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# EVALUATION OF SUBSTITUTED BENZAMIDES AS P2 LIGANDS FOR SYMMETRY-BASED INHIBITORS OF HIV PROTEASE

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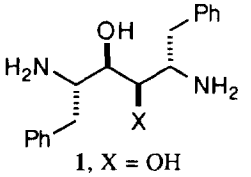
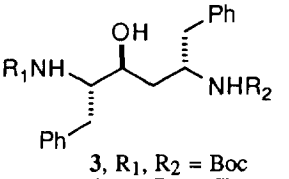
**Abstract.** Substituted benzamides were evaluated as P2 ligands for symmetry-based HIV protease inhibitors. In contrast to previous reports, 2-methyl substitution provided only a modest potency increase in combination with hydrogen bonding groups at the 3-position. Furthermore, hydrogen bonding functionality at the 4-position was well tolerated and independent of substitution at the 3-position.

The design of potent and specific inhibitors of the proteinase from human immunodeficiency virus (HIV protease) represents a promising approach for the therapeutic treatment of AIDS.[1] A variety of classes of peptidomimetic inhibitors have been discovered based both on the HIV protease substrate sequences and on the three-dimensional structure of the $C_2$-symmetric, homodimeric enzyme active site.[2,3] Recently, a variety of ligands optimized for binding in the symmetry-related S2 and S2' subsites of HIV protease have been reported for hydroxyethylamine[4-6] and hydroxyethylbenzamide[6] inhibitors. In particular, the 3-hydroxy-2-methylbenzoyl group provides an attractive P2 ligand due to its lack of stereocenters and ease of synthesis.[6] The *ortho*-methyl group provides additional binding over the corresponding unsubstituted analogs through increased hydrophobic interactions with the S2 pocket and/or stabilization of a non-coplanar bound conformation of the benzamide group.[6] This conformation, confirmed by X-ray studies,[6,7] allows potential hydrogen bonding interactions from the 3-hydroxyl group to either $Asp_{29}$ or $Asp_{30}$. In the search for inhibitors with reduced molecular weight and/or activity against mutant HIV which emerges upon passage with our clinical candidate ABT-538,[8] we have examined P2 ligands attached to the symmetry-based core diamines **1**[9] and **2**[10] by both rational design[11] and through combinatorial methods.[12] A recent report on the successful utilization of substituted benzamides for improving the activity of symmetry-based inhibitors[7] prompted us to report our additional findings.




**1**, X = OH
**2**, X = H



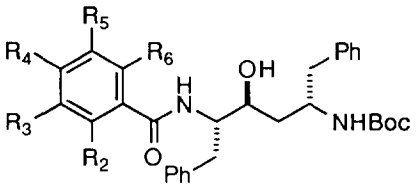

**3**, $R_1$, $R_2$ = Boc
**4**, $R_1$, $R_2$ = Cbz
**5**, $R_1$ = H, $R_2$ = Boc

We initiated our studies with the benchmark inhibitors **3** and **4**, which inhibited purified HIV protease in the lower nanomolar range.[13] We chose to modify the two non-equivalent ends of **3** and **4** independently, since the crystal structures of longer inhibitors derived from **2** had demonstrated an asymmetric mode of binding which allowed close interaction of the hydroxyl group with both catalytic aspartate residues.[14] As a result, we did not anticipate equivalent enhancements resulting from substitution either proximal or distal to the hydroxyl group.

Analogs of **3**, with P2 benzamide groups proximal to the hydroxyl group were prepared by acylation of the mono-Boc diamine **5**.[15] Alternately, **5** was further protected with the Cbz group, and the Boc group was removed to reveal the distal amino group for benzoylation. Substituted benzoic acids were derived by reduction and diazotization of commercially available materials. All inhibitors showed satisfactory purity by $^1$H NMR and mass spectral analysis.

The $IC_{50}$ values for analogs of **3** and **4** against purified HIV protease, determined as described previously,[16] are shown in Tables 1 and 2, respectively. Surprisingly, the unsubstituted benzamide **6** showed,

Table 1. Inhibition of HIV protease by benzamide analogs of **3**.




| No | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $IC_{50}$ (nM) | $EC_{50}$ (μM) | $CCIC_{50}$ (μM) |
|---|---|---|---|---|---|---|---|---|
| **3** | | | | | | 5.3 | nd | nd |
| **6** | H | H | H | H | H | 4.5 | 4.3 | 16 |
| **7** | OH | H | H | H | H | 145 | >75 | >100 |
| **8** | H | OH | H | H | H | 3.4 | 1.6 | 45 |
| **9** | H | H | OH | H | H | 3.3 | 4.8 | 29 |
| **10** | H | OH | OH | H | H | 4.1 | 2.9 | 21 |
| **11** | H | $OCH_3$ | OH | H | H | 3.3 | 2.8 | 43 |
| **12** | H | $OCH_3$ | H | H | H | 23 | >10 | 19 |
| **13** | H | OH | $NH_2$ | H | H | 11 | NE | 11 |
| **14** | H | $NH_2$ | OH | H | H | 7.6 | 5.0 | 57 |
| **15** | H | $NH_2$ | $NH_2$ | H | H | 9.6 | 7.6 | 81 |
| **16** | H | $OCH_3$ | $NH_2$ | H | H | 7.5 | 3.2 | 38 |
| **17** | $CH_3$ | OH | H | H | H | 1.6 | 1.2 | 47 |
| **18** | $CH_3$ | $NH_2$ | H | H | H | 2.6 | 1.4 | 56 |
| **19** | Cl | $NH_2$ | H | H | H | 5.3 | 3.1 | 55 |
| **20** | Cl | H | OH | H | H | 1.4 | 1.8 | 23 |
| **21** | Cl | H | $NH_2$ | H | H | 3.4 | 2.9 | 48 |
| **22** | H | $NH_2$ | H | H | Cl | 3.3 | 1.8 | >100 |
| **23** | H | OH | H | H | Cl | 12 | 3.7 | 19 |
| **24** | Cl | H | H | H | Cl | 67 | >10 | 19 |
| **25** | Cl | $NH_2$ | H | H | Cl | 31 | nd | nd |
| **26** | H | OH | H | OH | H | 51 | 11 | 53 |
| **27** | H | $NH_2$ | H | $NH_2$ | H | 250 | NE | 92 |

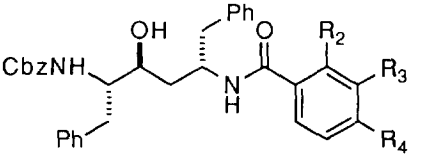
nd - no data; NE - no effect; ABT-538: Ki = 0.02 nM, $EC_{50}$ = 0.022 μM.[8]

within experimental error, equivalent activity to the di-Boc derivative **3**, suggesting extensive hydrophobic interactions of **6** with the S2 subsite. Whereas the 2-hydroxy analog **7** was a poor inhibitor, substitution of OH at either the 3 and/or 4 position (**8** - **10**) provided inhibitors of slightly greater potency than **6**. These results suggest that a single conformation of the benzoyl group allows hydrogen bonding of both hydroxyl groups with the S2 subsite. Modeling studies indicated that the 4-hydroxyl group of **9** - **11** may be positioned for hydrogen bond donation to the carboxylate of Asp 30. Interestingly, **10** and **11** were of similar potency, suggesting that the 3-hydroxyl group of **8** and **10** accepts a hydrogen bond from the NH of either Asp 29 or 30.[7] In the absence of a 4-hydroxyl group, however, the 3-methoxy group was incapable of sustaining an energetically favorable hydrogen bond, as evidenced by the lower activity of **12** compared to **8**. As observed previously,[6] amino groups at the 3 and 4-positions (**13** - **16**) were less effective than the more strongly hydrogen bonding phenolic groups.

Disappointingly, the degree of enhancement in binding affinity upon addition of a hydrophobic group *ortho* to the benzamide linkage was modest (compounds **17** - **21**). The measured difference between the binding constants of pairs of corresponding inhibitors (**8**:**17** and **9**:**20**) was only *ca.* 0.3 - 0.35 kcal/mol, in contrast to a 1.4 kcal/mol difference reported for a similar system.[7] Importantly, however, we continued to observe the equivalence of hydrogen-bonding functionality at either the 3 or 4-position of the benzamide. Furthermore, the 6-chloro-3-amino and 2-chloro-3-amino inhibitors **19** and **22** were equipotent, although the corresponding 6-chloro-3-hydroxyl inhibitor **23** was less active than **17**. It is unclear whether the benzamide rotates by 180° within the S2 pocket between the 2,3- and 3,6-disubstituted inhibitors,[6] since incorporation of either an additional *ortho*-hydrophobic group (compounds **24** and **25**) or *meta*-hydrogen bonding group (compounds **26** - **27**) resulted in a significant decline in activity.

We briefly examined analogs of Cbz-based inhibitor **4** with substituted benzamides distal to the hydroxyl group of the core diamine. Results are provided in Table 2. Interestingly, while the potency of unsubstituted benzamide **28** was significantly (sevenfold) lower than that of **6**, most of the substituted benzamides were *ca.* twofold less active than the corresponding "proximal" benzamides (*cf.* **29**:**13**, **30**:**16**, and **32**:**21**). The exception was **31**, which, in a manner similar to baseline inhibitors **3** and **4**, was indistinguishable from **20**.

Table 2. Inhibition of HIV protease by benzamide analogs of **4**.




| No | $R_2$ | $R_3$ | $R_4$ | $IC_{50}$ (nM) | $EC_{50}$ (μM) | $CCIC_{50}$ (μM) |
|---|---|---|---|---|---|---|
| **4** | | | | 5.6 | >30 | >100 |
| **28** | H | H | H | 31 | >10 | >100 |
| **29** | H | OH | $NH_2$ | 22 | >10 | 18 |
| **30** | H | $OCH_3$ | $NH_2$ | 10.5 | >8 | 18 |
| **31** | Cl | H | OH | 1.3 | 2.2 | 18 |
| **32** | Cl | H | $NH_2$ | 6.1 | >10 | 15 |

The anti-HIV activity ($EC_{50}$), and cytotoxicity ($CCIC_{50}$) of each inhibitor were determined in MT4 cells using a cytopathicity assay.[16] As observed previously for symmetry-based inhibitors,[17] $EC_{50}$ values (Tables 1 and 2), generally paralleled the $IC_{50}$ values for HIV protease inhibition but at 1000-fold higher concentrations. The structure-activity relationships were similar also, and little difference in activity was observed upon adding an *ortho* substituent to the P2 benzoyl group (*cf.* **17**:**8**).

In conclusion, this study has shown that substituted benzamides can function as useful P2/P2' groups for symmetry-based HIV protease inhibitors. In contrast to previous studies, however, the addition of a small, hydrophobic *ortho* group produced only a modest (*ca.* twofold) increase in activity. Furthermore, our results suggest that equal enhancement can be achieved not only with 3-substituted benzamides, as previously reported using structure-based design,[6,7] but also with hydrogen bonding substituents at the 4-position.

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