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Nucleotide competing reverse transcriptase inhibitors: Discovery of a series of non-basic benzofurano[3,2-*d*]pyrimidin-2-one derived inhibitors

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This manuscript is dedicated to the memory of a respected friend and colleague, Dr. Louis Morency.

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ABSTRACT

A HTS screen led to the identification of a benzofurano[3,2-*d*]pyrimidin-2-one core structure which upon further optimization resulted in **1** as a potent HIV-1 nucleotide competing reverse transcriptase inhibitor (NcRTI). Investigation of the SAR at N-1 allowed significant improvements in potency and when combined with the incorporation of heterocycles at C-8 resulted in potent analogues not requiring a basic amine to achieve antiviral activity. Additional modifications at N-1 resulted in **33** which demonstrated excellent antiviral potency and improved physicochemical properties.

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In 2011, the United Nations estimated that 34 million people worldwide were infected with the Human Immunodeficiency Virus (HIV).¹ Additionally, more than 2 million new cases of infection were reported, and the number of AIDS related deaths was estimated at 1.7 million.² Currently marketed treatments for HIV fall into six major categories: chemokine antagonists,³ fusion inhibitors,⁴ integrase inhibitors,⁵ protease inhibitors,⁶ nucleoside/ nucleotide (NRTI)⁷ and non-nucleoside inhibitors (NNRTI).⁸ Together the two classes of reverse transcriptase (RT) inhibitors make up almost half of all the available treatments. A few years ago, a new class of RT inhibitors has been reported.⁹ Similar to the NRTI's, biochemical studies showed these compounds to be competitive with the next incoming nucleotide and consequently they were termed nucleotide-competing reverse transcriptase

[†] Dr. Louis Morency passed away suddenly on July 24, 2012.

inhibitors (NcRTI's).¹⁰ Like the NNRTI class, these inhibitors are structurally distinct from the deoxyribonucleotide triphosphates (dNTPs), and in contrast to the NRTI's they are not chain terminators. As a result of these differences relative to the established classes of RT inhibitors, the NcRTI class presents a distinct resistance profile, rendering this an attractive mode of inhibition of this enzyme.

The tricyclic urea **1** (Fig. 1) arose as a promising lead based on initial optimization of the potency and cytotoxicity index of a hit derived from a high throughput screening campaign. Early SAR around this core revealed that all three pharmacophores were tolerant of basic functionality and this resulted in improved antiviral activity. Profiling data of inhibitor **1** showed promising metabolic stability in human and rat liver microsomal preparations but poor apical to basolateral (AB) permeability in Caco-2 monolayers, presumably as a result of the highly basic nature of two of the three pharmacophores.¹¹ Sequential removal of the basic amines showed that the presence of only one was sufficient to maintain satisfactory levels of antiviral potency. With this knowledge of the SAR, the initial strategy to optimize the series was to maintain

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Figure 1. Benzofurano[3,2-d]pyrimidin-2-one lead scaffold.

a single basic amine to drive the potency and attenuate the basicity of the inhibitors in order to improve the in vitro profile, in particular the permeability. This would also reduce the potential risk for undesirable off-target activities associated with highly basic and lipophilic compounds (e.g., phospholipidosis¹²).

Initially, the optimization of the series began with a detailed investigation of the N-1 position for which previous investigations had demonstrated the potential to improve potency. To facilitate generation of SAR, a model analogue was selected containing a benzylamine moiety at C8 as the only basic functional group (Table 1). The parent analogue **2** containing the 2-methoxyethyl substituent at N-1 (entry 1) showed weak activity on this unoptimized scaffold. Remarkably, extending the ether chain by one methylene unit resulted in a 60-fold increase in activity (entry 2). Further extending the chain resulted in **4**, which showed no additional advantage. In the hydrocarbon series, a more detailed investigation could be undertaken. Shorter analogues ranging from methyl to *i*-Bu (entries 4–8) showed relatively poor but similar potency

Table 1 N-1 SAR



Entry	Compd	R	$IC_{50} (nM)^a$
1	2	-(CH ₂) ₂ OMe	2330
2	3	–(CH ₂) ₃ OMe	39
3	4	–(CH ₂) ₄ OMe	86
4	5	Me	645
5	6	Et	890
6	7	n-Pr	>685 (45%)
7	8	<i>i</i> -Pr	800
8	9	i-Bu	655
9	10	<i>n</i> -Bu	42
10	11	<i>n</i> -Pentyl	27
11	12	$-(CH_2)_2CHMe_2$	190
12	13	$-(CH_2)_3CHMe_2$	55
13	14	(S)-CH ₂ CH(Me)CH ₂ OMe	635
14	15	(R)-CH ₂ CH(Me)CH ₂ OMe	195
15	16	$-(CH_2)_3Ph$	>365 (25%)
16	17	$-(CH_2)_3CO_2H$	>4700 (46%)
17	18	$-(CH_2)_3CO_2Et$	>1100 (14%)
18	19	Ph	245
19	20	<i>p</i> -Nitrophenyl	1.7
20	21	4-Methyl-3-cyanophenyl	7.3

^a Values in parentheses are the percent inhibition at the highest concentration achieved.

whereas a significant increase was observed with the five-atom *n*-pentyl group (entry 10). Interestingly, compound **10** containing an *n*-butyl group was found to be significantly more active than the corresponding ether analogue 2, however, there was no difference between the 5-atom analogues 3 and 11. The addition of branching substituents seemed to be less well tolerated as illustrated by the four fold loss of potency observed between the *n*-Bu analogue **10** and **12** (entries 9 and 11). This effect was also observed in the ether series as demonstrated by 14 (entry 13) and 15 (entry 14) which incorporated a methyl group on the chain and showed a 5 to 15-fold loss in activity. Interestingly, an eutomer effect was observed with these two analogues, the analogue with Rstereochemistry being more potent. Further exploration demonstrated that larger substituents such as 3-phenylpropyl (entry 15) and substituents containing a polar group such as acid (entry 16) and ester (entry 17), were poorly tolerated. Aromatic substitutions at the N-1 position resulted in very potent analogues: however, these compounds generally displayed unsatisfactory levels of cytotoxicity (entry 19). Taken together, these results demonstrated a clear preference for straight chain lipophilic groups at the N-1 position and that both the *n*-pentyl and 3-methoxypropyl groups would serve as useful substituents to elucidate the SAR at other positions.

Having demonstrated that the N-1 position allowed significant improvements in potency while maintaining a single basic amine, efforts were undertaken in order to improve the in vitro profile of monobasic analogues. Previous SAR had shown that the presence of a basic amine at the C8 position was associated with significant hERG inhibition as well as an increase in the number of hits in an off-target pharmacology panel. Since these issues were substantially less pronounced for analogues containing a basic group at C4, the initial strategy was to maintain the basic amine at this position in conjunction with the 3-methoxypropyl group at N-1 as the key pharmacophores to drive the potency (Table 2). Preliminary SAR demonstrated that constricting the N-ethylglycineamide into an alkylpiperazine (22-23) maintained potency and also afforded a modest increase in permeability. Therefore, subsequent SAR was aimed at decreasing the pK_2 of the amine to further improve the Caco-2 permeability. More than sixty compounds covering a range of pK_a values were prepared, several of which demonstrated significant improvements in Caco-2 permeability (e.g., entries 4, 5 and 7). In fact, consideration of a series of these compounds showed a trend indicating a dependence of the Caco-2 permeability on the calculated pK_a (data not shown).¹³ Analogs with a calculated pK_a of 8 or higher were associated with Caco-2 permeability less than 5×10^{-6} cm/s. Unfortunately, while it was clear that attenuating the pK_a of the compounds could afford significant improvements in permeability, no compounds were discovered that maintained both metabolic stability and acceptable permeability in this series. Additionally, the less basic compounds generally displayed reduced antiviral activity (entry 3 vs 7). Interestingly, it was shown that the piperazinyl pyridine analogues (e.g., 27) possessed similar activity and in vitro profiles relative to their non-aza counterparts (entry 2). Consequently, this modification was adopted in further SAR studies in order to mitigate the potential for genotoxicity associated with the presence of an aniline.¹⁴

Concurrent SAR focused at C-8 revealed that small heterocycles were viable replacements for the basic amine of **1** or the halogen of **22**. A number of heterocycles were introduced including various regioisomers of substituted pyrazoles, thiazoles, oxazoles and pyridines. The five-membered heterocycles were generally well tolerated and the 4-substituted regioisomers were usually preferred. In particular 1-methylpyrazole-4-yl became a preferred substituent at this position (e.g., **29**, Table 3). Not only did this group result in an analogue with excellent enzyme and cell based antiviral activity, a significant improvement in cytotoxicity was also

Table 2C4 SAR directed toward improving Caco-2 permeability



Entry	Compd	Х	R	cpK _a ^a	IC ₅₀ (nM)	EC ₅₀ (nM)	CC ₅₀ (µM) ^c	HLM CL (%Q _H) ^b	Caco-2 (10 ⁻⁶ cm/s)
1	22	СН	N H H NHEt	7.8	2.8	33	>8.6	46	<0.1
2	23	СН		8.2	9.1	35	2.3	51	2.7
3	24	СН		8.8	1.7	25	2.7	28	0.3
4	25	СН		8.1	13	290	>3.6 (20)	52	8.9
5	26	СН	N N N OMe	7.0	10	130	>4.2	61	8.0
6	27	N		8.0	3	37	>3.2	59	2.6
7	28	N		6.9	8	56	ND^{d}	78	12

^a pK_a Values were calculated using ACD/PhysChem Suite version 12, ACD Labs, Toronto.

^b Microsomal clearance is reported as the measured $t_{1/2}$ normalized with respect to the hepatic blood flow of the given species.

^c Values in parentheses are the percent inhibition at the highest concentration achieved.

^d ND = not determined.

observed. The excellent antiviral activity observed with the combination of optimized N-1 and C-8 substituents, along with the challenges encountered during our efforts to improve the physicochemical properties of the series by attenuating the basicity of the amine (vide supra), prompted us to consider the truncation of the C-4 piperazine group thereby completely eliminating the basic amine. Application of this strategy to piperazine **29** resulted in **30** which demonstrated a level of antiviral activity which while reduced was nonetheless encouraging for a non-basic inhibitor. Incorporation of the more potent *n*-pentyl fragment resulted in **31** which for the first time gave a compound with excellent antiviral activity but no strongly basic centers. Further profiling of these analogues revealed that the methoxypropyl fragment imparts good aqueous solubility whereas the *n*-pentyl analogue **31** results in significantly increased lipophilicity as well as poor solubility at pH 6.8. Given the disparity in the antiviral and solubility data between analogues containing these N-1 substituents, a hybrid structure was sought which would maintain the favorable properties of both. Further contemplation of the structural similarity of these two fragments led to the tetrahydrofurylethyl moiety as in **32**. Upon synthesis of the racemic analogue **32** we were delighted to observe that this compound, containing no basic amine, showed an EC_{50} value of 13 nM and excellent aqueous solubility at both pH 2 and 6.8.

Encouraged by these results we initiated a synthesis of the individual enantiomers of **32** (see Schemes 1 and 2). Given the poor metabolic stability of **32**, the chiral N-1 fragments were grafted onto the more optimized 6-aza core (Table 4). Previous SAR indicated that this modification had a beneficial effect on both the lipophilicity and microsomal stability of the series. In the

Table 3

Discovery of non-basic inhibitors



^b ND = not determined.

event, an eutomer effect was observed and the *S* enantiomer **33** proved to be slightly more potent. Additionally, the 6-aza modification showed the expected improvement in the metabolic stability in human liver microsomes and the excellent aqueous solubility of **32** was retained in the chiral analogue **33**. Despite the relatively poor Caco-2 permeability, the promising overall profile and good potency without the necessity of a basic functional group represented a significant advancement of the series.

Compound **33** was profiled against a number of recombinant viruses with resistant mutations to known reverse transcriptase

inhibitors (Table 5). As expected, the potency of analogue **33** was not affected by the NNRTI-induced resistance mutation Y188L. Similarly, the NNRTI-resistance mutation K103N/Y181C was fully susceptible to inhibition by analogue **33**. Consistent with observations made for other NcRTI's,^{9b,c} the K65R RT mutant virus (resistance mutation to NRTI's such as tenofovir) was hypersusceptible to inhibition by compound **33** compared to wild type virus. Limited cross-resistance was observed between compound **33** and the NRTI class. Viruses harboring the M184V mutation, displayed a fourfold shift in EC₅₀ values. In addition, the thymidine associated muta-



Scheme 1. Reagents and conditions: (a) Cs₂CO₃, DMF, 90 °C; (b) ClCO₂Et, Na₂CO₃, toluene, reflux; (c) DIAD, PPh₃, THF; (d) K₂CO₃, Pd(dppf)Cl₂, dioxane, H₂O, 90 °C; (e) 2-bromo-5-fluoropyridine, *n*-BuLi, THF, -78 °C; (f) (i) MeNH₂, DMSO, 90 °C; then (ii) NH₄OAc, 130 °C.



Scheme 2. Reagents and conditions: (a) (i) Ethyl chloroformate, Et₃N,THF, -20 °C; (ii) CH₂N₂ (b) PhCO₂Ag, Et₃N, MeOH, 0 °C; (c) LiAlH₄, THF.

Table 4

In vitro profiles of 33 and 34



^a Microsomal clearance is reported as the measured $t_{1/2}$ normalized with respect to the hepatic blood flow of the given species.

Table 5

Antiviral profile of 33 versus RT resistant mutations

Virus	EC ₅₀ (nM)	Fold (vs WT)		
WT	19.7	_		
Y188L	20	1		
K103N/Y181C	39	2		
K65R	3.7	0.19		
M184V	78.3	3.9		
TAM-1 ^a	147.5	7.4		
TAM-2 ^b	27	1.4		
Q151M complex ^c	3.7	0.19		

^a TAM-1 = M41L/T67N/L210W/T215Y/K219E.

^b TAM-2 = M41L/D67N/K70R/T215F/K219E.

^c Q151M complex = A62V/V75I/F77L/F116Y/Q151M.

tions (TAM-1) complex displayed a sevenfold loss in susceptibility to compound **33** while a mutant bearing the Q151M mutation complex was hypersusceptible (fivefold) to inhibition. Overall, compound **33** displays minimal to no cross-resistance with both NRTI and NNRTI classes which suggests that an NcRTI may be appropriate for dosing in combination with other HIV RT inhibitors.

Synthesis

The synthesis of compounds **33** and **34** is outlined in Scheme 1. A one-pot condensation-cyclization sequence of ethyl glycolate with the commercially available chloropyridine **35**, afforded the aminobenzofuran **36** in modest to good yield. Carbamoylation with ethyl chloroformate gave **37** onto which the N-1 substituent was then incorporated via the Mitsunobu¹⁵ reaction of either (*R*)- or (S)-2-(tetrahydrofuryl-2-yl)ethanol (Scheme 2). Suzuki–Miyaura¹⁶ coupling allowed introduction of the C-8 pyrazole in low to modest yield over two steps. The lower yield at this stage is presumably a consequence of the Mitsunobu reaction, the product of which was invariably contaminated with significant amounts of DIAD related by-products even after repeated chromatography. Consequently, purification was best achieved after the Suzuki–Miyaura coupling.¹⁷ Incorporation of the C-4 pyridine ring was accomplished by treatment of a mixture of **40** and 2-fluoro-5-bromopyridine in THF with *n*-BuLi followed by quench at low temperature (HOAc in THF) to afford ketone **41**. Treatment with methylamine was followed by heating the resulting product in fused NH₄OAc at 130 °C to accomplish the formation of the pyrimidone ring. Analogues contained in Tables 1–3 were prepared using similar methods.¹⁸

The preparation of the required chiral alcohols **38** was carried out as shown in Scheme 2. The Arndt–Eistert¹⁹ protocol was utilized as the key step of the sequence. Conversion of the commercially available acid **42** to the mixed anhydrides followed by treatment with excess diazomethane gave the diazoketones **43** in good yield. Silver mediated Wolff rearrangement^{19a} of **43** in MeOH gave the corresponding methyl esters in acceptable yield. Finally, LAH reduction of **44** afforded the desired chiral alcohols **38** in good yield.

In conclusion, we have optimized a series of benzofurano[3,2*d*]pyrimidin-2-one derived nucleotide-competing reverse transcriptase inhibitors. Incorporation of appropriate substitution at the N-1 position in combination with heterocycles at C-8 generated analogues with excellent antiviral potency and obviated the necessity for a basic amine. Further modifications showed that the (*S*)-2-(tetrahydrofuryl-2-yl)ethyl group provided a potent analogue with improved aqueous solubility. Efforts toward the further optimization of the in vitro profile of the series as well as the impact on the susceptibility to M184V and TAM-1 complex will be reported elsewhere.

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Supplementary data

Supplementary data (a description of the enzymatic and cell based assays (antiviral and cytotoxicity), as well as methods for Caco-2 permeability, solubility, metabolic stability in microsomes, log*D* and solubility. Schemes describing the general synthesis of the compounds not covered in Schemes 1 and 2 are also included) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.021. These data include

MOL files and InChiKeys of the most important compounds described in this article.

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