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STRUCTURE-BASED DRUG DESIGN OF NONPEPTIDIC P_2 SUBSTITUENTS FOR HIV-1 PROTEASE INHIBITORS

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Abstract. The cocrystal structures of LY289612 and LY297135 were used as a starting point in the design of nonpeptidic HIV-1 protease inhibitors. This report details the discovery of a series of novel aromatic P_2 replacement groups. The 3-hydroxy-2-methyl benzoic acid group, discovered in AG1254, was incorporated into the hydroxyethyl amine series to produce the potent antiviral compound (LY309391/ AG1310).

Early research provided evidence that the retrovirus designated as human immunodeficiency virus (HIV-1) was responsible for the debilitating disease known as acquired immunodeficiency syndrome (AIDS).[1] Tragically no cure or effective treatment is currently available and the number of reported cases of AIDS continues to expand. An intense research effort focused on the life cycle of HIV-1 has unearthed promising targets for controlling the progression of HIV-1 infection. It was demonstrated that processing of the gag-pol substrate by HIV-1 protease is essential for the formation of mature and infectious viral particles.[2] Thus, the protease became an obvious target for chemotherapeutic intervention in the HIV-1 life cycle.[3] We have previously reported the discovery of an initial benzamide lead compound **LY289612**.[4] However, this initial lead lacked oral bioavailability, possibly due to the peptidic nature of the ligand. This report describes the optimization of **LY289612**, utilizing information from cocrystal structures with HIV-1 protease, leading to the nonpeptidic phenol **AG1254** (Figure 1). In addition, the m-hydroxy-o-methyl P_2 group was incorporated into the hydroxyethyl amine series to afford a potent antiviral agent **LY309391/AG1310**.

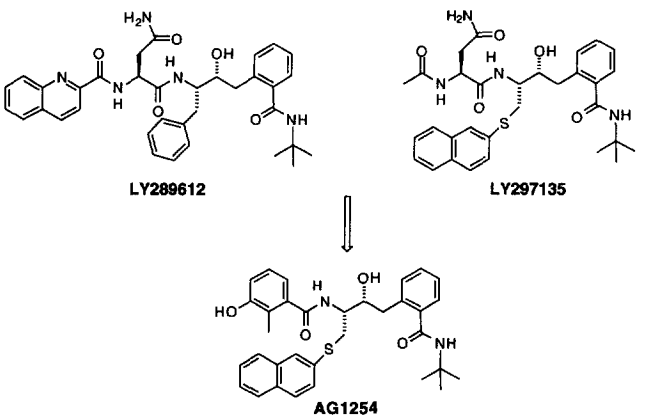
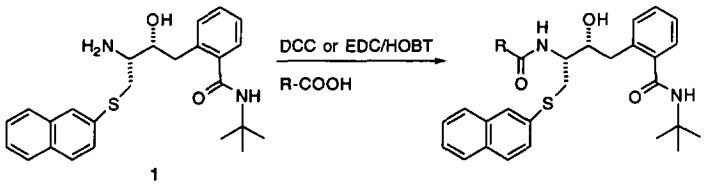


Figure 1. Design progression from initial lead to nonpeptidic compound AG1254

The project goal was to design novel structures that would inhibit HIV-1 protease and demonstrate better pharmacologic profiles. A modular design strategy was utilized to carry out these goals. In other words, one area of the molecule was varied, based on crystal structure information, while the rest of the molecule was held constant. Analysis of the new inhibitor in an HIV-1 protease inhibition assay and a subsequent cocrystal structure allowed iterative optimization of the series.[5] We have previously discussed the discovery of a novel S-Aryl P_1-P_3 spanning groups.[6] The sulfur atom enhanced the hydrophobic interaction of the inhibitor with the P_1 pocket residues. In the following study, nonpeptidic P_2 groups were designed utilizing the cocrystal structures of **LY289612** and S-2-Naphthyl **LY297135** as a starting point.

First a bicyclic aromatic ring system was designed to replace the asparagine-quinaldic acid. These compounds were assayed for inhibition of HIV-1 protease (Table 1).[7] In most cases saturation of the second ring resulted in enhanced enzyme inhibition. Synthesis of this series generally involved a simple peptide coupling of the unprotected amino-alcohol **1** with an appropriate aromatic acid using standard DCC or EDC/HOBT conditions to afford the coupled amides in 50-90% yield (Scheme 1). The saturated rings were prepared by direct reduction of the coupling products with sodium cyanoborohydride in acetic acid.[8]

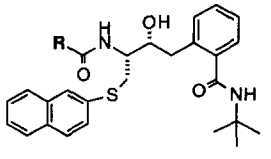
Scheme 1

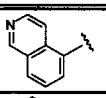
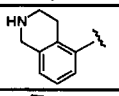
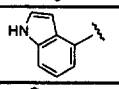
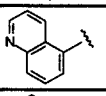
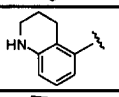
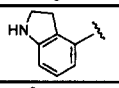
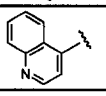


1

The most active compound in the bicyclic series was **AG1204**, which employed a tetrahydroquinoline group. Crystal structures of **AG1136** and **AG1204**[9] demonstrated that the bicyclic compounds bound as modeled, with the aromatic ring deep in the P_2 pocket and out of plane with the amide carbonyl. In addition, the saturated ring was perpendicular to the S-2-Naphthyl ring. The structure of **AG1136** revealed a potential hydrogen bond between the ring nitrogen, which is likely to be protonated, and the amide carbonyl oxygen of Gly48. The crystal structure information and activity data for **AG1204** led directly to the design of the simplified monocyclic series.

Table 1. Benzamide structure-activity relationships of bicyclic aromatic P_2 groups

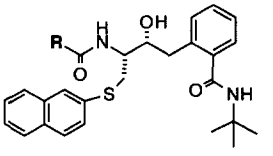


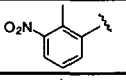
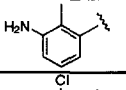
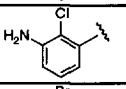
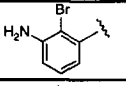
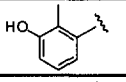
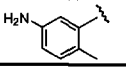
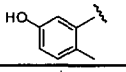
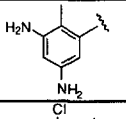
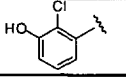
AG #	R	Ki (nm)	CEM IC_{50} (μg/mL)[10]
AG1135		800	16
1136		180	>50
1200		164	6.6
1203		113	5.7
1204		24	1.9
1206		166	>50
1210		265	5.6

The first monocyclic compounds were modeled in the same conformation observed in the bicyclic series. The ortho-methyl group was included in these designs to energetically favor the required phenyl ring conformation out of plane with the amide carbonyl. The first design excised two carbons from the **AG1204** structure to form a 2,3-substituted phenyl ring. The enzyme inhibition of **AG1232** was comparable to the best bicyclic compounds and a cocrystal structure was obtained. Unexpectedly, the structure indicated a unique binding mode when compared to the bicyclic aromatic series. The o-methyl group of **AG1232** was buried deep in the hydrophobic part of the P_2 pocket and the aniline nitrogen made a weak hydrogen bond (3.2 A°) to the Asp30 sidechain carboxylate at the back of the P_2 pocket. Thus the aromatic ring had flipped 180° relative to the bicyclic series and exhibited an edge to face interaction with the S-naphthyl ring. In fact this orientation is actually similar to that previously observed in the isophthalic acid series.[6] Replacement of the o-methyl group with chlorine or bromine resulted in slightly increased enzyme inhibition and antiviral activity. Replacement of the nitrogen substituent by an oxygen resulted in **AG1254**, which was 10x more active in the enzyme assay and 5x more active in the antiviral testing. The crystal structure of **AG1254** showed the phenol oxygen, a better hydrogen bond donor than the aniline nitrogen, made a stronger hydrogen bond to the Asp30 side chain. In contrast to the aniline series, changing the o-methyl substituent to chlorine did not improve activity in the phenol series. The crystal structures of both **AG1232** and **AG1256** showed the o-methyl group in the hydrophobic part of the P_2 pocket. Superposition of the two crystal structures suggested the 3,5-diamino pattern as a target for synthesis. As predicted the enzyme activity and antiviral activity of **AG1282** improved by 10x compared to **AG1232**. In contrast, the phenol was a poor inhibitor in the 2,5-substitution pattern (**AG1273**). Further efforts in the benzamide series did not produce an improved enzyme inhibitor or antiviral agent.

Overall a novel structure with decreased molecular weight and comparable enzyme activity to **LY289612** was achieved in this series.

Table 2. Benzamide SAR of monocyclic aromatic P_2 groups



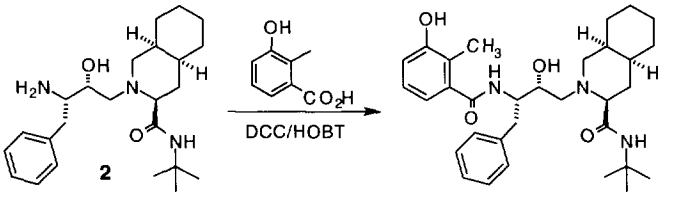
AG #	R	Ki (nm)	CEM IC_{50} (μg/mL)
1224		**540**	**>50**
1232		**49**	**5**
1240		**16**	**1.5**
1246		**33**	**1.6**
1254		**3**	**0.97**
1256		**17**	**1.3**
1273		**500**	**ND**
1282		**5**	**0.46**
1309		**15**	**ND**

The superposition displayed in Figure 2 was created by aligning the two protein crystal structures in the same frame of reference. The crystal structure of **AG1254** superimposed onto **LY289612** demonstrated that the modular design concept had been validated for this series. The unchanged benzamide portion of each molecule was nearly superimposable. A more detailed analysis of many HIV-1 protease cocrystal structures will be published separately. There was evidence in the literature that novel P_2 groups in the hydroxyethyl amine series exhibited high antiviral activity.[11] Thus, the novel o-methyl-m-phenol group discovered in the benzamide series was incorporated into the hydroxyethyl amine series.

Figure 2. Bound crystal structure of phenol **AG1254**[12] (violet) compared to **LY289612** (cyan)

The known hydroxyethyl amine tert-butyl amide **2**[3c,11] was coupled to 2-methyl-3-hydroxybenzoic acid using typical DCC/HOBT conditions to provide **AG1310** in good yield. The nonpeptidic compound exhibited strong inhibition of HIV-1 protease although slightly weaker than **AG1254** (Table 3.). However, the compound demonstrated greatly increased antiviral potency compared to **AG1254**.[13] In addition, **AG1310** showed plasma levels above the IC_{90} in an initial oral bioavailability screen.[14] Optimization and pharmacology of the hydroxyethyl amine series will be reported separately.

Table 3. Hydroxyethyl amine synthesis and inhibition data



Compound	Ki (nm)	CEM IC_{50} (μg/mL)
LY309391/ AG1310	21	0.010

In summary, starting from the crystal structure of **LY289612**, utilizing a modular approach, a novel series of HIV protease inhibitors was designed to change the properties of the original lead. An iterative process of design, synthesis, assay, cocrystal structure, and redesign resulted in a novel series of HIV-1 protease inhibitors and a potent antiviral development candidate.

Acknowledgments: We would like to thank Bonnie Bowden (SRI) for results of CEM cell assay. We also are indebted to Ken Su and Jeffrey Burgess for oral bioavailability data.

REFERENCES AND NOTES

1. (a) Gallo, R. C.; Salahuddin, S. Z.; Popovic, M. Shearer, G. M.; Kaplan, M.; Haynes, B. F.; Palker, T. J.; Redfield, R.; Oleske, J.; Safai, B.; White, G.; Foster, P.; Markham, P. D. *Science* **1984**, *224*, 550. (b) Barre-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axier-Blin, C.; Vezinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science* **1984**, *220*, 868.
2. (a) Debouck, C. *AIDS Res Hum Retrovir* **1992**, *8*, 153. (b) McQuade, T. J.; Tomasselli, A. G.; Liu, L.; Karacostas, V.; Moss, B.; Sawyer, T. K.; Heinrikson, R. L.; Tarpley, W. G. *Science* **1990**, *247*, 454. (c) 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) Wlodawer, A.; Miller, M.; Jaskolski, M.; Sathyanarayana, B.; Baldwin, E.; Weber, I.; Selk, L.; Clawson, L.; Schneider, J.; Kent, S. *Science* **198**, *245*, 616. (b) Huff, J. R. *J. Med. Chem.* **1991**, *34*, 2305. (c) Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Kröhn, A.; Lambert, R. W.; Merrett, J. H.; Mills, J. S.; Parkes, K. E. B.; Redshaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. J.; Machin, P. J. *Science* **1990**, *248*, 358. (d) Kempf, D. J.; Norbeck, D. W.; Codacovi, L. M.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Paul, D. A.; Knigge, M. F.; Vasavanonda, S.; Craig-Kennard, A.;Saldivar, A.; Rosenbrook, W.; Clement, J. J.; Plattner, J. J.; Erickson, J. *J. Med. Chem.* **1990**, *33*, 2687.
4. For information on LY289612: Kaldor, S. W.; Hammond, M.; Dressman, B. A.; Fritz, J. E.; Crowell, T. A.; Hermann, R. A. *Bioorg. Med. Chem. Lett* **1994**, *4*, 1385.
5. For complimentary approach to de novo design and optimization see: Appelt, K.; Bacquet, R. J.; Bartlett, C. A.; Booth, C. L.; Freer, S. T.; Fuhry, M. A. M.; Gehring, M. R.; Herrmann, S. M.; Howland, E. F.; Janson, C. A.; Jones, T. R.; Kan, C-C; Kathardekar, V.; Lewis, K. K.; Marzoni, G. P.; Matthews, D. A.; Mohr, C.;. Moomaw, E. W; Morse, C. A.; Oatley, S. J.; Ogden, R. C.; Reddy, M. R.; Reich, S. H.; Schoettlin, W. S.; Smith, W. W.; Varney, M. D.; Villafranca, J. E.; Ward, R. W.; Webber, S.; Webber, S. E.; Welsh, K. M.; White, J. *J. Med. Chem.* **1991**, *34*, 1925.
6. Kaldor, S. W.; Appelt, K.; Hammond, M.; Fritz, J. E.; Crowell, T. A. *Bioorganic & Medicinal Chemistry Letters*, previous paper in this issue
7. All new compounds were characterized by ^{1}H NMR, IR, MS and exhibited satisfactory elemental analysis.
8. Gribble, G. W.; Heald, P. W. *Synthesis* **1975**, 650.
9. K. Appelt and J. Davies, crystallography to be published in separate paper.
10. CEM cell assays were conducted at Southern Research Institute, according to the method described in Weislow, O. S.; Kiser, R.; Fine, D.L.; Bader, J. ; Shoemaker, R. H.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81(8)*, 577.
11. For recent example of optimization study of novel urethane P_2 group in hydroxyethyl amine series: Ghosh, A. K.; Lee, H. Y.; Thompson, W. J.; Culberson, C.; Holloway, M. K.; McKee, S. P.; Munson, P. M.; Duong, T. T.; Smith, A. M.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. *J. Med Chem.* **1994**, *37*, 1177.
12. Information about the x-ray crystal structure complex of **AG1254** with HIV-1 protease: space group $P2_12_12_1$, cell size a= 52.0 b= 59.1 c= 62.1, data 2.0 A°(87% complete) refined with X-Plor to R= 22.0% using all data between 6-2.0 A°, 52 water molecules.
13. Better antiviral activity could be due to increased cell penetration but no direct evidence was obtained.
14. Dosed in fed Fisher rats at 40 mg/kg (n= 3). Initial test showed plasma levels above IC_{90} (0.030 μg/mL) were obtained for 6 h with an oral availibility of 44%.

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