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Structural modifications of 5,6-dihydroxypyrimidines with anti-HIV activity

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ABSTRACT

A series of 5,6-dihydroxypyrimidine analogs were synthesized and evaluated for their anti-HIV activity in vitro. Among all of the analogs, several compounds exhibited significant anti-HIV activity, especially **1b** and **1e**, which showed the most potent anti-HIV activity with EC_{50} values of 0.14 and 0.15 μM, and TI (therapeutic index) values of >300 and >900, respectively. Further docking studies revealed that the representative compounds **1e** and **3a** could meet the HIV-1 integrase inhibition minimal requirements of a chelating domain (two metal ions) and an aromatic domain (π–π stacking interactions).

Acquired immunodeficiency syndrome (AIDS) and human immunodeficiency virus (HIV) infection represent global health hazards, complex scientific challenges, and obvious targets for drug discovery and vaccination, as well as having enormous social, economic and ethical ramifications.[1] HIV-1 integrase (IN) is one of the three virally encoded enzymes required for replication and therefore a rational target for chemotherapeutic intervention in the treatment of HIV-1 infection.[2] After years of sustained effort, Merck successfully developed raltegravir (RAL, also known as Isentress® or MK-0518, **1** Fig. 1), which was approved by the United States of America's Food and Drug Administration (FDA) in late 2007 as the first IN inhibitor.[3] The good pharmacokinetics, minimal side effects and safety of raltegravir expanded its broad use in AIDS patients, but RAL has limited intestinal absorption[4] and resistance cannot be overcome by increasing the administered dose. Elvitegravir (EVG, **2** Fig. 1), the next most advanced IN inhibitor appears to share cross-resistance with RAL.[5] Mutations that confer resistance to these inhibitors have been generated in cell culture and have emerged in clinical studies,[6] suggesting that IN inhibitors will be confronted with the same resistance issue that have plagued other HIV/AIDS chemotherapies.[7] An intensified search for structurally novel IN inhibitors to combat resistance is therefore urgently needed.

Since 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,[8] dihydroxypyrimidines (DHP, **3**, Fig. 2) that had been investigated as potential HCV NS5B polymerase inhibitors were screened in the HIV integrase assays.[9] Rencently, introduction and optimization of carboxamide substituents in the 4-position of the dihydroxypyrimidine core resulted in analogues effective in suppressing the spread of HIV-1 infection in cells.[10–12] To identify more potent scaffolds against HIV integrase, we designed a series of new DHP analogs (**1a**–**1l**, **2a**–**2c**, **3a**–**3c**) and assessed them as anti-HIV agents. Overall, the binding of IN inhibitors conforms to a previously proposed interfacial inhibition mechanism,[1] as they bind all three key elements: the enzyme by hydrophobic and Van Der Waals interactions; the metals by chelation; and the DNA by π–π stacking.[13] For example, *N*-methylpyrimidone (**4**, Fig. 2) which is the critical core skeleton of RAL can engage metal ion cofactors in the IN active site by interactions with uniquely positioned oxygen atoms of the pharmacophore.[14] RAL also makes hydrophobic interactions by the isopropyl group and stacking interactions by the methyl-oxadiazole group. Based on the above considerations, firstly the carboxamide and benzylureido substituents were introduced in the 4-position of the dihydroxypyrimidine core to get the two analogues of dihydroxypyrimidines (A and B). And we hypothesize that both of these dihydroxypyrimidines have the same binding behavior of the *N*-methylpyrimidone

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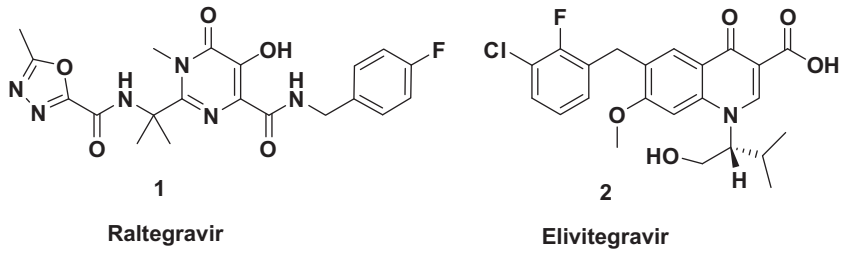


Figure 1.

core of RAL with the two metal ions (Mg^{2+}) (See Fig. 3). Secondly, the isopropyl and methyl-oxadiazole groups of RAL are involved in hydrophobic and stacking interactions with the side chains of Pro 214 and Tyr 212, respectively, further stabilizing this drug in the active site.[12] The target compounds (**1a**–**1l**, **2a**–**2c**, **3a**–**3c**) focused on the replacement of the methyl-oxadiazole group by benzyloxycarbonyl group and retention of the isopropyl group. Finally, hydrophobic or π–π stacking interactions can be achieved by aliphatic chain or aromatic groups.

As outlined in Scheme 1, target compounds containing the dihydroxypyrimidine core skeleton were successfully synthesized from commercially available reagents. The key dihydroxypyrimidine carboxylate intermediate **5** was prepared based on a reported method[15] and our optimized preparation of raltegravir[16], including aminonitrile formation, the addition of a benzyloxycarbonyl group, the conversion of nitrile to amidoxime, and cyclization to dihydroxypyrimidine. Firstly, treatment of the key DHP carboxylate intermediate **5** with the corresponding amine was performed in ethanol with microwave-assistance, leading to the formation of **1a**–**1l**, or with hydrazine hydrate in refluxing ethanol to generate **2a**. The reaction of **2a** with 4-fluorobenzaldehyde in refluxing THF yielded compound **2b**. Subsequently, **2a** was reacted with sodium nitrite in the presence of hydrochloric acid, and then refluxed in toluene via Curtius rearrangement yielding compound **2c**. **3a**–**3c** were prepared from **2c** reacted with the corresponding amine in THF. All target compounds were characterized by ^{1}H NMR and MS (see Supplementary Data).

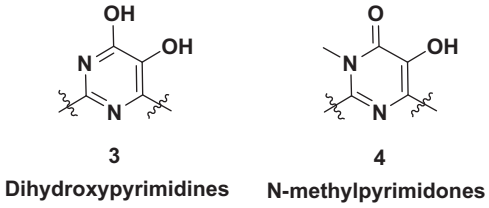


Figure 2.

Target compounds were evaluated for their inhibitory activity against HIV-1 replication in acutely infected C8166 cells in vitro according to the previously described method,[17–19] and AZT was used as a positive control. The assay results of the target compounds are presented in Table 1. Among all of the target compounds, **1b** and **1e** showed the most potent anti-HIV activity with EC_{50} values of 0.14 and 0.15 μM, and TI values of >300 and >900, respectively.

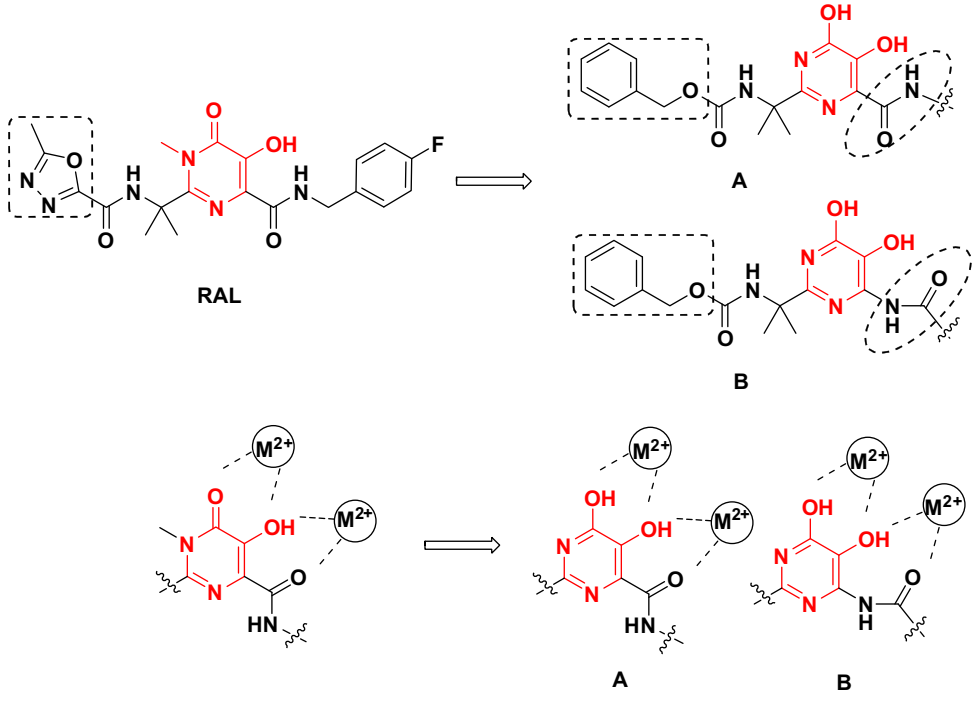
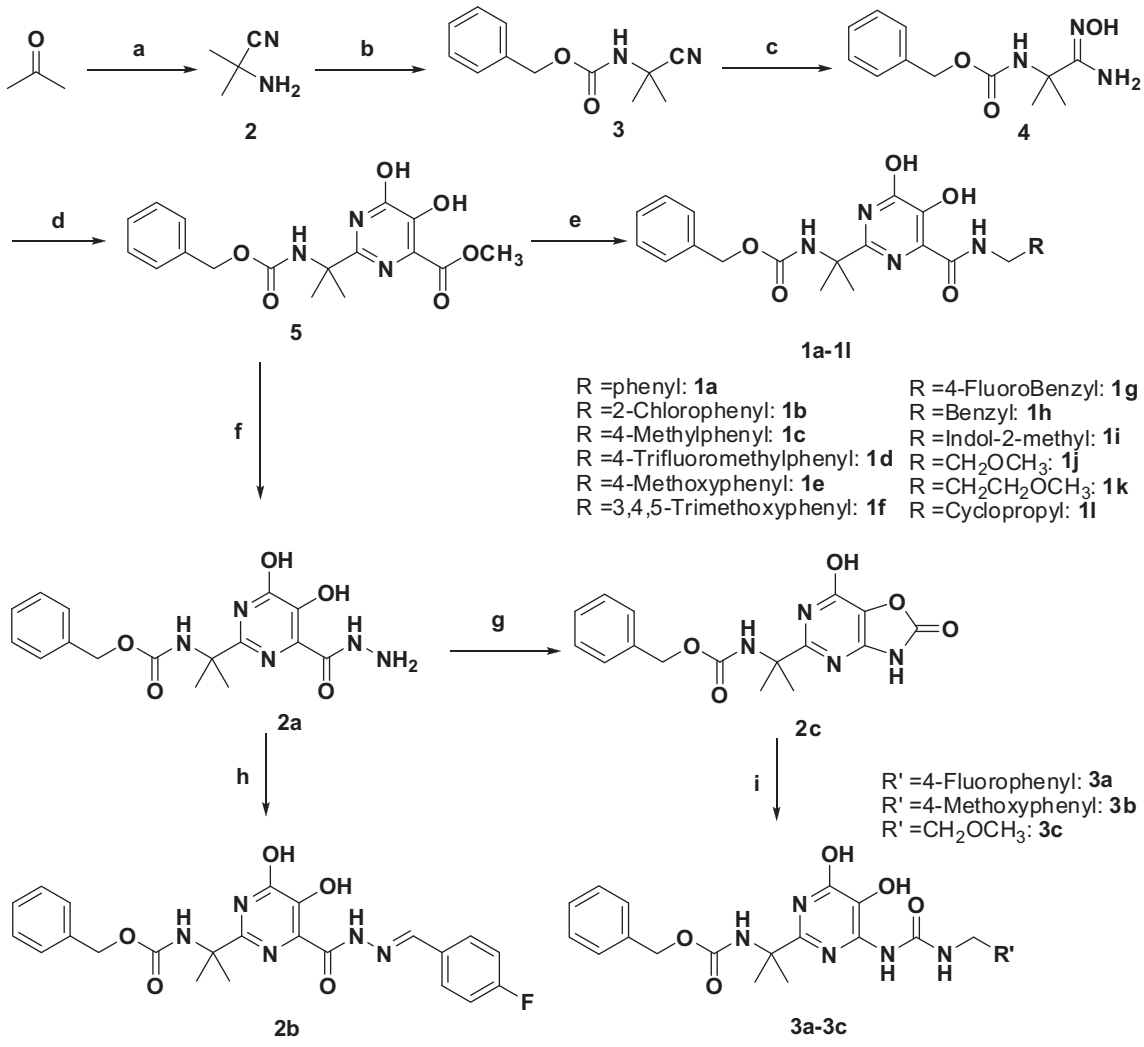


Figure 3.



Scheme 1. The synthetic route of target compounds. Reagents and conditions: (a) NaCN, NH_4Cl, ammonia (30%) in H_2O, rt; (b) Cbz–Cl, THF, $NaHCO_3$; (c) NH_2OH, IPA; (d) i: DMAD, MeOH, rt; ii: xylene, refluxed; (e) RCH_2NH_2, EtOH, microwave 90 °C; (f) $NH_2NH_2.H_2O$, EtOH, refluxed; (g) i: $NaNO_2$, 2 mol/L HCl; ii: toluene, refluxed; (h) 4-Fluorobenzaldehyde, THF, refluxed; (i) $R'CH_2NH_2$, THF.

In general, the introduction of a phenyl group at position R (**1a**–**1f**) leads to more potent anti-HIV activities than those of a benzyl group (**1g**–**1h**) or indol-2-methyl group (**1i**). It was evident that an aromatic ring separated by one sp^3 atom was essential for the activity since the aromatic ethanamine **1g**–**1i** (EC_{50} > 5 μM, TI <5) lost significant activity. Then the substitutions of benzyl groups were explored, when a non-substituted benzyl group (**1a**) or monosubstituted benzyl groups (**1b**–**1e**) were introduced, the EC_{50} and TI values of the corresponding compounds were <0.6 μM and >100, respectively. However, when the multisubstituted benzyl group was introduced at position R, the anti-HIV activities were reduced sharply (**1f**). No significant changes in the anti-HIV activities were observed with introduction of aliphatic chains at position R (**1j**–**1l**) of the amide. However among these compounds, the TI value of **1k** was >20. After optimization of the amide moiety, the conversion of amide to hydrazide (**2a**) and phenylmethylene hydrazide (**2b**) caused a substantial loss of the antiviral activity. On the other hand, the hydroxyl group on the dihydroxypyrimidine is crucial for the potency. When the hydroxyl group was cyclized to yield compound **2c**, the anti-HIV activity of compound **2c** markedly decreased. Furthermore, the benzylureido substituents were introduced from compound **2c**, the corresponding analogs (**3a**–**3c**) showed more potent activity than **2c**, especially the EC_{50} value of **3a**, which was approximately 9 μM.

Table 1
Anti-HIV activity of dihydroxypyrimidines in vitro[a]

Compounds	CC_{50}[b](μM)	EC_{50}[c](μM)	TI[d]
1a	41.44	0.32	128.16
1b	53.94	0.14	396.10
1c	233.82	0.55	383.04
1d	58.13	0.24	244.16
1e	164.03	0.15	1092
1f	109.50	12.32	8.89
1g	35.40	8.61	4.11
1h	38.49	11.58	3.32
1i	34.15	7.12	4.76
1j	348.19	25.99	13.40
1k	258.68	8.30	31.16
1l	203.72	24.45	8.33
2a	>500	164.35	>3.37
2b	377.82	27.66	13.66
2c	>500	74.19	>7.83
3a	81.10	9.87	8.21

Table 1 (*continued*)

Compounds	CC_{50}[b](μM)	EC_{50}[c](μM)	TI[d]
3b	>500	93.22	4.46
3c	>500	52.29	9.13
AZT[e]	5110	0.019	274200

[a] Values are means of two separate experiments.
[b] CC_{50} (50% cytotoxic concentration), concentration of drug that causes 50% reduction in total C8166 cell number.
[c] EC_{50} (50% effective concentration), concentration of drug that reduces syncytia formation by 50%.
[d] In vitro therapeutic index (CC_{50} value/EC_{50} value).
[e] AZT was used as a positive control.

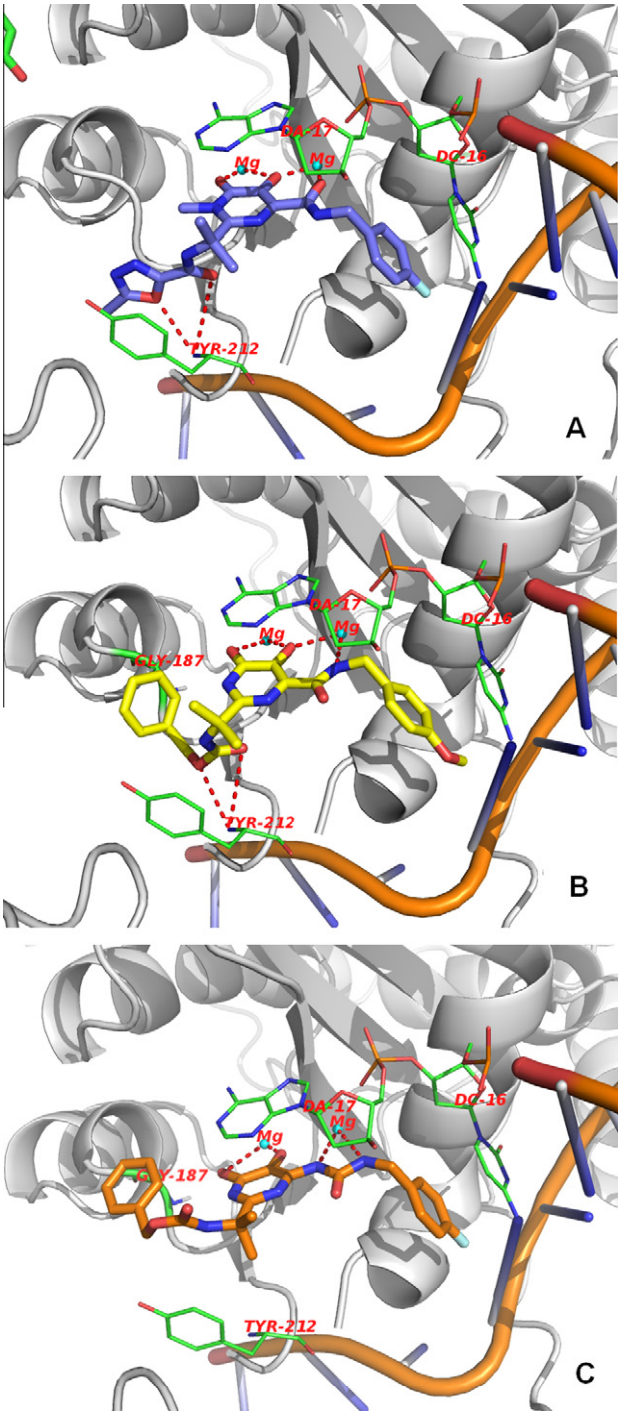


Figure 4. Docking of raltegravir (A), **1e** (B) and **3a** (C) in the active site of homoly model of HIV-1 IN.

Compounds **1e** and **3a** were chosen as representative of the target compounds and further analyzed. These compounds were docked into homologous models of the HIV integrase core domain based on original crystal structures of IN from prototype foamy virus (PFV) (pdb: 3OYA)[14,20] using GLIDE 4.0. The docking study revealed the following information: (1) the anti-HIV activity of compounds **1e** and **3a** involved the two-metal chelating mechanism, but the specific binding mode is different from raltegravir. The C-6 hydroxyl group, 5-ketone and 4-carbonyl group in raltegravir form the chelation (Fig. 4A), but for compound **1e**, the C-6, C-5 hydroxyl and the NH of the 4-carboxamide form the chelation (Fig. 4B). Moreover, for compound **3a**, the C-6, C-5 hydroxyl groups and the two NHs of 4-ureido form the chelation (Fig. 4C). (2) Similar to raltegravir, the substituted benzyl group and pyrimidine ring of compounds **1e** and **3a** exhibit π–π stacking interactions with DC16 and DA17, respectively. (3) No π–π stacking interactions could be detected between the benzyloxycarbonyl group in the two compounds and Tyr 212, but the benzyloxycarbonyl group in compound **1e** could form a hydrogen bond with Tyr 212, which could explain why the anti-HIV activity of compound **1e** is better than that of compound **3a**. Generally, compounds **1e** and **3a** could meet the requirements of the minimal pharmacophore embedded in major IN inhibitors: specifically, a chelating triad capable of binding two Mg^{2+} ions and a hydrophobic benzyl moiety.[7]

In summary, we designed and synthesized a series of dihydroxypyrimidine derivatives (**1a**–**1l**, **2a**–**2c**, **3a**–**3c**) that exhibited obvious anti-HIV activity in vitro. Among all of the analogs, compounds **1a**–**1e**, **1k**, and **3a** exhibited potent anti-HIV activities. Especially, **1b** and **1e** exhibited significant anti-HIV activities with EC_{50} values of 0.14 and 0.15 μM, and TI values of >300 and >900, respectively. Therefore, the dihydroxypyrimidine analogs afforded advantageous features of improved antiviral potency and decreased cytotoxicity with high therapeutic index, giving rise to the discovery of potent anti-HIV agents.

Acknowledgments

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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.2012.09.070.

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