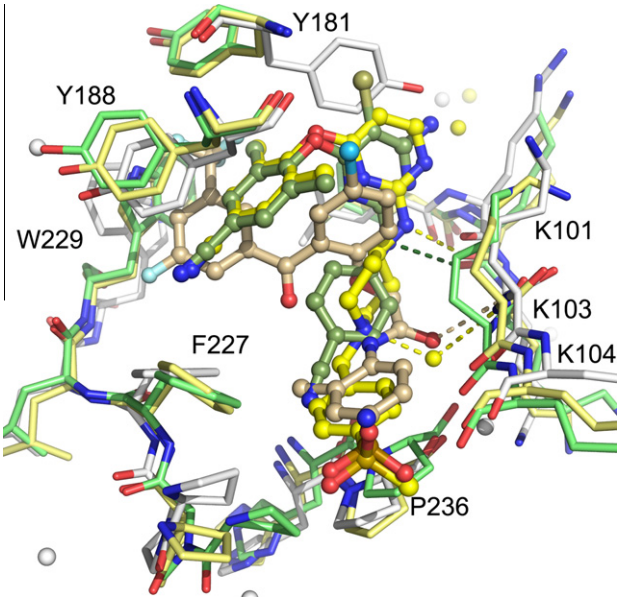


Figure 4. Overlay of crystal structures of **5h** (yellow), Etravirine (green) and GW-69564 (tan) in wild type HIV-RT enzyme.

Table 3
Antiviral potencies of **5k** and **5m** compared to reference NNRTIs[a]

	WT, 10% FBS	WT, 40% HuS[b]	K103N/ Y181C	K103N/ L100I	Y188L	G190A	V106A
Efavirenz	1.8	18.1	82.8	>500	>1K	6.0	5.7
5k	6.1	48.7	7.3	5.9	2.8	1.8	2.0
5m	9.9	61.5	18.9	9.1	3.2	6.0	2.3
Etravirine	2.1	25.9	9.2	9.5	3.1	1.1	2.0

[a] Antiviral IC_{50}'s are given in nM.
[b] WT activity in the presence of 40% HuS is an indicator of protein shifted potency.[17]

and **5m** is given in Table 3 and includes values for protein shifted WT potency,[17] antiviral activities versus further mutants of interest, and comparative data for selected reference NNRTIs. Analog **5k** was quite potent against all mutants tested, and as such displayed a highly promising profile for a next-generation NNRTI. **5m** was less active but the sulfonamide analogs remain of interest for their potential as prodrug substrates.

In summary, we have discovered a novel series of *N*-benzyl piperidine compounds which combine features of both K101 and K103 main-chain binding types of NNRTIs. The best compound **5k** has good potency against wild type RT and several important mutant strains in both enzyme and antiviral assays. The series has a unique binding mode which utilizes a bridging water molecule between the piperidine nitrogen and the K103 backbone. Further work is in progress towards improving overall antiviral profile and protein shifted potency for derivatives using the novel piperidinyl aminopyrimidine substructure. Thus, exploration starting from the secondary *N*-phenyl lead **6** has already yielded compounds with superior antiviral properties, and that work will be the subject of another publication.

Acknowledgements

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12. Atomic coordinates for the structures of Etravirine and **5h** bound to HIV/RT have been deposited with the RCSB Protein Data Bank (PDB) under the access codes 3M8P, and 3M8Q, respectively.
13. *Enzymatic assay*: IC_{50} values were obtained from a SPA heteropolymeric assay. RNA-dependent DNA polymerase activity was measured using a biotinylated primer oligonucleotide and tritiated dNTP substrate. Newly synthesized DNA was quantified by capturing the biotinylated primer molecules on streptavidin coated Scintillation Proximity Assay (SPA) beads (Amersham).
14. For a detailed description of the syntheses of aminopyrimidine inhibitors see: Kertesz, D. J.; Brotherton-Pleiss, C.; Yang, M. US 2008/0146595.
15. *Replication assay*: EC_{50} values were obtained following incubation of compound with infected MT4 cells in the presence of 10% fetal bovine serum (FBS). 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. For a detailed description of the anitiviral assay see: Pauwels, R.; De, C. E.; Desmyter, J.; Balzarini, J.; Goubau, P.; Herdewijn, P.; Vanderhaeghe, H.; Vandeputte, M. *J. Virol. Methods* **1987**, *16*, 171.
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17. Determination of potency versus wild type virus was repeated in the presence of 40% human serum (HS) for these compounds. The resulting 'protein shifted potency' as compared to the result with 10% FBS (see Ref. 15) is considered an indicator of potential for in vivo activity.