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N-(4-Fluorobenzyl)-3-hydroxy-9,9-dimethyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrazino[1,2-*a*]pyrimidine-2-carboxamides a novel class of potent HIV-1 integrase inhibitors

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ABSTRACT

A novel class of tetrahydro-pyrazinopyrimidine-2-carboxamides have been identified as HIV-1 integrase inhibitors. Optimization of the initial lead culminated in the discovery of a series of compounds with high potency on the enzyme and an antiviral cell-based activity equivalent to that showed by Raltegravir, the first in class HIV-1 integrase inhibitor.

HIV integrase is one of the three enzymes encoded by the HIV genome and is essential for HIV replication. Consequently, integrase inhibitors are emerging as an exciting class of novel therapies for the management of HIV infection. In our laboratories, we discovered Raltegravir (**1**), which is the first in class HIV integrase inhibitor and represents a new and improved treatment of AIDS.[1] We also conducted a wide structure activity relationship exploration around the pyrimidone core focused on constraining the *N*-methyl group to the pyrimidone C-2 substituent. These efforts resulted in a series of potent bicyclic compounds bearing a pendent amino group located at the *pseudo*-benzylic position (e.g., compound **2**, HIV strand transfer inhibition QUICKIN IC_{50} = 69 nM).[2,3]

Herein, an alternative cyclization strategy is described which capitalizes on closing the *N*-methyl group of the pyrimidone core directly to the basic nitrogen of the side chain to provide the general structure **A** (Fig. 1).

The gem dimethyl group of compound **1** (Raltegravir) was maintained in order to eliminate the chiral center and to block potential oxidative metabolism. Preliminary exploration on this new class of compounds was directed to modify the size of the aliphatic ring and the functional groups linked to the nitrogen within it (Table 1).

The closure to a six-membered lactam **5** or a cyclic sulfonamide **6** yielded more potent enzyme inhibitors with respect to the five-membered derivatives (compounds **3** and **4**), but they showed comparatively low antiviral activity. The corresponding bicyclic amine **7** proved to have not only a good activity on the enzyme but also significant efficacy in the cell based assay under both serum conditions. A tertiary amine was also tolerated as observed for compound **8** which was found nearly equipotent to **7**. A further ring expansion to give the corresponding seven-membered ring analog **9** did not improve the cellular potency.

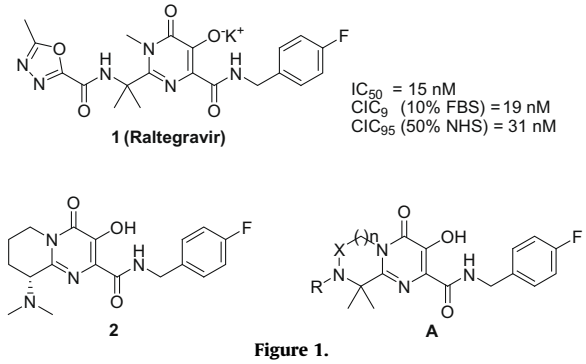

Figure 1.

The lack of a chiral center, the potency displayed in the viral spread assay and the excellent rat PK profile (Clp = 10 mL/min/kg, *F* = 57%) of compound **8** led to the investigation of further structural modifications of this new class of [6,6]-fused derivatives.

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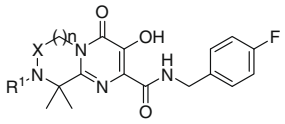
Table 1
Bicyclic pyrimidones

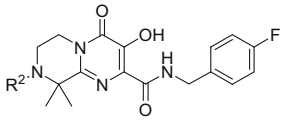


Compd	*n*	X	R^1	QUICKIN[a] IC_{50} (nM)	Spread CIC_{95}[b] (nM) (10% FBS)	(50% NHS)
3	0	CH_2	CH_3	30	500	1000
4	0	CO	H	32	>1000	>1000
5	1	CO	H	5	>1000	>1000
6	1	SO_2	H	7	5000	>10,000
7	1	CH_2	H	16	125	166
8	1	CH_2	CH_3	6	125	250
9	2	CH_2	H	20	500	250

[a] Results are the mean of at least three independent experiments. SD was always ±8% of the value. Assays were performed with recombinant HIV-1 integrase pre-assembled on immobilized oligonucleotides. Inhibitors were added after assembly and washings, and IC_{50} is the concentration of inhibitor that reduces HIV integrase activity by 50%. For details see Ref. 4.

[b] Spread assay: 95% inhibitory concentration for inhibition of the spread of HIV-1 infection in cell culture (HIV-1IIIB and MT-4 human T-lymphoid cells) in the presence of 10% heat-inactivated fetal bovine serum or 50% normal human serum. SD was always ±10% of the value. For details see Ref. 5.

Table 2
SAR on the nitrogen



Compd	R^2	QUICKIN[a] IC_{50} (nM)	Spread[b] (10% FBS) CIC_{95} (nM)
10	$COCH_3$	5	1000
11	$COCON(Me)_2$	6	1000
12	SO_2CH_3	10	1000
13	$CONHCH_2CH_3$	15	500
14	$SO_2N(CH_3)_2$	8	1000

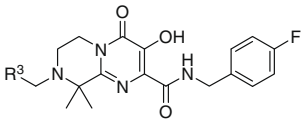
[a,b] For footnote details, see Table 1.

Capping the amine with different polar functionalities led to potent integrase inhibitors such as the acetamide **10**, the oxalamide **11**, the sulfonamide **12**, the urea **13** and the sulfamide **14**. However, these compounds exhibited significantly reduced antiviral activities (Table 2).

Subsequently, side chain substitution and the insertion of a further basic group were undertaken to see if a boost in activity could be achieved by the installation of a polar moiety maintaining the amino group on the ring (Table 3). The introduction of a second basic moiety resulted in little or no improvement; that is, **15** and **16**, which displayed a suboptimal activity both against the enzyme and in the cell based assay and the morpholine derivative **17** which exhibited an improved potency with modest cellular activity.

In contrast, the addition of a carboxamide group was tolerated and improved the efficacy in cell culture as shown for the amides **18** and **19**.

Table 3
Introduction of an amide group: reducing basicity

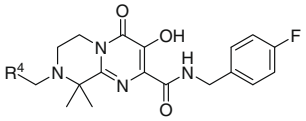


Compd	R^3	QUICKIN[a] IC_{50} (nM)	Spread CIC_{95}[b] (nM) (10% FBS)	(50% NHS)
15	$CH_2N(CH_3)_2$	62	1250	1250
16	$CH_2N(CHCH_3)_2$	76	625	625
17	CH_2-morpholine	3	500	1000
18	$CONH_2$	5	125	250
19	$CONHCH_3$	21	62	125
20	$CON(CH_3)_2$	8	62	125
21	CO-morpholine	7	62	250
22	CO-pyrrolidine	7	31	250
23	CO-(4-CH_3-piperazine)	21	62	125
24	COOH	8	500	1000
25	CN	5	31	125

[a,b] For footnote details, see Table 1.

The trend was more evident with **20** and **21** which displayed an eightfold improvement in Quickin activity and a 20-fold in the Spread assay (10% FBS) if compared to the amine analog **15**. Most noticeably, **22** displayed CIC_{95} (10% FBS) = 31 nM, but as detected for all the amides, exhibited decreased activity in presence of 50% NHS. This was probably due to their higher affinity for serum proteins, which reduced their effective concentrations toward the viral enzyme.

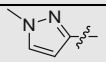
Table 4
Heterocyclic derivatives



Compd	R^4	QUICKIN[a] IC_{50} (nM)	Spread[B] CIC_{95} (nM) (10% FBS)	(50% NHS)
26	Ph	5	125	250
27		9	31	125
28		12	250	1000
29		6	31	125
30		5	62	250
31		7	31	125

(*continued on next page*)

Table 4 (*continued*)

Compd	R^4	QUICKIN[a] IC_{50} (nM)	Spread[B] CIC_{95} (nM) (10% FBS)	(50% NHS)
32		9	31	125
33		6	31	125
34		7	62	1000
35		3	31	93
36		9	15	125
37		4	31	125
38		25	31	62
39		21	15	62
40		5	7	62
41		8	15	62
42		62	31	125
43		13	15	62
44		6	15	62
45		2	31	62
46		6	15	125
47		2	500	1000
48		5	15	62
49		6	7	31

[a,b] For footnote details, see Table 1.

As before, the insertion of an additional basic group, to generate the piperazine derivative **23**, did not gain any further improvement in activity although it displayed a smaller serum shift. When the carboxamide moiety was replaced by carboxylic acid functionality (**24**), even if a potent integrase inhibitor was identified, it displayed low cellular activity demonstrating the desirability for a neutral moiety on the side chain region. This observation was confirmed by the excellent potency displayed by the nitrile **25** in both assays.

Given the encouraging results for **19**, **20** and **25**, heterocyclic amide isosteres were explored with a view to improve its biological activity and generate a more drug-like molecule.

Overall results highlighted that heterocyclic replacements for the amide bond usually displayed the improved potency in cell culture in the presence of low and high serum conditions (Table 4).

Generally, best results were obtained by five-membered heterocycles; although the six-membered counterparts such as compounds **26**, **27** and **29** maintained enzyme inhibitory activity, they exhibited a high shift between the in vitro and the cell based assay. No benefit came from increasing the distance between the aromatic moiety and the basic nitrogen as observed for pyridine **28**.

Several trends can be drawn from a closer analysis of the data. Pyrazoles (**30**, **31** and **32**) displayed similar inhibition of the spread of HIV-1 infection: acceptable cell permeability and a moderate shift in the presence of 50% of normal human serum. Interestingly, N-methyl substituted heterocycles usually showed improved cellular activity compared to those with NH substitution (as observed for **31** and **32** compared to **30**; **45** and **46** compared to **33**; **48** and **49** compared to **47**). Oxazoles (**35**, **37** and **41**) were found active on the enzyme displaying single digit nanomolar value. The triazole **38** showed virtually no shift between the in vitro and the cell based assay. In contrast, thiazole **36** exhibited similar enzymatic potency, but exhibited eightfold serum shift between low and high serum conditions. Oxadiazoles like **39**, **40**, **43** and **44**, while not displaying outstanding enzymatic activity, gave compounds with excellent cellular potency, with all four analogs showing CIC_{95} = 62 nM in high serum.

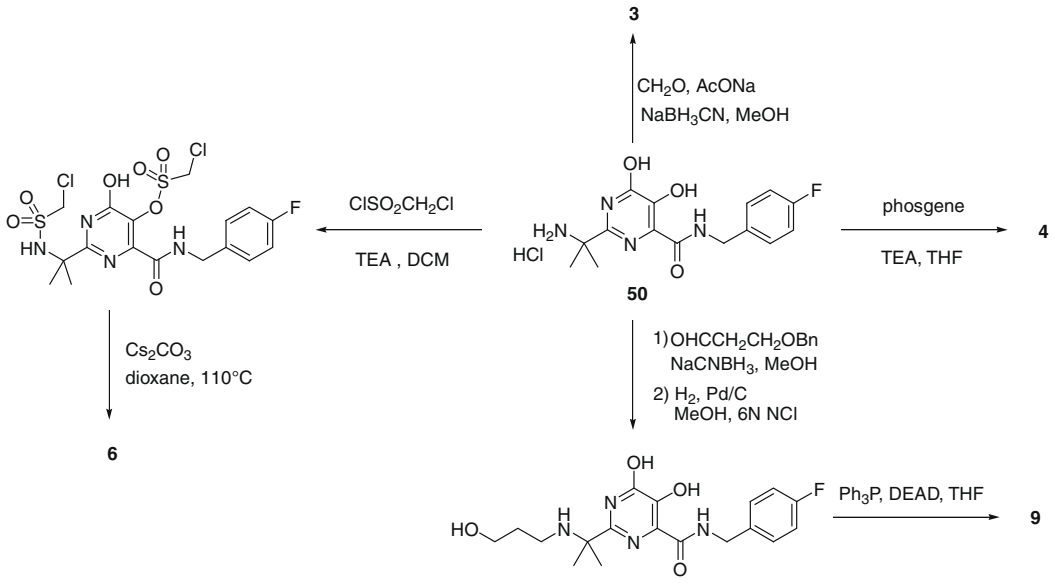
Specific individual compounds of interest, including the γ-methyltriazole **45**, were identified as exceptionally potent enzyme inhibitors with IC_{50} = 2 nM with more than acceptable cell penetration. The 2-methyl tetrazole **49** was the most potent inhibitor from this series displaying CIC_{95} = 31 nM in high serum.

In conclusion, tethering the *N*-methyl group of the pyrimidones onto the amino *gem*-dimethyl group was a successful strategy that led to a novel potent series of bicyclic HIV integrase inhibitors. These compounds have excellent antiviral activity equivalent to that showed by Raltegravir.

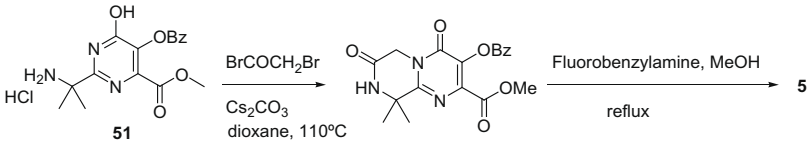
The synthetic route used for the advanced intermediate **50** is fully described in a separate publication.[6]

In Scheme 1 the synthetic routes for the assembly of the different sized bicycles are reported. A diverse approach to cyclize the [6,6]-fused ring, starting from the already known[6] hydrochloride salt **51**, is described in Scheme 2.

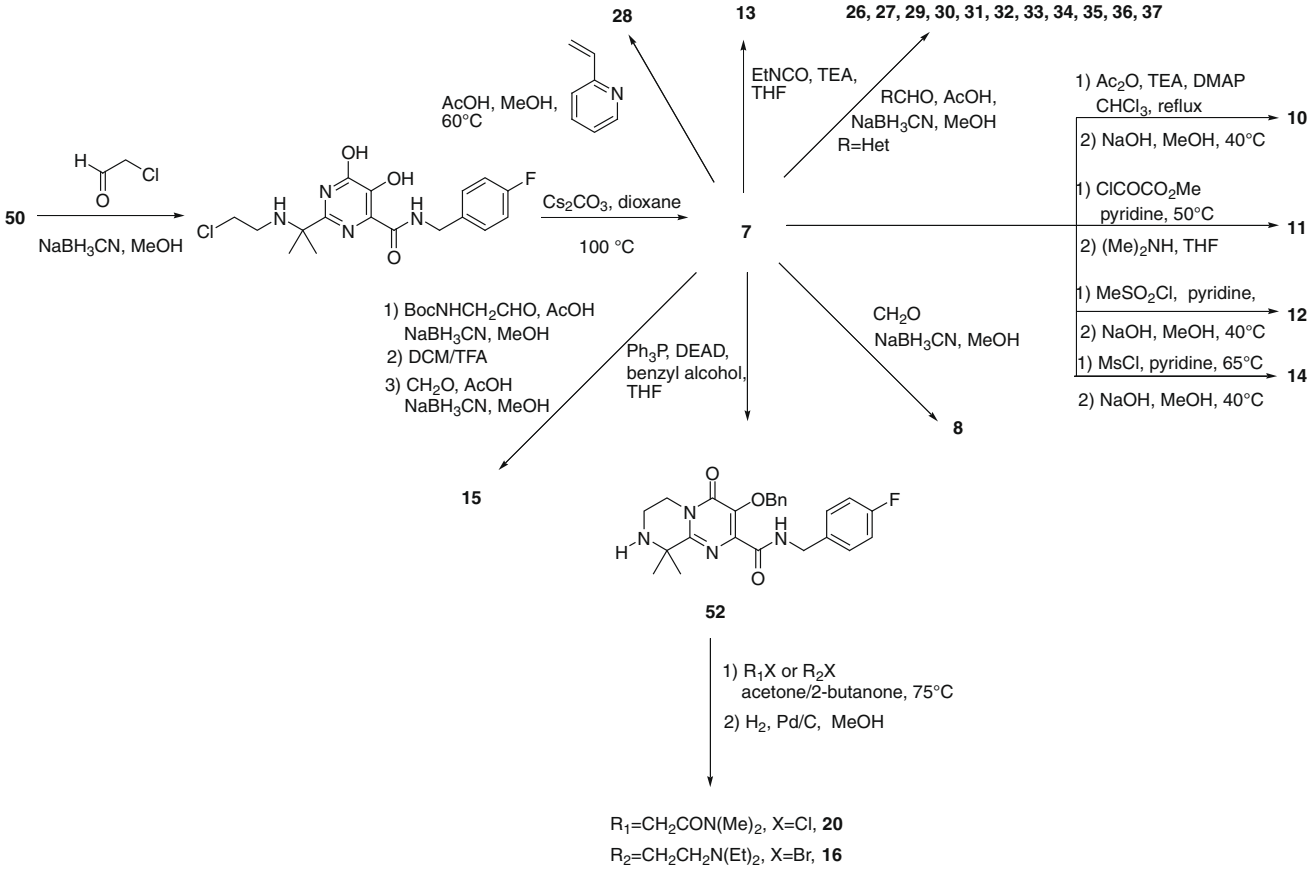
Furthermore, the preparation of the derivatives listed in Tables 2 and 3 is illustrated in both Schemes 3 and 4 as well as the preparation of the secondary amine **52**, which represents the common intermediate to provide almost all the heterocyclic compounds reported in Table 4.[7]



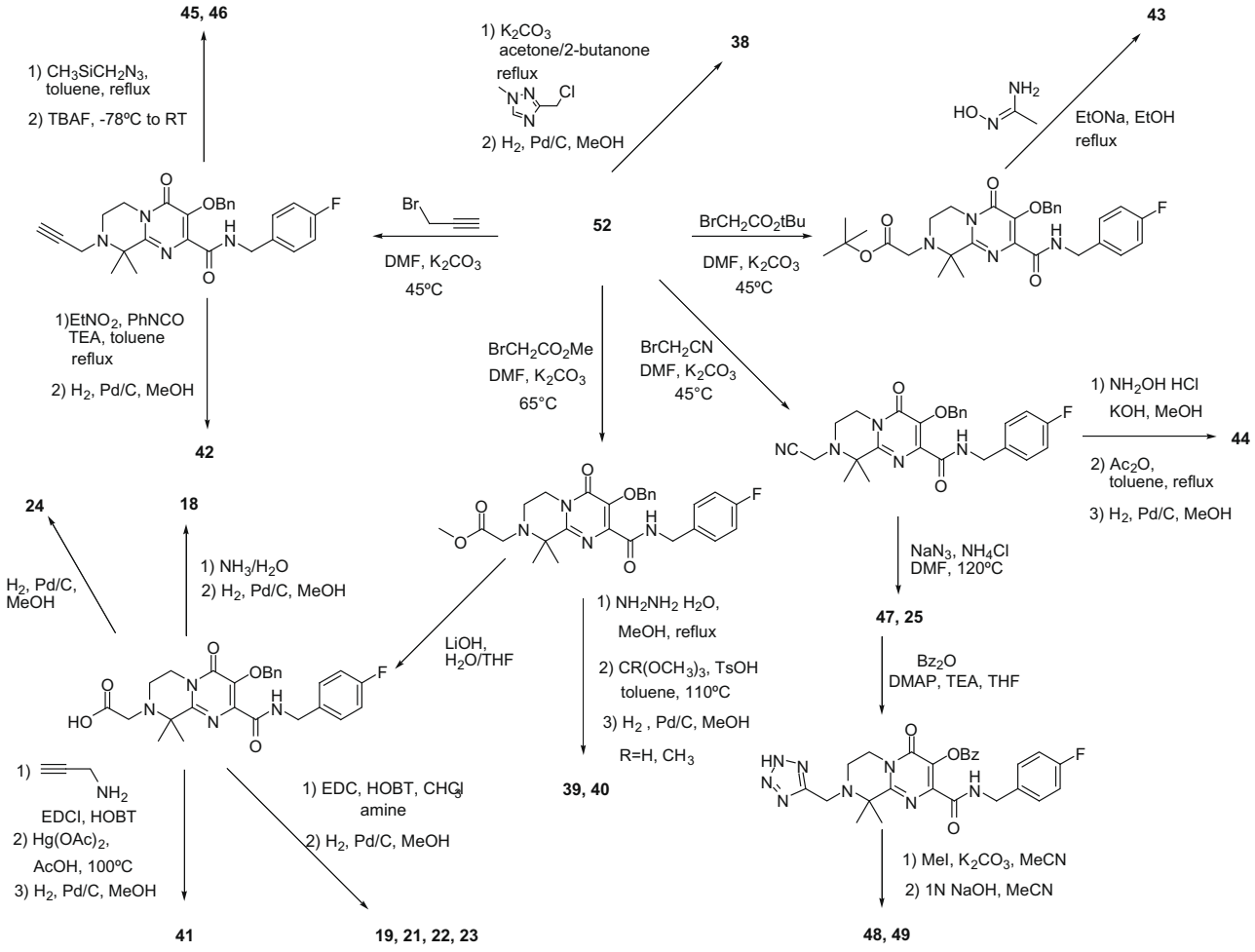
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.

Acknowledgments

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