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ISOPHTHALIC ACID DERIVATIVES: AMINO ACID SURROGATES FOR THE INHIBITION OF HIV-1 PROTEASE

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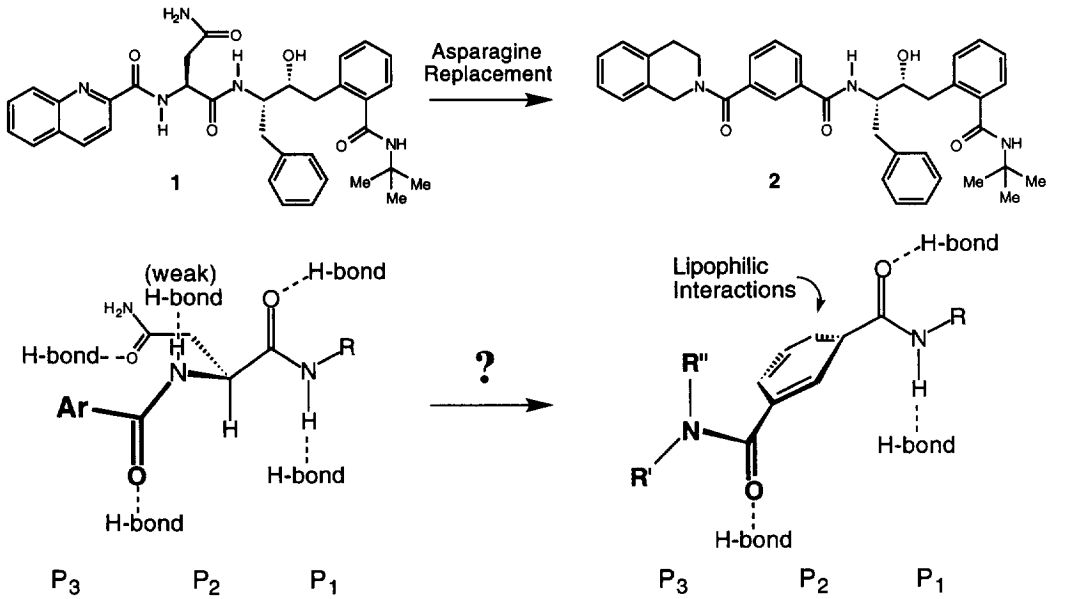
Abstract. Using the X-ray crystal structure of the inhibitor **1** complexed to HIV-1 protease, a new series of HIV-1 protease inhibitors was developed incorporating substituted isophthalic acid derivatives as amino acid surrogates. Through iterative structure-based design, the lead compound **2** was optimized to produce a variety of non-peptide HIV-1 protease inhibitors with significant antiviral activity. In contrast to **1**, several members of this series exhibit significant oral absorption in animals.

In an effort to combat human immunodeficiency virus type-1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS), many research groups have elected to target HIV-1 protease, the aspartyl protease encoded by the virus that has been demonstrated to be essential for viral replication.[1] As part of our program to develop potential clinical candidates for the treatment of AIDS, we sought to identify a potent inhibitor of HIV-1 protease with desirable whole cell antiviral activity and pharmacologic properties. Although our initial lead inhibitor **1** (Scheme 1) demonstrated good enzyme inhibition and antiviral activity, it exhibited poor oral bioavailability.[2] In this Letter, we disclose our efforts to improve the pharmacologic characteristics of **1** through the use of structure-based design.[3]

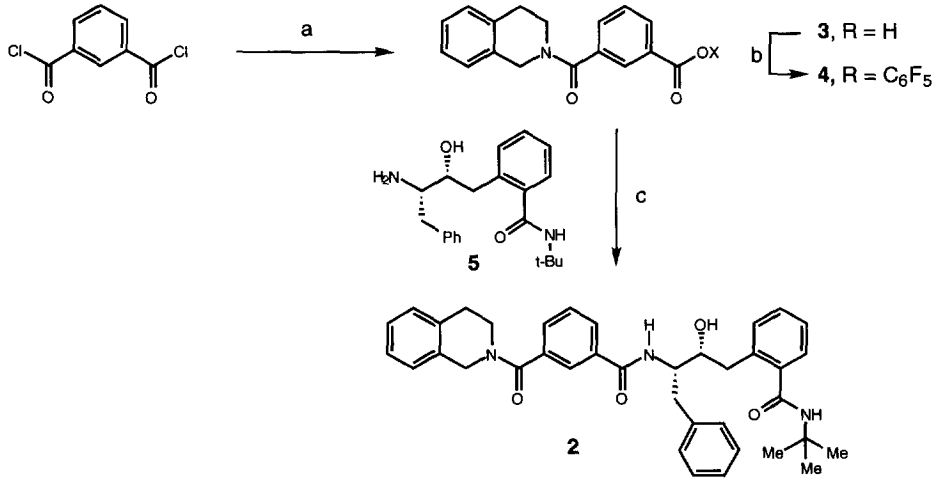
Scheme 1



The peptide-like nature, high molecular weight, and lipophilicity of **1** (MW = 609) were viewed as liabilities contributing to the compound's poor pharmacokinetic behavior.[4] Hence, the one remaining amino acid embedded in the structure, asparagine, became the focal point for our design efforts. The co-crystal structure of **1** complexed to HIV-1 protease indicates the the carboxamide side chain of the asparagine binds in the P_2 pocket of the enzyme (Scheme 1).[2] Significantly, substitution of valine for asparagine at this position produces only a modest drop in inhibitory activity, suggesting that both polar and liphophilic sidechains are well-tolerated in this region of the enzyme. With this observation in mind, we elected to model carbocyclic rings into the P_2 pocket of the enzyme which could be appropriately substituted to maintain both the lipophilic interactions with the P_3 pocket and the backbone hydrogen bonds achieved with **1**. Although saturated rings appeared problematic in modeling studies, isophthalic acid derivatives seemed well suited for the task.[5] In order to bias the molecule into a low energy conformation suitable for binding, a tertiary carboxamide was chosen for the P_3 region of the inhibitor. It was thought that prohibitive ortho interactions would serve to orient this moiety orthogonal to the aromatic ring, thus aligning the carboxamide carbonyl to gain a hydrogen bond to the enzyme.[6] As a result of these modeling exercises, **2** (Scheme 1) emerged as our first synthesis candidate.

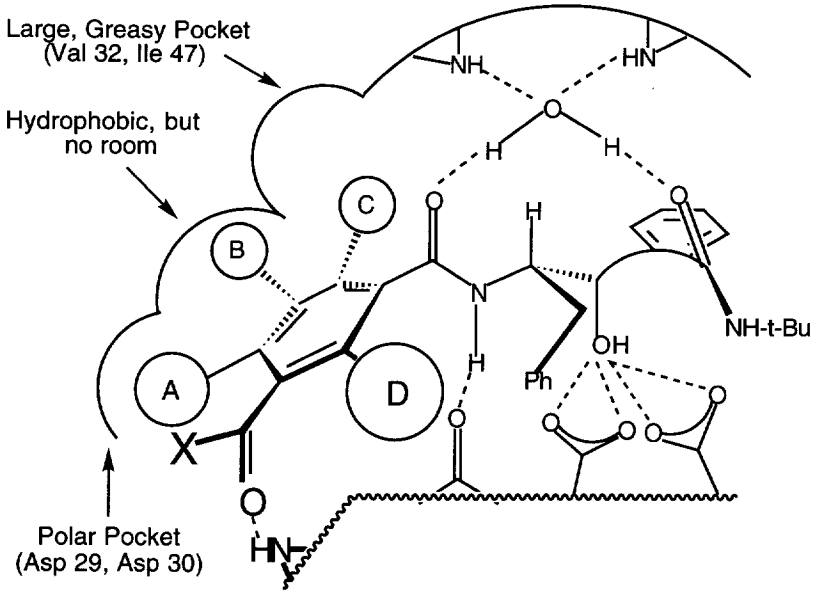
Inhibitor **2** was prepared as illustrated in Scheme 2.[7a,b] Treatment of isophthaloyl dichloride with one equivalent of tetrahydroisoquinoline followed by aqueous work-up delivered the acid **3**. Preactivation of **3** as its corresponding pentafluorophenyl ester **4** followed by reaction with known amino alcohol **5**[2] provided **2** for testing. **2** proved to be a moderate inhibitor of HIV-1 protease with an IC_{50} of 50 nM.[8] While this compound was substantially less potent than **1** (IC_{50} = 1.5 nM), it was viewed as a good starting point for further optimization efforts. To this end, the co-crystal structure of **2** complexed to HIV-1 protease was solved.[9] The inhibitor adopts the anticipated conformation and interacts with the P_3 through $P_{2'}$ pockets of the enzyme in a manner highly analogous to **1**. Significantly, the tetrahydroisoquinoline carboxamide portion of the inhibitor rotates orthogonal to the benzene ring to which it is attached to enable the predicted hydrogen bonding and lipophilic interactions with the P_3 pocket of the enzyme.

Scheme 2



Reagents: a) tetrahydroisoquinoline, Et_3N, Et_2O, -10°C (67%); b) C_6F_5OH, EDC, THF, 0°C (22%); c) NMM, **5**, CH_2Cl_2 (50%).

Figure 2

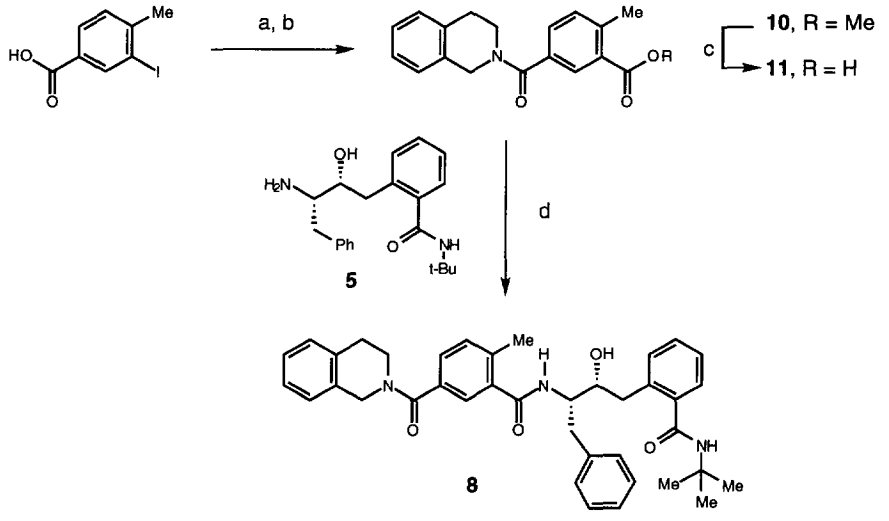


In an effort to increase the affinity of **2** for HIV-1 protease, we examined substitution of the isophthalic acid benzene ring. A cartoon depiction of our modeling predictions is shown in Figure 2. We concluded that methyl substitution at position C would lead to a better enzyme inhibitor as a result of enhanced interactions with lipophilic amino acid sidechains in the P_2 pocket of the enzyme. By contrast, methyl substitutions at positions A and B were viewed as counterproductive due to either mismatched interactions of polar and liphophilic groups (A) or prohibitive steric interactions (B). Methyl substitution at position D was not evaluated in our initial modeling exercises.

The synthesis of the required substituted isophthalic acid amide derivatives required development of a new synthesis route. By way of illustration, **8** (C = methyl) was prepared through the use of palladium-catalyzed carbonylation chemistry (Scheme 3). Commercially available 3-iodo-4-methyl-benzoic acid was reacted with tetrahydroisoquinoline in the presence of carbonyldiimidazole to form the corresponding amide. Palladium-catalyzed carbonylation in the presence of methanol yielded methyl ester **10**,[10] which was hydrolyzed to provide acid **11**. Finally, coupling with amino alcohol **5** gave access to **8**.

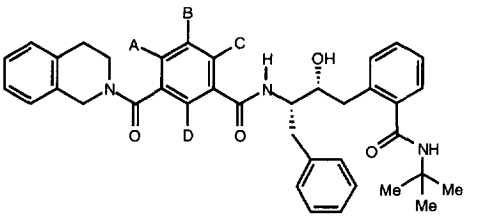
The four methyl substituted analogs of **2** were tested side-by-side with the parent compound in protease inhibition assays (Table 1). In accord with prediction, **8** (C = methyl) proved to be significantly more potent than **2**, with an IC_{50} of 6 nM. Furthermore, **6** (A = methyl) and **7** (B = methyl) were markedly inferior inhibitors, with IC_{50}s of 670 and 500 nM, respectively. Interestingly, **9** (D = methyl), an inhibitor not evaluated in our initial modeling studies, was a particularly poor inhibitor (IC_{50} >1000 nM). In retrospect, it is possible that prohibitive interactions between this inhibitor's methyl group and its P_1 phenyl substituent may be responsible for this complete erosion in inhibitory activity.

Scheme 3



Reagents: a) tetrahydroisoquinoline, CDI, THF (96%); b) cat. $Pd(PPh_3)_2Cl_2$, 1 atm CO, dicyclohexylamine, MeOH; c) LiOH, THF, H_2O (>95% for two steps); d) **5**, DCC, HOBt, DMF (53%)

Table 1

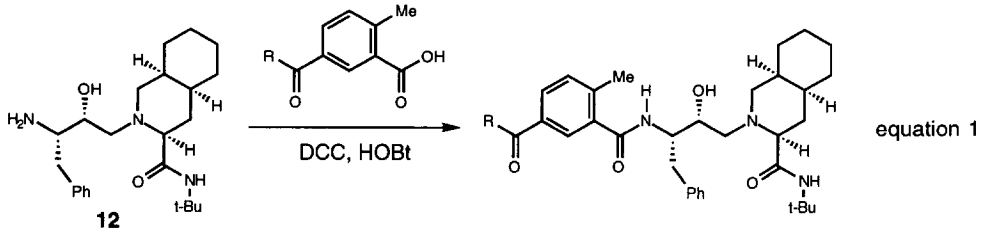


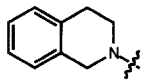
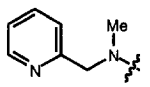
LY #	A	B	C	D	Prediction	HIVP IC50 (nM)
2	H	H	H	H	second	50 ± 12 (n=2)
6	Me	H	H	H	worst	670 (n=1)
7	H	Me	H	H	third	500 (n=1)
8	H	H	Me	H	best	6.1 ± 2.7 (n=2)
9	H	H	H	Me	not eval.	>1000 (n=1)

Although modification of our initial lead through the use of structure-based design had provided better enzyme inhibitors, these compounds were uniformly poor antivirals in whole cell assays, with IC_{50}s in the low micromolar range in HIV-infected CEM cells,[11] and displayed poor oral levels in whole animal studies. In order to improve the antiviral activity and pharmacology of the series, efforts were undertaken to modify the physical properties of the inhibitors by incorporating amine functionality at various positions in the inhibitors. It

was hoped that such salt-forming "handles" would increase the aqueous solubility of the relatively liphophilic lead inhibitors. The known tertiary amine-containing transition state mimic **12**[12] was used as a replacement for amino alcohol **5**. Coupling with acid **11** under standard conditions (Equation 1) gave **13**, a compound with disappointingly poor antiviral activity and pharmacology (Table 2). A second salt-forming group was then incorporated into the series, this time in the P_3-binding portion of the inhibitor.[13] The bismethanesulfonate salt of pyridyl analog **14** proved to have both significant antiviral activity (IC_{50} = 77 nM) and peak oral plasma levels more than 50-fold higher than its antiviral IC_{50} (C_{max} = 4400 nM) when dosed in rats.[14]

Table 2



Compound	R	HIVP IC50 (nM)	CEM IC50 (nM)	Oral Cmax (nM)
13		13.8 ± 3.0 (n=2)	1400 (n=1)	< 100 (n=2)
14		8.6 ± 4.4 (n=4)	77 ± 8.5 (n=2)	4400 ± 280 (n=2)
1		1.5 ± 1.1 (n=6)	27 ± 4.5 (n=2)	< 100 (n=2)
Ro-31-8959		1.7 ± 0.3 (n=2)	2.0 (n=1)	----

In summary, structure-based design has been used as a tool in improving the pharmacologic properties of the lead HIV-1 protease inhibitor **1**. Replacement of the one remaining amino acid in **1** with amino acid surrogates derived from isophthalic acid leads to an new series of non-peptide HIV-1 protease inhibitors with significant antiviral activity and greatly improved pharmacologic properties. Further studies on this series and related inhibitors will be reported separately.[15]

Acknowledgements. We wish to thank Theresa Gygi, Joe Manetta, and Joe Colacino for help in obtaining *in vitro* testing data.

References and Notes

1. Recent reviews: a) Huff, J.R. *J. Med. Chem.* **1991**, *34*, 2305. b) Thaisrivongs, S. *Annu. Rep. Med. Chem.* **1994**, *29*, 133.

2. Kaldor, S.W.; Hammond, M.; Dressman, B.A.; Fritz, J.A.; Crowell, T.A.; Hermann, R.A. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1385. Compound **1** is referred to a **LY289612** in this paper.

3. Preliminary account of this work: Kaldor, S. W.; Kalish, V.; Dressman, B.A.; Fritz, J.E.; Hammond, M.; Crowell, T.A.; Tatlock, J.; Reich, S.; Davies, J.; Appelt, K.; Shetty, B.; Su, K.S.; Campanale, K.M.; Burgess, J.A.; Lubbehusen, P.P.; Baxter, A.J.; Muesing, M.A.; Hatch, S.D. *Abstracts of Papers*, 208th National Meeting of the American Chemical Society, Washington, D.C.; American Chemical Society: Washington, D. C., **1994**; MED 17.

4. In general, analogous peptidomimetic renin inhibitors suffer similar problems in whole animal studies. See: Greenlee, W.J. *Med. Res. Rev.* **1990**, *10*, 173.

5. Very recently, reports have appeared describing saturated carbocycles and heterocycles which interact with the P_2 pocket of HIV-1 protease. See for example: a) Ghosh, A.K.; Thompson, W.J.; Fitzgerald, P.M.D.; Culberson, J.C.; Axel, M.G.; McKee, S.P.; Huff, J.R.; Anderson, P.S. *J. Med. Chem.* **1994**, *37*, 2506. b) Dorsey, B.D.; Levin, R.B.; McDaniel, S.L.; Vacca, J.P.; Guare, J.P; Darke, P.L.; Zugay, J.A.; Emini, E.A.; Schleif, W.A.; Quintero, J.C.; Lin, J.H.; Chen, I.-W.; Holloway, K.M.; Fitzgerald, P.M.D.; Axel, M.G.; Ostovic, D.; Anderson, P.S.; Huff, J.R. *J. Med. Chem.* **1994**, *37*, 3443.

6. Similar arguments were used in the design of the ortho-substituted benzamide portion of **1** (reference 2).

7. a) All new compounds provided satisfactory spectral data (^{1}H NMR, FAB exact MS or elemental analysis, IR, OR) and were homogeneous by TLC and/or HPLC. b) Full details of the chemistry, biology, crystallography, and pharmacology of this series will be the subject of a separate report.

8. Manetta, J.V.; Lai, M.-H.P.; Osborne, A.D. *Anal. Biochem.* **1992**, *202*, 10.

9. Appelt, K. (Agouron Pharmaceuticals, Inc.), unpublished results. A complex between **2** and HIV-1 protease was crystallized in the hexagonal space group $P6_1$ (a=b=62.9, c=83.1 Å, alpha=beta=90°, gamma=120°). Data were collected to 2.7 Å resolution using the structure of **1** as a starting model, and the structure refined to an R_{factor} of 15.2%. Full experimental details will be reported separately.

10. a) Schoenberg, A.; Bartoletti, I.; Heck, R.F. *J. Org. Chem.* **1974**, *39*, 3318. b) Hidai, M.; Hikita, T.; Wada, Y.; Fujikura, Y.; Uchida, Y. *Bull. Chem. Soc. Japan* **1975**, *48*, 2075.

11. Antiviral testing performed according to the method of Weislow, O.S.; Kiser, R.; Fine, D.L.; Bader, J.; Shoemaker, R.H.; Boyd, M.R. *J. Natl. Cancer Inst.* **1989**, *81(8)*, 577.

12. Parkes, K.E.B.; Bushnell, D.J.; Crackett, P.H.; Dunsdon, S.J.; Freeman, A.C.; Gunn, M.P.; Hopkins, R.A.; Lambert, R.W.; Martin, J.A.; Merrett, J.H.; Redshaw, S.; Spurden, W.C.; Thomas, G.J. *J. Org. Chem.* **1994**, *59*, 3656.

13. For a related functionalization of a pseudo-symmetrical HIV-1 protease inhibitor, see: Kempf, D.J.; Codacovi, L.; Wang, X.C.; Kohlbrenner, W.E.; Wideburg, N.E.; Saldivar, A.; Vasavanonda, S.; Marsh, K.C.; Bryant, P.; Sham, H.L.; Green, B.E.; Betebenner, D.A.; Erickson, J.; Norbeck, D.W. *J. Med. Chem.* **1993**, *36*, 320.

14. a) Dosed in fasted Fischer rats at 40 mg/kg. Full experimental details will be reported separately. b) Whereas **1** required dosing as a suspension despite extensive formulation efforts, **14** proved to be freely soluble in water (solubility > 10 mg/mL). It is tempting to speculate that the enhanced water solubility of **14** may be responsible for its improved pharmacokinetic properties.

15. See previous and subsequent papers in this issue.

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