Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 23, pp. 2897-2902, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00508-0

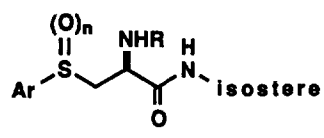
POTENT, ORALLY BIOAVAILABLE HIV-1 PROTEASE INHIBITORS CONTAINING NONCODED D-AMINO ACIDS

John E. Munroe*, Timothy A. Shepherd*, Louis N. Jungheim, William J. Hornback, Steve D. Hatch, Mark A. Muesing, MaryAnn Wiskerchen, Kenneth S. Su, Kristina M. Campanale, Angela J. Baxter and Joseph M. Colacino

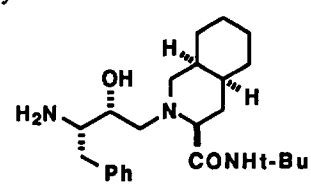
Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285.

Abstract. Novel noncoded D-amino acids have been combined with decahydroisoquinoline, octahydrothienopyridine, and urea hydroxyethylamine isosteres to provide potent HIV-1 protease inhibitors with excellent HIV-1 antiviral activity. LY314613 shows a promising combination of potency and oral bioavailability. Trends in the SAR and comparisons to other isostere derivatives will be discussed.

HIV-1 protease, an essential enzyme in the HIV life cycle, remains as an important target for AIDS chemotherapy.[1] Although numerous inhibitors have been reported, few can claim the combination of potent antiviral activity and oral bioavailability.[2] Compounds Ro 31-8959[3] and LY289612[4] are peptidomimetic inhibitors with excellent *in vitro* potency but poor oral bioavailability in animal models. We recently reported[5] on our attempts to improve the bioavailability of LY289612 by incorporating noncoded D-amino acids as P_2/P_3 ligands. Although we found compounds with improved antiviral potency, we saw only small improvements in oral bioavailability. Herein we report[6] on the combination of our D-amino acid ligand concept, represented by **1**, with the Roche[3] decahydroisoquinoline isostere **2**, high affinity *cis*-octahydrothienopyridine hydroxyethylamine isosteres[7] **3** and **4**, and the Monsanto[8] urea hydroxyethylamine isostere **5**.



1



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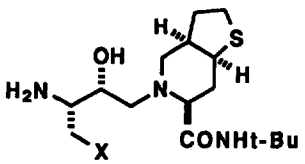
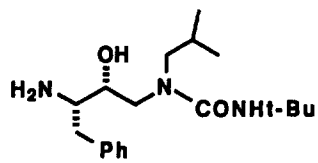


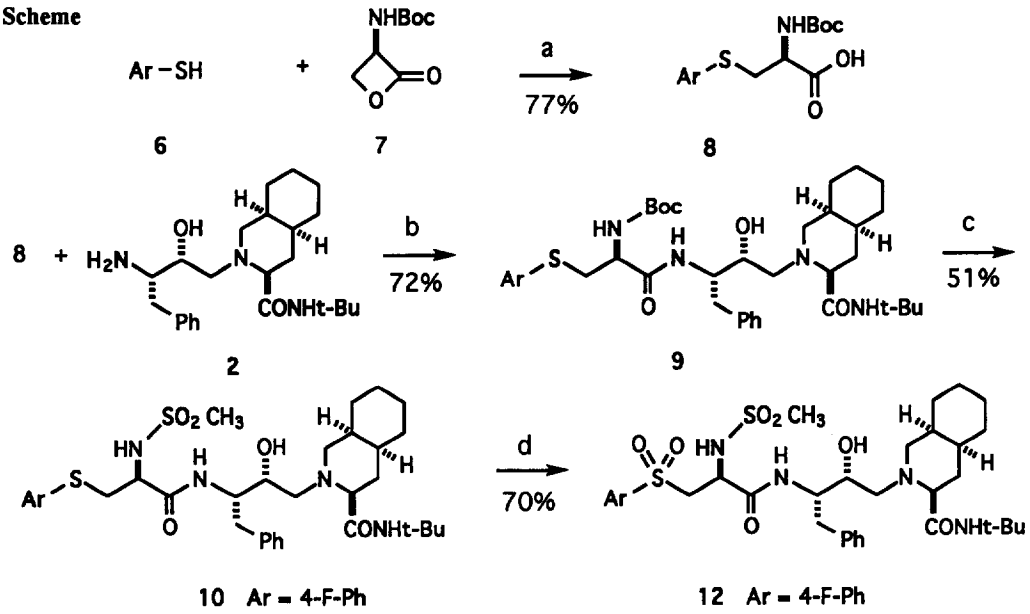
3: X = Ph; 4: X = SPh



5

The preparation of the D-amino acid substituted HIV protease inhibitors derived from these isosteres is described below and illustrated for one example utilizing isostere **2** (Scheme). The optically pure D-amino acids (**8**) were prepared by opening the N-tBoc-D-serine ß-lactone **7**[9] with the appropriate thiolate derived from **6**.

Scheme



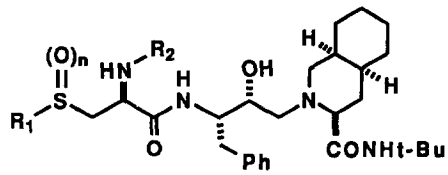
REAGENTS: a. NaH, THF; b. DCC, HOBt, THF; c. TFA, CH_2Cl_2 then MsCl, N-Methyl morpholine; d. Oxone®. Yields shown are for the synthesis of compound **12**.

The thiols (4-fluorothiophenol (4-F-Ph), 2-mercaptonaphthalene (2-Naph), and 8-mercaptoquinoline (8-Quin)) were chosen on the basis of earlier SAR studies[5] that showed the 4-fluorophenyl and naphthyl substituents to be particularly interesting. Coupling of these acids to isosteres **2** and **5** was accomplished using standard conditions (DCC/HOBt). The Boc group was removed by treatment with TFA and the amine sulfonylated with mesyl chloride or acylated with acetyl chloride. Oxidation with Oxone® selectively afforded the sulfones **12, 15, 16,** and **32**. The methane sulfonic acid salt of compound **12** is LY314163. Sulfone **17** was prepared by acylation of **2** by the D-amino acid side-chain already at the sulfone oxidation state (see below), with subsequent deprotection and acylation. Sulfoxides **11** and **14** were prepared by carefully monitoring Oxone® reactions at 0 °C. Sulfoxide **34** was obtained by using one equivalent of MCPBA at 0 °C. The relative configuration at the sulfur atom of these sulfoxides was not determined, but the compounds tested were obtained as single diasteriomers.

The HIV-1 protease inhibitors prepared from isosteres **3** and **4** focused on analogs having the D-amino acid side-chain at the sulfone oxidation state. Therefore, sulfides **8** were oxidized by treatment with Oxone® in methanol providing the corresponding aryl sulfones. This transformation was conducted prior to the acylation of **3** and **4** to avoid the potential oxidation of the sulfur atom(s) in the isosteres. Acylation of **3** and **4** by these sulfones, using the aforementioned conditions, provided the aryl sulfone analogs of **9** in good yield. Further manipulations to the final products (a. Boc cleavage; b. sulfonylation/acylation) proceeded in good yields.

The inhibition of both HIV-1 protease[10] and viral replication of HIV-1 infected CEM cells[11] by the D-amino acid modified decahydroisoquinoline compounds is shown below (Table 1). Some of these derivatives demonstrate the most potent antiviral activity we have observed to date utilizing this concept. Compounds **14**

Table 1. HIV-1 protease & antiviral activity (HXB2/CEM-SS) of D-amino acid inhibitors derived from **2**.

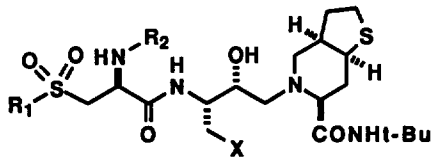


				HIV- 1 protease Enzyme Inhibition	HIV-1 / CEM Antiviral Activity	
compd.	R_1	R_2	n	IC_{50} (nM)	IC_{50}	IC_{95} (nM)
10	4-F-Ph	SO_2CH_3	0	24	150	550
11	4-F-Ph	SO_2CH_3	1	0.6	9.1	31
12	4-F-Ph	SO_2CH_3	2	2.0	17	54
13	2-Naph	SO_2CH_3	0	5.1	71	1900
14	2-Naph	SO_2CH_3	1	0.4	3.8	8.2
15	2-Naph	SO_2CH_3	2	0.5	3.2	10
16	2-Naph	$COCH_3$	2	1.6	9.5	22
17	8-Quin	$COCH_3$	2	1.7	1.9	64
18	$PhCH_2$	$COCH_3$	0	6.0	77	305

and **15**, for example, have potency roughly twofold better than Ro 31-8959.[12] We have found a consistent trend of antiviral potency for the isosteres to be decahydroisoquinoline > hydroxyethylphenyl[5] in this series. Bicyclic aromatics tended to be more active than the monocyclic 4-fluorophenyl. A trend is also observed with the oxidation state of the cysteine sulfur atom found in the P_3 ligand: sulfone, sulfoxide > sulfide.

The combination of the D-amino acid ligands with isosteres **3** and **4** resulted in potent enzyme inhibitors (Table 2). Many were excellent antivirals, having IC_{95}'s of less than 100 nM. In this SAR, a number of trends were also observed. As above, bicyclic aromatic containing inhibitors were more potent than their monocyclic counterpart. LY326910 (**24**) is particularly potent, demonstrating antiviral activity essentially equal to that of Ro 31-5989.[12] Trifluoroacetamido substituted D-amino acid ligands tended to be more potent enzyme inhibitors than their acetamido or methanesulfonamido analogs. However, this trend did not carry over uniformly to virally infected cells. Compounds prepared from isostere **3** were generally more potent (against both the enzyme and virus) than those prepared from isostere **4**, unlike what has been observed with other P_2 ligands.[7] One explanation could be the bulkier P_1 ligand in isostere **4** suffers unfavorable interactions with the large P_3 ligand of the D-amino acid. These interactions are absent in those compounds that show gains in potency going from isostere **3** to **4**. In addition, the antiviral potencies of compounds prepared from isostere **3** tended to be less potent than those from **2**. While compound **24** has a similar IC_{95} when compared to **16**, other compounds (**23**, **27**, **28**) show a 3- to 8-fold drop off in potency when compared to the corresponding analogs (**12**, **15**, **17**) derived from isostere **2**. The lack of improvement in antiviral potency of the D-amino acid modifications of **3**, and especially **4**, over that obtained by **2**, was disappointing considering our other experiences.[7]

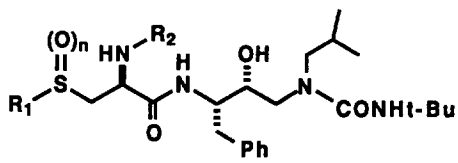
Table 2. HIV-1 protease & antiviral activity (HXB2/CEM-SS) of D-amino acid inhibitors derived from **3** & **4**.



				HIV- 1 protease Enzyme Inhibition	HIV-1 / CEM Antiviral Activity	
compd.	R_1	R_2	X	IC_{50} (nM)	IC_{50}	IC_{95} (nM)
19	4-F-Ph	$COCH_3$	Ph	1.2	70	730
20	4-F-Ph	$COCH_3$	SPh	1.8	57	180
21	4-F-Ph	$COCF_3$	Ph	<0.2	15	58
22	4-F-Ph	$COCF_3$	SPh	10	180	840
23	4-F-Ph	SO_2CH_3	Ph	0.22	43	190
24	2-Naph	$COCH_3$	Ph	0.5	1.9	23
25	2-Naph	$COCF_3$	Ph	<0.2	4.6	32
26	2-Naph	$COCF_3$	SPh	1.8	57	240
27	2-Naph	SO_2CH_3	Ph	<0.2	27	85
28	8-Quin	$COCH_3$	Ph	0.42	140	300
29	8-Quin	$COCF_3$	Ph	0.21	18	62
30	8-Quin	SO_2CH_3	Ph	0.71	72	250

A limited survey of compounds derived from the combination of the D-amino acid ligands with isostere **5** demonstrated the preference for bicyclic aromatic D-amino acid ligands (Table 3). While the 4-fluorophenyl

Table 3. HIV-1 protease & antiviral activity (HXB2/CEM-SS) of D-amino acid inhibitors derived from **5**.



				HIV- 1 protease Enzyme Inhibition	HIV-1 / CEM Antiviral Activity	
compd.	R_1	R_2	n	IC_{50} (nM)	IC_{50}	IC_{95} (nM)
31	4-F-Ph	SO_2CH_3	0	85	17,000	36,000
32	4-F-Ph	SO_2CH_3	2	10	10,000	21,000
33	2-Naph	SO_2CH_3	0	50	4,700	9,900
34	2-Naph	SO_2CH_3	1	2.1	14	62

analogs possess only micromolar antiviral activity, 2-naphthylsulfoxide **34** is a potent inhibitor of HIV-1 replication. These results point to the potential applicability of this concept to isostere **5** and further SAR studies are justified.

Selected members of the series of compounds derived from isosteres **2** and **3** were evaluated in a preliminary oral absorption screen.[13] Compounds were dosed at 40 mg/kg orally to fasted rats, followed by measurement of the total HIV-1 protease activity in the plasma and equated to parent by comparison to a standard curve. For a number of derivatives, the serum concentrations thus obtained tended to be low and/or variable. Whether this is due to poor absorption and/or metabolism to a less potent compound has yet to be determined. Studies varying formulations and the fed state of the rat are required to estimate more precisely the potential for absorption of these compounds.

The methanesulfonic acid salt of **12** (LY314163) performed well in this absorption screen and was chosen for further evaluation. The compound was formulated in water and dosed (orally at 40 mg/kg, I.V. at 20 mg/kg) to fed rats. The absolute oral bioavailability was calculated to be approximately 29%, based upon a comparison of the area under the curves (AUC) between the oral and I.V. routes. The maximum serum concentration observed was 1.6 μg/ml. This plasma concentration is at least ten times the IC_{95} from 15 min to 3 hr after exposure. The level of oral bioavailability for LY314164 combined with its potent antiviral activity is quite encouraging and we believe LY314163 warrants further preclinical study.

Acknowledgment: We thank Ms. Theresa Gygi and Mr. Joe Manetta for help with *in vitro* testing and Jeffrey Burgess and Penny Lubbehusen for preliminary bioavailability data. We acknowledge the support of Drs. Richard Jaskunas and Carlos Lopez. We also thank the Physical Chemistry Department at Lilly Research Laboratories for providing spectral and analytical data.

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12. Ro 31-8959 inhibited HIV-1 protease with an IC_{50} = 1 nM and inhibited HIV-1 replication in CEM cells with an IC_{50} = 6.6 nM and IC_{95} = 21 nM.

13. Plasma concentrations of test compounds were determined by analysis of the plasma sampled (which are composed of parent inhibitor as well as any metabolically derived HIV-1 protease inhibitors) for anti-HIV-1 protease activity with subsequent comparison to an anti-HIV-1 protease activity - concentration standard curve. Anti-HIV-1 protease activity was quantified from plasma using a fluorescence-HPLC enzymatic assay[10] that was performed on a Waters 660E with a Spectra Physics FL2000 fluorescence detector, using an APEC II C18 50 mm x 4.5 mm column.

(*Received in USA* 18 *August* 1995; *accepted* 30 *October* 1995)