Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1159–1162

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Novel Inhibitors of HIV Protease: Design, Synthesis and Biological Evaluation of Picomolar Inhibitors Containing Cyclic P1/P2 Scaffolds

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Received 17 December 1999; accepted 8 March 2000

Abstract—A novel series of HIV protease inhibitors containing cyclic P1/P2 scaffolds has been synthesized and evaluated for biological activity. The *trans* 3,5-dibenzyl-2-oxo pyrrolidinone ring system resulted in a 50 pM enzyme inhibitor against HIV protease in vitro when combined with an indanolamine derived P′-backbone. This compound also shows comparable activity to currently marketed drugs in the MT-4 cell-based antiviral assay.

Highly active antiretroviral therapy (HAART) is the currently recommended approach for the treatment of HIV infection.[1] HAART usually consists of a three-drug cocktail containing at least two components that inhibit different viral targets. While some drug combinations targeting exclusively the viral reverse transcriptase have become available recently,[2] protease inhibitors (PIs) still constitute the cornerstone of most treatment regimens. Such combination therapy has been strikingly successful over the past 5 years, drastically reducing AIDS related complications and deaths.[1,3] However, some aspects of the current generation PIs remain problematic. The generally large daily doses required for complete viral suppression, can lead to undesired side effects, limited patient adherence to therapy, and high cost.[4] Moreover, treatment failure due to resistance to the currently available PIs is increasing, which limits future treatment options because of cross resistance among the other available PI-therapies.[5] For these reasons we have been engaged in a next generation PI program aimed at identifying new, structurally diverse agents with significantly increased potency and unique resistance profiles.

Recently, we disclosed the design of a novel cyclic P1/P2 scaffold[6] that was incorporated into the hydroxyethylene isostere backbone of amprenavir (Fig. 1). Our initial success prompted us to investigate the use of these and similar cyclic sub-units in combination with other successful HIV PI backbones. In this paper we explore the initial structure–activity relationship (SAR) of a series of compounds that combine these novel cyclic units with the P1′–P3′ indanolamine-based arrangement first utilized by researchers at Merck.[7]

Our previous results in the sulfonamide series suggested that the five-membered 2-pyrrolidinone (**A**) scaffold may be superior to conformationally somewhat more flexible six-membered systems such as pyrimidinones or morpholinones. For this reason, we initially focused on the smaller ring lactam as well as a set of new, isosteric scaffolds such as 1λ6-isothiazolidine-1,1-dione (**B**), 2-imidazolidinone (**C**), and 1λ6,2,5-thiadiazolidine-1,1-dione (**D**) (Fig. 2).

Chemistry

The 2-pyrrolidinone **A** and 2-imidazolidinone **C** scaffolds were synthesized according to previously published procedures.[6] The synthesis of the 1λ6-isothiazolidine-1,1-dione **B** is outlined in Scheme 1. Cbz-phenylalanine was converted to the protected amino aldehyde via LAH

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0960-894X/00/$ - see front matter
PII: S0960-894X(00)00163-3

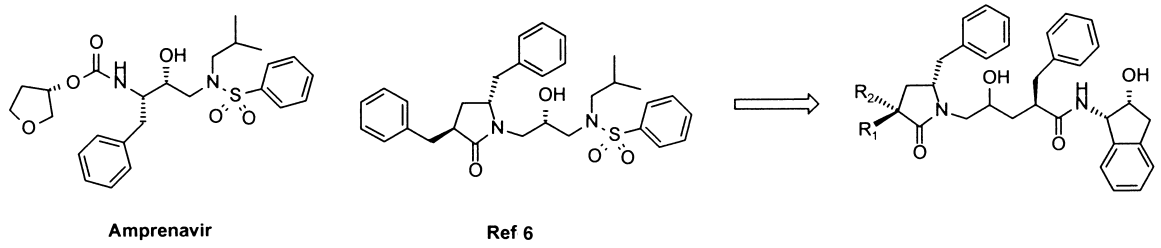


Figure 1.

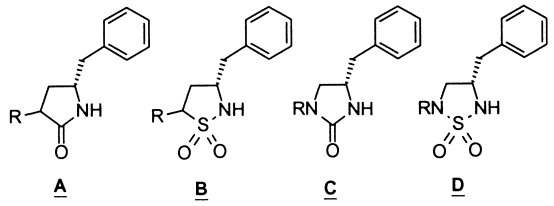
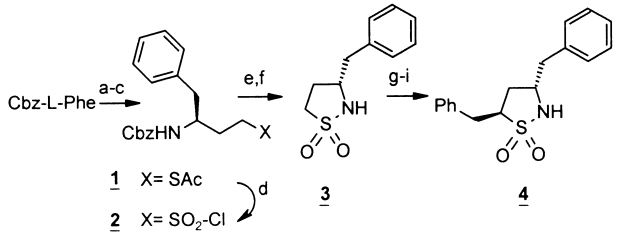


Figure 2.

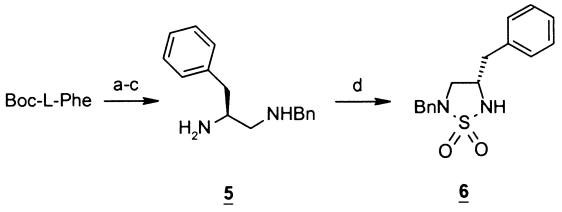
reduction of the corresponding Weinreb amide[8] (75%, two steps). Olefination, using the modified Peterson conditions published by Thompson et al.,[9] afforded the desired allyl amine (70%), which was converted to thioacetate **1** (79%) by radical induced sulfur addition to the terminal olefin position (thiolacetic acid/AIBN in CCl_4/hν = 254 nm).[10] Oxidation/chlorination with chlorine gas in acetic acid/aq hydrochloric acid[11] produced sulfonyl chloride **2** (95%).[12] The Cbz protecting group was removed with 48% HBr/AcOH and the resulting material was cyclized to the desired 1λ6-isothiazolidine-1,1-dione **3** by treating with triethyl amine in THF (58%, two steps). Introduction of the P2 benzylsubstituent to afford **4** followed the protection, alkylation, deprotection path used previously for the 2-pyrrolidinone ring system.[6]

The 1λ6,2,5-thiadiazolidine-1,1-dione scaffold **D** was prepared as detailed in Scheme 2, via borane reduction of L-phenylalanine benzylamide to the diamine **5**, followed by treatment with sulfamide in refluxing pyridine in the presence of excess triethylamine/DMAP,[13] affording **6** in 80% yield.

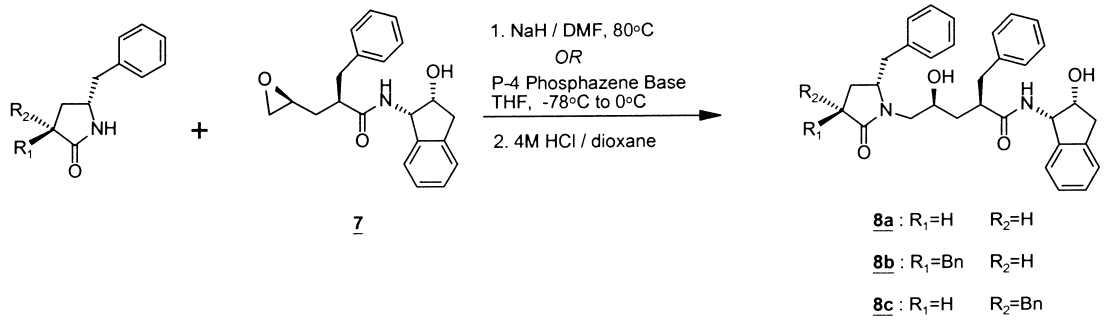
Coupling of the P1/P2 cyclic scaffolds to the known epoxide **7**[14] was initially accomplished in DMF at 80°C using sodium hydride (Scheme 3). Unfortunately, these harsh conditions took a heavy toll on the stereochemical integrity of the P2-substituent of scaffolds **A** and **B**, leading to equilibration (approximately 4:1 and 3:1 mixtures of *trans* to *cis* isomers for **A** and **B**, respectively). While this lack of stereochemical control was not acceptable for extended SAR studies, this route did, after careful chromatographic separation, provide an enriched sample of the *cis* isomer for comparison purposes. After considerable experimentation, a general solution to this epimerization problem was found with the use of the highly hindered P4 phosphazene base.[15] Thus combining all ingredients (phosphazene base added last) at low



Scheme 1. (a) (i) EDC/MeNHOMe/TEA; (ii) LAH/THF; (b) (i) $TMSCH_2MgBr$; (ii) $BF_3{\times}Et_2O$; (c) AcSH/AIBN/hv; (d) Cl_2/AcOH/HCl_{aq}; (e) HBr/AcOH; (f) TEA/THF; (g) Boc_2O/THF; (h) LDA/BnBr /THF; (i) TFA/CH_2Cl_2.

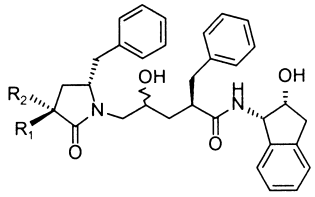


Scheme 2. (a) EDC/$BnNH_2$/TEA; (b) TFA; (c) $BH_3{\times}Me_2S$/THF; (d) $SO_2(NH_2)_2$/pyridine/TEA/DMAP, rfl.



Scheme 3.

Table 1. Inhibition of HIV protease and antiviral activity of **8a–c**



Entry	R_1	R_2	Conf. OH	K (nM)[a]	IC_{50} (μM)[b]
8a	H	H	[*S*]	330	>50
epi-**8a**	H	H	[*R*]	4800	>50
8b	Bn	H	[*S*]	0.05	0.3
8c	H	Bn	[*S*]	>0.5[c]	n/a[d]
Amprenavir				0.04	0.10
Ritonavir				0.02	0.21
Nelfinavir				0.01	0.05
Indinavir				0.07	0.04
Saquinavir				0.04	0.02

[a]K_i, enzyme inhibition constant.[20]
[b]IC_{50}, antiviral inhibition in MT-4 cell culture.[21]
[c]Represents lower limit estimate.[19]
[d]n/a, not available.

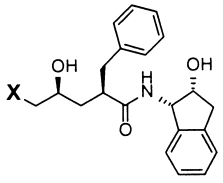
temperature and carefully warming to 0 °C over 2 h afforded the pure trans alkylation product **8b** in 65% yield with no trace of the undesired *cis* isomer **8c**.[20]

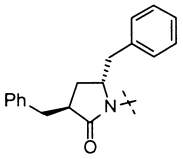
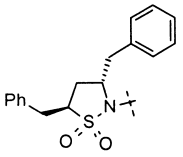
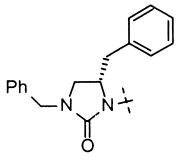
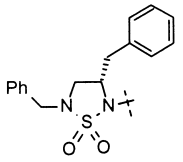
Results and Discussion

In order to establish the preferred stereochemistry of the central hydroxy functionality for these new backbones, we first synthesized the truncated cyclic scaffold (without P2 substituent) and coupled it to both epoxide isomers[17] **7** to afford **8a** and its backbone OH-epimer. Table 1 illustrates that there is at least a 10-fold preference of the *S* over the *R* configuration in terms of enzyme inhibition. This observation is consistent with the data reported for the related Indinavir backbone.[18] Encouraged by the considerable potency of these P2-truncated inhibitors, we predicted substantial room for exploration both in terms of a suitable P2 substituent as well as regarding the exact nature of the cyclic scaffold. As anticipated, introduction of a P2 benzyl substituent led to a dramatic increase in enzyme potency, producing a compound with 50 pM activity against HIV protease. This compound also showed 300 nM antiviral activity in a MT-4 cell-based assay. Table 1 furthermore confirms predictions derived from molecular modeling that the preferred configuration of the P2 substituent relative to the 5-benzyl substituent on the 2-pyrrolidinone ring system is *trans* (**8b** vs **8c**).

Attempts to optimize and simplify the cyclic P1/P2 scaffolds with isosteric replacements in the 2- and 3-positions proved more challenging (Table 2). The replacement of the carbonyl functionality by a sulfonyl group led to a 300-fold increase in K_i value. While the carbonyl oxygen has been shown[6] to participate in a crucial hydrogen bond to the 'flap water' in the active site, we had hoped that the pair of sp2 hybridized oxygens in the sulfonyl replacement might be able to maintain or even improve

Table 2. Biological activity of various P1/P2 scaffolds



X	K_i (nM)	IC_{50} (μM)
	0.05	0.3
	16	n/a
	1.2	0.3
	0.8	1.5

this interaction. Based on molecular modeling, the decreased potency of the 2-imidazolidinone (**C**) and 1λ6,2,5-thiadiazolidine-1,1-dione (**D**) ring systems was less surprising. In both cases, the P2 substituent is attached to a partially sp2 hybridized nitrogen atom which projects it into the corresponding active site pocket at a similar angle to that of the less active *cis* isomer in the 2-pyrrolidinone series. The 10-fold loss in potency for these isosteres is therefore consistent with the results obtained for **8c**.

In summary, we have discovered a new and highly potent cyclic HIV protease inhibitor scaffold. Lead compound **8b**, is roughly equipotent with the currently marketed HIV protease inhibitors and modeling studies indicate the potential for further optimization in the P1, P2 and P1′ positions. We have initiated work in all of these areas and will report our progress in future accounts.

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