# 4,5-Dihydroxypyrimidine Carboxamides and *N*-Alkyl-5-hydroxypyrimidinone Carboxamides Are Potent, Selective HIV Integrase Inhibitors with Good Pharmacokinetic Profiles in Preclinical Species

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**Abstract:** The dihydroxypyrimidine carboxamide **4a** was discovered as a potent and selective HIV integrase strand transfer inhibitor. The optimization of physicochemical properties, pharmacokinetic profiles, and potency led to the identification of **13** in the dihydroxypyrimidine series and **18** in the *N*-methylpyrimidinone series having low nanomolar activity in the cellular HIV spread assay in the presence of 50% normal human serum and very good pharmacokinetics in preclinical species.

Human immunodeficiency virus type 1 (HIV-1) is the etiological agent of the acquired immunodeficiency syndrome (AIDS). Inhibition of HIV-1 replication remains the fulcrum for AIDS treatment, and three viral enzymes have been identified as key targets to arrest the HIV life cycle: reverse transcriptase, protease, and integrase. All the currently used oral drugs for the treatment of HIV infection inhibit the first two of these enzymes. Unfortunately, the emergence of drug-resistant virus strains, limited oral bioavailability, and toxicity related to the dosing regimens drastically reduce the efficacy, compliance, and tolerability of the current treatments.

Inhibition of HIV integrase, responsible for inserting the viral DNA into the host cellular genome, results in arrest of the HIV life cycle and is, therefore, a very attractive therapeutic target. Upon inhibition of integrase, the viral DNA is converted into a circular DNA unable to be integrated into the host genome.[1] Recently, HIV integrase has been validated as an antiviral target in SHIV infected rhesus macaques,[2] proving that in vitro inhibitors of the strand transfer step are capable of inhibiting viral replication in vivo. Furthermore, proof of concept has also been achieved in man.[3] Several classes of HIV integrase inhibitors have been reported in the literature: for example, diketo acids,[4] naphthyridine ketones,[5] naphthyridine carboxamides,[6] quinolines,[7] and tri-cyclic pyrroloquinolines.[8] All share a common pharmacophore capable of binding to the magnesium ions present in the HIV integrase active site.

We recently reported three classes of HCV NS5b RNA-dependent RNA polymerase inhibitors, two of which were discovered by HTS: diketo acids[9] (Figure 1, **1**) and meconic acid (**2**) derivatives.[10] Both classes unfortunately suffer problems such as chemical instability, irreversible covalent binding to protein, and poor stability in plasma. The third class, dihydroxypyrimidine carboxylic acids (**3**) and *N*-alkyl hydroxypyrimidinone carboxylic acids (**3a**) were designed to have more drug-like properties and very importantly with the aim of maintaining the correct geometry to bind the $Mg^{++}$ ions in the active site of the HCV polymerase.[11] Unfortunately, most of these inhibitors showed a suboptimal activity in the HCV cell-based assay.[12] This property was mainly attributed to the free carboxylic acid. Several carboxylic acid isosteres and derivatives, including amides (**4**), were synthesized to solve this problem.

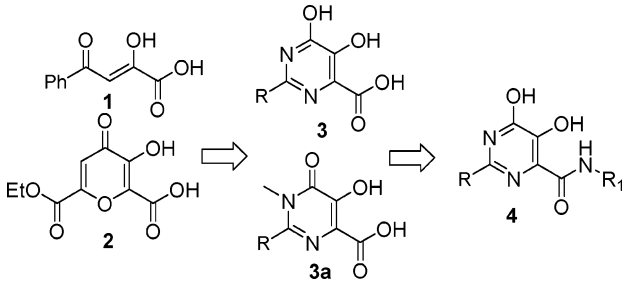
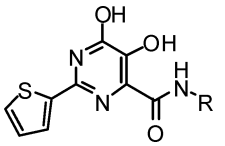



**Figure 1.** Discovery of dihydroxypyrimidine carboxamide **4** as an HIV integrase inhibitor.

**Table 1.** Optimizing the Amide



| cmpd | R | $IC_{50}$[a] ($\mu$M) | $CIC_{95}$[b] (10% FBS/$\mu$M) |
|---|---|---|---|
| **4a** | $CH_2Ph$ | 0.085 | >10 |
| **5** | $CH_2$4F-Ph | 0.010 | >10 |

[a] HIV strand transfer assay results are the mean of at least three independent experiments, SD was always ±8% of the value, and $IC_{50}$ is the concentration of inhibitor that reduces HIV integrase activity by 50%. For details of the assay, see ref 143. [b] Spread assay results are the mean of at least three independent experiments, SD was always <±10% of the value, and $CIC_{95}$ is the concentration of inhibitor that inhibits the HIV replication in cell-based assays by 95%. For details of the assay, see ref 14.

HIV integrase and HCV polymerase share the common feature of using two magnesium ions in the active site as a key component of the catalytic machinery. In spite of the fact that the two enzymes catalyze two different types of reaction, the architecture of the amino acids within the catalytic site and the geometry of the catalytic metals are conserved. This observation was the basis of an integrated drug discovery program, where we were screening compounds made as inhibitors of one viral target using this catalytic machinery, across enzymes belonging to the same superfamily from other viruses. While all the HCV polymerase inhibitors having a free carboxylic acid were almost completely inactive against HIV integrase, we were delighted to find that the benzyl amide **4a** (Table 1) was a potent, reversible and selective HIV integrase strand transfer inhibitor showing nanomolar activity in the enzymatic assay ($IC_{50}$ = 0.085 $\mu$M; Table 1). Compound **4a** was inactive against HCV NS5b polymerase at 50 $\mu$M. The rat pharmacokinetic profile of **4a** showed modest oral bioavailability ($F$ = 15%), low plasma clearance (Clp = 5 mL/min/kg) and good half-life ($t_{1/2}$ = 3 h; Table 2).

Extensive structure activity relationship studies on the amide moiety of **4a** led to the identification of the *para*-fluorobenzyl amide **5** (Table 1) as the optimal fragment, showing an $IC_{50}$ = 0.010 $\mu$M in the enzymatic assay.

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**Table 2.** Pharmacokinetics[a]

| cmpd | species | $F^b$ % | nAUC[c] $\mu$M·h·kg/mg | Clp[d] mL/min/kg | $t_{1/2}$[e] h |
|---|---|---|---|---|---|
| **4a** | rat | 15 | 0.40 | 5 | 3 |
| **5** | rat | 29 | 1.3 | 11 | 1.3 |
| **10** | rat | 59 | 1.8 | 14 | 1.7 |
| **10** | dog | 93 | 19.9 | 0.5 | 6.8 |
| **13** | rat | 28 | 0.8 | 16 | 2 |
| **13** | dog | 100 | 21.5 | 1.9 | 4.8 |
| **13** | rhesus | 61 | 1.9 | 15 | 6 |
| **14** | rat | 27 | 0.13 | 44 | 0.45 |
| **14** | dog | 90 | 20.0 | 2.0 | 6 |
| **18** | rat | 56 | 2.3 | 9 | 1.1 |
| **18** | dog | 69 | 13.6 | 2.2 | 7.3 |
| **18** | rhesus | 73 | 2.4 | 14 | 2.0 |

[a] See Supporting Information for PK details. [b] $F$ = oral bioavailability. [c] nAUC = dose normalized area under the curve. [d] Clp = plasma clearance. [e] $t_{1/2}$ = plasma half-life.

The rat pharmacokinetic profile of **5** showed improved oral bioavailability ($F$ = 39%) and similar plasma clearance (Clp = 11 mL/min/kg) compared to that of **4a**. Unfortunately, in spite of the very good potency in the enzymatic assay, these molecules did not show either activity or toxicity in the cellular assay (Spread) under two serum conditions [10% fetal bovine serum (FBS) and 50% normal human serum (NHS)]. This finding can probably be explained by a combination of different factors such as poor solubility, poor cell permeability, and high protein binding. To improve these parameters, effort was devoted to the replacement of the thiophene at the 2-position of the dihydroxypyrimidine carboxamide. Removal of the thiophene moiety gave the unsubstituted compound **6** (Table 3), which retained nanomolar activity in the enzymatic assay ($IC_{50}$ = 0.06 $\mu$M), thus indicating that the substituent at the 2-position is not involved in a strong interaction with the enzyme. Therefore, **6** represents the minimal scaffold of this new class of HIV integrase inhibitors. Replacement of thiophene with methyl **7**, phenyl **8**, and benzyl **9** generated compounds having the same level of potency as the lead **4a** ($IC_{50}$ = 0.05−0.07 $\mu$M). In spite of the similar potency on the enzyme, **9** showed an evident gain of activity in the cell-based assay ($CIC_{95}$ = 5.8 $\mu$M in 10% FBS). To optimize further this compound, it was decided to introduce a basic amine, which could improve solubility and which potentially could also balance the negative charge of the deprotonated hydroxyl group at the 5 position of the scaffold. This strategy led to **10** (racemic), one of the first compounds with submicromolar activity in the spread assay

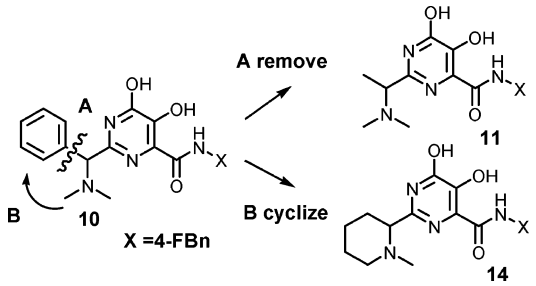


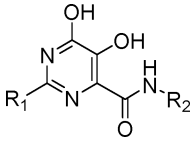

**Figure 2.** Strategies applied to reduce the protein binding.

($CIC_{95}$ = 0.3 $\mu$M 10% FBS), showing virtually no shift with respect to enzymatic activity ($IC_{50}$ = 0.2 $\mu$M). Unfortunately, a great potency shift was observed in the cell-based assay in the presence of 50% NHS ($CIC_{95}$ > 10 $\mu$M). The PK profile was improved with respect to **4** and **5**, with very good oral bioavailability ($F$ = 59% rat, 93% dog) and low plasma clearance (Clp = 14 mL/min/kg rat, 0.5 mL/min/kg, dog, Table 2). The high protein binding in human plasma (hPPB = 99.9%) explained the large shift in potency between low and high serum conditions in the cell-based assays. A reduction of hPPB was, therefore, mandatory to gain activity in the more physiological conditions. Given the well-established correlation between binding of lipophilic acids to albumin and log $P$, the strategy focused on reducing the lipophilicity of **10** in either acyclic or cyclized analogues (Figure 2.)

The first approach (Figure 2, **A**) was to remove the phenyl ring, generating compounds as **11**, where a simple methyl group replaces the phenyl moiety. This modification was beneficial in reducing the plasma protein binding (hPPB = 92.5%), and the compound showed only a 4-fold shift between low and high serum conditions ($CIC_{95}$ = 0.125 $\mu$M 10% FBS, 0.50 $\mu$M 50% NHS). The methylene analogue (**12)** and the gem dimethyl derivative (**13)** were synthesized to remove the chiral center of **11** (Table 3). No improvement in activity was achieved with **12**. However, **13** is an extremely interesting compound, being equipotent on the enzyme and in the cell-based assay in low serum conditions with a marginal shift in high serum concentrations due to the low hPPB = 88.7% ($IC_{50}$ = 0.05 $\mu$M; $CIC_{95}$ = 0.05 $\mu$M 10% FBS; 0.11 $\mu$M 50% NHS).

The oral bioavailability of **13** was good in rat (28%) and excellent both in dog (100%) and in rhesus (61%). Plasma clearance was low for dog and medium in rat and rhesus, with good plasma half-life (Clp = 16, 2, and 15 mL/min/kg; $t_{1/2}$ =

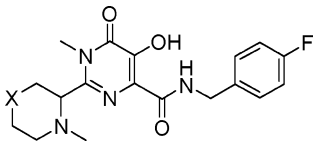
**Table 3.** Optimizing the Dihydroxypyrimidines




| cmpd | R1 | R2 | $IC_{50}$[a] ($\mu$M) | $CIC_{95}$[b] (10% FBS/$\mu$M) | $CIC_{95}$[b] (50% NHS/$\mu$M) |
|---|---|---|---|---|---|
| **5** | thiophene | $CH_24F$-Ph | 0.01 | >10 | >10 |
| **6** | H | $CH_24F$-Ph | 0.06 | >10 | >10 |
| **7** | Me | $CH_24F$-Ph | 0.06 | 10 | >10 |
| **8** | Ph | $CH_2$Ph | 0.07 | >10 | >10 |
| **9** | $CH_2$Ph | $CH_24F$-Ph | 0.05 | 5.80 | >10 |
| **10** | CH(Ph)$NMe_2$ | $CH_24F$-Ph | 0.20 | 0.30 | >10 |
| **11** | CH(Me)$NMe_2$ | $CH_24F$-Ph | 0.08 | 0.125 | 0.50 |
| **12** | $CH_2NMe_2$ | $CH_24F$-Ph | 0.20 | >1.0 | >1.0 |
| **13** | $C(Me)_2NMe_2$ | $CH_24F$-Ph | 0.05 | 0.05 | 0.11 |
| **14** | 2-NMe pip | $CH_24F$-Ph | 0.20 | 0.15 | 0.40 |
| **15** | 2-NMe morpholine | $CH_24F$-Ph | | 0.03 | 0.24 |

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

**Table 4.** *N*-Methylpyrimidones



| cmpd | X | $IC_{50}$[a] ($\mu$M) | $CIC_{95}$[b] (10% FBS/$\mu$M) | $CIC_{95}$[b] (50% NHS/$\mu$M) |
|---|---|---|---|---|
| **16** | $CH_2$(+/−) | 0.21 | 0.840 | 1.10 |
| **17** | O (+/−) | 0.06 | 0.065 | 0.100 |
| **18** | O (+) | 0.020 | 0.040 | 0.065 |
| **19** | O (−) | 0.025 | 0.09 | 0.19 |

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

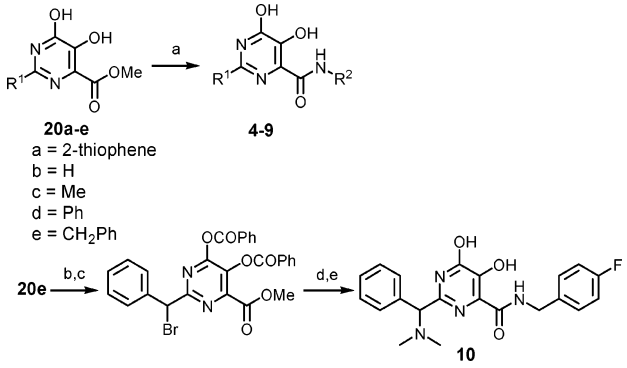
2, 5, and 6 h for rat dog and rhesus, respectively). Furthermore, **13** was found to be inactive against HIV RT, HCV NS5B polymerase and human $\alpha$, $\beta$, and $\gamma$ polymerases ($IC_{50}$ > 10$\mu$M).

The second approach (Figure 2, **B**) to reduce the lipophilicity and increase solubility of **10** was the simultaneous deletion of the phenyl moiety and cyclization of the amine to form *N*-methyl piperidine ring derivative **14** (Table 3). This again reduced the binding to human plasma protein (hPPB, 94.8%), and consequently, the shift between the low and the high serum conditions was only 3-fold in the spread assays ($CIC_{95}$ = 0.14 $\mu$M 10% FBS, 0.40 $\mu$M 50% NHS). The PK profile was again very good, especially in dog, displaying high oral bioavailability (90%) and low plasma clearance (2 mL/min/kg). Replacing the piperidine moiety of **14** with morpholine in **15** led to a further improvement in potency in the spread assay ($CIC_{95}$ = 0.03 $\mu$M 10% FBS, 0.24 $\mu$M 50% NHS). Capitalizing on the experience gained in the HCV polymerase inhibitor program, where the *N*-methylpyrimidinone **3a** showed similar or improved activity and pharmacokinetic profile with respect to the dihydroxypyrimidine, the *N*-methyl pyrimidinones **16** and **17** (Table 4) were prepared as racemates.

The human protein binding was drastically reduced for both compounds (hPPB **16** = 48%, **17** = 70%), and in the cell-based assays, **16** had reduced potency ($CIC_{95}$ = 0.840 $\mu$M 10% FBS, 1.1 $\mu$M 50% NHS). However, **17** proved to be very active in the cell-based assays ($CIC_{95}$ = 0.065 $\mu$M 10% FBS, 0.100 $\mu$M 50% NHS). Therefore, **17** was resolved into the single enantiomers **18** and **19**. The intrinsic activity on the enzyme was the same ($IC_{50}$ = 0.020 and 0.025 $\mu$M for **18** and **19**), further confirmation that the moiety at the 2-position is not involved in enzyme recognition. In the cell-based assay, **18** and **19** showed a 2-fold shift under the two serum conditions (**18**, $CIC_{95}$ = 0.04 $\mu$M 10% FBS, 0.065 $\mu$M 50% NHS; **19**, $CIC_{95}$ = 0.09 $\mu$M 10% FBS, 0.19 $\mu$M 50% NHS). Along with its very high potency, **18** had excellent pharmacokinetics in all preclinical species. It showed high oral bioavailability and exposure after oral dosing in rat dog and rhesus ($F$ = 56, 69, and 73%; nAUC = 2.3, 13.6, and 2.4 $\mu$M·h·kg/mg for rat, dog, and rhesus, respectively) with low plasma clearance in all species [Clp = 9 (rat), 2.2 (dog), and 14 (rhesus) mL/min/kg] and moderate to very good plasma half-lives ($t_{1/2}$ = 1.1 (rat), 7.3 (dog), and 2.0 (rhesus) h). Furthermore, **18** was inactive against HIV RT, HCV NS5B polymerase, and human $\alpha$, $\beta$, and $\gamma$ polymerases ($IC_{50}$ > 10 $\mu$M).

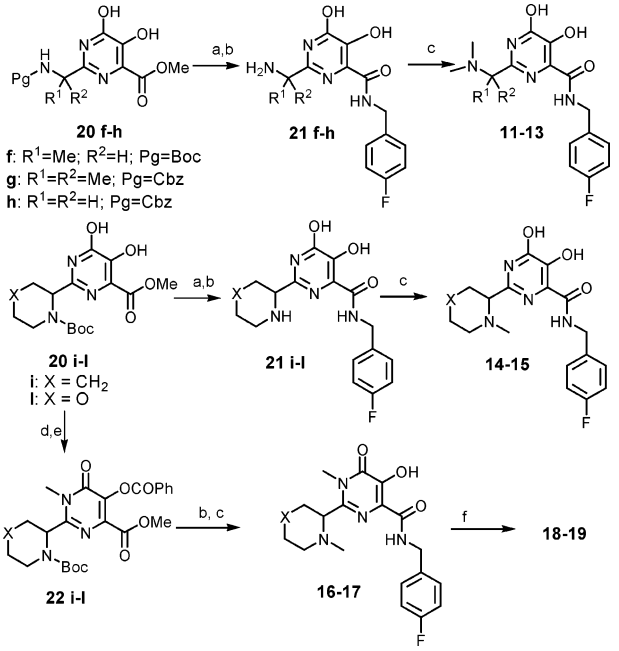
Schemes 1 and 2 depict the general procedures for the synthesis of compounds. 2-Substituted-5,6-dihydroxypyrimidine-4-carboxamides **4**−**10** were accessed by treatment of the corresponding methyl esters with the desired amine in solvents like methanol, DMF, or NMP (Scheme 1). 2-Substituted methyl-5,6-dihydroxypyrimidine-4-carboxylates **20a**−**e** were synthesized from the appropriately substituted amidoximes, either commercially available or prepared from nitriles, following the literature procedure.[15] Compound **10** was prepared from **20e** via bis-benzoylation, followed by bromination under standard conditions. Treatment of the resulting benzyl bromide with dimethylamine in THF gave displacement of the bromine with concomitant deprotection of the 6-hydroxyl group. Subsequent coupling with *p*-fluorobenzylamine in DMF gave compound **10**.

**Scheme 1.** Synthesis of Compounds **4**−**10**[a]




[a] Reagents and conditions: (a) $R^2NH_2$ (3 equiv), MeOH, DMF, or NMP, 90 °C; (b) PhCOCl (5 equiv), pyr, rt; (c) NBS (1.1 equiv), $(PhCO)_2O_2$ (0.1 equiv), $CCl_4$, reflux; (d) 2 M $Me_2NH$ in THF; (e) 4-F-$C_6H_4CH_2NH_2$ (3 equiv), DMF, 90 °C.

**Scheme 2.** Synthesis of Compounds **11**−**17**[a]




[a] Reagents and conditions: (a) 4-F-$C_6H_4CH_2NH_2$ (3 equiv), MeOH, 90 °C; (b) $CH_2Cl_2$/TFA (8:2), rt or $H_2$, Pd/C (10%), MeOH, rt; (c) 37% HCHO (5 equiv), $NaCNBH_3$ (1.5 equiv), AcOH (10 equiv), MeOH, rt; or 37% HCHO (6 equiv), $NaCNBH_3$ (5 equiv), NaOAc (6 equiv), MeOH, rt; (d) $(PhCO)_2O$ (2 equiv), pyr; (e) $Me_2SO_4$ (1.5 equiv), LiH (1.2 equiv) or $Cs_2CO_3$ (1.5 equiv), dioxane, 60 °C; (f) chiral HPLC.

Compounds **11**−**15** were prepared from the corresponding 2-substituted pyrimidine methyl esters **20f**−**l**, with the nitrogen of the 2-substitutent protected as Boc or Cbz (Scheme 2). Synthesis of the benzylic amide was performed as previously described and followed by removal of the protecting group to give intermediates **21f**−**l**. Alkylation of the nitrogen was achieved via reductive amination. A similar reaction sequence was applied to the *N*-methylpyrimidones **22i**−**l** prepared by

*N*-methylation of the monobenzoylated methyl ester, yielding compounds **16** and **17**. Resolution of **17** by chiral HPLC gave the single enantiomers **18** and **19**.

In summary, the knowledge of the common active site architecture of the HCV polymerase and HIV integrase and the fact that both enzymes use $Mg^{++}$ ions in their catalytic machinery led us to test designed HCV polymerase inhibitors against HIV integrase. The screening led to the identification of a new potent and selective class of HIV integrase strand transfer inhibitors represented by **5**. Optimization of potency, physical chemical properties, and pharmacokinetic profiles in preclinical species culminated in the identification of **13** and **18**. Compounds **13** and **18** showed $CIC_{95}$ equal or superior to the HIV drugs on the market and very good pharmacokinetic profiles in preclinical species. Further work in these promising series will be reported in due course.

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**Supporting Information Available:** Synthetic procedures, NMR, MS, and PK procedures are reported in detail. This material is available free of charge via the Internet at http://pubs.acs.org

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