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PEPTIDOMIMETIC INHIBITORS OF HUMAN IMMUNODEFICIENCY VIRUS PROTEASE (HIV-PR): DESIGN, ENZYME BINDING AND SELECTIVITY, ANTIVIRAL EFFICACY, AND CELL PERMEABILITY PROPERTIES

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Abstract: The structure-activity relationships and pharmacophore modeling aspects of a series of HIV PR inhibitors modified at the N- and/or C-terminus of the dipeptide isostere ChaΨ[CH(OH)CH_2]Val (Cha, cyclohexylalanine) are reported. The HIV PR binding affinity-selectivity (vs. human renin, pepsin, and cathepsins-D and E), antiviral efficacy (HIV-1/vVK-1 infected CV-1 cells) and cellular permeabilities (Caco-2) are noted.

INTRODUCTION

The aspartic proteinase (PR) encoded within the *pol* gene of human immunodeficiency virus (HIV) provides a target for therapeutic intervention in the treatment of acquired immunodeficiency syndrome (AIDS). This enzyme is indispensable for processing of the viral *gag* and *gag/pol* polyproteins which takes place during the final maturation step of the viral life cycle. Blocking of PR activity by peptidyl or peptidomimetic inhibitors[1-7] or by mutagenesis[8] results in the production of immature, non-infectious viral particles. Therefore, it is believed that HIV PR inhibitors may be of significance to AIDS drug discovery. The HIV PR cleaves several peptide bonds within *gag/pol* polypeptide precursors to liberate both structural proteins and enzymes (including reverse transcriptase and PR)[9]. The primary structures of these viral substrates vary substantially at their P_3-P_3' sites, and additional studies on nonviral HIV PR substrates have provided new insights towards understanding the discrete specificities for peptide (or protein) recognition by the enzyme[10]. Relative to our previously reported discovery[1] of the prototypic, peptidomimetic HIV PR inhibitor Tba-ChaΨ[CH(OH)CH_2]Val-Ile-Amp[2] (U-81,749E, Fig. 1; Tba = tert-butylacetyl and Amp = 2-aminomethypyridine) we have advanced the design and structure-activity analysis of a series its congeners having N- and/or C-terminal synthetic tailoring. In retrospect,

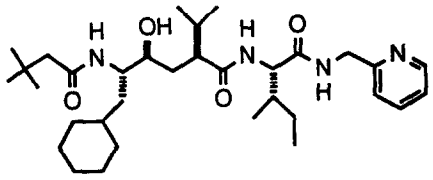


Figure 1. *Chemical structure of U-81,749E.*

the parent compound was the first disclosed HIV PR inhibitor having no traditional 'peptide' substructure as it contained only one amino acid. In addition, it is noted that U-81,749E was identified by systematic screening of a renin inhibitor database of which it was determined[10] to be a relatively low affinity inhibitor (IC_{50} *ca.* 10 μM).

The discovery of peptidomimetic (peptide-like and nonpeptide) inhibitors of HIV PR has been a fast-moving and exciting area of interdisciplinary research in which the design, structural analysis, and biological testing of these compounds has become quite sophisticated. Some representative lead compounds that have been previously disclosed by researchers at Upjohn (e.g., U-75,875)[5], Hoffman-LaRoche (e.g., Ro-31-8959)[4], and Merck (e.g., L-687,908)[6] are shown in Fig. 2, and these HIV PR inhibitors exemplify high affinity, peptidomimetic ligands which also are quite potent as antiviral agents against a number of HIV-infected cell lines and related cell assays. Improving the bioavailability properties of such compounds remains a challenge, considering the need for drug delivery by the oral route, and other issues such as hepatobiliary clearance, transport into the brain, solubility, etc., which pose difficult barriers for such compounds to overcome. Nevertheless, the systematic evaluation of such peptide-like compounds may address these issues, and development of structure-activity/bioavailability databases may provide insight into the rational design of new HIV PR inhibitors. In this report we describe the *in vitro* cellular permeability properties of selected U-81749E analogs using a human colon adenocarcinoma (Caco-2) cell monolayer model system which has been advanced by a number of research groups for exploring the potential oral bioavailability characteristics of peptide-like compounds[11].

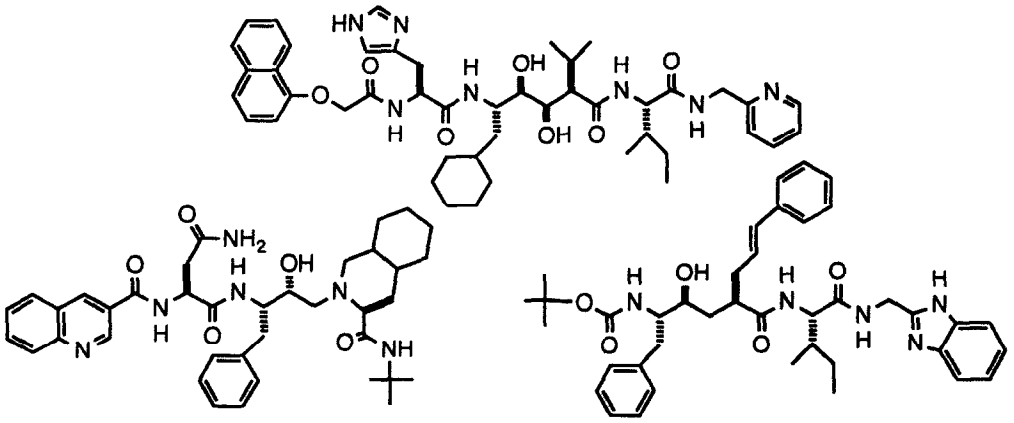


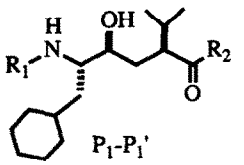
Figure 2. *Chemical structures of U-75875E (top), Ro-31-8959 and L-687,908 (lower left and right, respectively).*

RESULTS AND DISCUSSION

The biological testing of U-81,749E analogs of the generic template R_1-ChaΨ[CH(OH)CH_2]Val-R_2 included: (1) HIV-1 and HIV-2 PR inhibition (K_i); (2) aspartyl protease inhibition selectivity relative to human renin, pepsin, cathepsin-D and cathepsin-E; (3) antiviral efficacy as measured by a hybrid HIV-1/vaccinia virus infected monkey cell line (HIV-1/vVK-1 infected CV-1) which produces HIV-1 gag-pol derived polyproteins and

subsequent release of noninfectious, HIV-like particles from the host CV-1 cell; and (4) intestinal transport efficacy as determined using Caco-2 cell culture monolayers. Table I summarizes the structure-activity relationships of fifteen compounds of which subsets were selected for secondary biological evaluation (Tables II and III). The chemistry logic underlying these studies included amino acid substitutions at the P_2 and/or P_2' sites, non-amino acid N- and/or C-terminal functionalization, and HIV PR active site computer-assisted molecular modeling (CAMM) to probe inhibitor binding interactions. We have previously described both the biological assay methodologies[1,5,10,12-14] as well as the synthetic chemistry procedures[15,16] used to prepare the above compounds and/or intermediates. NMR, FAB-MS and HPLC criteria were met for each analog reported here.

Table I. *HIV-1 PR Inhibition and vVK-1/CV-1 antiviral structure-activity studies.*



ChaΨ[CH(OH)CH_2]Val

Entry	R_1	R_2	HIV-1 PR K_i, nM	vVK-1/CV-1* Inh.@ 10 μM (IC_{50})
1	Ac	Ile-Amp	8	50% (10 μM)
2	Ac	D-Ile-Amp	>1000	22%
3	Ac	Ile-Amp(O)	16	20%
4	H	Ile-Amp	800	0%
5 (U-81,749E)	Tba	Ile-Amp	75	75% (3 μM)
6	Hxa	Ile-Amp	47	67% (3 μM)
7	Poa	Ile-Amp	61	90% (3 μM)
8	Pyc	Ile-Amp	8	100% (0.3 μM)
9	Boc	Ile-Amp	8	100% (0.3 μM)
10	Boc-His	Ile-Amp	10	53% (10 μM)
11	Noa-His	Ile-Amp	5	100% (0.3 μM)
12	Noa-NMeHis	Ile-Amp	30	85% (0.5 μM)
13	Noa-Val	Ile-Amp	61	93% (0.3 μM)
14	Noa-Asn	Ile-Amp	4	93% (0.3 μM)
15	Qnc-Asn	Ile-Amp	9	97% (0.3 μM)
16	Qnc-Asn	Ile-OH	>1000	0%
17	Qnc-Asn	Npt	14	70% (0.3 μM)
U-75875	Noa-His-ChaΨ[CH(OH)CH(OH)]Val-Ile-Amp		<1	100% (0.2 μ M)

Abbreviations: Cha, cyclohexylalanine; Amp, 2-aminomethyl-pyridine; Amp(O), Amp-N-oxide; Tba, tbutylacetyl; Hxa, hexanoyl; Poa, phenoxyacetyl; Pyc, pyridyl-2-carboxyl; Boc, tbutyloxycarbonyl; Noa, naphthyloxyacetyl; NMeHis, N^{α}-methyl-His; Qnc, quinoline-2-carbonyl; Npt, neopentylamine.
*Inhibition of cellular p24 expression (versus control; 24 h) as previously reported (1).

Highlights of the biological evaluation of compounds **1-17** are as follows: (1) relative to U-81,749E, an Ac moiety was found to be as effective as a Boc group at R_1 in terms of enhancing HIV-1 PR binding affinity; however, the lipophilicity of the N-terminal (R_1) functionality was important for antiviral potency; (2) the stereochemical and stuctural integrity of the C-terminal (R_2) Ile-Amp moiety of compound **1** was required for retention of both HIV-1 PR binding and antiviral potency; (3) N-terminal elaboration to insert P_2 amino acids such as His, NMe-His, Val, and Asn within the analog subset **11-14** exhibited discrete variations in HIV-1 PR

binding affinity, and the two optimal residues in this series were His and Asn; (4) relative to compound **15**, C-terminal modification markedly affected the HIV-1 PR binding affinity and antiviral potency; it was determined that a simple alkylamine (i.e., Npt) moiety at R_2 could substitute for Ile-Amp; (5) aspartyl protease inhibition, K_i, ranged 10- to 100-fold for compounds **6**, **11** and **15**; U-75,875 exhibited reasonable HIV PR selectivity to human pepsin and cathepsins-D and E, but not with respect to human renin; and (6) the comparative Caco-2 cell permeability[17], cLogP[18], and HIV-1 PR binding affinity properties of compounds **1**, **5**, **8** and **9** were found to generally correlate to the observed antiviral potencies, albeit these results represent a limited database and further studies are required to substantiate this relationship. These data extend and confirm previous reports[4-6] of HIV PR inhibitors of similar peptidomimetic structures in terms of the structure-activity properties of N-terminal (R_1) and C-terminal (R_2) functionalization relative to such P_1-P_1' dipeptide isosteres.

Table II. *Aspartyl protease inhibition-selectivity studies.*

Entry	HIV-1 PR K_i, nM	HIV-2 PR K_i, nM	Renin K_i, nM	Pepsin K_i, nM	Cathepsin-D K_i, nM	Cathepsin-E K_i, nM
9	8	---	1200	330	100	90
11	5	170	2	290	200	50
15	9	65	212	12	2	3
U-75,875	<1	30	2*	265	>1000	142

*IC_{50}.

Table III. *Comparative Caco-2 cell permeability, cLogP, HIV-1 PR inhibition and antiviral properties.*

Entry	Caco-2 permeability Pe, cm s^{-1} x 10^6	cLogP	HIV-1 PR K_i, nM	vVK-1/CV-1 IC_{50}, μM
1	1.2 (±0.5)*	2.76	8	10
5	11.5 (±1.9)	4.62	75	3
8	17.0 (±1.0)	3.66	30	3
9	18.8 (±1.4)	5.45	8	0.3

*±S.D. (≥ triplicate determination)

Relative to U-81,749E these studies further exploit the P_1-P_1' ChaΨ[CH(OH)CH_2]Val dipeptide isostere by chemical elaboration at the N- and/or C-terminii. A series of U-81,749E analogs were examined by CAMM to provide insight to the rational design of second-generation compounds having improved biological and/or physical properties, including aqueous solubility. Specifically, a 3-D model of U-81,749E (Fig. 3) complexed to HIV-1 PR active site was advanced[19] using a recently described[20] CAMM technique, MOSAIC/GROW, which is applicable to the *de novo* construction of peptidyl (or peptidomimetic) compounds interacting a macromolecular "host" (*e.g.*, enzyme). U-81,749E adopted an extended conformation with H-bonding interactions between each of its backbone amide groups and H-bonding donor/acceptor groups at the enzyme active site in a manner consistent with known[7] X-ray structures of inhibitor bound HIV-1 PR complexes, including a recent report by Thompson and co-workers[21] for a chemically-related, P_1-P_1' Ψ[CH(OH)CH_2] substituted inhibitor. A more

detailed structure-activity and CAMM analysis of these and other compounds will be disclosed elsewhere.

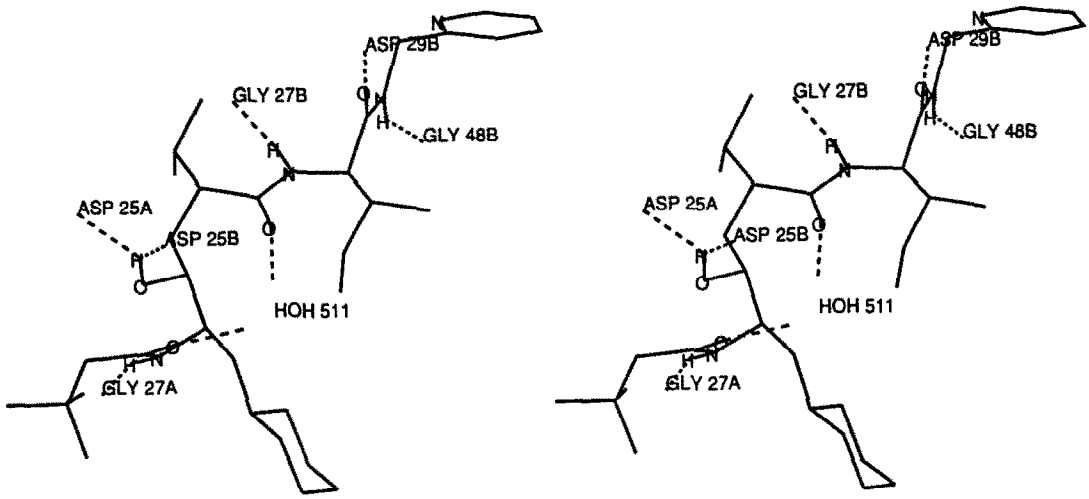


Figure 3. *Stereo view of CAMM model of U-81,749E bound to HIV-1 PR. Intermolecular H-bonds are shown (dashed lines), and participating enzyme active site residues and an active site H_2O molecule are identified.*

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