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α-HYDROXYAMIDE DERIVED AMINODIOLS AS POTENT INHIBITORS OF HIV PROTEASE

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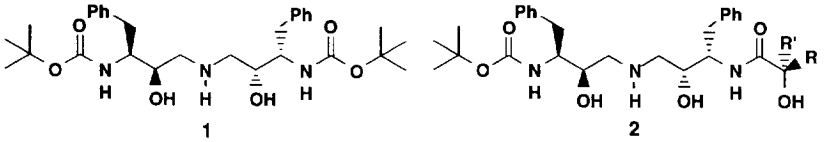
Abstract: A novel series of HIV protease inhibitors has been prepared. Replacement of the P_2 carbamate of compound **1** [IC_{50} = 125 nM] with an α-hydroxy amide moiety results in a significant increase in anti-HIV protease activity [e. g., compound **25a**; IC_{50} = 15 nM]. Furthermore, isomers with (R) absolute configuration at the P_2 site show greater inhibitory activity than the corresponding (S)-isomers. A proposed binding mode based on molecular modeling is used to rationalize the structure-activity relationships.

The human immunodeficiency virus (HIV) is the causative agent of Acquired Immune Deficiency Syndrome (AIDS). An HIV-encoded aspartyl protease is essential for the production of infectious virus, and inhibitors of this enzyme are attractive candidates for AIDS chemotherapy.[1] Recently, we published the design and synthesis of a novel class of HIV protease inhibitors containing an aminodiol transition-state isostere.[2] The lead compound in this series (**1**) has enzyme IC_{50} and HIV-1 ED_{50} values of 125 nM and 80 nM respectively. Extensive efforts have been made to optimize the potency of this compound. Herein, we describe a series of P_2 α-hydroxyamide analogs **2** with improved anti-HIV protease and antiviral activity.



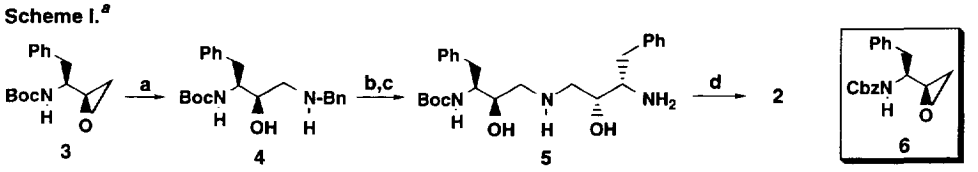
1 **2**

Figure I

Chemistry: All inhibitors were derived from the key intermediate **5** (Scheme I) *via* an amide coupling with appropriate chiral or racemic α-hydroxy carboxylic acids. In cases where racemic acids were used, the resulting mixtures of diastereomeric products were generally separated by chromatography, and each isomer was tested independently for its biological activity.

The synthesis of **5** was carried out *via* Boc-L-phenylalanine derived epoxide **3**.[2] Thus **3** was converted to the secondary amine **4** by treatment with excess benzyl amine (5 equivalents) in the presence of 1 equivalent $LiClO_4$ in acetonitrile (95% yield). Reaction of this compound with Cbz-L-phenylalanine derived epoxide **6**[2] in refluxing methanol followed by deprotection *via* Pd-catalyzed hydrogenolysis afforded the key intermediate **5** (90% yield, 2 steps). Coupling reactions of the various hydroxy acids with amine **5** were carried out using BOP reagent[3] in the presence of N-methylmorpholine (NMM) to give products **2** in 60-85% yield.

Scheme I.[a]



[a]a) 5 Eq. benzyl amine/1 eq. $LiClO_4/CH_3CN$, 95%; b) compound 6 (see ref. 2)/MeOH, 97%; c) $Pd(OH)_2/H_2/HCl$ followed by K_2CO_3, 93%; d) appropriate α-hydroxycarboxylic acid in the presence of BOP reagent/NMM/DMF

The requisite hydroxy acids or lactones used were either obtained from commercial sources or synthesized by methods described below. The synthesis of the (R)- and (S)-enantiomers of the acid **7** was carried out as described by Ito and Kibayashi.[4] Analogs **22a** and **22b** (Table 1) were initially prepared by resolving a diastereomeric mixture prepared by coupling **5** with the lithium salt of racemic acid **8**. Racemic **8** was prepared by the addition of *t*-butylmagnesium chloride to ethyl pyruvate at -78 °C in THF to afford a *ca.* 40% yield of the corresponding hydroxy ester. Saponification with 1 equivalent of LiOH in 3:1 dioxane-water at 75 °C afforded a quantitative yield of **8** as its lithium salt. Subsequently, an enantioselective synthesis was developed leading to **(R)-8**, which was required for the synthesis of **22a**.[5,6] The trifluoro acid **9** was prepared according to the procedure of Burstein and Ringold.[7] Compound **10** was synthesized *via* the corresponding cyanohydrin in 2 steps. Thus treatment of 2,2-dimethylcyclopentanone with TMSCN in the presence of ZnI_2 in methylene chloride,[8] followed by hydrolysis of the crude cyanohydrin with HCl in refluxing aqueous-dioxane, gave a 16% yield of the corresponding hydroxy acid **10**.

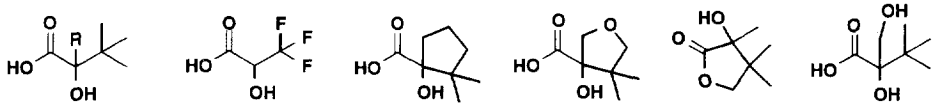


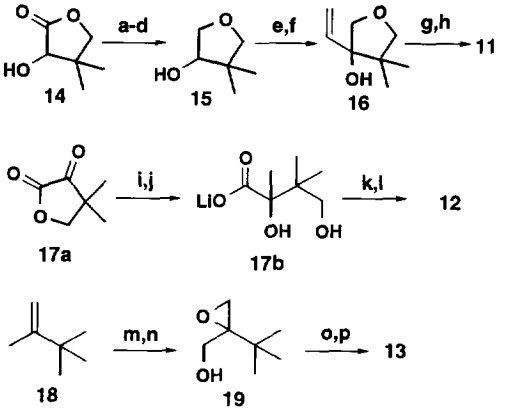
Figure 2

Preparation of the tetrahydrofuran derived acid **11** was carried out in 8 steps from pantolactone **14** (Scheme II). Thus benzylation of the secondary hydroxyl group, followed by a 2-step reduction of the lactone carbonyl and Pd catalyzed reductive removal of the benzyl group, afforded the hydroxytetrahydrofuran **15**. Oxidation of the secondary hydroxyl group gave the corresponding ketone, which was converted to **16** by treatment with vinyl magnesium bromide in the presence of $CeCl_3$. The allylic alcohol **16** was subjected to ozonolysis followed by treatment with methyl sulfide, and the resulting crude aldehyde was converted to the target acid **11** *via* Lindgren oxidation.[9]

Compound **12** could be prepared from dihydro-4,4-dimethyl-2,3-furandione (**17a**) *via* treatment with methylmagnesium chloride in THF at -78 °C, and the crude product was treated with LiOH in refluxing THF-water. The resulting crude lithium salt of 2,4-dihydroxy-3,3-dimethylbutanoic acid (**17b**) was acidified and subjected to silica gel chromatography to afford pure lactone **12** (40% yield from **17a**). The tetrahydroxy analogs **29a** and **29b** were isolated by chromatographic resolution of the 1:1 mixture of diastereomers obtained by coupling lactone **12** (Figure 2) with the amine **5** in DMF at 100-145 °C (42% yield). The isomeric dihydroxy acid

13, used for the preparation of analogs **30a** and **30b**, was synthesized in 4 steps (Scheme II) from 2,3,3-trimethylbutene (**18**) by SeO_2 catalyzed oxidation,[10] epoxide formation with MCPBA, $RuCl_3$ catalyzed oxidation,[11] and subsequent acid catalyzed hydrolysis.

Scheme II[a]

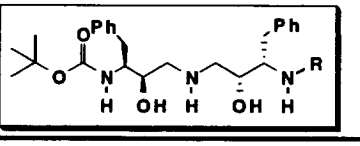


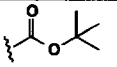
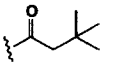
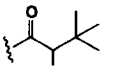
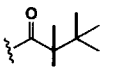
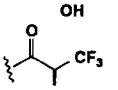
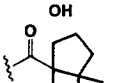
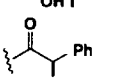
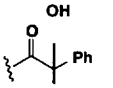
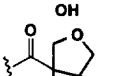
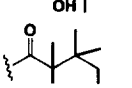
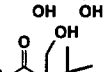
[a] **a) NaH/$PhCH_2Br$/cat. Bu_4NI/THF, 60%; b) DIBAL/ toluene-CH_2Cl_2-hexane/-78 °C, 94%; c) Et_3SiH/ BF_3•Et_2O/CH_2Cl_2, -78 to 0 °C, 88%; d) $Pd(OH)_2$-C/ H_2/EtOH, 91%; e) PCC/4Å molecular sieves, 59%; f) vinylmagnesium bromide/ $CeCl_3$/THF/-78 °C, 90%; g) O_3/Me_2S/MeOH, 97%; h) $NaClO_2$/sulfamic acid, 68%; i) CH_3MgCl/THF/-78 °C; j) LiOH/THF-H_2O/ reflux; k) dil. HCl; l) silica gel chromatography, 40% yield from 17a; m) SeO_2/ t-BuOOH/salicylic acid, 33%; n) MCPBA/CH_2Cl_2, 77%; o) $RuCl_3$/$NaIO_4$/ CH_3CN-H_2O, 58%; p) dil. H_2SO_4, 53%**

Biological Results and Discussion: We had previously reported the synthesis of the amide **20** (Table I) in which a carbamate oxygen of **1** is replaced by a methylene group.[2] This modification results in a 32-fold loss of HIV protease inhibitory activity, suggesting the critical role of this linkage. Introduction of an α-hydroxyl group afforded the corresponding diastereomeric α-hydroxy amides **21a** and **21b**. The (R)-hydroxyamide **21a** exhibits a 24-fold increase in intrinsic activity over the (S)-isomer **21b**, and an 80-fold increase over the parent unsubstituted amide **20**. The potent antiviral activity of the α-methyl analog **22a** provides access to a potentially more metabolically stable amide.

The crucial nature of the P_2 *t*-butyl group is demonstrated by the 11-fold decrease in the intrinsic activity of the geminal dimethyl analog **23**. Furthermore, the retention of activity by the trifluoromethyl analog **24a** suggests a similar hydrophobic effect for this substitutent. Constraining the *t*-butyl and methyl groups of **22a** to form a 5 membered ring provides compound **25a**, which shows a 2-3 fold increase in intrinsic activity presumably due to diminished entropy. Phenyl and tetrahydrofuranyl groups have been known to act as suitable P_2 groups for HIV protease inhibitors;[12] however, incorporation of these groups in the aminodiols results in 5-8 fold loss in intrinsic activity (compounds **26a**, **27a** and **28a**). These results, along with previously published data[2] suggest that a properly positioned *t*-butyl or an analogously branched group may be the most suitable hydrophobic P_2 substituent for this class of inhibitors. The enhanced intrinsic activity exhibited by the (R)-isomer **21a** compared to the (S)-isomer **21b** is also observed with several additional pairs of aminodiols (e.g., **22a,b**; **26a,b** and **27a,b**). A binding mode is proposed (Figure 3) to explain these results using molecular modeling based on the enzyme bound X-ray crystal structure of **1**.[13]

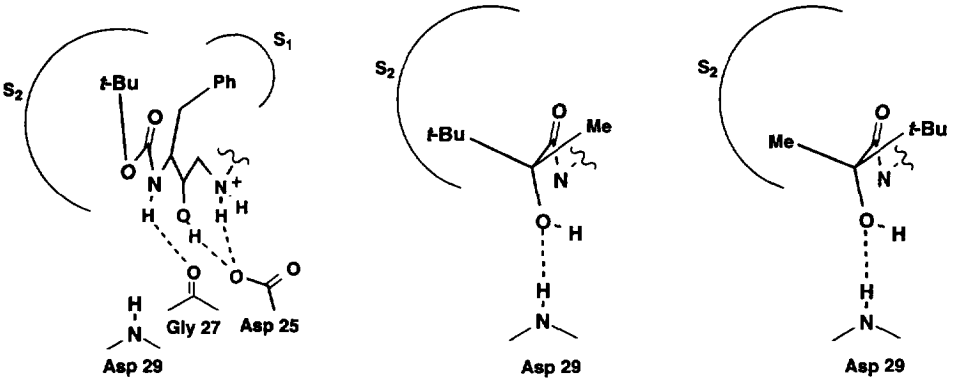
Table 1. Structure-Activity-Relationships of α-Hydroxyamide Derived Aminodiols.



Compd. No.	R	IC_{50} (nM)[a]	ED_{50} (nM)[b]
1		125[c]	80
20		4,000	1,200
21a (R isomer)		50	55
21b (S isomer)		1,200	nd
22a (R isomer)		40[d]	60
22b (S isomer)		900	1,380
23		450	970
24a (isomer A)		40	60
24b (isomer B)		2,200	nd
25a (isomer A)		15	30
25b (isomer B)		250	375
26a (R isomer)		320	650
26b (S isomer)		>10,000	nd
27a (R isomer)		260	1,350
27b (S isomer)		6,500	nd
28a (isomer A)		220	230
28b (isomer B)		3,500	nd
29a (isomer A)		130	160
29b (isomer B)		120	110
30a (isomer A)		60	25
30b (isomer B)		10,000	nd

[a]Concentration of compound needed to inhibit cleavage of V-S-Q-N-(b-naphthylalanine)-P-I-V by 50%.[2] [b]Concentration needed to inhibit virus replication by 50% as determined by an XTT endpoint;[2] average of at least 2 determinations. [c]K_i = 100 nM. [d]K_i = 24 nM.

The crystal structure of carbamate **1** reveals that the P_2 *t*-butyl group is in the plane of the carbonyl group (Figure 3). Molecular dynamics simulations[14] performed on **22a** and **22b** suggest that the most stable conformation in these amides places the *t*-butyl group roughly perpendicular to the plane of the carbonyl group. Consequently the (R)-isomer **22a** can interact simultaneously with Asp 29 as well as the S_2 hydrophobic site. However, the *t*-butyl group shifts completely out of the S_2 site in order for the OH group to form a hydrogen bond to Asp 29 in case of the (S)-isomer (Figure 3). The loss of the hydrophobic interactions with the amino acid side chains at the S_2 site is energetically unfavorable, thus resulting in diminished activity for the (S)-isomer.



Carbamate **1** (R)-Isomer **22a** (S)-Isomer **22b**

Figure 3: Three dimensional representation of the lowest energy conformations of α-hydroxyamides 22a and 22b at the hydrophobic S_2 site of the HIV-1 protease as determined by molecular modeling based on the X-ray crystal structure of enzyme bound carbamate 1.

This model reveals several additional potential interactions at the S_2 site (e. g. with Asp 30 and Gly 48).[15] Several attempts were made to utilize these interactions by incorporating additional hydroxyl groups at the P_2 site of **22a**. For example, the tetrahydroxy analogs **29a,b** and **30a,b** were prepared and evaluated for their intrinsic and antiviral activities but showed no significant enhancement in potency.

Most of the analogs in Table 1 with significant intrinsic activity ($IC_{50} < 1.0$ μM) were tested for antiviral activity against HIV-1 in cell culture and exhibit comparable enzyme and antiviral activities as had been noted previously for other members of the aminodiol class.[2a] Furthermore these compounds did not inhibit cell growth at the concentrations used to determine antiviral activity ($CC_{50} > 10.0$ μM).

In conclusion, we have prepared a novel series of HIV-protease inhibitors with favorable ED_{50}/IC_{50} ratios. It has been shown that aminodiols possessing α-hydroxyamide P_2 groups with appropriate absolute stereochemistry are potent HIV protease inhibitors. It remains to be shown if this moiety is appropriate for other classes of protease inhibitors as well. Molecular modeling strongly suggests involvement of the Asp 29 residue of the enzyme.

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14) The molecular dynamics simulation used the GB/SA solvent model for water (Still W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem . Soc.* **1990**, *112*, 6127.) with the Kollman united atom AMBER force field (Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem . Soc.* **1984**, *106*, 765.) as implemented in the BatchMin V4.0 program. The coordinates of the crystal structure of the parent compound were used as the initial coordinates of the simulation. The entire enzyme complex was included in the simulation. However, to maintain the integrity of the structure, 186 Ca atoms (not in the active site) were tethered to their original coordinates. Also, distance constraints were applied to 12 hydrogen bonds between the inhibitor, three water molecules and the enzyme. The distance constraints included a 0.4 Angstrom flat bottom well. The annealing involved seven picoseconds of molecular dynamics at 300 degrees followed by 3 picoseconds ramping the temperature down to 100 degrees. The final energy of each orientation was taken after 300 steps of energy minimization with the distance constraints removed.
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