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Structure-Based Design of Achiral Anthranilamides as P2/P2' Surrogates for Symmetry-Based HIV Protease Inhibitors: Design, Synthesis, X-ray Structure, Enzyme Inhibition and Antiviral Activity.

Ramnarayan S. Randad,* Lucyna Lubkowska, Anna Bujacz, Rajan H. Naik, Sergei V. Gulnik, Betty Yu, Abelardo Silva, Sanjeev Munshi, Tracy M. Lynch[a], David J. Clanton[a], T. Narayana Bhat, and John W. Erickson.

Structural Biochemistry Program, [a]AIDS Drug Screening and Development Laboratory, SAIC Frederick, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD 21702

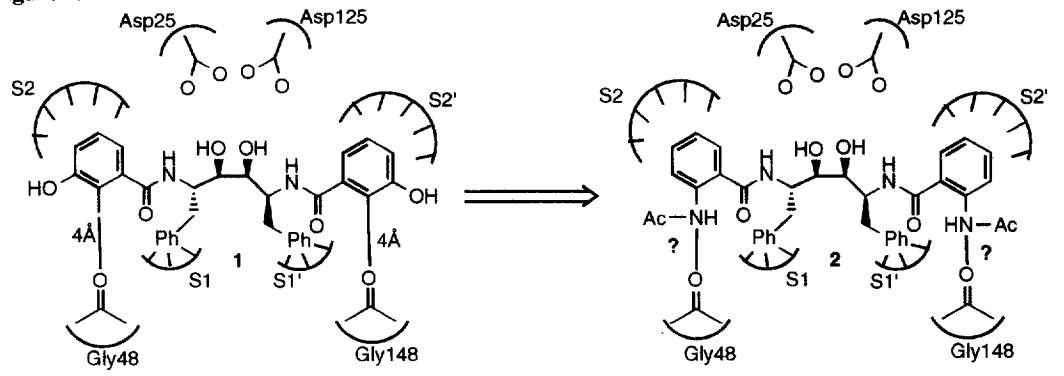
Abstract: Guided by the structure of HIV PR complexed with 2S,3R,4S,5S-2,5-bis[N,N'-((3-hydroxy-2-methylphenyl)carbonyl)amino]-3,4-dihydroxy-1,6-diphenyl hexane (**1**), a novel, achiral, non-peptidic anthranil (Ant) group was designed as a P2/P2' ligand. Symmetry-based inhibitors containing N-(2-pyridinylmethoxy-carbonyl)anthranil group are potent anti viral agents. Compounds **12** and **14** exhibited protease inhibitory activity of 60 and 70 pM, anti viral activity of 13 and 56 nM and cellular toxicity of >10 uM respectively.

The development of the inhibitors of a human immunodeficiency virus type 1 protease (HIV PR) is of great interest because of their potential for the treatment of Acquired Immunodeficiency Disease Syndrome (AIDS).[1] Recently we have reported the design and synthesis of a series of symmetry-based HIV PR inhibitors containing achiral, non-peptidic *ortho*-substituted benzamides as P2/P2' ligands (**1**).[2] In this communication, we describe a series of novel nonpeptidic, achiral anthranilamide-containing, symmetry-based HIV PR inhibitors. We report here on the design, synthesis, enzyme inhibition and anti viral activity of these compounds along with a crystal structure of HIV PR/**12** complex.

Design of Anthranilamide as a P2/P2' Ligand: Structural studies on the HIV PR/**1** complex revealed that the polar carbonyls of Gly48/148 do not make complementary hydrogen bonds with inhibitor **1** unlike the case for most peptidic inhibitors.[1] We further noted that the 2-methyl group of benzamide in inhibitor **1** is within 4Å of the Gly48/148 carbonyl oxygen atoms.[2] Thus, we considered modifying the 2-Me group of the benzamide with an NH group for example, that would in principle be capable of hydrogen bonding with the Gly48/148 backbone carbonyl oxygen atoms (**Figure 1**). In addition N-acetylation or carbamylation of the anthranil (Ant) group offers possibilities for the addition of P3/P3' substituents. The 3-hydroxy group of inhibitor **1** interacts with the NH of Asp29/129[2]. In the anthranilamide containing inhibitors, the Ant-N-acetyl carbonyl group was expected to provide hydrogen bonding interactions with the Asp29/129 NH. Modeling of the symmetry-based N-acetyl-anthranil analog, **2**, revealed an excellent complementarity for inhibitor-enzyme interactions, whereby the anthranil carbonyl group could interact with the "flap" water, the N-H group is positioned to interact with the Gly48/148 carbonyl groups, the N-acetyl carbonyls interact with the Asp29/129 NH atoms, and the aromatic

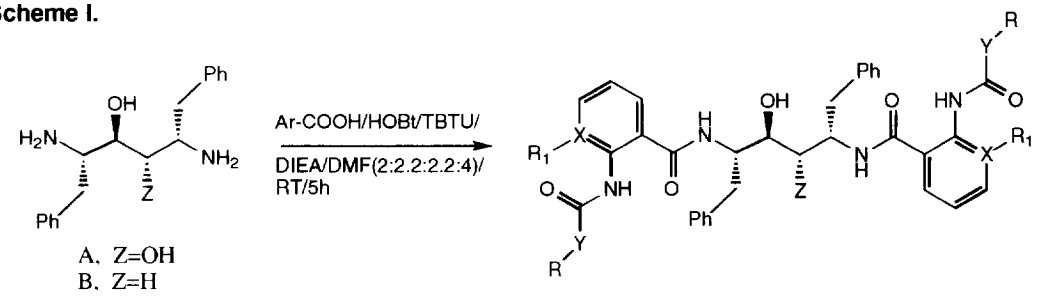
ring of anthranil group provides hydrophobic interactions between the compound and the S2/S2' subsite residues of HIV PR. The compound **2** and its analogs **3-16** were prepared and analyzed for HIV protease inhibition.

Figure 1.



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 reported procedure.[3a] The 2,5-diamino diol **A** was condensed with suitably substituted N-acyl/carbamyl-anthranilic acid using a standard peptide coupling procedure (TBTU/HOBt/ DIPEA) to provide compounds **2-12** (**Scheme I**) in a 70-80% yield; poor yields (10-20%) were observed in the case of **2**. The N-acylanthranilic acids were prepared by reaction of the anthranilic acid with the corresponding acid chloride. The N-benzyloxycarbonylanthranilic acid was prepared by the condensation of the anthranilic acid with the benzyoxycarbonyl chloride and the N-(pyridinyl-methoxycarbonyl)anthranilic acids were prepared by reaction of the methyl 2-isocyanatobenzoate with the corresponding alcohols. Reaction of methyl anthranilate with triphosgene following the Majer and Randad procedure[3b] provided methyl 2-isocyanatobenzoate. Hydrogenolysis of compound **8** using 10% Pd/C provided compound **9**. Deshydroxy analogs **13-16** were similarly prepared using SSS-2,5-diamino-1,6-diphenyl-3-hexanol core (**B**).[4] The structures of all newly synthesized compounds were established by ^{1}H NMR spectroscopy and mass spectral (FAB or/and HRMS) analysis.

Scheme I.



Results and Discussion: The inhibitory potencies of compounds **1-16** are presented in **Table 1**. Compound **2** (**Table 1**), in which the P2/P2' groups are achiral, non-peptidic, N-acetylanthranilamide, inhibited HIV PR with a K_i value of 41 nM. In comparison, inhibitor **1**, containing 3-hydroxy-2-methylbenzamide as the P2/P2' ligands, exhibited a K_i value of 1.2 nM. Substitution of N-acyl with N-ethylcarbamate (compound **3**) exhibited low solubility. Inhibitor **2** spans from the S2 to S2' subsites of the enzyme leaving most of the S3, and S3' subsites unoccupied. The introduction of the N-benzyloxycarbonyl-Ant (Ant = anthranil) group to the symmetry-based cores **A** or **B**, was expected to provide an inhibitor that could interact with the S3 to S3' subsites. Accordingly, compound **4** was prepared and it exhibited a 20-fold improvement in enzyme inhibitory potency (K_i = 2.4 nM) compared to **2**.

Compound **4** was co-crystallized with HIV PR and the structure was used to design analogs. Investigation of the HIV PR/**4** complex suggested that an appropriate *meta*-substitution of the anthranilamide group can produce additional interaction between the inhibitor and the Asp30 side chain carboxylate. The *meta*-hydroxy containing compound (**5**) was prepared and found to possess a K_i value of 20 nM. The 3-chloro and 3-methyl derivatives **6** and **7** respectively, did not improve the protease affinity of the anthranilamide-containing inhibitors.

Compound **4,** although a potent inhibitor of protease, did not show any anti viral activity. We presumed that the inactivity of compound **4** in the anti viral assay may be related to its poor cell permeability, as predicted by its calculated $logP^5$ value of 8.2 (**Table 1**). Compound **9**, possessing the Ant-N-CO-CH_2OH group, was designed to provide a specific interaction with the polar guanidinium side chains of Arg8/108 and also to improve solubility. This compound exhibited a K_i of 90 nM and an EC_{50} of 60 uM. The corresponding O-benzyl analog (**8**) was inactive. The nicotinyl analog **10**, exhibited a K_i value of 3 nM and only a marginal improvement in the calculated logP value (7.9).

We next considered changes to the P3/P3' benzyloxy group of **4** with the aim of improving solubility and designed compounds **11** and **12** . The piperazine-containing inhibitor, **11,** had a deleterious effect on inhibitory potency. Compound **12**, containing the 2-pyridinylmethoxy group as a P3/P3' ligand, exhibited a 400-fold improvement in inhibition of HIV protease, K_i value of 60 pM, compared to **4** and a calculated logP value of 6.5. Consistent with our predictions, compound **12** exhibited anti viral activity in the low nM range with an EC_{50} value of 13 nM and only moderate cytotoxicity (TC_{50} = 10 uM).

Compound **12** was co-crystallized with HIV PR and its three dimensional structure was determined using X-ray crystallography to 2.1Å resolution(**Figure 2**). The X-ray crystallographic analysis reveals that inhibitor **12** binds in an extended conformation spanning from S3 to S3' subsites of the enzyme. As predicted from modeling studies, the aromatic ring and the amide carbonyl groups of Ant are not co-planar; the dihedral angles between the Ant carbonyl and aromatic ring are -79° on the R-OH side and -143° on the S-OH side. The group is involved in several polar and hydrophobic interactions. The aromatic ring of the Ant residue projects into the S2/S2' subsite, making extensive Van der Waals interactions with the hydrophobic side chains of the residues Ala28/128, Val32132, Ile47/147, Ile50/150, and Ile84/184. As predicted from modeling studies the Ant-NH group interacts with the carbonyl's of the Gly48/148, and the Ant-NH-carbonyl interacts with the Asp29/129 NH's. The hydrogen bonding distances and angles with, Gly48 carbonyl are 2.9Å and 129°; with Gly148 carbonyl are 3.2Å and 132°; with Asp29 NH are 3.0Å and 165°; and with Asp129 NH are 2.8Å and

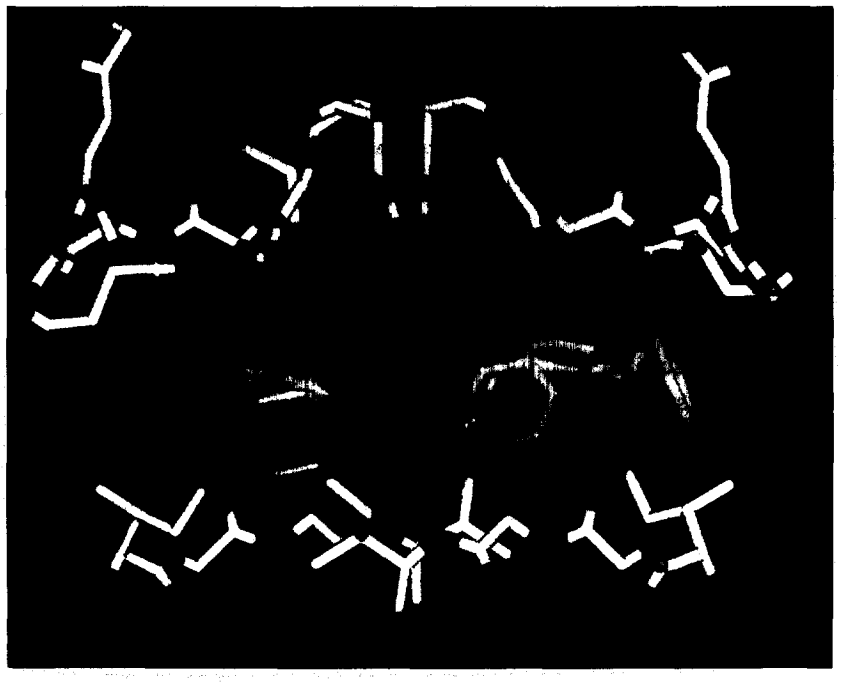
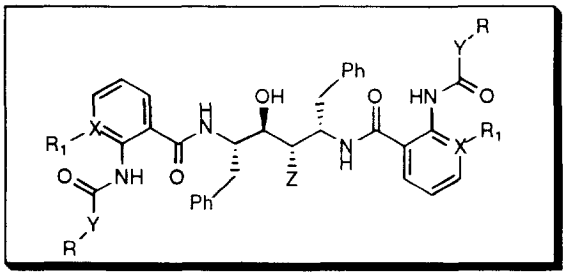


Figure 2. X-ray structure of inhibitor **12** complexed with HIV PR. Atoms of the inhibitor **12** are colored yellow and the atoms of HIV PR are colored by type. Important hydrogen bonding interactions are drawn in dashed white lines.

164". Other polar interaction between the Ant group and the enzyme involve interactions with the localized water molecule observed near the "flap" region of the enzyme.

The P3/P3' pyridinyl group stacks against the charged guanidinium group of Arg8/108, similar contacts are also observed in the HIV PR/A-77003 complex.[6] The nitrogen atom of the 2-pyridinyl group is involved in interactions with the ordered water molecules in the S3/S3' subsite of the enzyme. On the contrary, the P3/P3' benzyl side chains of **4** are rotated away and do not form stacking interactions with the guanidinium group of Arg8/108. The stacking of P3/P3' pyridinyl groups combined with the water mediated interactions of the 2-pyridinyl nitrogen atoms with the protease may account for the 60-fold increase in potency of inhibitor **12** compared to **4**. The interactions of the P1/P1' benzyl side chain with the HIV PR are similar to those observed with other HIV PR/inhibitor complexes possessing the same core (*i.e.*, A77003.[6]). The R-hydroxyl group of the inhibitor core points into the active site pocket, whereas the S-OH group is pointed away from the active site pocket. The deshydroxy compounds **13** and **14**, in which the poorly positioned S-OH group of the diol core is replaced by hydrogen, exhibited a inhibition constants of 1.2 nM and 70 pM, respectively. In contrast to the 10-fold increase in potency observed for the deshydroxy analog over the potent diols,[6] the deshydroxy anthranilamide-containing compounds did not provide analogous potency enhancements; compare K_i values of

Table 1: P2/P2' anthranilamide-containing symmetry-based HIV PR inhibitors and their inhibitory potencies.



Comp.	R	X	R_1	Y	Z	K_i (nM)[b]	logP (cal)[a]	EC_{50} (M)[c]
1					OH	1.2	4.889	9×10^{-7}
2	CH_3	C	H	-	OH	41	4.320	IN
3	CH_2CH_3	C	H	O	OH	insoluble	6.140	ND
4	CH_2Ph	C	H	O	OH	2.4	8.160	IN
5	CH_2Ph	C	OH	O	OH	20	7.959	ND
6	CH_2Ph	C	Cl	O	OH	91	9.340	ND
7	CH_2Ph	C	CH_3	O	OH	12	9.240	ND
8	OCH_2Ph	C	H	CH_2	OH	>10 μM	6.910	ND
9	OH	C	H	CH_2	OH	20	2.515	6×10^{-5}
10	CH_2Ph	N	-	O	OH	3	7.868	ND
11	5-Me-Pi[2]	C	H	-	OH	>10 μM	5.664	ND
12	CH_2-2-Py	C	H	O	OH	0.06	6.491	13×10^{-9}
13	CH_2Ph	C	H	O	H	1.2	8.282	ND
14	CH_2-2-Py	C	H	O	H	0.07	6.614	56×10^{-9}
15	CH_2-3-Py	C	H	O	H	0.26	5.863	1.4×10^{-7}
16	CH_2-4-Py	C	H	O	H	0.45	5.393	2.6×10^{-6}

2-py = 2-pyridinyl, 5-Me-Pi = 5-methyl-2-pyrazinyl. ND = not determined; IN = >100 uM. [a] ref. 5; [b]Inhibition of HIV PR was measured using a fluorogenic substrate[8]; [c]EC_{50} = the concentration of compound required to produce 50% inhibition of HIV-1 infection in CEM cells and are average of three experiments.

compounds **4** vs **13** and **12** vs **14**. Analogs possessing the 3-pyridinyl group (**15**) and 4-pyridinyl group (**16**) were less potent. The decreased potency of compounds **15** and **16** may be attributed to the inability of the P3/P3' 3- and 4-pyridinyl nitrogen to participate in the water-mediated stabilizing interactions.

Anti Viral Activities: Compounds **9**, **12** and **14-16** were evaluated for their ability to inhibit the spread of HIV-1 in human T-lymphocyte cell culture.[7] In general, the anti viral activity for this series of compounds appears to be 100 to 1000-fold less than their protease inhibitory activity. Compounds **12** and **14** exhibited EC_{50} values of 13 nM and 56 nM, respectively. The deshydroxy analog **14** exhibited low cytotoxicity (>50 uM) compared to **12** (TC_{50} of 35 uM). Furthermore, compound **14** completely suppressed p24 synthesis at 10 nM whereas compound **12** was 10-fold less potent in the same assay.

Conclusions: SAR data and an X-ray crystallographic analysis of HIV PR/**12** complex reveal that achiral, non-peptidic anthranilamides are excellent P2/P2' peptide surrogates in symmetry-based diol inhibitors. The anthranilamide group participates in both the polar and hydrophobic interactions that are commonly observed between the enzyme and peptidic inhibitor in the vicinity of the S2/S2' subsites. Both inhibitors **12** and **14** exhibited good anti viral activity and low cellular toxicity. The anti viral activity of compound **12** compares favorably with other potent HIV PR inhibitors currently undergoing clinical trials. Pharmacokinetic optimization studies are underway.

Supplementary Material Available: Experimental details, crystallographic details and spectroscopic data for the compounds reported here are available from the authors.

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