Bioorganic & Medicinal Chemistry Letters 18 (2008) 3865–3869

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

4-Hydroxy-5-pyrrolinone-3-carboxamide HIV-1 integrase inhibitors

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ARTICLE INFO

Article history:
Received 6 June 2008
Revised 16 June 2008
Accepted 16 June 2008
Available online 20 June 2008

Keywords:
AIDS
HIV-1 integrase
Diketoacids
Pyrimidines
Pyrrolinones

ABSTRACT

The viral enzyme integrase is essential for the replication of HIV-1 and, after the discovery of Isentress™, represents a validated target for anti-retroviral therapy. Incorporation of the dihydroxycarbonyl pharmacophore into a pyrrolinone scaffold led to the discovery of 5-pyrrolinone-3-carboxamides as a structurally diverse class of HIV-1 integrase inhibitors.

A variety of anti-HIV agents rely on inhibition of key enzymes involved with the virus life cycle. The utility of compounds directed at single targets is greatly limited by the development of resistance. HAART (Highly active anti-retroviral therapy) has demonstrated that targeting multiple enzymes provides one means of circumventing this problem, with combination therapies directed against both the HIV reverse transcriptase and the protease enzymes showing good results in reducing viral loads in HIV-1 infected patients.[1] However, while HAART is undeniably effective, it can fail to control HIV replication in patients due to several limitations, such as lack of therapy adherence, toxicities issues, and the emergence of drug resistant viral strains. Recently, Isentress™ discovered in our laboratories has been approved by the FDA, an oral drug that inhibits HIV integrase, the third enzyme encoded by the HIV-1 genome and is expected to complement HAART offering a new treatment option and renewed hope to sufferers of this important disease.[2]

Integrase catalyzes the integration of double stranded viral DNA into the host cell's genomic DNA.[3] Integration consists of three biochemical steps: the assembly of integrase on viral DNA, endonucleolytic cleavage of the first two nucleotides from each 3′ terminal of the viral DNA, and strand transfer of the recessed viral DNA to the host cell DNA.[4,5] 1,3-Diketoacids **1** were reported to be effective integrase inhibitors and prevent HIV-1 replication in cell culture (Fig. 1).[6] The same dihydroxycarbonyl pharmacophore was recognized in the pyrimidines/pyrimidones **2** with tremendous improvements in drug-like properties and led ultimately to the discovery of Isentress™.[7] Continuing our work in this research field and in an attempt to identify new scaffolds featuring a suitable replacement for the 1,3-diketoacid motif, we designed and synthesized a novel series of substituted pyrrolinones **3** HIV-1 integrase inhibitors.

Other laboratories also demonstrated that the diketoacid pharmacophore can be replaced with 2-pyrrolinones having a ketone or an heteroaromatic substituent.[8] The structural requirements for inhibitory activity defined by our preceding studies on pyrimidine inhibitors[9] guided the preparation of this series of pyrrolinone inhibitors having a benzylic amide substituent. The diketoacid portion of the template is believed to bind to the Mg^{2+} cofactor located in the active site, while the *p*-fluorobenzyl participates in a specific interaction with an adjacent hydrophobic pocket. On pyrimidines/pyrimidones it was established that in terms of potency, a variety of substituents are tolerated in the 2-position of the heterocyclic core, the presence of a basic amine was required to modulate physical properties and, ultimately, achieve activity in cell culture.

The initial replacement of the pyrimidine core with the pyrrolinone, keeping fixed the *p*-fluorobenzyl amide moiety, resulted in **4** which inhibited the strand transfer process of HIV-1 integration with an IC_{50} = 100 nM (Table 1). A dihydroxypyrimidine with a comparable substitution pattern **2** (R′ = 4-F, R″ = 4-$CH_3C_6H_4$) showed an IC_{50} = 20 nM. Molecular modeling showed a good superimposition of **4** with the above 2-arylsubstituted dihydroxypyrimidine (Fig. 2) and suggested the possibility to further explore the N-1 substituents, with the potential of changing physicochemical properties of the inhibitor as with the pyrimidines/pyrimidones. Conformational analysis and energy minimizations were carried out using the MMFFs force field, as implemented in Macro-

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doi:10.1016/j.bmcl.2008.06.056

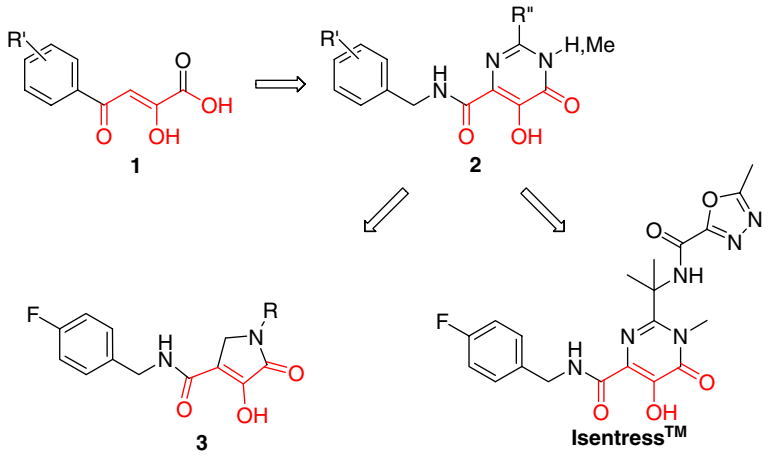


Figure 1. Conception of 4-hydroxy-5-pyrrolinone-3-carboxamide inhibitors.

Table 1
Inhibition of HIV-1 integrase catalytic activities and HIV-1 replication in cells by compounds **4**–**12**

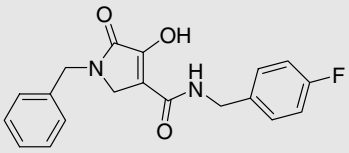
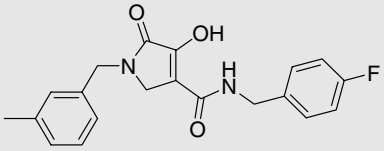
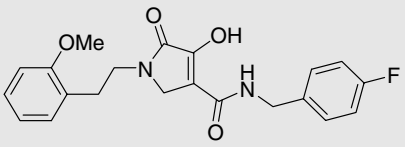
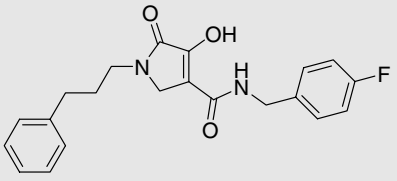
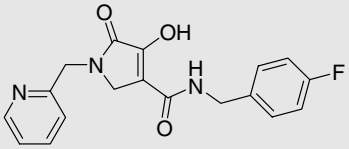
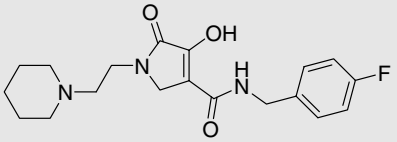
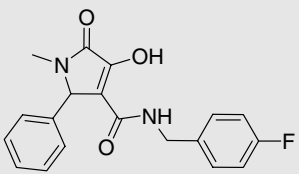
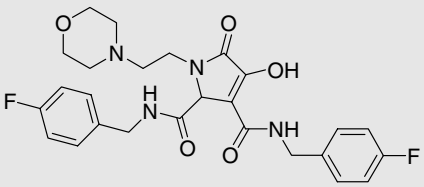
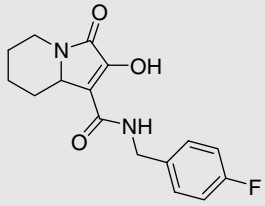
Compound	Structure	Inhibition of strand transfer IC_{50}[a] (μM)	Antiviral activity CIC_{95}[b] (μM)
4		0.1	na[c]
5		0.006	na[c]
6		0.004	na[c]
7		0.005	na[c]
8		0.094	na[c]
9		>1.0	na[c]
10		>1.0	na[c]

Table 1 (*continued*)

Compound	Structure	Inhibition of strand transfer IC_{50}[a] (μM)	Antiviral activity CIC_{95}[b] (μM)
11		>1.0	na[c]
12		0.018	35% @ 25 μM

[a] Assays were performed with recombinant HIV-1 integrase (0.1 μM) preassembled on immobilized oligonucleotides.[12] Values are means of three experiments.
[b] Cell culture inhibitory concentrations (CIC_{95}) are defined as those which inhibited by >95% the spread of HIV-1 infection in MT-4 human T-lymphoid cells maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum.[13] Cytotoxicity is not observed in cell culture at concentrations up to 20 μM.
[c] Not active at 25 μM.

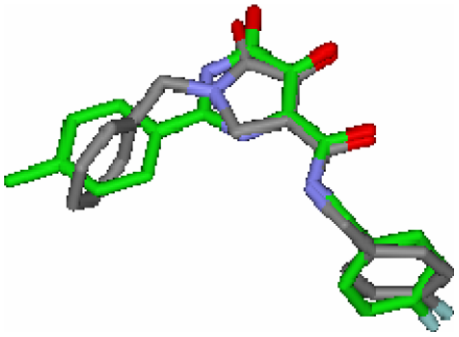


Figure 2. Superimposition of **4** with a dihydroxypyrimidine.

Model v9.0. Schrodinger's maestro front end was used to build initial conformers and to superimpose molecular structures.[10]

A significant increase in affinity was observed upon introduction of a methyl on the benzyl substituent (compound **5**) or by increasing the length of the carbon chain between the aryl ring and the pyrrolinone nitrogen (compounds **6** and **7**), with IC_{50}s in the low nanomolar range.

Reasoning that perhaps the lack of cellular activity was due to suboptimal physical chemical properties that could affect cell permeability and/or binding to intracellular proteins and also plasma proteins present in the cell medium, we tried to include polar or ionizable groups into the molecules.

However, this strategy was not successful since we did not observe any improvement in cellular activity. Moreover, while the pyridyl-substituted compound **8** retained potency with respect to its phenyl counterpart (IC_{50} = 94 nM), the piperidine-derivative **9** did not show significant inhibitory activity. Protein binding of **8** was very high indeed since the compound was ≪1% unbound in human plasma.

Apart from N-1 substitution, we went on to explore substituents at the 2-position. However, as illustrated by comparison of compounds **10** and **4**, moving the phenyl ring to the 2-position was detrimental, indicating that there is limited tolerance for substitution at this site. The more functionalized compound **11** was inactive too. The introduction of a substituent in this position results in the loss of oxygen atoms co-planarity, which is required for Mg^{2+} chelation. As a bicyclic tetrahydropyridopyrimidone was found to be an important scaffold for HIV-1 integrase activity,[11] we prepared the pyrrolinone analog **12**, which rescued the in vitro activity to low nanomolar values. Furthermore, with this compound for the first time we started to see some inhibition in the cell-based assay, although weak. This more rigid bicyclic core superimposes well with the corresponding pyrimidinone thus offering additional opportunities for SAR exploration (Fig. 3).

None of the novel pyrrolinones showed cellular toxicity at a maximum tested concentration of 20 μM.

The target *N*-substituted-4-hydroxy-5-oxo-2,5-dihydro-*1H*-pyrrole-3-carboxamides **4**–**10** were prepared with minimal modifications of a literature procedure.[14] Michael addition of the appropriate amine **13** on *N*-(4-fluorobenzyl)acrylamide **14** gives the β-aminopropionate 4-fluorobenzylamide **15** which is then condensed with diethyloxalate in the presence of sodium ethoxide. The synthetic sequence employed is shown in Scheme 1. Synthesis of compound **11** was achieved by a different reaction sequence, based on the alkylation of 2-(4-morpholinyl)ethanamine **16** with diethyl (*2Z*)-2-butenedioate **17**, base promoted condensation with diethyl oxalate and final installation of the 4-fluorobenzylamide by reaction with *p*-fluorobenzylamine in ethanol, in the presence of NaOEt (Scheme 2). For the synthesis of **12**, we used an imine-addition method based on condensation of imine **19** with ethyl oxalacetate.[15] As reported in the literature, imine **19** (Δ^1-piperidine) is an elusive species, therefore was prepared by dehydrohalogenation of *N*-chloropiperidine and refluxed with a benzene solution of diethyloxalacetate. The *p*-fluorobenzylamide moiety was installed upon microwave irradiation in a *N*-methylpyrrolidone solution (Scheme 3).

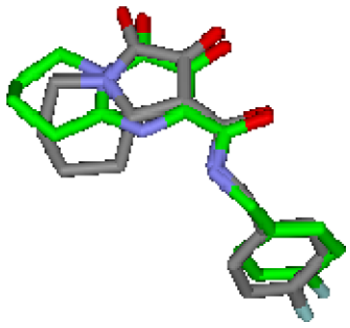
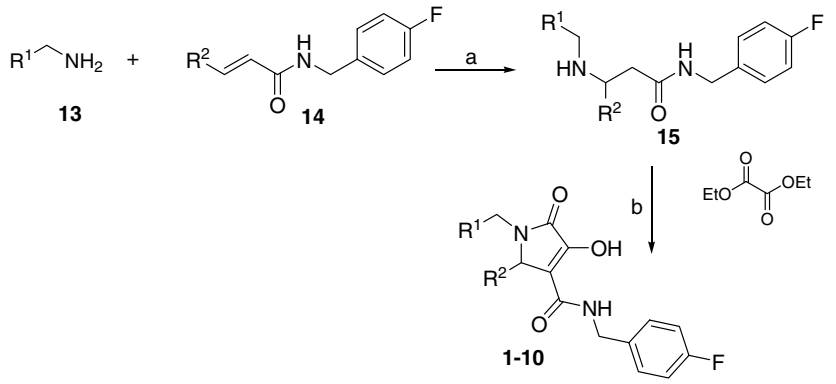
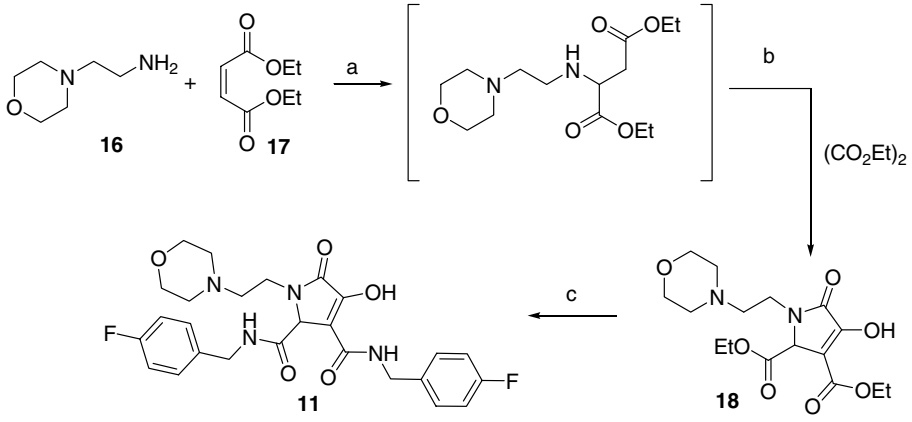


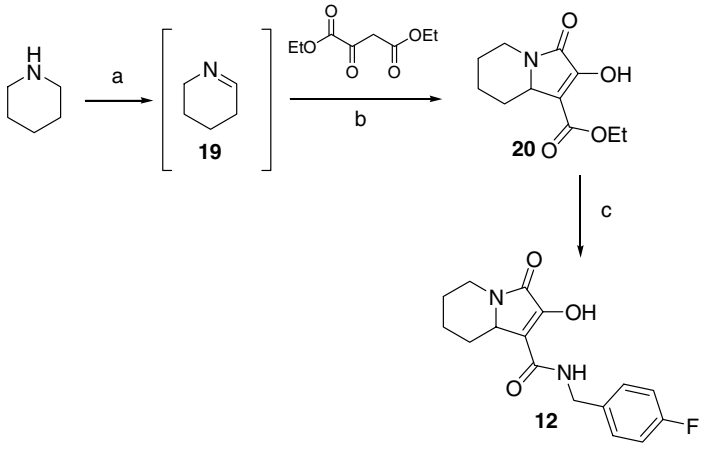
Figure 3. Superimposition of **12** with a bicyclic-pyrimidinone.



Scheme 1. Reagents and conditions: (a) EtOH, 60 °C, 12 h; (b) NaOEt (1.2 equiv), EtOH, reflux, 2 h.



Scheme 2. Reagents and conditions: (a) EtOH, 60 °C, 12 h; (b) NaOEt (1.2 equiv), EtOH, reflux, 2 h (20%, 2 steps); (c) 4-F-$C_6H_4CH_2NH_2$ (2.2 equiv), NaOEt (2 equiv), EtOH, refl. (45%).



Scheme 3. Reagents and conditions: (a) i—*N*-chlorosuccinimide (1.8 equiv), Et_2O; ii—KOH (1 equiv), EtOH, 0 °C; (b) benzene, reflux, 3.5 h (30%, 2 steps); (c) 4-F-$C_6H_4CH_2NH_2$ (3 equiv), NMP, 200 °C, 300ss (60%).

In conclusion, we have identified a series of 4-hydroxy-5-pyrrolinone-3-carboxamide HIV-1 integrase inhibitors incorporating the co-planar diketoacid pharmacophore as a novel class of compounds reasonably having the same mode of binding to the enzyme as the dihydroxypyrimidines/pyrimidones. A limited SAR around the pyrrolinone core resulted in the discovery of compounds with inhibitory activity in the low nanomolar range. Further chemical optimization of the substituents would better define the potential of this template for the development of inhibitors which may have different profiles than other known inhibitors.

Acknowledgments

We thank Kara A. Stillmock, Daniel J. DiStefano, Peter J. Felock, and William A. Schlief for HIV biological testing, and Michael Rowley for discussion.

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