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6-Benzoyl-3-hydroxypyrimidine-2,4-diones as dual inhibitors of HIV reverse transcriptase and integrase

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ABSTRACT

N-3-Hydroxylation of pyrimidine-2,4-diones was recently found to yield inhibitors of both HIV-1 reverse transcriptase (RT) and integrase (IN). An extended series of analogues featuring a benzoyl group at the C-6 position of the pyrimidine ring was synthesized. Through biochemical studies it was found that these new analogues are dually active against both RT and IN in low micromolar range. Antiviral assays confirmed that these new inhibitors are active against HIV-1 in cell culture at nanomolar to low micromolar range, further validating 3-hydroxypyrimidine-2,4-diones as a viable scaffold for antiviral development.

Human immunodeficiency virus (HIV) infects an estimated 33 million people globally and poses a huge healthcare challenge with high mortality and morbidity rates.[1] As effective HIV vaccines remain elusive despite tremendous efforts,[2,3] chemotherapy continues to provide the main leverage in battling HIV/AIDS. Current standard therapy, the highly active antiretroviral therapy (HAART),[4,5] involves the use of multiple antivirals with orthogonal mechanisms of action to create a large genetic barrier to resistance. Its success in clinically managing HIV/AIDS notwithstanding, HAART requires nearly perfect adherence[6,7] which can be difficult to achieve with the complex dosing of multi-target therapy. Suboptimal compliance generally leads to treatment failure and development of multi-drug resistance.[6,7] Drug–drug interactions are among the most prominent complications of HAART.[8] Simplifying HAART regimens to improve adherence has been a subject of tremendous research efforts and interests.[9,10] Conceptually the simplest form of multi-target therapy would be a single structure compound inhibiting multiple viral targets. We have previously reported various examples[11–15] of single structure dual inhibitors of HIV-1 RT and IN. Of particular interest was our recently disclosed RT/IN dual inhibitor scaffold[14] based on pyrimidine-2,4-dione non-nucleoside RT inhibitors (NNRTIs). In this case, N-3 hydroxylation of known 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio) thymine (HEPT) type NNRTIs yielded new compounds active against IN while retaining inhibition against RT (Fig. 1, chemotype **I**).[14] Interestingly, similar to HEPT, pyrimidine-2,4-diones featuring a C-6 benzoyl group (chemotype **IIa**) also represent an important class of NNRTIs.[16–22] It is easily conceived that introducing an N-3 hydroxy group to these NNRTIs could generate a new molecular scaffold for inhibitors of RT (RTI) and IN (INI) (Fig. 1, chemotype **IIb**). Here we report the synthesis and biological evaluation of this extended series of 6-benzyol-3-hydroxypyrimidine-2,4-diones.

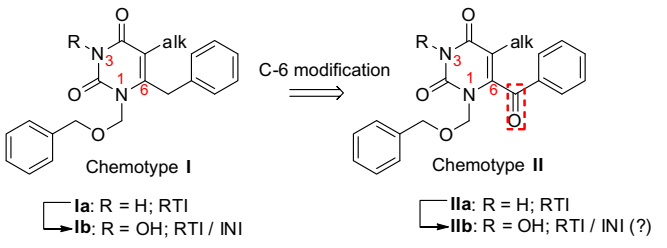

Figure 1. Design of the new series of N-3-hydroxypyrimidine-2,4-diones featuring a C-6 benzoyl group.

Abbreviations: HIV, human immunodeficiency virus; RT, reverse transcriptase; IN, integrase; HAART, highly active antiretroviral therapy; IN, integrase; NNRTI, non-nucleoside RT inhibitor; HEPT, 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio) thymine; 3′-P, 3′ processing; ST, strand transfer; CCD, catalytic core domain; CPE, cytopathic effect.

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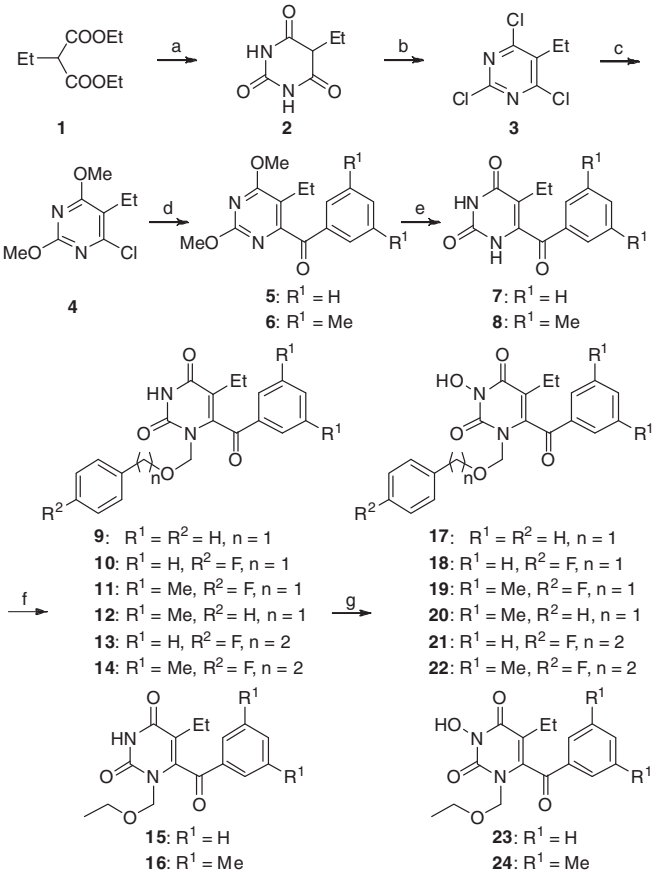

Scheme 1. Synthesis of compounds **17–24**. [a]Reagents and conditions: (a) urea, NaOMe, MeOH, reflux, 75%; (b) $POCl_3$, dimethylaniline, 89%; (c) MeONa, MeOH, 81%; (d) (i) substituted phenyl acetonitrile, NaH, DMF, rt; (ii) NaH, DMF, air, 84–87%; (e) HCl, MeOH, reflux, 85–93%; (f) $CH_3C(OTMS)$═NTMS (BSA), benzyl alcohol substrate, $(HCHO)_n$, TMSCl, TBAI (cat.), CH_2Cl_2, rt; or (for **15**, **16**) BSA, chloromethyl ethyl ether, TBAI (cat.), CH_2Cl_2, rt, 74–94%; (g) NaH, *m*-CPBA, THF, rt, 38–68%.

The new chemotype was synthesized via a route described in Scheme 1. The synthesis involves key intermediates **7** and **8** which were prepared starting from readily available malonate **1** following reported procedures.[16–18] The N-1 ether linkage was then introduced through a regio-selective alkylation with a chloromethyl ether to give compounds **9–16**. N-3-hydroxylation was achieved via a base-mediated *m*-CPBA oxidation[14] to produce final compounds **17–24**.

Final compounds **17–24** were biochemically evaluated against recombinant HIV-1 RT and IN. For the RT assay, all compounds were first screened at two different concentrations (100 and 10 μM) and the promising ones were then assayed in dose response fashion. It was observed from these assays that except for **21** these newly synthesized analogues generally inhibit RT at low micromolar concentrations (Table 1). Notably, the 3,5-dimethyl group on the benzene ring of the C-6 substituent appears to significantly benefit RT binding (**19** vs **18**, **20** vs **17**, **24** vs **23**). The same effect was observed with the HEPT NNRTI GCA-186[23] where the dimethyl group on the C-6 benzene provides additional van der Waals interactions with the roof of the binding pocket. Meanwhile, the fluorine atom on the benzene ring of the N-1 side chain was found to hamper RT inhibition (**18** vs **17**, **19** vs **20**), and the extra carbon in the N-1 linker also conferred a substantial decrease in RT inhibition (**21** vs **18**, **22** vs **19**).

The ability of these new analogues to inhibit HIV-1 IN was assessed using a biochemical gel assay. Both the 3′ processing (3′-P) and the strand transfer (ST) functions were evaluated in this assay. The results clearly demonstrate that these compounds selectively inhibit ST at low micromolar range (Table 1). Most intriguingly, IN ST inhibition seems to have a high dependence on the benzene ring in N-1 side chain as the two analogues with an aliphatic N-1 side chain (**23** and **24**) showed no inhibition. Another major observation was that the extra carbon on the N-1 linker appears to considerably benefit IN binding as compounds **21** and **22** exhibit significantly higher potency against IN. It was also found that the fluorine atom on the N-1 benzene tends to slightly favor IN inhibition (**18** vs **17**, **19** vs **20**). These findings conform to the common pharmacophore of IN ST inhibitors in which a fluorobenzyl group with a specific spatial arrangement to a chelator is a required structural determinant.[15,24–28]

Table 1
Biochemical assay results of compounds **17–24** against HIV-1 RT and IN

Compd	RT			IN IC_{50}[b] (μM)	
	100 μM[a]	10 μM[a]	IC_{50}[b] (μM)	3′-P	ST[c]
17	84	61	14	>111	37 ± 6
18	78	41	23	>111	25 ± 3
19	85	68	7.6	>111	21 ± 3
20	92	79	1.0	>111	41 ± 4
21	46	4	––	>111	4.1 ± 0.2
22	74	60	10	>111	7.0 ± 0.8
23	61	37	43	>111	>111
24	94	61	0.5	>111	>111

[a] % Inhibition under single concentration.
[b] Concentration inhibiting enzyme activity by 50%.
[c] Mean value ± standard deviation from three independent measurements.

To confirm the binding of our compounds to both RT and IN, a representative inhibitor (**20**) was docked into both the NNRTI binding pocket (A)[14] and the IN catalytic core domain (CCD) (B)[29] using Glide v2.5 at Standard Precision.[30] Significantly, the N-3 OH of **20** is hydrogen bonded to the K101 backbone in the NNRTI binding pocket, allowing the inhibitor to adopt a conformation where the C-6 benzene ring interacts favorably with the Y188, and the N-1 side chain sits in an open channel (Fig. 2,A). On the other hand, the same inhibitor was found to fit nicely into the IN CCD in complex with Mg and viral DNA (Fig. 2,B). The deprotonation of the N-3 OH group of the pyrimidine ring allows the chelation of two Mg^{2+} ions and the placement of the N-1 benzyl group into the protein–DNA interfacial hydrophobic pocket. These docking studies corroborate the observed dual inhibitory activities and suggest that this chemotype has the ability to engage with both RT and IN.

Finally, the antiviral activity of these new compounds was evaluated in CEM-SS cells with the IIIB strain of HIV-1 using an assay based on viral cytopathic effect (CPE). The reduction of CPE was used to indicate the inhibition of viral replication. Compounds were first tested at a single concentration (10 μM) for antiviral activity and cell viability. The active ones were then tested in dose response fashion.

As shown in Table 2, except for compound **22**, all new compounds yielded 100% inhibition against HIV-1 at 10 μM without appreciable cytotoxicity (Table 2). Further testing in dose–response fashion found that these compounds inhibit HIV-1 at low micromolar to low nanomolar range. The parallel cytotoxicity assay showed that all final compounds are generally safe with favorable therapeutic indices. Interestingly, compound **24** which inhibited RT with the highest potency in the series while lacking anti-IN activity, and compound **21** which showed the best anti-IN activity with only marginal inhibition against RT, both demonstrated excellent antiviral activity. The overall anti-HIV-1 activity of the rest of the compounds likely reflects the dual inhibitory activities against both RT and IN.

In summary, based on recently disclosed N-3-hydroxypyrimidine-2,4-diones as a new chemotype for dual inhibitors of HIV-1

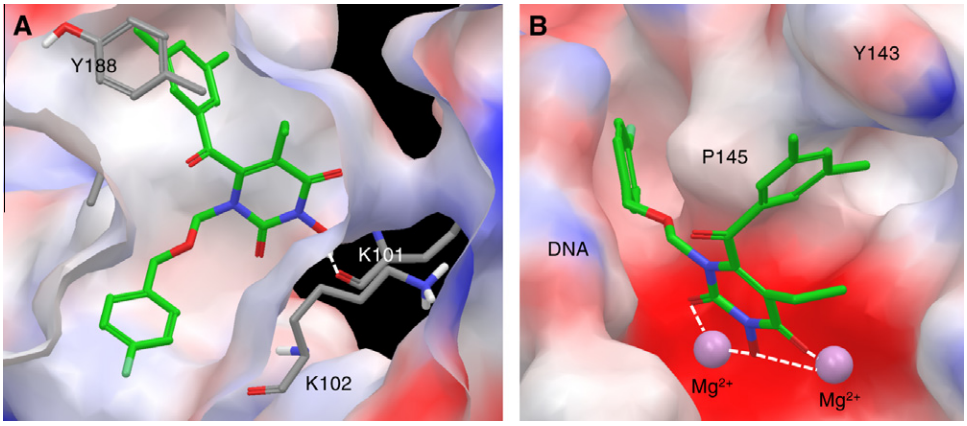

Figure 2. Inhibitory modes of binding for compound **20** within (A) the NNRTI binding pocket and (B) the active site of IN CCD in complex with Mg^{2+} and DNA. The electrostatic potential surface of the binding pocket is shown to highlight the hydrophobic regions (white) in both enzymes.

Table 2
Antiviral assay results of compounds **17–24** against HIV-1

Compd	10 μM		Dose response		
	% CPE[a] reduction	% Cell viability	EC_{50}[b] (μM)	CC_{50}[c] (μM)	TI[d]
17	100	98	1.4	>100	>71
18	100	100	2.0	>100	>50
19	100	100	0.36	28	78
20	100	100	0.061	33	540
21	100	100	0.34	>100	290
22	56	81	––	––	––
23	100	100	2.3	94	41
24	100	100	0.030	77	2600

[a] Viral cytopathic effect.
[b] Concentration inhibiting viral replication by 50%.
[c] Concentration causing 50% cell death.
[d] Therapeutic index, defined by CC_{50}/EC_{50}.

RT and IN, we have synthesized an extended series of analogues featuring a benzyol group at the C-6 position of the pyrimidine. Through biochemical studies and cell culture antiviral assays it was found that these new analogues inhibit RT, IN and HIV-1 at nanomolar to low micromolar range. Molecular modeling also suggests that these compounds can fit nicely into both the NNRTI binding pocket and IN CCD. These results provide further support on 3-hydroxypyrimidine-2,4-diones as a valid scaffold for antiviral development.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.02.069.

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