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Dihydroxy-pyrimidine and *N*-methylpyrimidone HIV-integrase inhibitors: Improving cell based activity by the quaternarization of a chiral center

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ABSTRACT

In the context of HIV-integrase, dihydroxypyrimidine and *N*-methyl pyrimidone inhibitors the cellular activity of this class of compounds has been optimized by the introduction of a simple methyl substituent in the α-position of the C-2 side chains. Enhanced passive membrane permeability has been identified as the key factor driving the observed cell-based activity improvement. The rat PK profile of the α-methyl derivative **26a** was also improved over its *des*-methyl exact analog.

The human immunodeficiency virus 1 (HIV-1) is the causative agent of acquired immune deficiency syndrome (AIDS), a disease that results in infection and death rates of 2.7 and 2 million per year, respectively.[1] The standard HIV treatment is a triple therapy commonly referred to as highly active antiretroviral therapy (HAART).[2] While HAART is undeniably effective, it can fail to control HIV replication in patients due to several limitations, such as lack of therapy adherence, toxicity, and the emergence of drug resistant viral strains. Therefore there is a continuous need for new agents with improved properties.

The HIV-1 retroviral integrase (IN) is an enzyme required to catalyze the integration of double stranded viral DNA into host cellular genomic DNA.[3] Integration consists of three biochemical steps: assembly of IN on viral DNA, endonucleolytic cleavage of the first two nucleotides from each 3′ terminal strand of the viral DNA, and strand transfer of the recessed viral DNA to the host cell DNA.[4,5] We have recently reported the discovery of 5,6-dihydroxypyrimidine and related *N*-methyl pyrimidone[6,7] carboxamides as potent and selective inhibitors of the HIV-IN-catalyzed strand transfer process. Optimization of these scaffolds led to the finding that the presence of a saturated nitrogen-heterocyclic substituent at the position 2 of the pyrimidine ring (as in **1**, Fig. 1) gave good cell based potency.[7,8]

It has also been shown[8] that a tetrasubstituted sp^3-carbon atom adjacent to the heterocyclic core is a critical feature of Raltegravir (**2**, Fig. 1) a first-in-class HIV-1 IN inhibitor that was discovered in our laboratories.[9] In the present report, we detail our evaluation of a hybrid compound class that retains the cyclic amine functionality found in **1** together with the additional methyl group at the α-position of the C-2 side chain (i.e. **3**, Fig. 1). This apparently minor structural change provided a general approach towards improving cell-based antiviral efficacy for these classes of HIV-integrase inhibitors.

The chemistry used to prepare the compounds in this study has been previously described[7–10] and starts from the appropriate nitriles **4a–b** and **5a–b** (Scheme 1).[8–11] Conversion to the

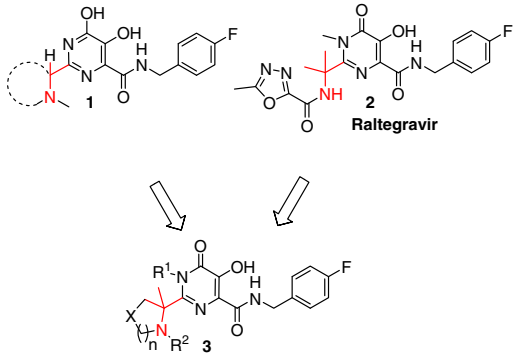

Figure 1. Dihydroxy-pyrimidines and *N*-methylpyrimidones with a quaternary α-substituent at position 2.

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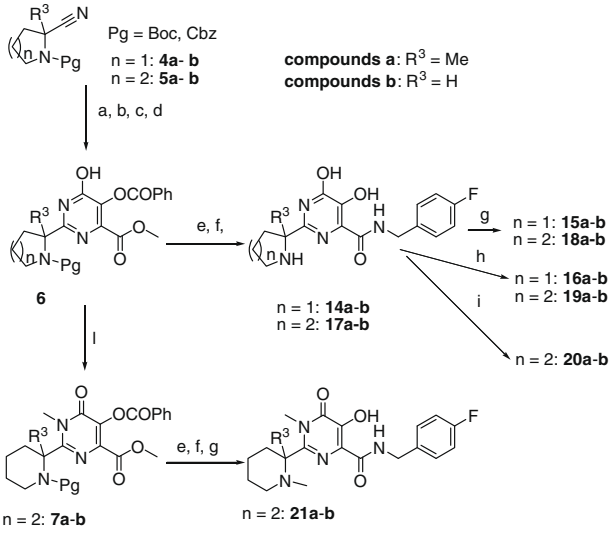
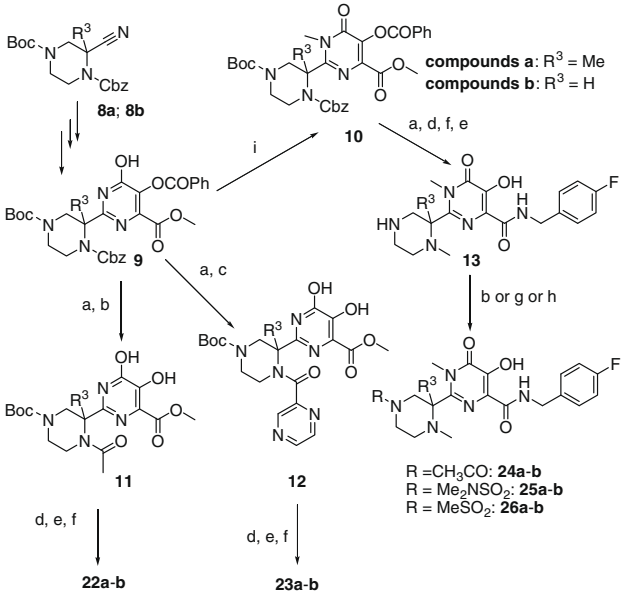


Scheme 1. Synthesis of compounds **14a–b** to **21a–b**. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, Et_3N, EtOH, reflux; (b) DMAD, $CHCl_3$, reflux; (c) xylene, reflux; (d) Bz_2O, Py, rt; (e) 4F–$BnNH_2$, MeOH, reflux; (f) TFA–DCM, rt, or H_2, Pd/C, MeOH, rt; (g) H_2CO, $NaCNBH_3$, NaOAc, MeOH, rt; (h) Ac_2O, Py, rt; (i) 2,4-dimethyl-1,3-thiazole-5-carboxylic acid, EDC, HOBT, DCM, rt; (l) LiH, dioxane, Me_2SO_4, 60 °C.



Scheme 2. Synthesis of piperazine **22a–b** to **26a–b**. Reagents and conditions: (a) H_2, Pd/C, MeOH, rt; (b) Ac_2O, Py; (c) 2-pyrazine carboxylic acid, HOBT, EDC, DIPEA, DCM; (d) 4F–$BnNH_2$, MeOH, reflux; (e) TFA–DCM (9/1), rt; (f) H_2CO, $NaCNBH_3$, NaOAc, MeOH; (g) Me_2NSO_2Cl, Et_3N, DCM; (h) MsCl, Et_3N, DCM; (i) LiH, dioxane, Me_2SO_4, 60 °C.

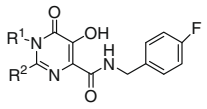
corresponding amidoximes followed by reaction with dimethylacetylene dicarboxylate and cyclization in refluxing xylene afforded the dihydroxypyrimidine methyl esters which were reacted with benzoic anhydride in pyridine to accomplish the regioselective 5-OH protection (compounds **6** and **9**, yields 23–52% over 4 steps). The synthesis of the *N*-methyl pyrimidones **7a–b** featured the use of LiH as base and dimethyl sulfate as electrophile followed by separation from the 6-*O*-methyl byproduct via silica gel chromatography: the ratio of *N*- versus *O*-alkylated product was 5:1. Reaction of either the pyrimidines **6** or pyrimidones **7a–b** with 4-fluorobenzylamine in MeOH gave the corresponding 4-carboxamides. Removal of the protecting group from the nitrogen atom of the heterocycle at C2 gave the secondary amines **14a–b** and **17a–b**. These compounds were reductively alkylated to give the corresponding tertiary amines (**15a–b**,**18a–b**, and **21a–b**) or used in amide coupling (Ac_2O, Py, rt or 2,4-dimethyl-1,3-thiazole-5-carboxylic acid, EDC, HOBT) to generate **16a–b, 19a–b**, and **20a–b**. The piperazine analogs **22a–b**/**26a–b** were prepared from the orthogonally protected nitriles **8a**–**b**[8,11c] as outlined in Scheme 2. The synthesis of intermediate **9** was carried out as previously described and was followed by Cbz deprotection of the piperazine N1 nitrogen by hydrogenolysis then formation of amides **11** and **12** using, respectively, acetic anhydride in pyridine or 2-pyrazine carboxylic acid under amide coupling conditions. TFA mediated removal of the N4 *t*-butoxycarbonyl group followed by reductive methylation and benzyl amide formation afforded compounds **22a–b** and **23a–b**. The synthesis of *N*-methyl pyrimidone analogs bearing a methyl group on the piperazine N1 nitrogen (compounds 24a–**b** to **26a–b**) was performed through preparation of the common intermediate **13**. Thus after Cbz removal from **10** (which was isolated from a 1:1 mixture with the *O*-alkylated byproduct), reaction with 4-fluorobenzylamine followed by reductive methylation and treatment with TFA gave the amine **13** in readiness to be treated with acetic anhydride in pyridine to obtain **24a–b**. Alternatively treatment of **13** with dimethylsulfamyl chloride or methanesulfonyl chloride and triethylamine in DCM afforded **25a–b** and **26a–b**, respectively.

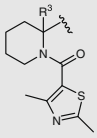
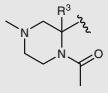
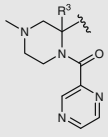
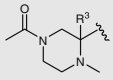
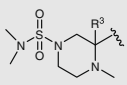
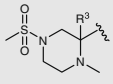
A set of 13 *des*-methyl dihydroxypyrimidines and *N*-methylpyrimidones **14b**–**26b** which had strand transfer IC_{50}'s below 500 nM were selected for investigation, and their corresponding α-methyl analogues **14a**–**26a** were synthesized as outlined above. Table 1 summarizes the enzyme inhibition activity and cell-based potency for this compound set. As a general trend it can be observed that α-methyl derivatives **14a**–**26a** were more active than the corresponding *des*-methyl analogs in the HIV Spread cell-based assay. Spread CIC_{95} values under low serum concentration (10% FBS) ranged from either 4 to 600 or from 40 to 2500 nM for α-methyl and *des*-methyl series respectively; the statistical significance of this observation was judged high by Student's *t*-test analysis (*P*-value <0.05).[12] As already observed in the program the enhanced cell-based potency apparently does not correlate with improved in vitro enzyme inhibition, and moreover no significant effect on activity (*P*-value >0.8) in the strand transfer inhibition assay was observed as a consequence of introducing the α-methyl group.

We decided to investigate other parameters such as distribution coefficient (Log $D_{7.4}$), solubility, plasma protein binding, and membrane permeability of these IN inhibitors to assess whether trends emerged that might account for the improved cell-based activity observed.

As expected α-methyl derivatives were more lipophilic with respect to their *des*-methyl analogs: a net increase in their Log *D* values ranging from 0.3 to 1.7 log unit has been experimentally determined (Table 2). Although increased lipophilicity is often associated with reduced aqueous solubility, for the present compound set no solubility issues were encountered: all compounds, regardless of the series they belonged to, were soluble at the upper limit of the solubility assay[15] (200 μM, data not shown) in phosphate buffered saline (PBS) at pH 2 and 7. The propensity of the α-methyl/*des*-methyl analogs to bind to plasma proteins was determined by measurement of their free fractions in human serum (Table 2). For the most part, there was a little change in free fraction for the α-methyl analogs and this translated to a similar plasma shift in the Spread assay for α-methyl/*des*-methyl compound pairs between Fetal Bovine Serum (FBS) and Normal Human Serum (NHS, results not shown). A clear exception was compound **26a** which had 8-fold lower free fraction in human plasma proteins than the *des*-methyl analog **26b**. This higher hPPB for **26a** resulted

Table 1
In vitro and in cell activity



R^1	R^2	Compd	R_3 = Me (α-methyl)		Compd	R_3 = H (*des*-methyl)	
			Strand transfer inhibition[a] IC_{50} (nM)	Spread[b] 10% FBS CIC_{95} (nM)		Strand transfer inhibition[a] IC_{50} (nM)	Spread[b] 10% FBS CIC_{95} (nM)
H		**14a**[c]	80	390	(±)-**14b**	120	2500
H		**15a**[c]	90	190	(**±**)-**15b**	70	150
H		**16a**[c]	20	260	(**±**)-**16b**	40	>1000
H		(**±**)-**17a**	20	160	(**±**)-**17b**	130	1460
H		(**±**)-**18a**	250	90	(**±**)-**18b**	220	150
H		(**±**)-**19a**	14	80	(**±**)-**19b**	20	270
H		(**±**)-**20a**	20	60	(**±**)-**20b**	50	>1000
Me		(**±**)-**21a**	550	>1000	(**±**)-**21b**	440	830
H		(**±**)-**22a**	100	600	(**±**)-**22b**	30	790
H		(**±**)-**23a**	100	160	(**±**)-**23b**	60	350
Me		(**±**)-**24a**	4	40	(**±**)-**24b**	6	60
Me		(**±**)-**25a**	20	20	(**±**)-**25b**	20	40
Me		(**±**)-**26a**	15	4	(**±**)-**26b**	30	240

[a] Assay was performed with recombinant HIV-1 IN (0.1 mM) preasseambled on immobilized oligonucleotides.[13] Inhibitors were added after assembling and washings, and IC_{50} is the concentration of the inhibitor that reduces HIV-integrase activity by 50%. Results are the mean of at least three independent experiments. SD was ±35% of the value.

[b] Spread assay results are the mean of at least three independent experiments; SD was always ±35% of the value. CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 10% of fetal bovine serum (FBS).[12–14]

[c] **14a**–**16a** *S*-enantiomer.

Table 2
Log *D*, human protein binding and permeability

Compd α-methyl/*des*-methyl	Log *D*[a]	hPPB f_u[b] (%)	Permeability[c] (10^6cm/s)
14a/**14b**	2.7/nd	21/20	6.4/0.7
16a/**16b**	1.2/nd	7/8	12.2/3.5
17a/**17b**	nd/0.4	29/34	1.9/0.07
18a/**18b**	nd/0.8	8/5	2.2/0.7
19a/**19b**	1.7/1.2	9/1	6.3/3.74
20a/**20b**	1.9/1.5	13/10	40.5/5.7
21a/**21b**	nd/nd	38/39	15.3/15.1
22a/**22b**	0.9/0.4	13/10	21.3/0.4
23a/**23b**	0.9/0.6	18/10	5.4/0.4
24a/**24b**	2.1/0.5	11/29	5.7/2.3
25a/**25b**	2.9/1.2	19/20	57.6/3.2
26a/**26b**	nd/nd	4/31	34.3/2.4
Verapamil[d]	—	—	49.9
Theophylline[e]	—	—	5.4

[a] For details see Ref. 19.
[b] Fraction unbound to human plasma proteins as determined by ultrafiltration method. For details see Ref. 20.
[c] For each pre-coated plate-based permeability assay two different measurements of pH (6–7.4) have been performed in quadruplicate.[21,22]
[d] Positive control.
[e] Negative control.

in a 10-fold activity shift when the cell-based Spread assay was conducted in the presence of high serum condition (**26a** CIC_{95} = 4/40 nM in 10% FBS/50% NHS, Table 4); in contrast **26b** was equipotent at the two different serum concentrations (CIC_{95} = 240/220 nM in 10% FBS/50% NHS). Taken together, the reasonably high free fractions for the entire compound set, the similar protein binding values for α-methyl and *des*-methyl analogs and the expectation that protein binding effects are less prominent in FBS suggested that improved potency for the α-methyl analogs does not stem from reduced protein binding.

Parallel artificial membrane permeability assays (PAMPA) were conducted to measure passive membrane permeability (results in Table 2) of our compound set using a pre-coated plate-based method.[21,16] Notably, for all but one of the compound pairs (**21a**/**21b**) the α-methyl derivative had higher permeability than the corresponding *des*-methyl analog. Permeability for the α-methyl compounds was usually high, and only for two of these compounds (**17a** and **18a**) was below the negative control threshold. In contrast, all the *des*-methyl derivatives except **21b** had permeability values that are similar to or (more usually) below the negative control. Thus for the class of α-methyl derivatives increased permeability appears to be responsible for enhanced cell based potency, confirming the well documented correlation between membrane permeability and lipophilicity.[17,18] It is noteworthy how consistently the introduction of the α-methyl group influences the physicochemical properties and ultimately the cell-based potency across this range of different chemical structures.

The introduction of a methyl substituent also proved beneficial to the pharmacokinetic properties of the inhibitors, as is illustrated in Table 3 for representative compound pairs **18a–b** and **26a–b**. As reported elsewhere[9] these inhibitors have low oxidative turnover in liver microsomes (from both human and pre-clinical species) and their major route of metabolism is 5-O-glucornidation mediated by UDPGA. When the α-methyl derivative **18a** was dosed intravenously it showed longer half life (17 h) and reduced plasma clearance (8 mL/min/kg) with respect to its *des*-methyl analog **18b** which had high clearance and rather short half life (75 mL/min/kg and 0.4 h, respectively). The AUC of **18a** and **18b** after oral dosage (3 mg/Kg) were respectively 1.8 and 0.4 (μM × h). When **26a** was administered orally and intravenously to rat, it showed good oral bioavailability (73%), AUC of 2.2 (μM × h), moderate clearance (33 mL/min/kg), and plasma half life of 2.1 h. In contrast, compound **26b** had plasma clearance similar to rat liver blood-flow (75 mL/min/kg), moderate bioavailability (38%), a shorter half-life (0.8 h), and AUC was 0.6 (μM × h).[7]

Table 3
Rat pharmacokinetic parameters for **18a**–**b** and **26a**–**b**

Compd	Clp[a]	$t_{1/2}$[b]	AUC[c] PO
18a	8	17	1.8
18b	75	0.4	0.4[d]
26a	33	2.1	2.2
26b	75	0.8	0.6

[a] Plasma clearance (mL/min/kg).
[b] Plasma half life following iv administration (h).
[c] Area under the curve following oral administration (μM × h): dose 3 mg/kg, (n = 3); vehicle: 1%methylcellulose.
[d] Vehicle: 10%DMSO/90%PEG_{200}.

Table 4
In vitro and cell based activity of racemate **26a** and its two enantiomers compared to Raltegravir™

Compd	Inibition of strand transfer[a]	Antiviral Activity	
	IC_{50} (nM)	10% FBS[b] CIC_{95} (nM)	50% NHS[c] CIC_{95} (nM)
(±)-**26a**	15	4	40
(+)-**26a**	20	<8[d]	60
(−)-**26a**	20	<8[d]	20
Raltegravir[e]	15	19	31

[a,b] For footnote details see Table 1.
[c] Spread assay results are the mean of at least three independent experiments; SD was always ±35% of the value. CIC_{95} is the concentration of compound that inhibits HIV replication in the cell-based assay by 95% in the presence of 50% of normal human serum (NHS).
[d] n = 1.
[e] For details see Ref. 9.

In summary, the introduction of a methyl substituent at the α-position of our previously reported C2 saturated nitrogen-heterocycle substituted dihydroxypyrimidine and *N*-methyl pyrimidone HIV IN inhibitors has been explored. This structural change consistently resulted in augmented cell based activity, and enhanced passive membrane permeability was identified as the key factor driving this improvement. In both compound pairs analyzed (**18a–b** and **26a–b**) the α-methyl derivatives showed also a better pharmacokinetic profile than the corresponding *des*-methyl analogs, suggesting that this structural change may provide a route to modulating in vivo properties. The piperazine derivative **26a** has attractive cell based activity (CIC_{95} is 20 nM in 50% NHS) and shows improved potency and pharmacokinetic properties with respect to **26b**.

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