Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 11, pp. 1391-1396, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00163-4

D-AMINO ACIDS AS NOVEL P_2/P_3 LIGANDS FOR INHIBITORS OF HIV-1 PROTEASE

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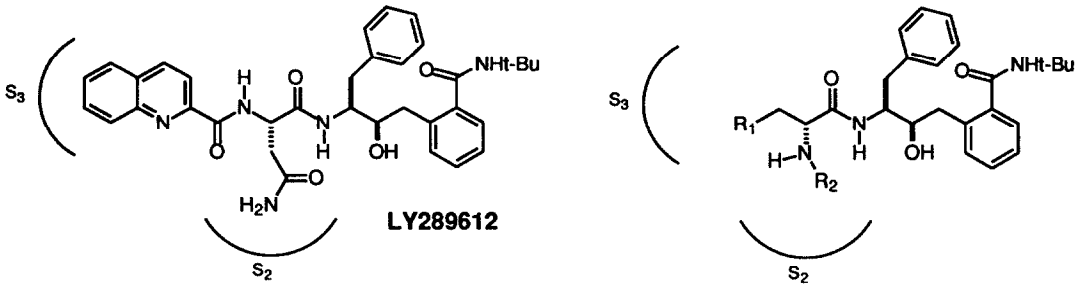
Abstract: Noncoded D-amino acids have been synthesized which effectively replace the P_2/P_3 ligands of the HIV-1 protease inhibitor **LY289612**. Several analogues are shown to have potent enzyme inhibitory and antiviral activities.

HIV-1 protease, an essential enzyme in the life cycle of the HIV virus, continues to be an exciting target for AIDS therapy.[1] In particular, an enormous effort has gone into finding peptidomimetic inhibitors of HIV-1 protease. Several potent inhibitors have been reported such as **Ro-31-8959**[2] and **LY289612**[3] (see Fig. 1). The goal of this work was to improve the potency and pharmacokinetic properties of our lead inhibitor **LY289612**. One can sometimes improve the pharmacokinetic profile of a peptide or peptidomimetic compound by enhancing its proteolytic stability. One potential approach to accomplish this is to replace the natural L-amino acids with noncoded L-amino acids. Another approach is to replace one or more of the peptide's natural L-amino acids with its D-isomer.[4] The above HIV-1 inhibitors contain only one natural amino acid, the asparagine which binds in the S_2 site of the target enzyme. Finding new P_2 ligands which serve as asparagine surrogates using the first approach has been the focus of several investigations.[5] Our approach is a hybrid of the two, specifically to use noncoded D-amino acids to replace the asparagine. It was postulated that if the stereochemistry of the asparagine asymmetric center was inverted in **LY289612** it would require the substituted amino group to serve as the P_2 ligand (R_2 in Fig.1) and the aromatic P_3 ligand would be derived from the D-amino acid side chain (R_1). In order for this concept to be successful a nitrogen substituent which was relatively small and capable of hydrogen bonding ($CONH_2$ mimic) was needed. Also, the amino acid side chain would now become the peptidomimetic's backbone, thus it needs to mimic the large, lipophilic nature of the quinaldic amide moiety in the parent inhibitors. Herein we report that an appropriately designed noncoded D-amino acid can be incorporated into inhibitors of HIV-1 protease and retain potent antiviral activity.

Figure 1. "Role reversal" of the D-amino acid. S_2 and S_3 represent binding pockets of the HIV-1 protease.



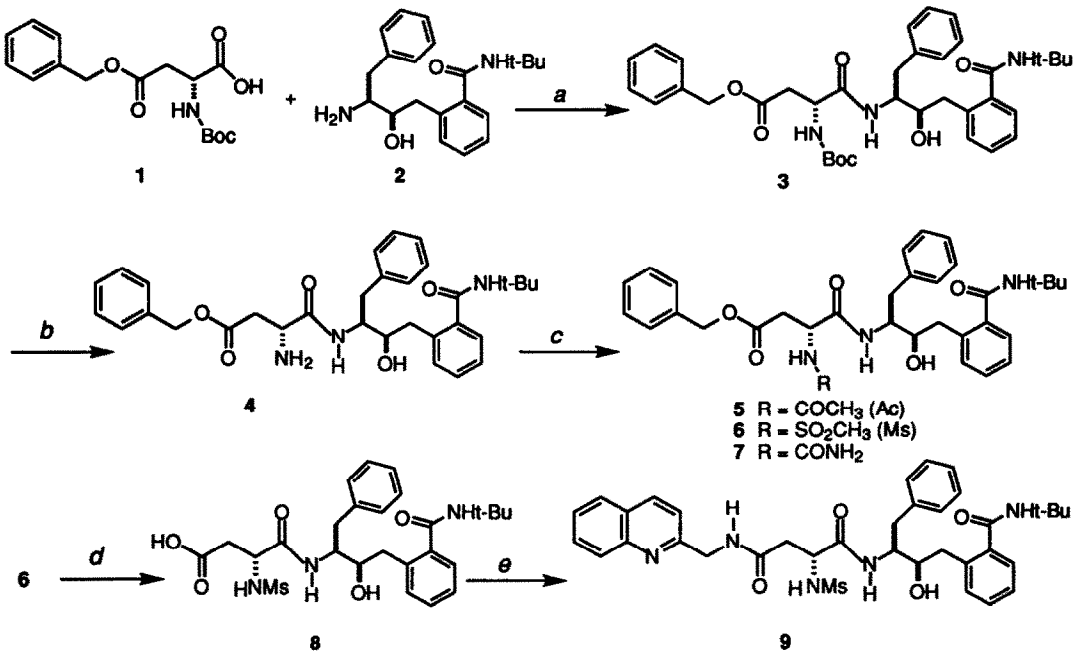
In order to test the D-amino acid concept quickly, a commercially available, optically pure, Boc protected amino acid which contained an aromatic moiety in the side chain, N-tBoc-D-aspartic acid ß-benzyl ester[6] (**1**) was selected as our starting point (Scheme 1). This compound was coupled with the optically pure amine **2**[3] portion of our lead inhibitor **LY289612** to give compound **3**.[7] Unfortunately **3** and its deprotected analogue **4** showed only weak inhibition of the HIV-1 protease. However, intermediate **4** represents a critical starting material in a search for a new P_2 ligand which could serve as an asparagine side chain surrogate. A number of N-substituted analogues, eg. **5**, **6**, **7**, were prepared and the amine substituted with acetyl or mesyl groups produced potent enzyme inhibitors which were also weak antiviral agents (see Table 1). To show that this activity was unique to the D-amino acid, the synthesis of their isomeric L counterparts, compounds **21** and **22**, was undertaken. They were synthesized in the same fashion starting from N-tBoc-L-aspartic acid ß-benzyl ester.[6] As expected these compounds showed very weak enzyme inhibitory activity.

Since it appeared that an N-acetyl moiety was an acceptable P_2 ligand, and many N-acetyl substituted optically pure amino acids are commercially available, a quick search for groups which could serve as new P_3 ligands was made. N-acetyl-S-benzyl-D-cysteine,[8] for example, when coupled to compound **2** gave **23** which exhibits an enzyme inhibitory IC_{50} = 45 nM. The commercially available N-acetyl-S-benzyl-L-cysteine[9] used to synthesize isomeric L analogue **24** confirmed that the activity was specific to the D-isomer (Table 1).

While these results were exciting, none of these compounds exhibited the antiviral potency of our lead inhibitor. It appeared that a P_3 ligand which more closely resembled the quinaldic amide portion of **LY289612** was needed. To this end quinaldic amide **9** was synthesized by first removing the benzyl group of ester **6** and then coupling with 2-(aminomethyl)quinoline[10] (Scheme 1). Unfortunately this

compound had activity essentially equivalent to compound **6** from which it was derived.

Scheme 1.



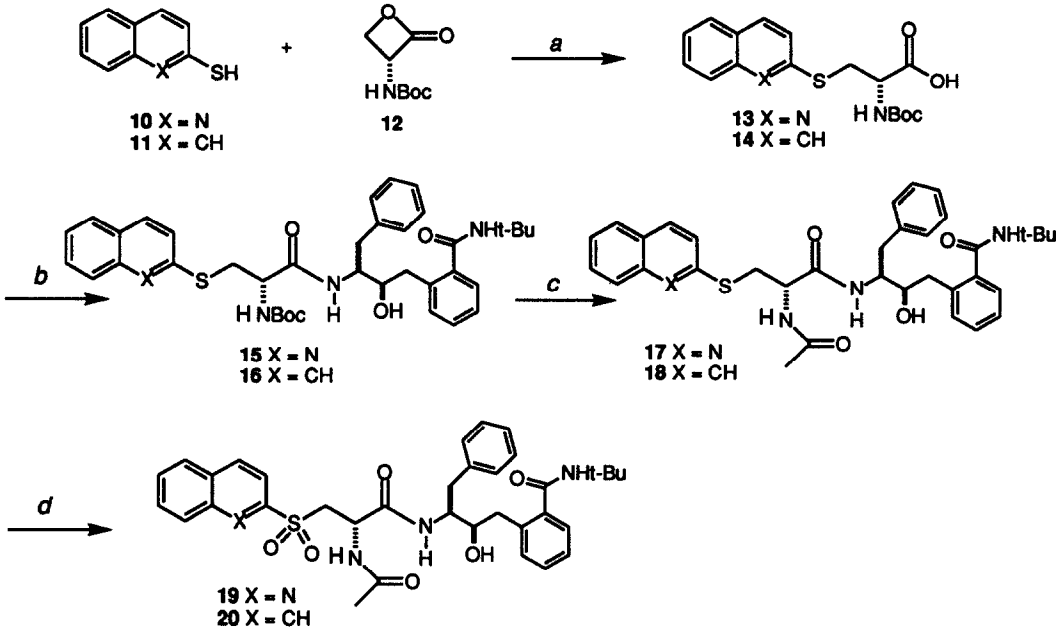
(a) DCC, HOBt, 87%; (b) TFA, CH_2Cl_2, quant.; (c) Ac_2O, pyr., 40% or MsCl, Et_3N, 54% or TMSNCO, 68%; (d) 5% Pd/C, NH_4OCHO, MeOH, 95%; (e) DCC, HOBt, 2-(aminomethyl)quinoline, 53%

Our attention turned to synthesizing a P_3 ligand which not only placed the aromatic group at the appropriate position in the amino acid backbone but also incorporated an isostere for the amide group. Due to the recent interest in the development of peptidomimetic enzyme inhibitors, a number of amide isosteres have been developed including sulfones.[11] Sulfones **19** and **20** (Scheme 2) were selected as our synthetic targets. To accomplish this N-tBoc-D-serine ß-lactone[12] **12** was opened with 2-quinolinethiol to give optically pure D-amino acid **13**. Amide bond formation under the normal conditions (DCC, HOBt) gave **15**. The Boc group was removed and the amine acylated with acetyl chloride to furnish **17** in 84% yield. In the final step the sulfide was oxidized to the sulfone using Oxone®.[13] It was gratifying to find that **19** had both improved enzyme inhibitory and whole cell antiviral activity (Table 1). Napthyl analogues **18** and **20** were also synthesized. Sulfone **20** is one of the most

potent HIV-1 protease inhibitors of this molecular size (MW = 644) known. It is interesting to note that upon oxidation of sulfide **18** to sulfone **20** one can achieve a significant improvement in activity. This may be due to the fact that the sulfone moiety more closely mimics the amide backbone of the natural substrate.

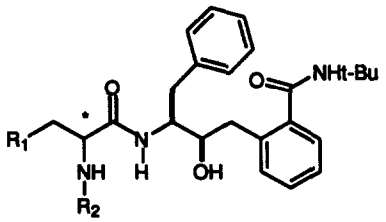
In summary, noncoded D-amino acids have been synthesized which effectively replace the P_2/P_3 ligands of the HIV-1 protease inhibitor **LY289612** as evidenced by improved enzyme inhibitory and whole cell antiviral activity. This concept may find applications in peptidomimetic inhibitors of other proteolytic enzymes. We continue to explore this concept and additional studies including evaluation of the pharmacokinetic properties of these novel agents will be reported in due course.

Scheme 2.



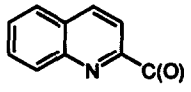
(a) NaH, THF, 72%; (b) **2**, DCC, HOBt, 71%; (c) TFA, CH_2Cl_2 then Et_3N, AcCl, 68% two steps; (d) Oxone®, 88%. (Yields reported are for the synthesis of **20**)

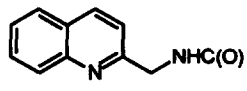
Table 1.

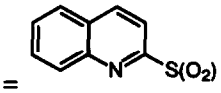


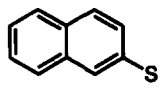
compd	R_1	R_2	*	Enzyme Inhibition[14] IC_{50} (nM)[a]	Antiviral Activity[15] IC_{50} (nM)[b] CEM	MT2
LY289612	$CONH_2$	QC	L	1.5(5)	23(42)	52(34)
3	$PhCH_2OC(O)$	t-BOC	D	>1,000	NT[c]	NT
4	$PhCH_2OC(O)$	H	D	>1,000	NT	NT
5	$PhCH_2OC(O)$	$C(O)CH_3$	D	85	NT	NT
21	$PhCH_2OC(O)$	$C(O)CH_3$	L	>1,000	NT	NT
7	$PhCH_2OC(O)$	$C(O)NH_2$	D	51	22,400(1)	11,900
6	$PhCH_2OC(O)$	SO_2CH_3	D	5.4	1,090(1)	1,270
22	$PhCH_2OC(O)$	SO_2CH_3	L	>1,000	NT	NT
9	QA	SO_2CH_3	D	8.0	580	1,440
23	$PhCH_2S$	$C(O)CH_3$	D	45	2,910	3,120
24	$PhCH_2S$	$C(O)CH_3$	L	>1,000	NT	NT
19	QO	$C(O)CH_3$	D	0.3	53	180
18	NS	$C(O)CH_3$	D	42	1,130	1,000(1)
20	NO	$C(O)CH_3$	D	0.3	11(4)	31(3)

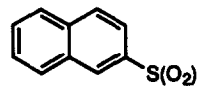
* D and L designation used rather than R and S for clarity of concept [a] Values are a single determination unless noted with a (n). [b] Values are the average of two determinations unless noted with a (n). [c]NT= Not tested.

QC= 

QA = 

QO= 

NS = 

NO = 

Acknowledgment: The authors wish to thank Theresa Gygi, Joe Manetta, and Joe Colacino for *in vitro* testing. Thanks to Ed Smith, James Fritz and Tom Crowell for helpful discussions. We acknowledge the support of Dr. Richard Jaskunas and Dr. Carlos Lopez. We would also like to thank the physical chemistry department at Lilly Research Labs for providing analytical and spectral data.

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9. Purchased from Janssen
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(*Received in USA* 10 *February* 1994; *accepted* 29 *April* 1994)