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Development of 2-^{t}butyl-*N*-methyl pyrimidones as potent inhibitors of HIV integrase

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Abstract—A series of novel 2-^{t}butyl-*N*-methyl pyrimidone HIV-1 integrase inhibitors have been identified. Optimization of the initial lead resulted in compounds such as **9d** and **14a**, which showed high levels of activity in cell culture inhibiting viral replication with CIC_{95} of 10 nM in the presence of 50% normal human serum.

Together with protease and reverse transcriptase, integrase is one of the three virally encoded enzymes required for the replication of the human immunodeficiency virus (HIV-1).[1] HIV integrase catalyses the insertion of the reverse-transcribed viral genome into the host DNA in a multistep process. After assembling onto viral DNA, HIV integrase cleaves the two terminal nucleotides from each 3′-end. Following nuclear entry, the viral 3′-ends are ligated into the host genome (strand transfer).[2] Inhibition of HIV integrase results in arrest of HIV life cycle, and represents therefore an attractive target for the treatment of HIV infection.[3]

Previous studies in our group had established that 5,6-dihydroxypyrimidine-4-carboxamides are potent inhibitors of HIV integrase and efficiently suppress virus spread in HIV-infected cells.[4–6] Initial structure-activity relationship studies in the 2-thienyl series identified 4-fluorobenzylamide **1** as the optimal residue for enzyme inhibition (Table 1).[5] It was also established that, although in terms of intrinsic potency a variety of residues were well tolerated as replacements of the 2-thiophene ring, the introduction of a basic amine within the C-2 substituent was required to enhance activity in cell culture by improving cell permeability and reducing binding to cell medium plasma proteins (analogue **2** CIC_{95} = 1.0 μM in 10% fetal bovine serum, FBS).[6] Interestingly, further studies within this and other series of amine analogues showed a good correlation between an increase in the degree of substitution at the C-atom directly attached to the pyrimidine scaffold and the boost in potency observed in the enzyme and cellular assays.[6,7] Indeed, the *gem*-dimethyl analogue **4** was amongst the most potent compounds in the series, with CIC_{95} value of 0.06 μM (10% FBS) and no shift in the more physiological high serum conditions (CIC_{95} = 0.08 μM, 50% normal human serum, NHS).[6]

These findings prompted us to further investigate the importance of a tetrasubstituted sp^3 carbon at the C-2 position of the pyrimidine scaffold, and the ^{t}butyl group seemed an ideal starting point. We were pleased to find that, despite the absence of any basic substituent, carboxamide **5a** was a potent enzyme inhibitor (IC_{50} = 0.01 μM) and maintained good levels of cellular activity with a CIC_{95} value of 0.25 μM in 10% FBS. Despite this encouraging result, the high shift in potency observed for **5a** in high serum conditions (CIC_{95} =2.5 μM) prompted us to evaluate a series of structurally related benzylamides on the right-hand side of the scaffold, with the primary goal of improving cellular activity while reducing the serum shift.

The analogues **5a**–**f** were prepared by the displacement of ester **7** with a variety of benzylamines, as described in Scheme 1. The synthesis of 5,6-dihydroxypyrimidine-

Keywords: HIV integrase; *N*-methyl pyrimidones; Dihydroxypyrimidines.
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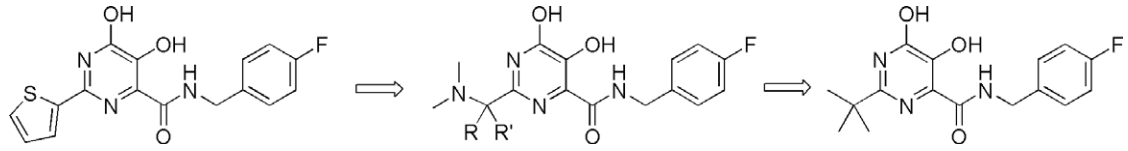
Table 1. Development of the 2-^{t}butyl-dihydroxypyrimidine scaffold

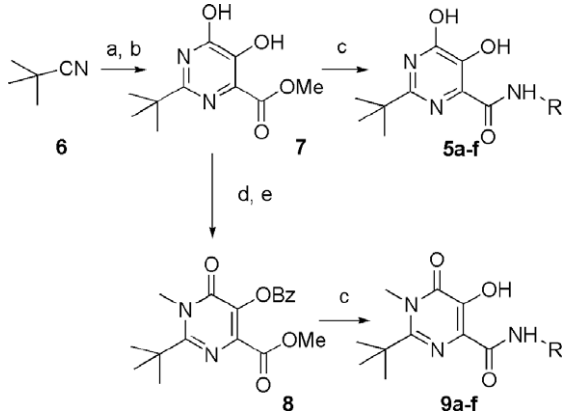


	1	2 R, R′ = H, H	3 Me, Me	4	5a
Inhibition of strand transfer IC_{50}[a]	0.01	0.2	0.01	0.005	0.01
Antiviral activity in cell culture CIC_{95}[b]					
10% FBS	>10	1.0	0.12	0.06	0.25
50% NHS	nd[c]	>1.0	0.50	0.08	2.5

[a] Assays were performed with recombinant HIV-1 integrase preassembled 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%. Results are the mean of at least three independent experiments. SD was always ±8% of the value. For details see Ref. 9 (μM).

[b] Spread assay: 95% cell culture inhibitory concentrations for inhibition of the spread of HIV-1 infection in cell culture, using HIV-1IIIb and MT-4 T-lymphoid cells, in a medium containing 10% heat-inactivated fetal bovine serum. Results are the mean of at least 3 independent experiments, SD was always < ± 10% of the value. For details see Ref. 10 (μM).

[c] Not determined.



Scheme 1. Preparation of compounds **5a**–**f** and **9a**–**f**. Reagents and conditions: (a)NH_2OH, MeOH, 55 °C (60%); (b) Dimethylacetylenedicarboxylate, $CHCl_3$, 60 °C; then xylene, reflux (27%); (c) RNH_2, MeOH, 70 °C (43–76% after preparative HPLC purification); (d) Bz_2O, Pyr, DCM (81%); (e) LiH, Me_2SO_4, dioxane, 60 °C (77%).

4-carboxylate core has been previously reported[5,6] and features the conversion of nitrile **6** into the corresponding amidoxime, followed by reaction with dimethylacetylenedicarboxylate and cyclisation to the desired pyrimidine methyl ester **7**. Amongst the various analogues synthesised both *N*-methyl amide **5d** and sulfonamide **5f** (Table 2) retained high levels of inhibition of viral spread in infected cells, with low shift between 10% FBS and 50% NHS (CIC_{95} = 0.06/0.12 μM for **5d** and CIC_{95} = 0.06/0.06 μM for **5f**).[8]

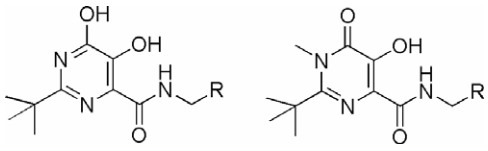
Contemporarily, a similar approach was undertaken with a series of analogues based on the structurally related *N*-methyl pyrimidone scaffold, the previous work of which in our group showed to have similar or improved activity profile to the dihydroxypyrimidine one (Scheme 1, **9a**–**f**).[7] The required ester **8** was prepared from **7** via regioselective 5-benzoylation followed by LiH-promoted N-alkylation (N- vs O-alkylation 4:1 after chromatographic purification). Treatment of **8** with a choice of amines gave the desired *N*-methyl pyrimidone benzylamides **9a**–**f**.

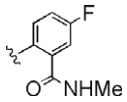
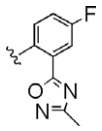
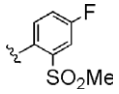
Interestingly, 4-fluorobenzylamide **9a** (Table 2) showed a comparable level of potency in low serum conditions with the corresponding dihydroxypyrimidine analogue **5a** (CIC_{95} = 0.12 μM vs CIC_{95} = 0.25 μM, respectively) but **9a** had only a 3-fold shift in 50% NHS instead of the 10-fold shift previously observed for **5a** (CIC_{95} = 0.37 μM vs CIC_{95} = 2.5 μM, respectively).

This first result was further supported by other *N*-methyl pyrimidone derivatives, which were found to be consistently more potent than their corresponding dihydroxypyrimidine analogues and with lower serum shift (Table 2, analogues **9b**, **9d**–**f**). In particular *N*-methyl amide **9d** and sulfone **9f** showed potency in the low nanomolar range and virtually no shift between the enzyme and cellular assays, even in high serum conditions (**9d** CIC_{95} = 0.01/0.01 μM; **9f** CIC_{95} = 0.01/0.02 μM in 10% FBS/50% NHS).

Prompted by these findings, further evaluation of the two most promising *N*-methyl pyrimidone analogues **9d** and **9f** was continued in vitro. Metabolic stability studies of the two analogues in rat, dog and human liver microsomes showed minimal turnover in the presence of NADPH, suggesting a marginal involvement of oxidative metabolism in the clearance of both compounds. In contrast, high turnover was observed in the presence of UDPGA, with sulfone **9f** having a higher glucuronidation rate than **9d** in all species (ranking order: rat > human > dog; rat intrinsic clearance 63 μL/min/mg for **9f** vs 16 μL/min/mg for **9d**).[11] The pharmacokinetic profile of both analogues was then evaluated in Sprague–Dawley rat (3 mpk iv and po, Table 4), where **9f** showed a higher level of plasma clearance than **9d** (37 ml/min/Kg vs 24 ml/min/Kg, respectively), thus substantiating the findings from the in vitro met-

Table 2. Benzylamide SAR in the dihydroxypyrimidone and *N*-methylpyrimidone series



R	Compound	IC_{50}[a] (μM)	CIC_{95}[a] (μM)		Compound	IC_{50}[a] (μM)	CIC_{95}[a] (μM)	
			10% FBS	50% NHS			10%FBS	50% NHS
	**5a**	0.01	0.25	2.5	**9a**	0.01	0.12	0.37
	**5b**	0.01	1.0	>1.0	**9b**	0.02	0.12	0.5
	**5c**	0.01	>1.0	>1.0	**9c**	0.02	1.0	>1.0
	**5d**	0.02	0.06	0.12	**9d**	0.02	0.01	0.01
	**5e**	0.02	0.12	>1.0	**9e**	0.02	0.02	0.12
	**5f**	0.06	0.06	0.06	**9f**	0.02	0.01	0.02

[a] For footnote details see Table 1.

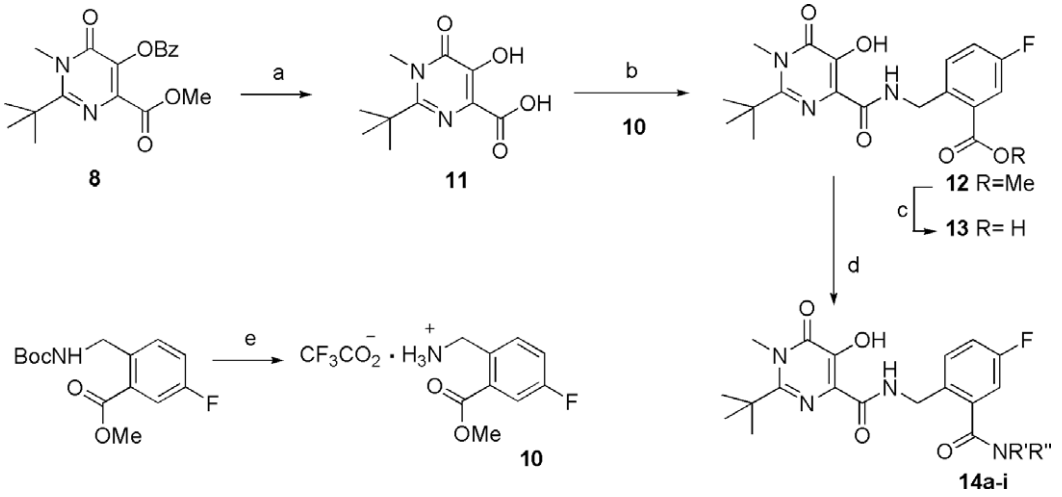
abolic stability experiments. Moreover, while both compounds had a comparable behaviour in terms of terminal plasma half lives and acceptable bioavailability ($T_{1/2iv}$ = 1.6 h; F = 18% for **9 d**; $T_{1/2iv}$ = 1.8 h; F = 17% for **9f**), exposure after oral administration for sulfone **9f** was less than half the exposure of amide **9d** (AUC 0.6 μM h vs 1.4 μM h, respectively).

Given the overall more favourable profile of **9d** over **9f**, we set out to explore if possible replacements of the 2-*N*-methyl carboxamide could offer an improvement to the pharmacokinetic behaviour of inhibitors in this series while retaining the same levels of cellular activity observed in high serum conditions for **9d**.

To allow maximum flexibility in the preparation of the 2-carboxamide analogues, we settled on the synthesis of 2-carboxy methyl ester **12**, a stable intermediate which, upon conversion to the corresponding carboxylic acid **13**, could give a variety of 2-amides analogues (Scheme 2). Thus, acid **11** was pre-activated with EDCI/HOBt and treated with benzylamine **10**[13] and a base to give ester **12**. Hydrolysis in standard conditions afforded the required acid **13** (89% yield over three steps) which could then be progressed to amides **14a**–**i** either via conversion to the corresponding acid chloride or pre-activation with EDCI/HOBt.

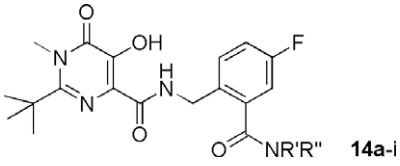
The results for analogues **14a**–**i** are reported in Table 3.

The unsubstituted amide **14a** retained both the intrinsic potency against integrase (strand transfer IC_{50} = 0.02 μM) and the antiviral activity in cell culture (CIC_{95} = 0.01 μM in 50% NHS) in the same range of *N*-methyl amide analogue **9d**. Increasing the length of the amide substituent, branching at the α-position and introducing a tertiary amide all proved to be detrimental in terms of intrinsic potency. Simple replacement of the *N*-methyl residue of **9d** with the *N*-ethyl or *N*-^{i}propyl as in **14c** and **14e** resulted in a 3- and 4-fold loss in potency respectively (strand transfer IC_{50} = 0.07 μM for **14c** and IC_{50} = 0.09 μM for **14e** vs IC_{50} = 0.02 μM for **9d**), while with the *N*-dimethyl amino group of **14g** a more dramatic 9-fold loss was observed (strand transfer IC_{50} = 0.18 μM). Diminished activity in cell culture in the presence of 50% NHS was observed for the most lipophilic analogues (**14f**: CIC_{95} = 0.12 μM and **14e**: CIC_{95} = 0.06 μM, 1% and 2% fraction unbound, respectively). Conversely, a lower protein binding can explain the re-established cellular activity for compounds such as **14g**, only moderately active in the strand assay but amongst the most potent of the series in high serum conditions (**14g**: 18% fraction unbound, IC_{50} = 0.18 μM; CIC_{95} = 0.03 μM 10% FBS and 50% NHS).

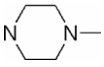


Scheme 2. Preparation of compounds **14a–i**. Reagents and conditions: (a) NaOH, THF/H_2O; (b) EDCI·HCl, HOBt, $^{i}Pr_2EtN$, DCM; (c) NaOH, THF/H_2O (89% from **8**); (d) EDCI·HCl, HOBt, $^{i}Pr_2EtN$, DCM, R′R″NH or $(COCl)_2$, cat. DMF, DCM, 0 °C 1 h then R′R″NH, DCM 0 °C to rt (24–36% after preparative HPLC purification); (e) CF_3CO_2H, DCM.

Table 3. SAR of 2-carboxamide analogues **14a–i**



14a-i

Compound	NR′R″	Inhibition of strand transfer IC_{50}[a] (μM)	Antiviral activity in cell culture CIC_{95}[b] (μM)		Protein Binding f_u (%)[c]
			10% FBS	50% NHS	
14a	NH_2	0.02	0.01	0.01	6
9d	$NHCH_3$	0.02	0.01	0.01	7
14c	$NHCH_2CH_3$	0.07	0.02	0.02	3
14d	$NH(CH_2)_2CH_3$	0.07	0.03	0.06	2
14e	$NHCH(CH_3)_2$	0.09	0.06	0.06	1
14f	$NHCH_2CF_3$	0.09	0.03	0.12	1
14g	$N(CH_3)_2$	0.18	0.03	0.03	18
14h		0.23	0.12	0.12	nd[d]
14i		0.49	0.12	0.12	34

[a] For footnote details see Table 1.
[b] For footnote details see Table 1.
[c] Fraction unbound to human plasma proteins as determined by ultrafiltration method. For details see Ref. 12.
[d] Not determined.

The pharmacokinetic profile of the most promising amide analogues was determined in Sprague–Dawley rat (Table 4). Carboxamide **14a** showed a marginal improvement in bioavailability but suffered from a substantial increase in plasma clearance and reduction in exposure over *N*-methyl amide **9d** (*F*% 23 vs 18; Cl 55 vs 24 ml/min/kg for **9d**; AUC 0.6 μM·hr for **14a** vs 1.4 μM h for **9d**). On the other hand the mono-substituted amides **14c** and **14e** had comparable levels of clearance to **9d**, and showed a promising increase in bioavailability (*F* = 30% and 40% for **14c** and **14e**, respectively).

In summary, optimization of **5a** resulted in the identification of a series of potent HIV integrase inhibitors such

Table 4. Pharmacokinetic profile of **9f**, **9d** and further 2-carboxamide analogues in rat[a]

Compound	*F*[b] (%)	$T_{1/2}$[c] (h)	Cl[d] (mL/min/kg)	AUC[e] (μM h)
9f	17	1.8	37	0.6
9d	18	1.6	24	1.4
14a	23	3.6	55	0.6
14c	30	2.6	21	1.8
14e	44	1.6	35	3.5

[a] 3 mg/Kg (*n* = 3); vehicle iv 20% DMSO/60% PEG400/20% Water, po 1%methylcellulose (suspension).
[b] Oral bioavailability.
[c] Terminal phase plasma half life following iv administration.
[d] Plasma clearance.
[e] Area under the curve following po administration.

as sulfone **9f** and carboxamides **14a** and **9d** characterized by a minimal scaffold and excellent cellular activity in the presence of 50% NHS ($CIC_{95} = 0.01$ μM for **9d** and **14a**). Studies in this series are ongoing to identify the optimal combination of potency, activity in cell culture and pharmacokinetic properties.

Acknowledgment

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