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Discovery of potent pyrrolidone-based HIV-1 protease inhibitors with enhanced drug-like properties

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Abstract—We have developed efficient syntheses of the HIV-1 protease inhibitor **4** and its analogues, which incorporate the pyrrolidone scaffold **2** as P1–P2 moiety. Evaluation of these analogues in the HIV-1 protease enzyme assay resulted in discovery of potent and more water soluble *meta*-amino- and *meta*-hydroxy inhibitors **17b** and **19b**. The SAR observed in this class of PIs could be rationalized with aid of the X-ray structure of inhibitor **28** co-crystallized with the HIV-1 protease, which suggested that the polar *meta*- (but not *para*-) benzyl substituents in P2 could side-step the hydrophobic S2 enzyme active pocket by rotating the P2 moiety around its Cβ–Cγ bond. Such reorientation allows to engage the unsubstituted, hydrophobic edge of benzyl moiety in P2 in the requisite P2/S2 hydrophobic interaction, and projects polar *meta*-substituent into the bound water. It appears that the *meta*-position can be chemically derivatized without potency loss of thus resulting inhibitors, as evidenced by potent **22**–**26**. We thus identified pyrrolidone **2**-based inhibitors exemplified by **17b** and **19b**, which uniquely accommodate both high enzyme potency and which provide a platform for fine-tuning of drug-like properties in this class of PIs by additional chemical manipulations on the *meta*-position.

Recent publications from several laboratories including ours disclosed novel and potent HIV-1 protease inhibitors incorporating various P1–P2 scaffolds such as 1,2,5-thiadiazolidine 1,1-dioxide, 2-imidazolidinone, isothiazolidine 1,1-dioxide, 5*S*-(phenylmethyl)-3-morpholinone, and 2-pyrrolidone.[1–5] The key driver for that work has been to discover potent inhibitors with improved drug-like properties, especially aqueous solubility, which could help to alleviate some of the pill burden issues encountered with the standard HAART therapy.[1]

Our previous letter described the synthesis of PIs of the type **3** incorporating the 2-imidazolinone scaffold **1** as P1–P2 moiety.[1] One of the conclusions reached in that work was that the *trans* relationship of both the P1 and P2 substituents was required in this class of PIs for high potency against the HIV-PR. We thus redirected our medicinal chemistry efforts into PIs utilizing 2-pyrrolidone **2** as P1–P2 scaffold, exemplified by lead inhibitor **4**, which features *trans* P1 and P2.

Our initial targets included the picolyl derivatives **10**–**12** (Fig. 1), which were predicted to be more potent than analogous P2-picolyl derivatives in the 2-imidazolidinone scaffold **1** series.[6] These derivatives were obtained by the condensation of 3- and 4-pyridinecarboxaldehydes with pyrrolidone **5**,[5] followed by a spontaneous dehydration and subsequent loss of the *N*-Boc-protecting group, resulting in respective derivatives **6** and **7** (Fig. 2).

Hydrogenation of **6** and **7** with stoichiometric H_2 (1 atm, Pd·C/$CaCO_3$ catalyst) exclusively yielded *cis* products **8**

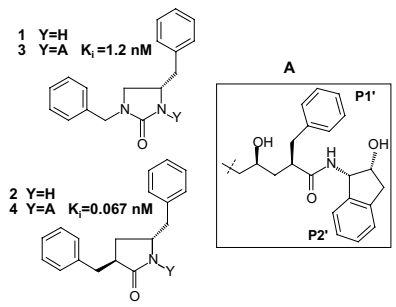

Figure 1. 2-Imidazolidinone **1** and pyrrolidone **2** P1–P2 scaffolds and corresponding PIs **3** and **4**.

Keywords: HIV-1 protease inhibitor; Aspartyl protease inhibitor; AIDS; Peptide mimetic; Peptidomimetic.
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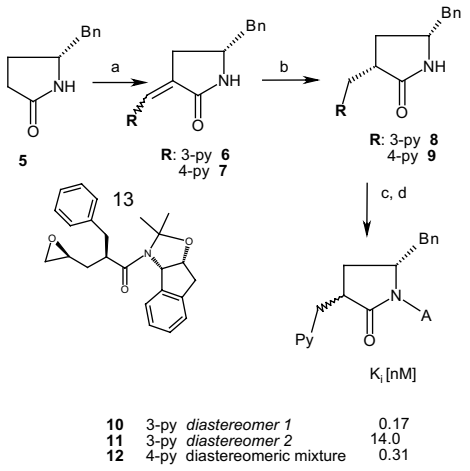


Figure 2. Reagents and conditions: (a) LDA, THF −78 °C, 3- and 4-pyridinecarboxaldehydes; 73–87% (b) 5% Pd·C, $CaCO_3$, H_2 titration; 99%; (c) NaH/DMF 80 °C, epoxide **13**; (d) 4 N HCl in dioxane/water (95:5, v/v), silica purification, 31–69%.

and **9**.[7] Since our objective was to synthesize the *trans* P1–P2 derivatives, *cis* intermediates were coupled to epoxide **13** under conditions known to epimerize the 3-position in 2-pyrrolidone (NaH/DMF, 80 °C).[5] Products were then chromatographically separated into the individual diastereomers **10** ($K_i = 0.17$ nM) and **11** ($K_i = 14$ nM) (Fig. 2). The 4-picolyl analogue **12** was synthesized in a similar fashion yielding equimolar mixture of diastereomers, which was assayed as such and proved to also be potent in the HIV-1 protease assay ($K_i = 0.31$ nM).

We also explored aliphatic P2-substituents in the context of pyrrolidone P1–P2 scaffold **2**. The rationale for this was based on SAR established in another scaffold series; 5*S*-(phenylmethyl)-3-morpholinone, in which the P2-allyl substituted analogue was found to be more potent than the P2-benzyl-substituted analogue.[8] Following this lead, the synthesis of the P2-allyl pyrrolidone-based inhibitor **14** was then accomplished by a diastereoselective alkylation of lactam **5** with allyl bromide and Boc-deprotection, followed by coupling of epoxide **13** and acetonide deprotection (Fig. 3).[5]

Perhaps surprising in light of prior SAR, the allyl-P2 inhibitor **14** (Fig. 3, $K_i = 0.45$ nM) was found less potent than the benzyl-P2 lead **4** ($K_i = 0.067$ nM).[5] This finding underscores different SAR in both the 5*S*-(phenylmethyl)-3-morpholinone and the 5*R*-(phenylmethyl)-2-pyrrolidinone (**2**) scaffold series and emphasizes a necessity to optimize SAR in each series independently.

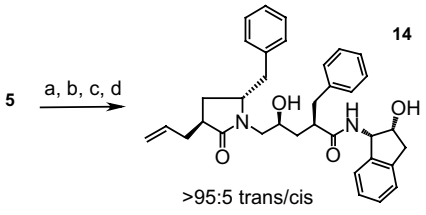


Figure 3. Reagents and conditions: (a) LHMDS −78 °C, allyl bromide, 78%; (b) TFA; (c) P4-phosphazine base, 1 equiv, THF, epoxide **13**, −78 to 0 °C; (d) 4 N HCl in dioxane/water (95:5, v/v), 81% for (b)–(d).

We optimized P2-benzyl motif even further by alkylating **5**[2,3] with various benzyl bromides X-Bn-Br, which generally proceeded with a high *trans* diastereoselectivity (ratio of 3*S*,5*R* to 3*R*,5*R* diastereomers ~4:1). Alkylating agents with masked functionalities, such as *m*-methoxy-benzyl bromide, *m*- and *p*-nitro-benzyl bromide were used towards syntheses of **19a**, **17a**, and **18a**, respectively. The resulting intermediates were then progressed as outlined in Figure 4, yielding pyrrolidones **15a**–**20a**. These derivatives were then coupled to epoxide **13** under either equilibrating or non-equilibrating conditions,[5] followed by the acetonide deprotection.[9] When feasible, mixtures of diastereomers were then chromatographically enriched to >95% optical purity, yielding PIs **15b**, **17b**, **19b**, and **20b** (Fig. 4, if diastereomers were separated, data is shown only for the more potent 3*S*,5*R* (*trans*) diastereomer).[5] Couplings of **16a** and **18a** to epoxide **13** were performed under equilibrating conditions, yielding equimolar mixtures of both diastereomers **16b** and **18b** (Fig. 4), which were evaluated as such in the HIV-1 protease assay.[10]

Enzyme inhibition data for **15b**–**20b** strongly suggests that *meta*-substituents in **15b**, **17b**, and **19b** yielded inhibitors, which were practically equipotent to lead molecule **4**, with the exception of methyl ester derivative **20b**. On the other hand, *para*-substituents in **16b** and **18b** caused a significant decrease in potency against HIV-1 protease (Fig. 4), far beyond when their K_i values are adjusted for 50% diastereomeric contents. We then attempted to expand the SAR by directly derivatizing these PIs. Thus, the oxidation of **15b** with urea–hydrogen peroxide (UHP) provided potent carboxamide **21** ($K_i = 0.1$ nM), while the alkylation of **19b** with bromoacetonitrile resulted in an approximately equipotent inhibitor **22** ($K_i = 0.08$ nM), oxidation of which also yielded potent carboxamide **23** ($K_i = 0.09$ nM) (Fig. 5).

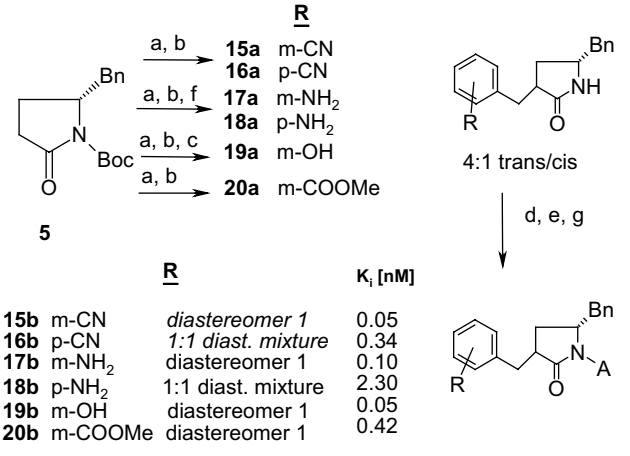


Figure 4. Reagents and conditions: (a) LHMDS −78 °C, R-Bn-Br, 79–93%; (b) TFA; (c) BBr_3; (d) P4-phosphazine 1 equiv, THF, epoxide **13**, −78 to 0 °C or NaH, DMF, 80 °C; (e) 4 N HCl in dioxane/water (95:5, v/v), 56%–87% for (b)–(e); (f) Pd·C/H_2; (g) silica gel chromatography, 51–73% for (f)–(g).

Capitalizing on the finding that even quite large *meta*-substituents were apparently compatible with binding to the S2 enzyme pocket, we then attempted to incorporate additional solubility modifiers into P2 by transforming ester **20b** to glycol- and morpholine-amides **24** and **25**, both of which were found to be subnanomolar inhibitors. In addition, amine **17b** could be converted to urea-based inhibitors **26** and **27**, both essentially equipotent to **17b** (Fig. 5).

Importantly, several potent analogues appeared to offer the potential for improved aqueous solubility. This was confirmed by the use of well-established computational methods, which indeed predicted marked improvements in aqueous solubilities of **10**, **17b**, **19b**, and **21** as compared to lead **4**.[11] Moreover, increased solubility of these compounds was not only compatible with their potent inhibition of the HIV–PR enzyme, but also the inhibition of live HIV-1 virus in MT4 cells (0.32, 0.21, 0.32, 0.20, and 0.14 μM for **4**, **10**, **17b**, **19b**, and **21**, respectively).

The key finding of this work is that of a dual role assumed by *meta*-substituents in scaffold **2**, which can *simultaneously* support high potency of inhibitors and accommodate polar, solubilizing groups. This outcome is unique and in contrast to our experience with the other scaffolds synthesized and examined in the course of this work,[1–5] for which attempts to introduce solubilizing groups into variety of position of the scaffold invariably resulted in a decrease of potency in the HIV-1 protease assay.[10] Of all the scaffolds examined,[1–5] only pyrrolidone **2** eventually allowed us to discover a specific *meta*-site, which supported both high enzyme potency and improved solubility, when polar substituents are used.

We next attempted to rationalize these unexpected findings with the aid of the X-ray structure of a co-crystal of HIV-1 protease and of inhibitor **28** (K_i = 0.05 nM) (Fig. 6a). This structure revealed that the hydrophobic edge of the P2-benzyl makes the critical hydrophobic interaction with the S2 enzyme pocket. To stay solvated, the relatively hydrophilic P2-*meta*-carboxamide benzyl in **28** sidesteps the hydrophobic S2 by rotating 180° around

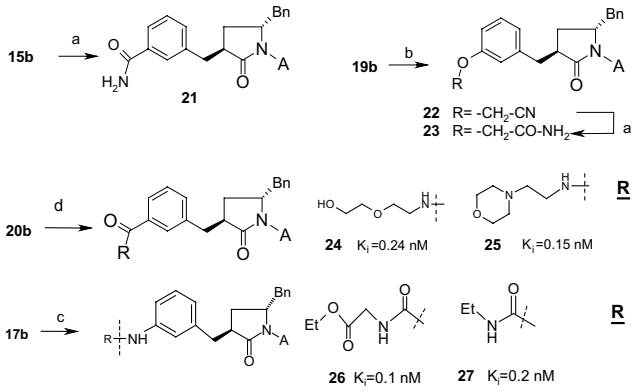


Figure 5. Reagents and conditions: (a) UHP, pH = 10, 2 h, 87–93%; (b) Br-CH_2CN, 79%; (c) isocyanate, 87–92%; (d) neat amine, 67–79%.

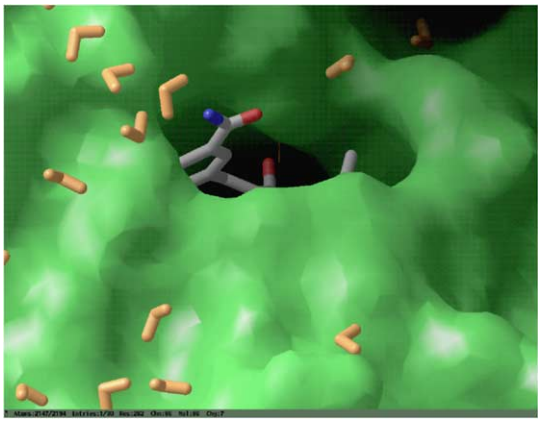
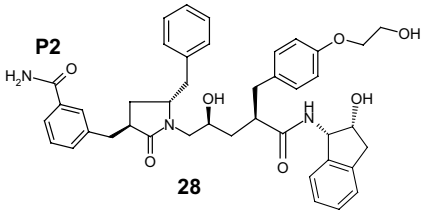


Figure 6. (a) The structure of inhibitor **28**; (b) close-up of the X-ray structure of **28** co-crystallized with HIV-PR, which shows P2 in **28** interacting with S2 and bound water (yellow).

the Cβ–Cγ bond. This positions the *meta*-substituent toward bound water at the mouth of the S2 site (Fig. 6b). Importantly, this model explains the relative lack of influence of the substituent size on inhibitor's K_i value. Thus, inhibitors with small polar *meta*-substituents, such as **17b** and **19b**, as well as with larger substituents such as **26**, are essentially equipotent (Figs. 4 and 5). Somewhat more hydrophobic *meta*-ester **20b** is too big to snugly fit the S2 subsite, but the solvation forces driving polar substituents (as in **19b**) away from the S2 by rotating P2 around the Cβ–Cγ bond, are absent in **20b**. Therefore, we further speculate that it is the steric clash between bulky *m*-$COOCH_3$ in **20b** and the S2 site, which results in approximately 8-fold loss of potency (K_i = 0.42 nM) compared to **19b**. The X-ray structure also suggests that *para*-substituents would be positioned in a way which results in their head-on collision with the S2 subsite (Fig. 6b), which cannot be avoided by rotating P2 around the Cβ–Cγ axis. Indeed, *para*-**16b** and **18b** are less potent than their *meta*-analogues **15b** and **17b**, respectively.



In summary, polar *meta*-substituents in the (3*S*,5*R*)-3,5-bis(phenylmethyl)-2-pyrrolidinone **2**, exemplified by inhibitors **17b** and **19b**, are unique in their ability to support both high potency against HIV-1 protease and an improved aqueous solubility. The relative lack of sensitivity of K_i to various polar *meta*-substituents in the (3*S*,5*R*)-3,5-bis(phenylmethyl)-2-pyrrolidinone P1–P2 moiety should allow further fine-tuning of drug-like properties in this series by chemical modifications of the *meta*-position. This opens the opportunity to discover other potent and soluble inhibitors, potentially with other improved drug-like properties, which could lower the pill burden in HAART. Work from our laboratories to that effect will be disclosed in due course.

Acknowledgements

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References and notes

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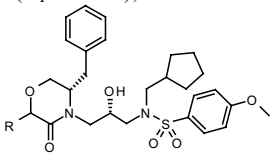
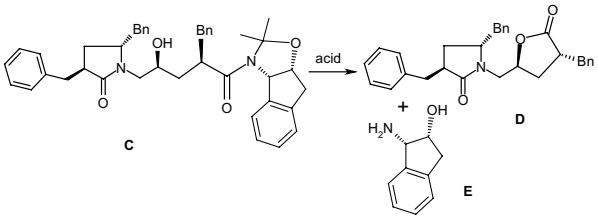
6. Several pairs of analogues in both **1** and **2** series suggested that the *trans* relationship of P1 and P2 enabled by the pyrrolidone scaffold **2** improves potency approximately by a factor of 20.
7. Pd·C catalyst and/or higher pressure of H_2 result in overreduction of pyridines to piperidines.
8. The coupling of P1–P2 allyl-morpholinone scaffold to Amprenavir-like P1′–P2′ resulted in 1:1 mixture of diastereomers **A** (K_i = 2 nM), Ref. 10.



R= Allyl IC_{50}=2 nM **A**
Benzyl IC_{50}= 30 nM **B**

Similarly synthesized benzyl morpholinone-based diastereomeric mixture **B** was much less potent (K_i = 30 nM), suggesting preference of allyl-P2 over benzyl-P2 in the morpholino series.

9. Deprotection of *N*-, *O*-acetonide **C** with TFA gives substantial amounts of the cyclization cleavage products, lacton **D** and indanolamine **E**. Additional experimentation allowed us to dramatically suppress the formation of **D** and **E** by the use of 4 N HCl in dioxane (95%) and water (5%)



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11. We utilized methods described in Klopman, G.; Wang, S.; Balthasar, D. M. *Chem. Inf. Comput. Sci.* **1992**, *32*, 474. Comparison of relative predicted solubilities for **10**, **17b**, **19b**, and **21** (concentration at saturation 0.526, 0.126, 0.079, and 0.063 μM) indicate that these compounds are predicted to be up to 10-fold more soluble than the reference inhibitor **4** (concentration at saturation 0.0525 μM).