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SYMMETRY-BASED HIV PROTEASE INHIBITORS: RATIONAL DESIGN OF 2-METHYLBENZAMIDES AS NOVEL P2/P2' LIGANDS.

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ABSTRACT: Readily accessible, non-peptidic, achiral 2-methylbenzamides were designed to serve as P2/P2' ligands for symmetry-based inhibitors of HIV-1 Protease. Introduction of 3-hydroxy substituent provided a potent inhibitor **7** (K_i = 0.8 nM).

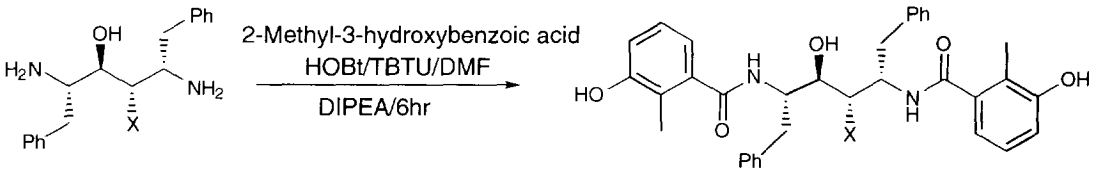
The human immunodeficiency virus type 1 (HIV) encodes an aspartic protease (HIV PR) which is essential for processing of viral polyproteins.[1] Inhibition of HIV PR results in the production of non-infectious virons.[2] Thus, the development of HIV PR inhibitors is an attractive strategy for the treatment of AIDS.[3] X-ray crystallography has revealed that HIV PR adopts a conformation in which an approximate 2-fold symmetry axis extends through the active site of the enzyme.[4] Erickson *et al.*[5] and Kempf *et al.*[6] have described the design of C_2 symmetry-based inhibitors to mimic the symmetry of the enzyme. Such inhibitors may provide advantages over more traditional, substrate-based inhibitors in terms of potency and specificity. Several C2 symmetry-based compounds are undergoing clinical trials.[6,7] During recent work aimed at reducing the structural complexity and peptidic nature of symmetry-based diol containing inhibitors, we observed that the P2/P2' carbamate units could be replaced by functionalized non-peptidic, achiral 3-hydroxybenzamides.[8]

BACKGROUND AND DESIGN CONSIDERATIONS: Structure of the HIV PR complexed with the mono-substituted 3-hydroxybenzamide, **1**, revealed that the aromatic ring of the benzamide and the amide carbonyl are not co-planar.[8] Presumably, this conformation facilitates optimal interaction of the amide carbonyls with the "flap" water (Wat301), and maximizes the S2/S2' hydrophobic contacts. Energy calculations on N-methyl-3-hydroxybenzamide showed that the most stable conformation is one in which the aromatic ring and the amide carbonyl groups are co-planar.[9a] Since accommodation of the 3-hydroxybenzamide group in the enzyme active-site requires the aromatic ring of the benzamide and the amide group to be in a higher energy, non co-planar conformation, enhanced affinity could presumably be obtained by suitable modifications that stabilize the bio-active conformations. *Ortho*-substitution of the benzamide ring[9b] has been shown to favor a conformation where the aromatic ring and the carbonyl groups are non co-planar. This report describes the effect of the P2/P2' *ortho*-methylbenzamide replacements on the potency of symmetry-based HIV PR inhibitors.

CHEMISTRY: The inhibitor core unit **A** (SRSS-2,5-diamino-1,6-diphenyl-3,4-hexanediol) was synthesized by McMurry coupling of natural Z-phenylalaninal following a previously reported procedure.[10] The 2-methyl-5-hydroxybenzoic acid was prepared by using Lewis acid catalyzed Diels-Alder reaction of 2-methylfuran and ethyl propiolate followed by saponification,[11] and the 2-methyl-3-hydroxybenzoic acid was prepared from corresponding amino compounds by the Sandmeyer reaction. Condensations of diol **A** with

suitably subsituted benzoic acid was performed using standard peptide coupling procedure (TBTU/HOBt/DIEA). This provided compounds **2-6**, respectively in a 70-80% yield (Scheme 1). Condensation of the deshydroxy compound **B** (SSS-2,5-diamino-1,6-diphenyl-3-hexanol)[12] with 2-methyl-3-hydroxybenzoic acid provided compound **7** (Scheme 1). The structures of all new compounds thus prepared were established by ^1H NMR spectroscopy and mass spectral (FAB or/and HRMS) analysis.

Scheme 1

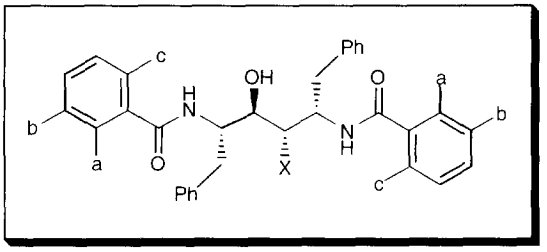


A, X = OH
B, X = H

5, X = OH
7, X = H

RESULTS AND DISCUSSION: The inhibitory potencies of compounds **1-7** are presented in Table 1. Compound **3** possessed the 2-methylbenzamide group as a P2/P2' ligand. This group was predicted to stabilize the bioactive conformation of the benzamide, and was about 5 fold more potent than **2**. Introduction of the

Table 1: Symmetry-based HIV PR inhibitors and their inhibitory potencies.



Comp.	a	b	c	X	K_i (nM)[a]
1	H	OH	H	OH	22
2	H	H	H	OH	40
3	CH_3	H	H	OH	8
4	H	OH	CH_3	OH	74
5	CH_3	OH	H	OH	1.2
6	CH_3	NH_2	H	OH	2.5
7	CH_3	OH	H	H	0.8

[a]K_i's were determined using a fluorogenic substrate-based assay for HIV PR inhibitors.[13]

hydroxyl or amino group in the *meta*-position of the benzamide was expected to provide polar interactions with the Asp29/129 and the Asp30/130 residues in the S2/S2' domain. In 3-hydroxybenzamide, two possibilities exist for *ortho*-substitution; we prepared both 2-methyl-5-hydroxy and 2-methyl-3-hydroxy benzamide-containing inhibitors. Inhibitor **5**, possessing a 2-methyl-3-hydroxy benzamide,[9c] further improved inhibitor potency (K_i of 1.2 nM). However, compound **4** (Table 1), possessing a 2-methyl-5-hydroxy benzamide, showed an unexpected 60-fold decrease in binding affinity (K_i of 74 nM) compared to **5**.

STRUCTURE OF HIV PR/5 COMPLEX. To gain further insight into inhibitor binding, a three dimensional structure of compound **5** complexed to HIV PR was determined by X-ray diffraction to 2.0Å. The X-ray crystallographic analysis reveals that inhibitor **5** binds in an extended conformation spanning from the S2 to S2' subsites. As predicted from modeling studies, the aromatic rings of the hydroxybenzamides and the amide carbonyl groups are not co-planar. The rotation of the aromatic ring of the benzamide out of the amide plane is 60° on the R-OH side and 44° on the S-OH side. Contrary to the 3-hydroxybenzamide groups of compound **1**[8], the 2-methyl-3-hydroxybenzamide groups of compound **5** makes symmetric interactions with HIV PR.

Several intermolecular hydrogen bonds are formed between inhibitor **5** and the enzyme. The conformation and interactions of the central diol core with HIV PR are similar to those observed with other inhibitor/HIV PR complexes possessing the same core *i.e.* A77003[14] (Figure 1). The R-hydroxyl group of the inhibitor core interacts in a nearly symmetric fashion with the active site aspartic acids, whereas the S-OH group is pointed away from the active site pocket.

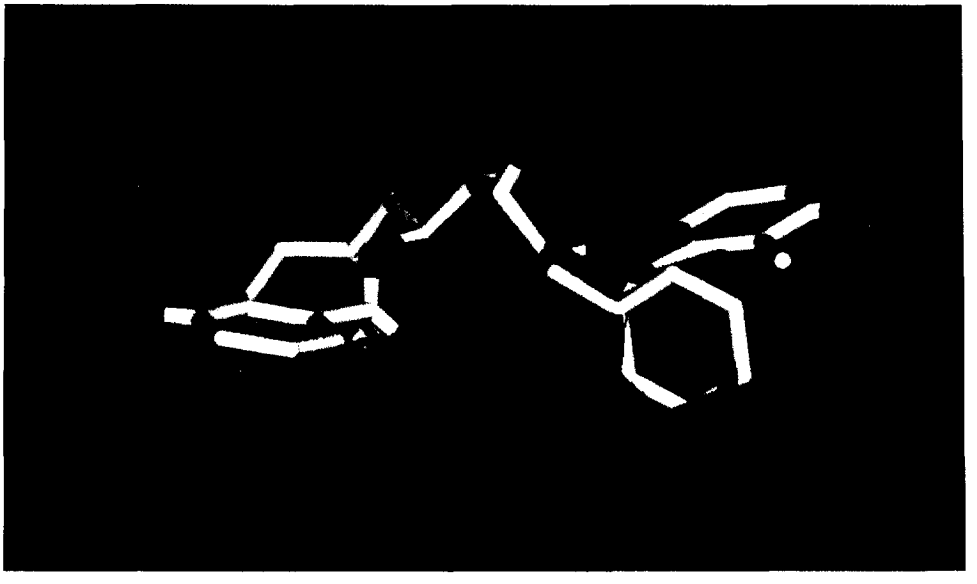


Figure 1. Superposition of the HIV-1 protease-bound conformations of **5** and A77003. Atoms of **5** are colored by type; atoms of A77003 are brown.

The deshydroxy compound **7**, in which the poorly positioned S-OH group of the diol core is replaced by hydrogen, exhibited an inhibition constant of 0.8 nM. The slight increase in inhibitory potency of compound **7** can be contrasted with the 10-fold increase in potency of the deshydroxy analog of A77003 which was attributed to a decreased desolvation penalty.[14] The hydroxyl substituent of the benzamide residue is positioned equidistant from the NH's of Asp29 (3.2Å)/129 (3.4Å), Asp30/130[16] (3.5Å) and the side chain carboxylate oxygen of Asp30/130 (2.8Å) (Figure 2). However, the distances and angles are not optimal for all three hydrogen bonds to each hydroxyl of the benzamide. An additional hydrogen bond is formed between the amide carbonyls and the "flap" water. In addition, several well-ordered water molecules are observed that solvate polar residues in the S3/S3' binding pocket. In contrast to peptidic inhibitors, the backbone carbonyls of Gly48/148 point towards the center of the aromatic ring of the benzamide residue and may form weak polar inteactions.[15]

Extensive hydrophobic interactions are observed between inhibitor **5** and the enzyme. The core benzyl groups of the inhibitor project into the S1/S1' binding site of the protease. The aromatic ring of the 2-methyl-3-hydroxybenzamide residue projects into the S2/S2' subsite, making extensive Van der Waals interactions with the hydrophobic side chains of the residues Ala28/128, Val32/132, Ile47/147, Ile50/150 and Ile84/184 (Figure 2). The structure of HIV PR/**5** complex suggests a deleterious close contact between the 2-methyl group of the benzamide and the side chain of Ile84 which may account for the low potency of **4**.

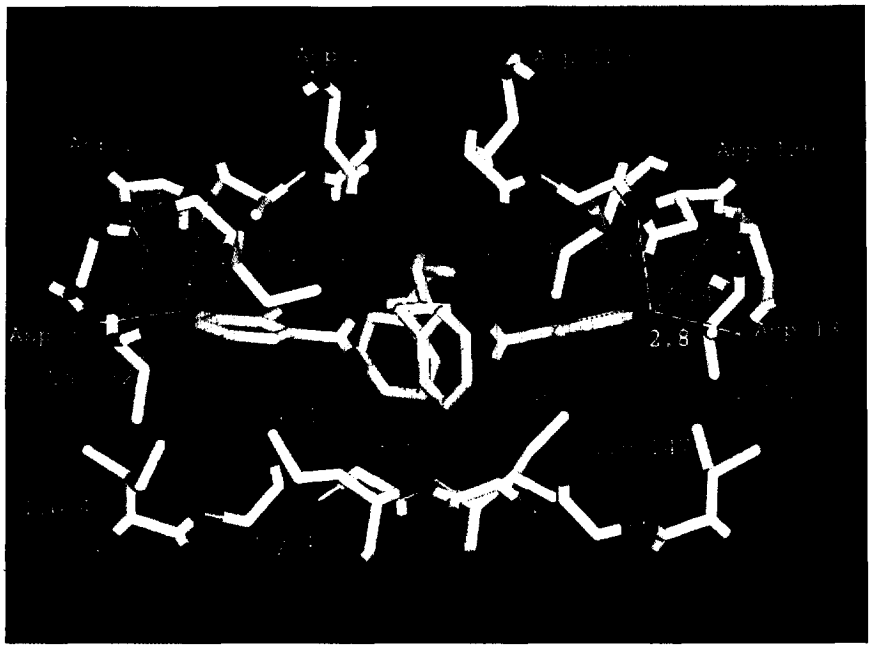


Figure 2. Interactions of compound **5** with HIV PR . HIV PR residues interacting with the 3-hydroxy-2-methylbenzamide group are labeled and the distances from the benzamide hydroxyl to the backbone NH's of Asp29/129 and side chain carboxylate of Asp30/130 are indicated in white. Atoms of **5** are colored yellow and the atoms of HIV PR are colored by type.

CONCLUSIONS: Using information gained from analysis of X-ray structures we have designed a P2-P2' inhibitor **7** with a high binding affinity (K_i of 0.8 nM) that interacts with the protease in a nearly symmetric manner. The high potency observed for this series might be due to the stabilization of a productive conformation for binding to the active site of HIV PR. The achiral, non-peptidic 2-methyl-3-hydroxybenzamide group is a novel P2/P2' replacement that simultaneously provides good hydrophobic interactions and hydrogen bonding capabilities with the enzyme.

SUPPLEMENTARY MATERIAL AVAILABLE: Experimental details and spectroscopic data for compounds reported here are available from the authors.

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