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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 350–353

From dihydroxypyrimidine carboxylic acids to carboxamide HIV-1 integrase inhibitors: SAR around the amide moiety

Alessia Petrocchi,[a,*] Uwe Koch,[a] Victor G. Matassa,[†] Barbara Pacini,[a] Kara A. Stillmock[b] and Vincenzo Summa[a]

[a]*Department of Medicinal Chemistry and Drug Metabolism, IRBM-MRL Rome, Via Pontina, Km 30.600, 00040 Pomezia, Rome, Italy*
[b]*Department of Antiviral Research, MRL West Point, Sumneytown Pike, PO Box 4, 19486, PA, USA*

Received 21 September 2006; revised 20 October 2006; accepted 20 October 2006
Available online 25 October 2006

Abstract—4,5-Dihyroxypyrimidine carboxamides, which evolved from a related series of HCV NS5b polymerase inhibitors, have been optimized to provide selective HIV integrase strand transfer inhibitors. Extensive SAR around the benzylamide moiety led to the identification of the *p*-fluorobenzylamide as optimal in the enzymatic assay.

Human immunodeficiency virus type 1 (HIV-1) is the etiological agent of the acquired immunodeficiency syndrome (AIDS). AIDS[1] is a major epidemic with more than 38 million infected people worldwide. FDA-approved therapies target three steps of the HIV life cycle: reverse transcription (NRTI, nucleoside reverse transcriptase inhibitor and NNRTI, non-nucleoside reverse transcriptase inhibitor), proteolytic maturation, and fusion. Triple therapy, commonly referred to as highly active antiretroviral therapy (HAART), is now the standard for treatment and consists of a protease inhibitor or an NNRTI in combination with two NRTIs. HAART, however, is often not well tolerated, requires stringent compliance, and leads to multidrug resistance. Therefore, novel therapies are needed. Integrase is an enzyme encoded by the HIV genome and represents a potential alternative target. It catalyzes the insertion and the integration of the proviral DNA into the genome of the host cell in two steps: 3′-processing, the endonucleolytic sequence-specific hydrolysis of 3′-ends of the viral cDNA and strand transfer, the ligation of the viral 3′-OH cDNA ends to the phosphate backbone of the host DNA acceptor.

The first generation integrase inhibitors reported approximately 12 years ago[2,3] blocked the whole assembly process. More recently, 1,3-diketoacids have been described which specifically inhibit the strand transfer reaction catalyzed by HIV integrase: their evolution to a molecule with more drug-like properties led to the naphthyridine series.[4,5]

HIV integrase together with the related enzyme hepatitis C virus (HCV) NS5b polymerase share a common mechanism of action with catalytic activity that is mediated through Mg^{2+} ions believed present in their active sites. Recently, we reported the discovery of 1,3-diketoacids[6] (e.g., **1**) and meconic acid derivatives[7] (e.g., **2**) as potent inhibitors of HCV NS5b polymerase (Fig. 1). These classes lacked drug-like characteristics: meconic acid derivatives were almost completely decarboxylated in acidic media, while DKAs showed a high level of irreversible covalent binding to proteins.[4] Dihydroxypyrimidine carboxylic acid (**3**), a designed hybrid of these two series,[8] provided a novel class of inhibitors of HCV NS5b polymerase with improved chemical and biological stability. Despite their potent activity against HCV NS5b polymerase, and a mechanism of action that involves direct interaction with the active site metal ions, dihydroxypyrimidine carboxylic acids **3** showed no activity against the HIV integrase enzyme.

However, as part of the optimization work to identify viable replacements for the carboxylic acid **3**,[9] carboxa-

Keyword: HIV integrase inhibitors.
* Corresponding author. Tel.: +39 06 91093500; fax: +39 06 91093654; e-mail: alessia_petrocchi@merck.com
† Present address: Almirall SA, Calle Treball 2-4, Sant Just Desvern, Barcelona 08960, Spain.

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doi:10.1016/j.bmcl.2006.10.054

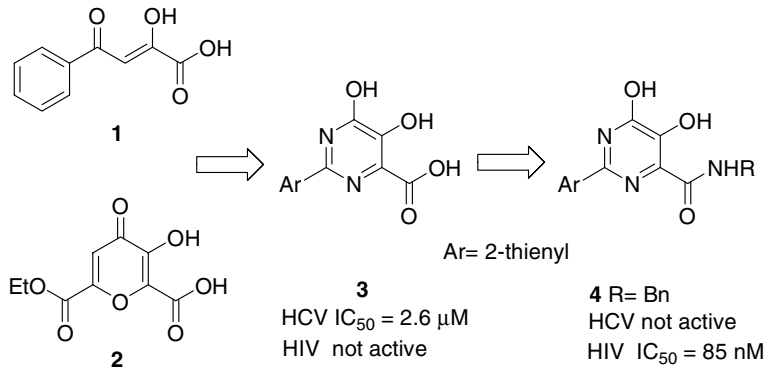


Figure 1. From dihydroxypyrimidine carboxylic acid to carboxamide.

mides were found to be inactive against HCV NS5b polymerase but selective inhibitors of the HIV integrase enzyme.

Here, we report our initial exploration of this series which focused exclusively on structure–activity relationships (SAR) at the benzylamide portion of the inhibitor.

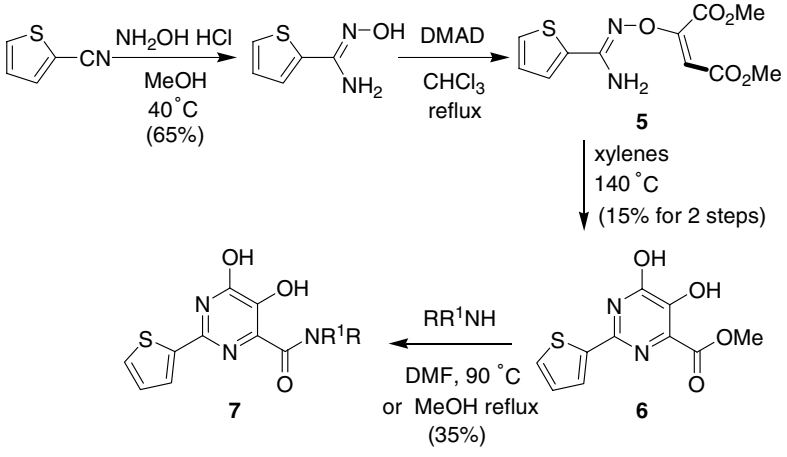
Scheme 1 describes a general procedure[10] for the synthesis of the dihydroxypyrimidine carboxamide **7** contained in this study. *N*′-Hydroxythiophene-2-carboximidamide, generated from thiophene-2-carbonitrile, was reacted with dimethyl acetylendicarboxylate in chloroform at 90 °C, to produce the Michael adduct as a mixture of cis–trans isomers. Heating in refluxing xylenes the foregoing mixture afforded the methyl ester **6**. The final carboxamide **7** was easily obtained by reacting **6** with the proper amine in *N*,*N*-dimethylformamide at 90 °C (or in refluxing methanol) and isolating the final product by trituration with diethyl ether, without any further purification.

The preliminary investigation of SAR at the carboxamide **7** is shown in Table 1. Replacement of the benzyl group with a cyclohexylmethyl substituent completely abolished activity (**8**) underlining the importance of the aromatic moiety in this part of the molecule. Varying the distance between the amide nitrogen and the aryl moiety produced the following results: elongating by an additional methylene unit (**9**) provided 4-fold gain in potency compared to the benzyl analog, whereas shortening the linker distance produced the weakly active anilide **10** (IC_{50} = 1 μM).

Insertion of the 1-naphthalene ring (**11**) increased potency 3-fold with respect to the lead. Similar enhancement was observed when the benzylic methylene was substituted with a methyl group, but only for the *S*-enantiomer (**12**, IC_{50} = 40 nM). The corresponding 1-naphthyl N-methylated (tertiary) amide **14** provided 7-fold drop in activity with respect to **11** that emphasizes the importance of the secondary amide moiety in the molecule.

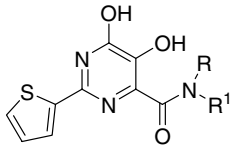
Indane chiral derivatives (**15**, *S*, IC_{50} = 20 nM; **16**, *R*, IC_{50} = 610 nM) displayed different inhibitory activity indicating again that stereochemistry at the benzylic position is vital for potency of branched compounds.

Efforts aimed at replacing the phenyl ring with a heterocycle are shown in Table 2. Compounds bearing polar heterocycles were completely inactive (**17**, **18**, and **19**). Substitution of the phenyl ring with thiophene (**20**) or with thiazoles (**21** and **22**) produced less active compounds. Potency was improved 10-fold and 2-fold, respectively, for benzothiophenes **23** and **24**, and 5-fold for indole **25** confirming the presence of enough space



Scheme 1. Synthetic procedure.

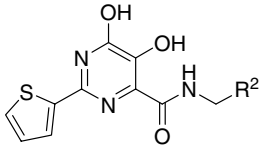
Table 1. Preliminary SAR at the carboxamide



Compound	R	R^1	QUICKIN IC_{50}[a] (nM)
4	CH_2Ph	H	85
8	CH_2-cyclohexane	H	50,000
9	CH_2CH_2Ph	H	20
10	Ph	H	1000
11	1-Naphthalene	H	30
12	(*S*)-$CH(CH_3)$-2-naphthyl	H	40
13	(*R*)-$CH(CH_3)$-2-naphthyl	H	610
14	1-Naphthalene	CH_3	210
15	(1*S*)-1-Indane	H	20
16	(1*R*)-1-Indane	H	610

[a] HIV strand transfer assay, see Ref. 4.

Table 2. SAR at the heteroaryl amide



Compound	R^2	QUICKIN IC_{50}[a] (nM)
17	4-Pyridine	16,600
18	1*H*-1,2,4-Triazole	50,000
19	1*H*-Imidazole	17,000
20	2-Thiophene	100
21	2-(1,3-Thiazole)	200
22	5-(1,3-Thiazole)	2500
23	2-Benzothiophene	10
24	3-Benzothiophene	50
25	3-1*H*-Indole	20

[a] HIV strand transfer assay, see Ref. 4.

around the benzyl amide, crucial for additional lipophilic interactions with the enzyme.

A rationale behind the observed potency trends for the heterocyclic analogs was found by correlating IC_{50} data versus calculated log *P* (Fig. 2).[11] Less polar heterocycles placed in the benzylic position yielded compounds with increased inhibitory activity suggesting that these bind in an apolar environment.

In order to further investigate the space available around the aromatic portion of benzylamides an extensive variety of substituents were explored (Table 3). Substituents in the *ortho* and *meta* positions were generally tolerated but did not lead to significantly improved compounds (**26**–**27**, **29**–**30**, and **32**–**33** all have potency within 2-fold of **4**). Tighter SAR was found around the *para* position where bulky groups dramatically diminished the inhibitory activity (e.g., **28** and **34**). Apparently the space available for enzyme interaction is reduced at this position and only smaller groups like a methyl (**31**) were

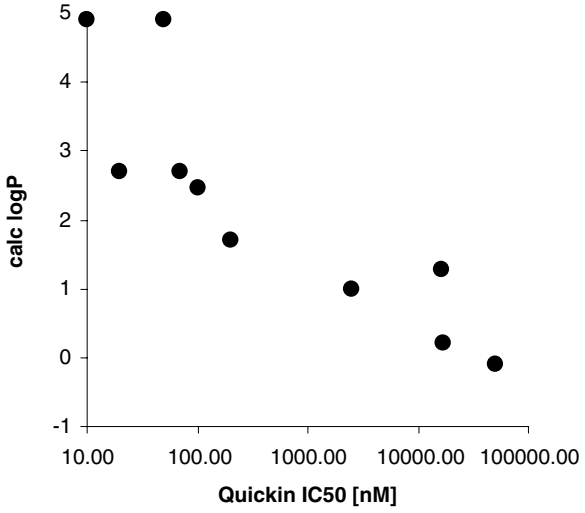
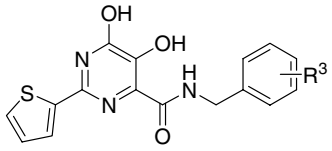


Figure 2. log *P* versus QUICKIN IC_{50}.

Table 3. SAR at the benzylamide



Compound	R^3	QUICKIN IC_{50} [a] (nM)
4	H	85
26	2-OMe	90
27	3-OMe	50
28	4-OMe	550
29	2-Me	110
30	3-Me	40
31	4-Me	70
32	2-Ph	130
33	3-Ph	50
34	4-Ph	50,000
35	4-Cl	20
36	2-F	160
37	3-F	70
38	4-F	10
39	3,4-Cl	10

[a] HIV strand transfer assay, see Ref. 4.

tolerated. When halogen atoms were located in *para* (**35** and **38**) inhibitory activity was significantly enhanced, and the *p*-fluorine derivative **38** emerged as the optimal substituent displaying an 8-fold improvement in potency compared to **4**.

A range of di-substituted compounds were investigated (e.g., **39**), but they provided no further gain over compound **38**. Best compounds ($IC_{50} < 1$ μM) were tested in cellular assay (Spread) under two serum conditions (10% fetal bovine serum and 50% normal human serum) showing no significant activity in the micromolar range.

Compound **38** was selected for further evaluation and interestingly displayed acceptable rat pharmacokinetics showing 39% oral bioavailability (*F*) and low plasma clearance (11 ml/min/kg). Counterscreening studies

proved that **38** did not inhibit human DNA polymerase α, β, and γ or HIV-RT. The efforts made to improve the potency of this novel class of HIV integrase inhibitors in cell-based assay will be reported elsewhere.[12]

In conclusion, dihydroxypyrimidine carboxamides were identified as a novel and selective class of HIV-1 integrase inhibitors. SAR around the carboxamide moiety generated low nanomolar inhibitors which have potential for future development as anti-HIV agents.

Acknowledgments

We thank Odalys Paz Gonzalez and Ralph Laufer for PK studies; William B. Schleif and Peter J. Felock for biological testing; Steven Harper and Michael Rowley for their helpful support and suggestions. This work was supported in part by a grant from the MIUR.

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