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UNSYMMETRIC NONPEPTIDIC HIV PROTEASE INHIBITORS CONTAINING ANTHRANILAMIDE AS A P2' LIGAND

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Abstract: A series of novel unsymmetrical anthranilamide-containing HIV protease inhibitors was designed. The structure-activity studies revealed a series of potent P2-P3' inhibitors that incorporate an anthranilamide group at the P2' position. A reduction in molecular weight and lipophilicity is achieved by a judicious choice of P2 ligands (i.e., aromatic, heteroaromatic, carbamate, and peptidic). A systematic investigation led to the 5-thiazolyl carbamate analog **8m**, which exhibited a favorable C_{max}/EC_{50} ratio (>30), plasma half-life (>8 h), and potent in vitro antiviral activity (EC_{50} = 0.2 uM). © 1998 Published by Elsevier Science Ltd. All rights reserved.

HIV-1 protease (HIV PR) plays a pivotal role in the maturation of virus particles, and thus is an attractive target for the treatment of Acquired Immunodeficiency Syndrome (AIDS).[1,2] HIV PR is a member of the aspartic protease family and is structurally different from mammalian aspartyl proteases. HIV PR exists as a homodimer with one active site which is C2 symmetric. The Abbott group initially described the design of potent symmetry-based peptidomimetic transition state isosteres for the inhibition of this critically important enzyme.[3] We have employed a similar strategy for the design of potent, symmetry-based HIV protease inhibitors that contain an achiral, nonpeptidic, substituted benzamide **1**[4a] or anthranilamide (Ant) **2**[4b] moieties as novel P2/P2' amino acid replacements. Inhibitor **2** exhibited potent protease inhibition (K_i = 70 pM) and antiviral activity (EC_{50} of 60 nM), but lacked oral bioavailability in rats. The major liabilities in this regard were thought to include its high molecular weight and high lipophilicity. In an effort to identify inhibitors that possess high potency, reduced molecular weight and lower lipophilicity, we elected to prepare and study the structure-activity relationships (SAR) of an unsymmetrical series HIV PR inhibitors **5-8**, that contain the Ant group in the P2 or P2' position. This strategy allowed us to vary the size and lipophilicity of the P2 and P2' substituents independently. Herein we describe the results of these studies as well as the X-ray structure analysis of one of these analogs, **7b**.

To support the concept that hybrid compounds derived from **1** and **2** could serve as novel protease inhibitors, we analyzed the protease-bound conformations of symmetry-based HIV PR inhibitors **1**[4a] and **2**.[4b] Modeling studies revealed that the P2 to P2' portions of these inhibitors overlap well onto one another, and the

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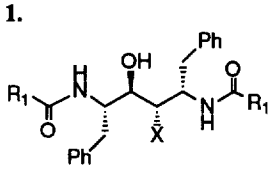
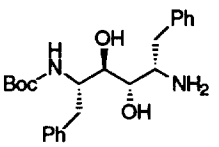
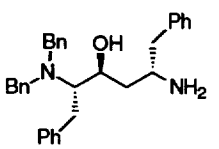
pyridinyl groups of **2** extend into the S3 and the S3' pockets of the enzyme. The PR bound conformations of **1** and **2** were used as starting geometries to construct hybrid inhibitors **6c** and **8d** (Sybyl™). Analysis of these structures suggested that both inhibitors fill the same hydrophobic pockets from S2 to S2', with compound **6c** extending into the S3 pocket and **8d** extending into the S3' binding domain of the protease. The energy minimized hybrid inhibitors aligned well onto the X-ray structures of **1** and **2** and maintained the proper alignment for polar interactions. No significant differences in the binding modes of **6c** and **8d** were observed. We then turned our attention towards synthesis of these targets.

Chemistry: The intermediates for the asymmetric acylation [2*S*-(*N*-*tert*-butyloxycarbonylamino)-5S-amino-3,4-dihydroxy-1,6-diphenyl-hexane **3**[5] and 2*S*-(*N*,*N*-dibenzylamino)-5*S*-amino-3-hydroxy-1,6-diphenylhexane **4**[6]] were prepared as described. The aromatic and hetero-aromatic acids were attached to the core amines **3** or **4** by N-hydroxybenzotriazole-mediated peptide coupling. To attach the desired heterocyclic carbamate, the intermediates were acylated with mixed the N-succinyl carbonate of the corresponding heterocyclic carbinol. The N-((2-pyridinylmethoxy)carbonyl)anthranilic acids[4b] and the heteroaromatic acids[7] were prepared as described.

Figure 1.







1, X = OH; R_1 = 2-Methyl-3-hydroxyphenyl. **3** **4**

2, X = OH; R_1 = 2-(N-((2-pyridinylmethoxy)carbonyl)amino)phenyl.

Structure-activity relationship and Discussion: Inhibitors **5** and **6** (Table 1) are designated as P3 to P2' inhibitors and inhibitors **7** and **8** (Table 1) are designated as P2 to P3' inhibitors. Compounds **5** and **7** possess the diol core, **3**, whereas compounds **6** and **8** incorporate the monohydroxy core, **4** (Figure 1). The inhibitors were evaluated for HIV PR inhibition activity using a fluorogenic substrate[8a] and for their ability to block the spread of HIV-I_{3b} in CEM cells[8b] (Table 1). Plasma concentrations of selected inhibitors in rats, were determined by HPLC analysis following IV and PO administration in vehicles of DMSO and PEG300, respectively.

P3-P2' inhibitors: Inhibitors **5** and **6** possess a pyridinyl-Ant group at the P3-P2 and a benzamide at the P2' positions. Compound **5a**, possessing Boc at the P2' position, exhibited a K_i of 0.45 nM, an antiviral EC_{50} of 0.49 uM and cellular toxicity (IC_{50}) of 21 uM. Contrary to our observations with related symmetrical compounds,[4a] the substitution of P2' Boc with substituted benzamide did not improve protease inhibition (compare **6a** vs **6c**). Analogs **5** and **6** exhibited protease inhibition K_i's that are one to two orders of magnitude worse than the initial lead, **2**.

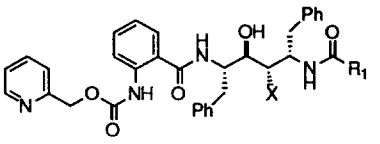
P2-P3' inhibitor: Concurrent with the above studies, we prepared P2 to P3' inhibitors **7** and **8**, which have a pyridinyl-Ant group at the P3'-P2' positions. Compound **7a**, possessing a Boc group at P2, exhibited about a three fold improvement in protease inhibition (K_i = 0.13 nM) over the corresponding P3-P2' analog **5a** (K_i = 0.45 nM). Analogs **7b** and **7c** possessing a 3-hydroxybenzamide and 2-methyl-3-hydroxy-benzamide at P2 position displayed PR inhibition K_i's of 0.5 and 0.09 nM, respectively.

To understand the nature of the binding of the P2-P3' analogs, we have obtained the high resolution, 2.1 Å, X-ray structure of the **7b**/HIV PR complex. Inhibitor **7b** binds to the enzyme in an extended conformation. The 3-hydroxybenzamide portion of the molecule occupies the S2 pocket, with the aromatic ring making hydrophobic interactions with Val32, Ile47 and Ile84, and the 3-hydroxyl group hydrogen bonding to the Asp30 carboxylate. The anthranilamide group interacts with the S2' and the pyridinyl group with the S3' subsite residues. Other interactions are similar to those seen in previously reported X-ray structures.[9] From the structure it is not clear why P2 to P3' inhibitors possessing the Ant group at the P2' position are more potent than P3 to P2' analogs possessing an Ant group at P2. Attempts to generate a crystal structure of a P3 to P2' inhibitor with HIV PR are in progress.

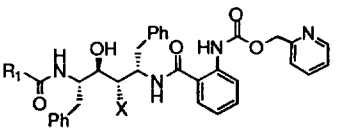
Inhibitor **8d** possessing a monohydroxy core, **4**, exhibited a K_i of 60 pM. The protease inhibitory activity of **8d** is comparable to **2** despite the lack of a P3 substituent in **8d**. Compound **6c**, the comparable monohydroxy analog with the benzamide group in the P2' position was about an order of magnitude less potent than **8d**. These results suggests that the anthranilamide and benzamide groups make optimal interactions with the S2' and S2 subsite residues of the enzyme, respectively (compare **8b** and **6b**, and **8d** and **6c**). Inhibitor **8d** exhibited an antiviral EC_{50} of 33 nM. Following PO administration of **8d** (37 mg/kg) in rats, a Cmax of 30 nM was achieved in 15 min, total bioavailability was around 1%. The IV profile of **8d** suggested a slow clearance with a $t_{1/2}$ of 133 min. Pursuant to these results, various analogs of **8d** were prepared to improve bioavailability. The anilino derivatives **8e**, **8f**, and **8g**, were prepared to improve solubility. The 2-methyl substituted-anilino derivative **8e** was a highly potent antiviral agent (EC_{50} = 60 nM), however it exhibited low oral bioavailability (F = <2%) as a hydrochloride salt. Aminobenzamides **8f** and **8g** exhibited reduced protease inhibitory and antiviral activity. Analogs **8h** and **8i** possessed a 5-membered heteroaryl moiety at the P2 position. These compounds were prepared to provide lower lipophilicity, but resulted in poor inhibitors of HIV PR.

P2 carbamates: A second series of analogs of **8d** was designed that incorporated carbamate moieties[11] at P2 position. In the carbamate series, the most potent protease inhibitor **8k** (K_i = 10 pM; calc. logP[10] = 4.9) was only weakly potent antiviral agent. Analog **8l**, featuring a tetrahydrofuranyl group at P2, exhibited a K_i of 40 pM, an antiviral EC_{50} of 30 nM, and a calculated logP of 5.1. Following PO administration at 38 mg/kg in rats, compound **8l** reached a peak plasma concentration (C_{max}) of 1037 nM in 15 min. The oral bioavailability of **8l** was estimated to be 6%. In an effort to further improve oral bioavailability of these compounds, we examined thiazolylmethyl carbamates, **8m** and **8n**. Among these analogs the 5-thiazolyl derivative (**8m**) retained the potency of **8l** and gave peak plasma levels >30-fold in excess of the antiviral EC_{50} (0.2 uM) after oral

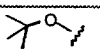
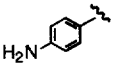
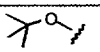
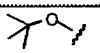
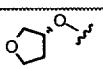
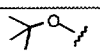
Table 1. Protease inhibition and antiviral activity of anthranilamides.

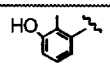
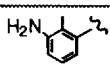
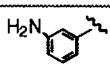
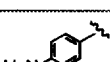


5 and 6.



7 and 8

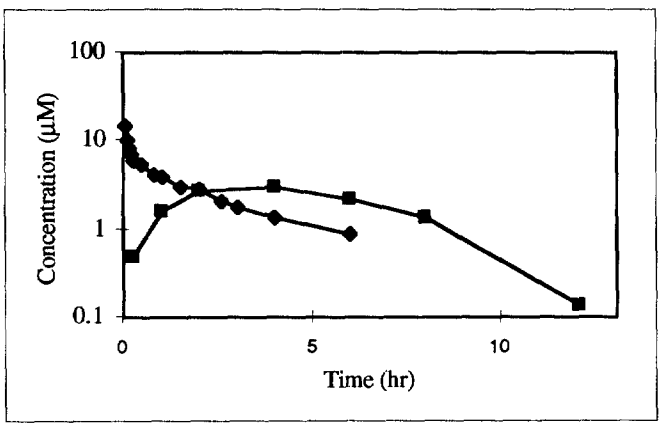
No.	R_1	X	K_i (nM)	EC50 (uM)
2	Py-Ant	OH	0.07	0.06
5a		OH	0.45	0.49
5b		OH	1.7	0.9
5c		OH	6.6	na
6a		H	0.85	0.49
6b		H	8.6	>10
6c		H	0.68	>1
7a		OH	0.13	0.4
7b		OH	0.5	na
7c		OH	0.09	0.02
7d		OH	0.4	0.8
7e		OH	0.07	0.06
8a		H	0.13	0.6
8b		H	0.85	0.3

No.	R1	X	K_i (nM)	EC50 (uM)
8c		H	0.27	0.5
8d		H	0.06	0.03
8e		H	0.12	0.06
8f		H	0.40	0.25
8g		H	1.5	0.5
8h		H	1.2	2
8i		H	4.7	3
8j		H	61%@ 10uM	>10
8k		H	0.01	0.35
8l		H	0.04	0.03
8m		H	0.03	0.2
8n		H	0.62	0.6
8o		H	0.03	0.007
8p		H	0.02	0.05

administration in rats.[12] The levels of **8m** in plasma declined slowly over a 12-h period and exceeded the in vitro EC_{50} even after 8 h following a single 40 mg/kg dose (Figure 2). The 4-thiazolylmethyl analog **8n** exhibited reduced HIV PR inhibitory potency (K_i = 0.62 nM).

Ethoxycarbonyl amino acid derivatives: Compounds **8o** and **8p** possess N-ethoxycarbonyl valine and N-ethoxycarbonyl isoleucine respectively as a P2 ligand. These compounds were prepared to compare the inhibitory potencies of the analogs possessing peptidic P2 ligands with those possessing nonpeptidic P2 ligands (**8a-n**). The protease inhibitory potencies of **8o** and **8p** (K_i = 30 and 20 pM, respectively) are comparable to the analogs possessing a P2 carbamate ligand (**8k**, **l**, and **m**). In general, the trend in the inhibitory potencies for this series is P2 carbamates ≥ P2 amino acid > P2 benzamides. Although a potent antiviral agent, no appreciable level of **8o** was detected in plasma following PO administration in rat.

Figure 2. Plasma concentration of **8m** following IV (◆) and PO (■) doses of 5 and 40 mg/kg respectively in rats (n = 2).



In summary, we have designed and synthesized a series of unsymmetrical anthranilamide-containing HIV PR inhibitors **5-8**. The structure-activity data suggest that the HIV PR inhibitory potency of the P2-P3' inhibitors, possessing an Ant group at the P2' position, is comparable to the longer symmetry-based inhibitor, **2**. This strategy allowed us to lower both molecular weight and lipophilicity. Analogs possessing benzamide, carbamate and peptidic groups at P2 demonstrated potent antiviral activity, and the carbamate analogs exhibited improved oral bioavailability in rat. The 5-thiazolylmethyl carbamate analog **8m**, upon oral dosing in rats, provided plasma levels >30-fold above the antiviral EC_{50} (F = 30%).

Acknowledgments and Disclaimer:

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References and Notes

1. Pearl, L. H.; Taylor, W. R. *Nature* **1987**, *329*, 351.
2. Kohl, N. E.; Emini E. A.; Schleif W. A.; Davis, L. J.; Heimbach J. C.; Dixon, R. A. F.; Scolnick, E. M.; Sigal, I. S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4686.
3. (a) Erickson, J. W.; Neidhart, D. J.; VanDrie, J.; Kempf, D.; Wang, X. C.; Norbeck, D. W.; Plattner, J. J.; Rittenhouse, J. W.; Turon, M.; Wideburg, N.; Kohlbrenner, W. E.; Simmer, R.; Helfrich, R.; Paul, D. A.; Knigge, M. *Science* **1990**, *249*, 527. (b) Kempf, D. S.; Codacovi, L.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Saldivar, A.; Vasavanonda, S.; Marsh, C. K.; Byrant, P.; Sham, H. L.; Green, B. E.; Betebenner, D. A.; Erickson, J. W.; Norbeck, D. W. *J. Med. Chem.* **1993**, *36*, 320.
4. (a) Randad, R. S.; Lubkowska, L.; Bhat, T. N.; Munshi, S.; Gulnik, S. V.; Yu, B.; Erickson, J. W. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1707. (b) Randad, R. S.; Lubkowska, L.; Bujacz, A; Naik, R. H.; Gulnik, S. V.; Yu, B.; Silva, A.; Munshi, S.; Lynch, T. A.; Clanton, D. J.; Bhat, T. N.; Erickson, J. W. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2557.
5. Kempf, D. S.; Marsh, C. K.; Codacovi, L.; Byrant, P.; Craig-Kennard, A.; Sham, H. L.; Zhao, C.; Vasavanonda, S.; Kohlbrenner, W. E.; Wideburg, N. E.; Saldivar, A.; Green, B. E.; Herrin, T.; Norbeck, D. W. *Bioorg. Med. Chem.* **1994**, *2*, 847.
6. Stuk, T. L.; Haight, A. R.; Scarpetti, D.; Allen, M. S.; Menzia, J. A.; Robbins, T. A.; Parekh, S. I.; Langridge, D. C.; Tien, J. J.; Pariza, R. J.; Kerdesky, F. A. *J. Org. Chem.* **1994**, *59*, 4040.
7. (a) Isooxazole-5-carboxylic acid: Spiegler, W., Gotz, N. *Synthesis*, **1986**, 69. (b) Thiazole-4-carboxylic acid: Hall, G. E.; Walker, J., *J. Chem. Soc.* **1966**, 1357. (c) 2-Methyl thiazole-4-carboxylic acid: Fuller, W. *J. Chem. Soc.* **1968**, 1526. (d) Thiazole-4-methyl carbinol: Kerdesky, F. A. J.; Seif, L. S.; *Synth.Commun.*; **1995**, *25*, 2639. (e) Thiazole-5-methyl carbinol: Michailow; B. *Obshch.Khim.*; **1957**, *27*; 726, 729.
8. (a) Kageyama, S.; Momoto, T.; Murakawa, Y.; Nomizu, M.; Ford, H.; Shirasaka, T.; Gulnik, S.; Erickson, J. W.; Takada, K.; Hayashi, H.; Broder, S.; Kiso, Y.; Mitsuya, H. *Antimicrob. Agents Chemother.* **1993**, *37*, 272. (b) Weislow, O. W.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. *J. National Cancer Inst.* **1989**, *81*, 577.
9. Hosur, M. V.; Bhat, N. T.; Kempf, D. J.; Baldwin, E. T.; Liu, B.; Gulnik, S.; Wideburg, N. E.; Norbeck, D. W.; Appelt, K.; Erickson, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 847.
10. Kellogg, G. E.; Joshi, G. S.; Abraham, D. J. *Med. Chem. Res.* **1992**, *1*, 444.
11. Kempf, D. S.; Sham, H. L.; Marsh, C. K.; Flentge, C. A.; Betebenner, D.; Green, B. E.; McDonald, E.; Vasavanonda, S.; Saldivar, A.; Wideburg, N. E.; Kati, W. M.; Ruiz, L.; Zhao, C.;Fino, L.; Patterson, J.; Molla, A.; Norbeck, D. W.; Norbeck, D. W. *J. Med. Chem.* **1998**, *41*, 602.
12. Thiazole analogs have been demonstrated to inhibit cytochrome P_{450}, which can contribute to enhance oral bioavailability. Analysis of this potential aspect of activity of **8m** is planned.