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Identification of benzofurano[3,2-*d*]pyrimidin-2-ones, a new series of HIV-1 nucleotide-competing reverse transcriptase 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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Benzofurano[3,2-*d*]pyrimidin-2-one

ABSTRACT

Screening of our sample collection led to the identification of a set of benzofurano[3,2-*d*]pyrimidine-2-one hits acting as nucleotide-competing HIV-1 reverse transcriptase inhibitors (NcRTI). Significant improvement in antiviral potency was achieved when substituents were introduced at positions N1, C4, C7 and C8 on the benzofuranopyrimidone scaffold. The series was optimized from low micromolar enzymatic activity against HIV-1 RT and no antiviral activity to low nanomolar antiviral potency. Further profiling of inhibitor **30** showed promising overall in vitro properties and also demonstrated that its potency was maintained against viruses resistant to the other major classes of HIV-1 RT inhibitors.

According to the United Nations, 34 million individuals were living with the human immunodeficiency virus (HIV) at the end of 2010. In the same year, it was estimated that 2.5 million people contracted HIV and the number of Acquired Immune Deficiency Syndrome (AIDS) related deaths worldwide was estimated to be 1.7 million.[1] Over 30 years of HIV research has resulted in more than 25 approved antiretrovirals. Additionally, combination antiretroviral therapy (cART) has significantly improved the life expectancy of HIV positive patients over the years.[2] The currently approved drugs belong to six classes: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors, CCR5 inhibitors and integrase inhibitors. Almost half of these drugs target HIV-1 reverse transcriptase (RT), a key multifunctional enzyme involved in post-entry viral replication.[3] These functions include both RNA-dependent DNA polymerase and ribonuclease H strand transfer catalytic activity. The chronic use of RT inhibitors associated with the high error rate of HIV-1 RT during the replication process has resulted in the emergence of multi-drug resistant viruses.[4] Consequently, there is a need for the development of potent and less toxic[5] RT inhibitors that retain high potency against wild type HIV-1 reverse transcriptase and drug-resistant variants.

A few years ago, two groups identified indolopyridones with antiretroviral activity from cell-based screening campaigns.[6] They found that these inhibitors targeted the polymerization activity of RT, with a mechanism of action that involves a competitive binding with the incoming deoxyribonucleoside triphosphate (dNTP) but without being chain terminators. The following year a third group identified a set of 4-dimethylamino-6-vinylpyrimidines which also inhibited the RT polymerization with a related mechanism.[7] The antiviral potency of the indolopyridones remained unaffected by most of the NNRTI and NRTI resistant strains and could be potentially used both in naïve and in treatment-experienced patients or combined with other classes of RT inhibitors.[8] This novel third class of RT inhibition is now referred as nucleotide-competing reverse transcriptase inhibitors (NcRTIs).[9]

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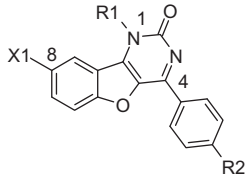
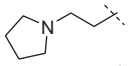
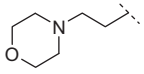
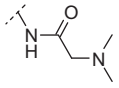
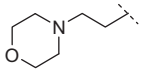
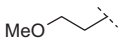
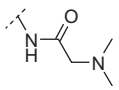
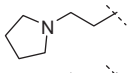
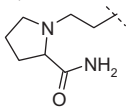
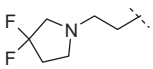
Inspired by these results, we carried out a screen of our compound collection in order to identify novel compounds displaying this mode of action.[10] A total of 7335 primary hits were identified. A series of subsequent profiling assays were performed including counter-screens against sensitivity to well characterized NNRTI resistance RT mutations and RT enzyme steady state kinetics. As a result, several benzofuranopyrimidone hits acting as NcRTIs (Table 1, inhibitors **1**, **2** and **3**) were identified.[11] Upon testing related analogues from our compound database, we realized that enzymatic activity was lost when the 2-pyrrolidinylethyl chain of inhibitor **1** was replaced by a 2-morpholinylethyl (**4**) or a methyl (**5**).[12] A greater than fourfold improvement in intrinsic potency was observed when a chlorine atom was introduced at position C8 (cf. **2** and **4**). The 2-dimethylaminoacetamide group of **3** seemed important because this compound was found to be at least 26-fold more potent than **5**. Upon screening different N1 substituents, we found that replacing the N1 methyl of **3** by a 2-methoxyethyl (**6**) resulted in a small gain in intrinsic potency. Since we observed that inhibitor **2** was more potent than compound **4**, we prepared inhibitor **7** which has a chlorine atom at C8. In this case, the modification did improve the IC_{50} value by sevenfold compared to **1**. Keeping a chlorine atom at C8, we further evaluated the impact of modifying the N1 pyrrolidinylethyl side chain on potency. Even though amide **8** and difluoropyrrolidinyl **9** were found to be equipotent to **7**, these side chains presented different physicochemical properties. For instance, inhibitors **8** and **9** have a reduced pK_a compared with **7** (calcd pK_a[13] of 9.9, 8.1 and 5.3 for **7**, **8** and **9,** respectively) which might have an impact on in vitro proprieties upon combining with other appendages. Clearly, substitution at positions N1, C4 and C8 on the benzofuranopyrimidone scaffold was found to play a key role for potency. Unfortunately, no antiviral activity was discernable for the compounds in Table 1 due to the poor cytotoxicity index (CC_{50}/EC_{50} <5-fold). We thus decided to further evaluate this series with the primary focus on establishing unambiguous antiviral potency and its potential to yield a lead optimization NcRTI series.

Using compound **6** as a starting point, we verified if productive SAR could be obtained upon modifying position C4 (Table 2). Incorporation of a small substituent, such as a methyl at the alpha position to the carbonyl, was tolerated (cf. **6** and **10**). Additionally, modification of the amino group had a modest impact on potency. Rigidifying the dimethylamino group to the corresponding pyrrolidine containing derivative **11** improved potency by threefold. It seemed that a hydrogen bond donor was preferred at this position since replacing the dimethylamino group of **6** with an *N*-ethyl substituent (**12**) improved intrinsic potency to 73 nM. Incorporation of a chlorine atom at position C8 of inhibitor **12** had a modest impact on potency (compound **13**). Reducing the carbonyl anilide of **11** to the corresponding aniline **14** improved intrinsic potency by threefold.

Replacing the anilide linker of **6** with ethers (**15** and **16**) or an amide (**17**) was not well tolerated since these modifications resulted in at least threefold antiviral potency loss. Upon screening other N-linked analogues, we found that *N*-arylpiperazines were also favored (**18** and **19**). As observed earlier, incorporating hydrogen bond donors improved antiviral potency (cf. **18**, **19** and **20**). Inhibitor **20** was one of the most potent compounds prepared in this study with an antiviral potency of 87 nM. This example

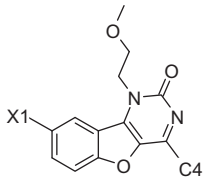
Table 1
Potency and cytotoxicity of compounds identified during uHTS campaign and related analogues

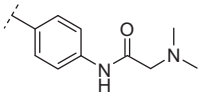
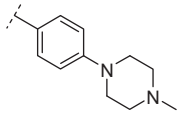
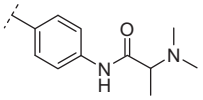
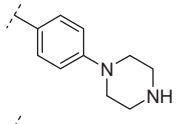
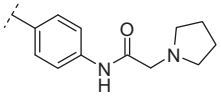
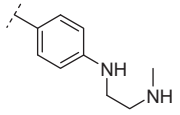
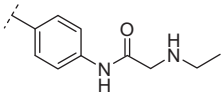
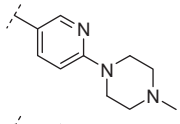
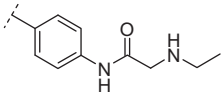
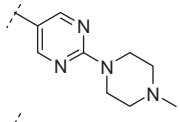
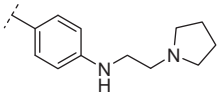
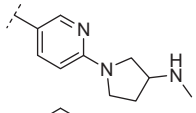
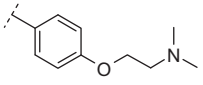
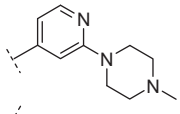
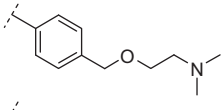
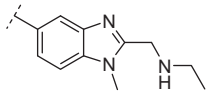
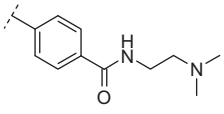


Compd	R1	R2	X1	IC_{50} (nM)	EC_{50} (nM)	CC_{50}[a] (nM)
1		H	H	4200	11,000	18,000
2		H	Cl	6500	–	–
3	Me		H	3100	1600	7700
4		H	H	>26,000	–	–
5	Me	H	H	>83,000	–	–
6			H	1200	3600	>11,000 (15%)
7		H	Cl	590	3700	>7300 (6%)
8		H	Cl	650	2600	>6700 (–)
9		H	Cl	320	2900	>4500 (–)

[a]The number in parentheses is the percent inhibition at the highest concentration achieved.

Table 2
Impact of C4 modifications on potency and cytotoxicity



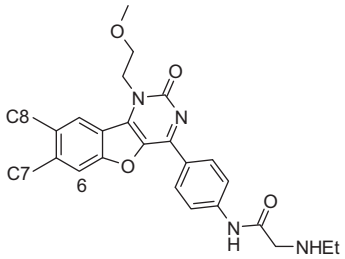
Compd	X1	C4	IC_{50} (nM)	EC_{50} (nM)	CC_{50}[a] (nM)	Compd	X1	C4	IC_{50} (nM)	EC_{50} (nM)	CC_{50}[a] (nM)
6	H		1200	3600	>11,000 (15%)	**18**	Cl		24	430	>4500 (18%)
10	H		1400	4400	>11,000 (14%)	**19**	Cl		10	175	2700
11	H		390	1100	5950	**20**	Cl		10	87	960
12	H		73	680	5100	**21**	Cl		75	890	>5800 (37%)
13	Cl		46	550	3000	**22**	H		155	690	>9500 (40%)
14	H		120	2400	>7400 (–)	**23**	Cl		40	210	4300
15	H		630	14,000	–	**24**	Br		>5000	–	–
16	H		818	>11,000	–	**25**	Br		42	540	>4400 (20%)
17	H		945	>9300	–						

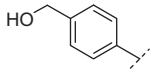
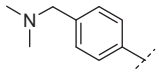
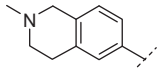
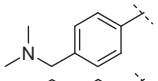
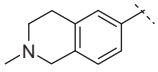
[a] The number in parentheses is the percent inhibition at the highest concentration achieved.

showed the synergistic effect on potency of reducing the anilide carbonyl (cf. **6**), incorporating two hydrogen bond donors (cf. **18**) and having an optimal *N*-alkyl substituent (cf. **14**). Knowing the risks associated with potential bioactivation of anilines[14] (e.g., **18** and **19**), we prepared the corresponding 2-piperazinylpyridine and the 2-piperazinylpyrimidine analogues (**21** and **22**, respectively). These compounds were found to be equipotent with **18** in our cell-based assay. Antiviral potency could be further improved when different amines were incorporated on the pyridine (e.g., **23**). *para* substituted piperazinylpyridines were favored over the *meta* isomer (cf. **21** and **24**).[15] Bicyclic heterocycles could also be introduced at position C4. For example, rigidification of the 2-ethylaminoacetamide of **13** to the corresponding benzimidazole **25** was well tolerated. From this study, we established that considerable improvement in potency and cytotoxicity index could be achieved when modifications (e.g., increasing hydrogen bonds and reducing anilide carbonyl) were performed at position C4.

After studying the effect of C4 and N1 modifications on potency and cytotoxicity index, we turned our attention toward evaluating the impact of C7/C8 substitution on potency (Table 3). The introduction of a phenyl group (**26**) or a 3-pyridyl substituent (**27**) at position C8 resulted in a 2- to 3-fold improvement in intrinsic and antiviral potencies compared to **12**. Incorporation of a hydroxymethyl appendage on the C8 phenyl (**28**) had no impact on potency, but introduction of a dimethylamino group (**29**) was beneficial. At this point, we investigated whether rigidification of the dimethylamino functionality would improve potency further. It was gratifying to obtain an IC_{50} value of 4.2 nM for the *N*-methyltetrahydroisoquinoline derivative **30**. In addition, **30** turned out to be the most potent analogue of this series with an EC_{50} of 30 nM. Interestingly, we realized that some of the most potent C8 substituents were also tolerated at C7 (e.g., **31** and **32**). A similar exercise was performed at position C6, but this did not lead to a significant improvement in potency. From these results, it seemed

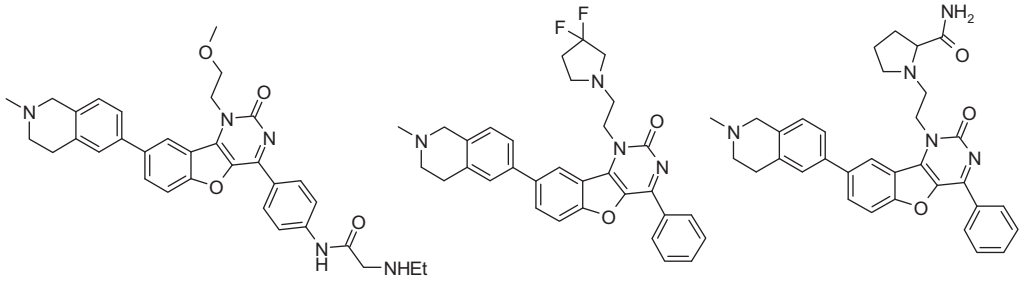
Table 3
Impact of C8 and C7 modifications on potency and cytotoxicity



Compd	C8	C7	IC_{50} (nM)	EC_{50} (nM)	CC_{50}[a] (nM)
12	H	H	73	680	5100
26		H	33	360	2300
27		H	27	200	>5100 (33%)
28		H	34	300	>1300 (–)
29		H	12	94	3900
30		H	4.2	30	4800
31	H		7.4	53	1000
32	H		8.6	88	2100

[a] The number in parentheses is the percent inhibition at the highest concentration achieved.

Table 4
Cross-resistance profile of selected C8 *N*-methyltetrahydroisoquinolinyl derivatives[a]



	30	**33**	**34**
IC_{50} (nM)	4.2	20	5.9
EC_{50} WT (nM)	30	25	52
CC_{50}/EC_{50}	137	>46	>127
Serum shifted EC_{50} (fold)	1.5	7.3	8.4
EC_{50} K65R (fold)	0.1	0.3	0.3
EC_{50} Y115F (fold)	2	–	–
EC_{50} M184V (fold)	1.1	4.8	5.8
EC_{50} Y115F/M184V (fold)	3.3	14.8	14.2
EC_{50} K103N/Y181C (fold)	1.1	1.7	1.7

[a] See Supplementary data for assay details.

potency can be improved when basic groups were incorporated at various positions around the core.

Having discovered the impact of the presence of an *N*-methyltetrahydroisoquinolinyl substituent at C8 on potency, we introduced this group on previously prepared inhibitors with different pyrrolidinylethyl N1 side chains (Table 4). Interestingly, the C4 2-ethylaminoacetamidylphenyl group was not required to provide similar level of antiviral potency for **33** and **34** compared to **30**.

Table 5
In vitro profile of selected C8 *N*-methyltetrahydroisoquinolinyl derivatives[a]

	30	33	34
Sol pH 2.0/6.8 (μg/mL)	>822/650	>1000/<0.1	781/725
Caco-2 ($\times 10^{-6}$ cm/s)	<0.1	0.55	0.17
HLM/RLM $t_{1/2}$ (min)	93/140	19/6	73/60
Log *D*	2.4	3.9	3.0

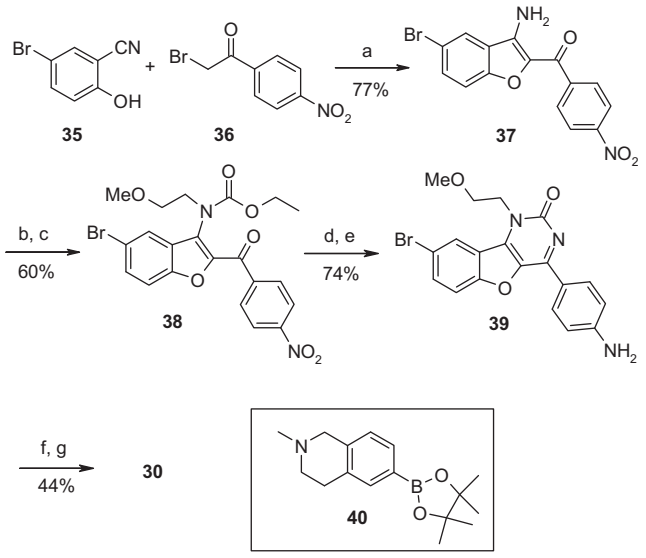
[a] See Supplementary data for assay details.

Although these compounds were equipotent, the antiviral potency of **30** was less shifted in the presence of 50% human serum. This might be related to the higher lipophilicity (log*D*, see Table 5) of the latter analogues. Inhibitors **30**, **33** and **34** were further profiled against recombinant viruses expressing clinically relevant RT resistance mutations. As observed with other NcRTIs, an NRTI resistant virus bearing the RT K65R mutation showed a 3- to 10-fold hypersusceptibility to our compounds.[6] NRTI drug-resistant viruses (having Y115F, M184V and Y115F/M184V mutations) exhibited minimal to no cross-resistance to compound **30**. The potency of **33** and **34** decreased 5- to 6-fold and 14- to 15-fold against recombinant viruses carrying the M184V or Y115F/M184V mutations, respectively. Consistent with their NcRTI mode of action, the potency of the tested compounds was not affected by NNRTI drug-resistant mutations, such as K103N/Y181C. We also confirmed a competitive mode of action with the incoming dNTP upon performing kinetic studies with inhibitors **12**, **30** and **33**.[16]

Further in vitro profiling of compounds **30**, **33** and **34** showed excellent aqueous solubility at pH 2.0 (Table 5) but poor caco-2 permeability. This could be rationalized considering the basicity (calculated pK_a: ~8.8) of the *N*-methyltetrahydroisoquinoline moiety and that molecules carrying a charge at physiological pH are known to show poor permeability in the caco-2 assay.[17] Inhibitors **30** and **34** were reasonably stable in the presence of human (HLM) and rat (RLM) liver microsomes. Presumably as a result of its higher log*D*, compound **33** was much less stable with a short $t_{1/2}$ of 19 and 6 min in the same in vitro assays. Metabolite determination identified the difluoropyrrolidine as the major site of oxidative metabolism.

The synthesis of inhibitor **30** started with the alkylation of 4-bromo-2-cyanophenol (**35**) with alpha-bromoketone **36** to give the aminobenzofuran analogue **37** (Scheme 1). The primary amine was carbamoylated and the resulting carbamate was N-alkylated with 1-bromo-2-methoxyethane to give compound **38**. The urea ring was formed by heating **38** in melted ammonium acetate and the nitro group was reduced to aniline **39** upon treatment with iron in HCl/EtOH at reflux. The resulting amine was acylated with bromoacetyl bromide followed by displacement with ethylamine and subsequent Suzuki–Miyaura[18] cross-coupling with boronic ester **40** provided inhibitor **30**. The other inhibitors described in this Letter were prepared in a similar fashion.[10]

In conclusion, we have identified a novel NcRTI series displaying excellent antiviral potency. This was accomplished by optimizing an uHTS hit from micromolar enzymatic activity lacking antiviral activity to inhibitors with low nanomolar antiviral potency. This work demonstrated the optimization potential of the scaffold by identifying four points of diversity where potency and in vitro/in vivo parameters could potentially be improved. Modification of the C4 position had a profound impact on antiviral potency when N-substituted 4-aminoaryls were investigated. We also found that aromatic basic groups were preferred at positions C8 and C7. This portion of the RT binding site can accommodate a variety of substituents with different sizes. We discovered that an *N*-methyltetrahydroisoquinolinyl substituent at C8 provided inhibitors with the best antiviral and intrinsic potencies. We did attempt to model the binding mode of our inhibitors using the available structural information[19] on HIV RT. Due to the highly flexible nature of the polymerase active site and the requirement of the ATP moiety, we judged that docking models would not be reliable enough to support a structure-based rational design approach. Further profiling of inhibitor **30** showed desirable lipophilicity, liver microsome stability and aqueous solubility. Additionally, it maintained its potency against other classes of RT mutant viruses. Our efforts aimed at improving the permeability and pharmacokinetic profile will be reported in subsequent publications.



Scheme 1. Reagents and conditions: (a) Na_2CO_3, acetone, reflux; (b) ethyl chloroformate, K_2CO_3, toluene, 100 °C; (c) NaH, $CH_3OCH_2CH_2Br$, DMF, 90 °C; (d) NH_4OAc, 130 °C; (e) Fe, EtOH, HCl, H_2O, 90 °C; (f) bromoacetyl bromide, CH_2Cl_2,then 2 M ethylamine in THF, DMF; (g) boronic ester **40**, K_2CO_3, CsF, Pd(dppf)Cl_2, dioxane, H_2O, microwave, 120 °C.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.042.

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11. IC_{50} represents the concentration of a drug that is required for 50% inhibition in vitro and EC_{50} corresponds to the concentration required for obtaining 50% of a maximum effect in vivo. CC_{50} is defined as the concentration of a drug resulting in the death of 50 percent of the host cells.
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