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SYNTHESIS AND BIOLOGICAL EVALUATION OF A SERIES OF HIV-1 PROTEASE INHIBITORS

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Abstract. A series of HIV-1 protease inhibitors was prepared and evaluated against the free enzyme for inhibition properties, and for their anti-viral properties in human T lymphoid cells infected with HIV_{IIIB}. Compounds **12**, and **19** are the most potent anti-viral agents prepared in this study and are compared to Ro 31-8959, a compound currently in clinical trials for the treatment of AIDS.

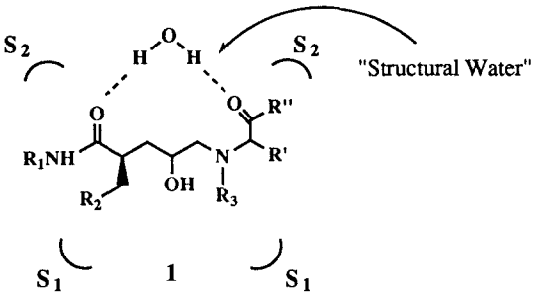
Acquired Immune Deficiency Syndrome (AIDS) is an ever increasing disease of global proportions. It has been estimated[1] that there are currently 10 million patients worldwide infected with HIV, the causative virus of AIDS, and that by the year 2000, there could be 20 million patients that are HIV-positive. Because of the highly infectious nature of this disease, and the high mortality associated with AIDS, there is an urgent need to develop chemotherapeutic agents to combat this virus.

HIV-protease is the enzyme responsible for maturation of non-infectious HIV virions to mature infectious virions. This occurs following proteolysis of the viral polyproteins (*gag* and *gag-pol*) into the essential viral enzymes and structural proteins.[2] Mutagenesis studies have shown that mutants which lack protease function are non-infectious, lending credence to the hypothesis that inhibition of the HIV-protease will stop proliferation of virus.[3]

With information gleaned from other aspartyl protease inhibition programs (eg. renin), standard medicinal chemistry principles have been applied to the design and synthesis of HIV-1 protease inhibitors;[4] at least one of these is currently in clinical evaluation for the treatment of AIDS.[5] Several X-ray crystallographic analyses of HIV-1 protease and HIV-1 protease/inhibitor complexes are published,[6] and a number of groups have embarked on the *de novo* design and synthesis of potent inhibitors of this critical enzyme.[4,7,8] We report, herein, a new series of potent HIV-1 protease inhibitors and their anti-viral properties.

We chose to utilize the substructure depicted in **1**, as a transition state mimic for the naturally occurring hydrolytic event. The rationale for this approach was that the secondary hydroxyl group of **1** would displace the catalytic water found in the active site of the protease, and in so doing the process would be entropically favorable.[9] Additionally, proper stereochemical alignment of the secondary hydroxyl group should be mandatory for maximal binding.[10]

The choice of the R_1, R_2, R_3, and R_4 groups would be dictated by molecular modelling experiments and standard medicinal chemistry principles.[8] In particular, we concentrated on polyamide derived inhibitors which contain lipophilic side-chains that were designed to fit into the S_1 and S_2 pockets of the HIV-1 protease. Lastly, it is important to maintain a 1,8-dicarbonyl functionality to permit hydrogen bonding to the so-called "structural water" present in the X-ray crystal structures of all protein/inhibitor complexes available to date.[6] Within these structural confines, we designed and synthesized a series of potential HIV-1 protease inhibitors. The compounds presented, herein, structurally contain two carboxyl termini; there is no amine terminus. We then evaluated these agents for their ability to inhibit HIV-1 protease activity *in vitro*, and their ability to act as anti-viral agents in cultured human cells infected with HIV_{IIIB} virus (**Table 1**).



Chemistry.

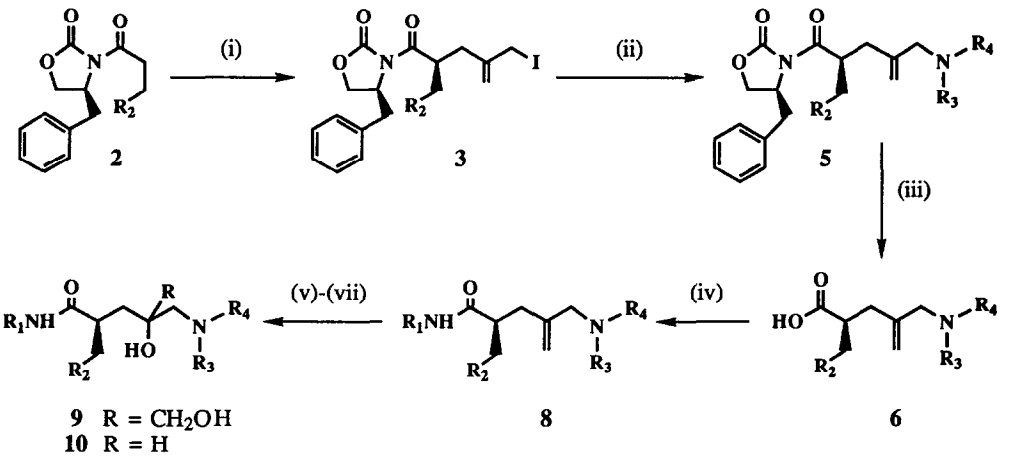
A general synthetic pathway is presented (**Scheme 1**) which was used to prepare each of the inhibitors described herein. Appropriately substituted allyl iodide **3** was prepared by reaction of the sodium enolate[11] of the keto-oxazolidinone derivative **2** with 3-iodo-2-iodomethyl-1-propene.[12] Allyl iodide **3** was then allowed to react with amines **4** in the presence of *N,N*-diisopropylethylamine (Hunig's base) to give allyl amines **5**. The chiral auxiliary within **5** was removed by facile hydrolysis with lithium hydroxide and hydrogen peroxide[13] to give acid **6**, which was directly coupled to amines **7** with benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP Reagent)[14] in the presence of Hunig's base. Allyl amine **8** was most efficiently transformed into alcohol **10** by a three step procedure involving first dihydroxylation of the 1,1-disubstituted olefin with stoichiometric osmium tetraoxide (OsO_4). The derived diol **9**, obtained as a mixture of two diastereomers, was oxidatively cleaved to the ketone with sodium periodate ($NaIO_4$) and directly reduced to the secondary alcohol **10** by reaction with sodium borohydride ($NaBH_4$). The isolated alcohol **10** was obtained as a mixture of diastereomers (the ratio of which was dependent on the particular case), and was usually tested as the mixture.

Biology.

The enzyme inhibition assay used was a scintillation proximity assay (^{125}I-SPA).[15] Utilizing limiting amounts of the enzyme, we incubated test compounds with the bead suspension for 5 min. Following termination of the experiment, we measured the radioactivity of the assay mixture. Enzyme inhibition was measured as a function of substrate concentration; 50% inhibition (IC_{50}) values are reported in **Table 1**.

Our anti-viral assay consists of treating human T lymphoid (MT-2) cells that are infected with HIV_{IIIB} virus with test compounds. After an incubation period of 5 days at 37 °C, the number of viable cells was ascertained by standard MTT dye assay.[15] A compound concentration that provides 50% inhibition (IC_{50}) of cell death (with respect to untreated control) is reported in **Table 1.**

Scheme 1.[a]



[a] (i) NaHMDS, THF; 3-iodo-2-iodomethyl-1-propene, (ii) R_3R_4NH (**4**), $(i\text{-Pr})_2EtN$, Toluene, Δ (iii) LiOH, H_2O_2, H_2O, THF, 0 °C, (iv) R_1NH_2 (**7**), BOP Reagent, $(i\text{-Pr})_2EtN$, CH_2Cl_2 (v) OsO_4, Pyr, Toluene, (vi) $NaIO_4$, THF, H_2O, (vii) $NaBH_4$, Ethanol

Results and Discussion.

A number of structural parameters were examined with respect to HIV-1 protease inhibition and anti-HIV_{IIIB} properties. We observed that when R = CH_2OH enzyme inhibition was slightly diminished compared to R = H (**11** vs. **12**). There was, however, a 10-fold decrease in the anti-viral IC_{50} for **11** perhaps due to decreased cellular bioavailability.

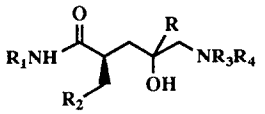
Variation of the R_1 group had an interesting effect on the protease inhibition. For example, comparison of the series **12-15** shows a 25-fold range in enzyme inhibition. Compound **13**, which lacks a lipophilic P_3 binding group, still maintains moderate enzyme inhibition. The most significant difference within this series was observed in the anti-viral IC_{50} values which span a range of > 500-fold. Further variation of R_1, as in **16** and **18** resulted in protease inhibitors that are particularly potent, IC_{50} = 0.07 μM; disappointingly, these compounds were found to be inactive as anti-viral agents, IC_{50} > 1750 nM.

Modification of R_2 was examined with respect to only phenyl and isopropyl with no observed difference in biological activity, compare **12** and **19**. Apparently, some structural variability here is permitted without deleterious effects.

We modified R_3 and R_4 extensively; consider the series of compounds **12**, **20-22**, and **24**. Within this series (excluding **21**), protease inhibition spans 5-fold; however, as anti-viral agents, the same group

differs by > 500-fold with compound **12** exhibiting the most potent IC_{50}'s. Compound **21** is inactive both as a protease inhibitor and anti-viral agent. Within this series, we observed that the bicyclic amine (DIC) gave inhibitors that provided the most potent anti-virals. Modification of R_3 and R_4 by removal of one of the rings (Pip, **22**), replacement by proline (Pro, **20**), or replacement with the less constrained analogue **24** all resulted in less active anti-virals. Lastly, a comparison of **16** (which is equipotent to **12** as a protease inhibitor) to **17**, which differ in R_3 and R_4 by having a less constrained group, reveals a deterioration of enzyme inhibition by > 20-fold, and inactivity as anti-viral agents. Many of the compounds described herein are equipotent to Ro 31-8959 (**25**)[5] as enzyme inhibitors, but disappointingly none are as potent as anti-viral agents.

Table 1. HIV-1 Protease Inhibition and Anti-HIV_{IIIB} Activities for Compounds **11**-**25**.[a,b]

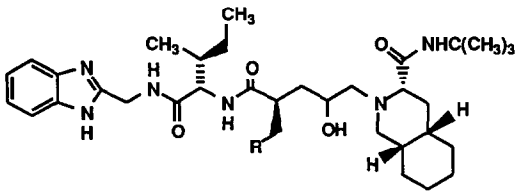


Compound Number	R_1NH	R_2	NR_3R_4	R	Enzyme Inh.[c] IC_{50} (μM)	Anti-viral[c] IC_{50} (nM)
11	AMB-L-Ile	Ph	DIC[16]	CH_2OH	0.07	280
12a	AMB-L-Ile	Ph	DIC	H[d]	0.03	21
12b	AMB-L-Ile	Ph	DIC	H[e]	0.03	29
13	CH_3O-L-Ile	Ph	DIC	H	0.87	> 1750
14	AMP-L-Ile	Ph	DIC	H	0.15	310
15	AEB-L-Ile[17]	Ph	DIC	H	0.13	850
16	QC-DAC	Ph	DIC	H	0.07	3600
17	QC-DAC	Ph	CME[18]	H	> 1.4	> 1750
18	IQC-DAC	Ph	DIC	H	0.07	1750
19	AMB-L-Ile	*i*-Pr	DIC	H	0.03	31
20	AMB-L-Ile	Ph	L-Pro-NH-*t*-Bu	H	0.08	490
21	AMB-L-Ile	Ph	L-Pro-L-Ile-O-*t*-Bu	H	> 1.4	> 1750
22	AMB-L-Ile	Ph	L-Pip-NH-*t*-Bu	H	0.03	480
23	AMP-L-Ile	Ph	L-Pip-NH-*t*-Bu	H	0.25	> 1750
24	AMB-L-Ile	Ph	CMA[19]	H	0.14	> 1750
25	Ro 31-8959[5]				0.03	3

[a] Abbreviations:[20] AMB = [(1H-benzimidazol-2-ylmethyl)amino]; DIC = [3S-(3α,3aβ,8aβ)]-[3-[[(1,1-dimethylethyl)amino]-carbonyl]octahydro-2(1H)-quinolyl]; AMP = [(2-pyridinylmethyl)amino]; AEB[17] = [(R)-(1H-benzimidazol-2-ylethyl)amino]; QC = 2-quinolinecarbonyl; DAC = [(1S)-*trans*-1,2-cyclohexanediamino]; CME[18] = [(S)-[1-(cyclohexylmethyl)-2-[(1,1-dimethylethyl)amino]-2-oxoethyl]methylamino]; IQC = 3-isoquinolinecarbonyl; L-Pip = L-pipecolinic acid; CMA[19] = [(cyclohexylmethyl)-[2-[(1,1-dimethylethyl)-amino]-2-oxo-ethyl]amino]. [b] All new compounds presented gave satisfactory ^1H NMR and FAB mass spectral data in accord with the assigned structure. [c] For an explanation of the biological assays see ref 15. [d](R)-configuration at carbinol carbon. [e](S)-configuration at carbinol carbon.

Conclusions.

We have shown the synthesis and biological evaluation of a series of HIV-1 protease inhibitors. Within the series presented, compounds **12a**, **12b**, and **19** are the most potent anti-viral agents and contain an (aminomethyl)benzimidazole group at the P_3 site, an L-isoleucine and *t*-butyl groups at the P_2 sites, a benzyl (or *iso*-butyl) group and a *cis*-decahydroisoquinoline group at the P_1 sites. In all cases, we maintained a polyamide backbone, a 1,8-dicarbonyl functionality and a secondary hydroxyl group to interact with the enzyme in hydrogen bonding. Perhaps most interestingly, we found that the two epimeric alcohol isomers **12a**, and **12b** (which were separated by silica gel chromatography), showed equal enzyme inhibition and anti-viral properties. A rationale for this may be that the protease/inhibitor complex is sufficiently flexible to accommodate each isomer equally well.[21]



12a,b R = Ph
19 R = *i*-Pr

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10. For example, compare the HIV-protease inhibition of several of the inhibitors presented in references 5a and 5b, which differ only in the stereochemistry of the secondary hydroxyl center. Invariably, one isomer is significantly more potent than the other. See also: Rich, D. H.; Sun, C.-Q.; Vara Prasad, J. V. N.; Pathiasseril, A.; Toth, M. V.; Marshall, G. R.; Clare, M.; Mueller, R. A.; Houseman, K. *J. Med. Chem.* **1991**, *34*, 1225-1228.
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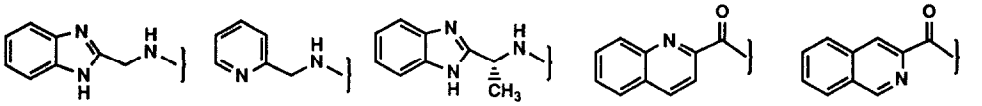
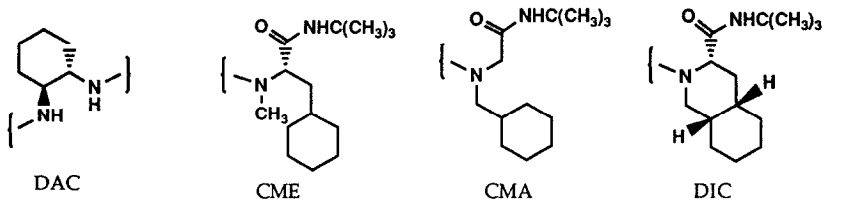
17. (D)-*N*-CBZ-alanine and 1,2-phenylenediamine were coupled with carbonyl diimidazole in THF. The product was cyclized by refluxing in *o*-xylene.The CBZ-protecting group was removed by catalytic hydrogenation to give (R)-α-methyl-1H-benzimidazole-2-methanamine (AEB group).
18. The CME group was introduced by reaction of iodide **3** (R_2 = Ph) with (S)-2-amino-*N*-(1,1-dimethylethyl)cyclohexanepropanamide, followed by reductive amination of the product with paraformaldehyde and sodium borohydride.
19. Reductive amination of cyclohexanecarboxaldehyde and 2-amino-*N*-(1,1-dimethylethyl)acetamide with sodium borohydride provided 2-[(cyclohexylmethyl)amino]-*N*-(1,1-dimethylethyl)acetamide (CMA group) which was allowed to react with iodide **3** (R_2 = Ph).
20. Structural description of the abbreviations used:





21. Independent asymmetric synthesis, molecular modeling, and X-ray analysis of **12a**, and **12b** will be reported in due course. Trova, M. P.; Babine, R. E.; Xu, Z.-B. Unpublished results.