3-Tetrahydrofuran and Pyran Urethanes as High-Affinity P_2-Ligands for HIV-1 Protease Inhibitors

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Received November 4, 1992

Acquired immunodeficiency syndrome (AIDS) resulting from infection with the human immunodeficiency virus (HIV),[1] continues to be one of the most challenging problems in medical history. Through substantial advances in molecular and cellular biology, much has been learned with unprecedented rapidity about the virus HIV-1. Since the virally encoded HIV-1 protease is responsible for proteolytic processing of the *gag* and *gag-pol* polyproteins to form infectious virions,[2] inhibition of this enzyme has become an attractive therapeutic target for AIDS chemotherapy.[3]

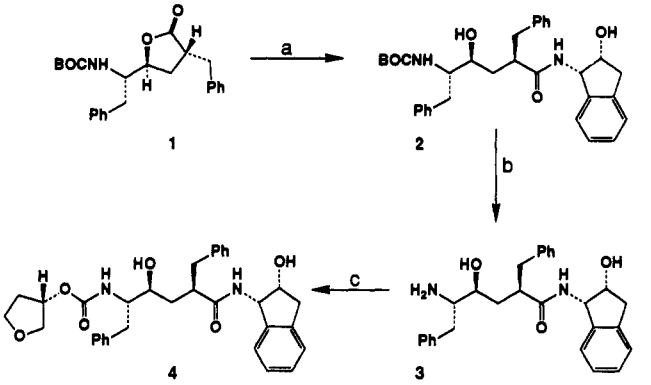
Consequently, several reports of novel cyclic and conformationally constrained ligands that mimic natural amino acid side chains have aided the design of HIV-1 protease inhibitors with improved pharmacokinetic properties. To date, perhaps the most notable are the *cis*-decahydroisoquinoline for the P_1' binding region,[4] the 1(*S*)-amino-2(*R*)-hydroxyindan as P_2' ligand,[5] and the 2-quinolinoyl moiety as the P_3 ligand.[4] As part of our continuing efforts in the design of novel, cyclic ligands for the HIV-1 protease substrate binding site, we have incorporated various constrained urethanes in place of N-terminus acyclic *tert*-butylurethane. In this communication, we report that the urethanes of commercially available 3-tetrahydrofurans and easily accessible 3-tetrahydropyrans are high affinity ligands for the S_2 binding domains of the HIV-1 protease.

The known lactone 1 has been previously converted to compound 2 by our laboratories.[5,6] As depicted in Scheme I, treatment of compound 2 with trifluoroacetic acid in methylene chloride at 0 °C for 30 min furnished the amine 3 in good yield. Urethanes 4–9, as shown in Table I, were then prepared by alkoxycarbonylation[7] of the corresponding alcohol with amine 3. For example, active carbonate, obtained by reaction of 3(*S*)-hydroxytetrahydrofuran, disuccinimidyl carbonate, and triethylamine in acetonitrile, readily reacted with amine 3 to provide the urethane 4 in good yield after silica gel chromatography.

Various urethanes in the hydroxyethylamine series were prepared as shown in Scheme II. Previously reported[8] azido epoxide 10 and decahydroisoquinoline[9] derivative 11 were heated at reflux in 2-propanol for 12 h to furnish the azido alcohol 12 in 83% yield after silica gel chromatography. Catalytic hydrogenation of this resulting azide with 10% palladium on charcoal in a mixture of tetrahydrofuran and methanol (4:1) in the presence of acetic acid afforded the amine 13 in 98% yield. The amine 13 was then converted to the urethanes 14 and 16–20 using the alkoxycarbonylation reaction as described above. The structures of all new compounds, thus obtained, were established by ^{1}H NMR spectroscopy, elemental analysis, and mass spectral analysis.[10]

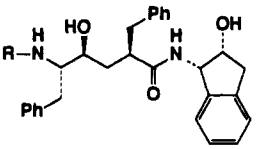
From Table I, it can be seen that the 3-tetrahydrofuranyl- or 3-tetrahydropyranylurethanes are very high affinity ligands into the S_2 binding region of the enzyme HIV-1 protease. Replacement of BOC urethane of compound 2 with 3(*S*)-tetrahydrofuranylurethane (compound 4, IC_{50} < 0.03 nM) resulted in over a 10-fold increase

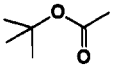
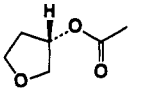
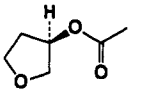
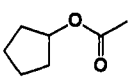
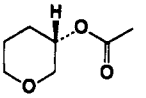
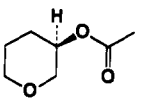
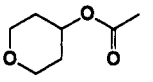
Scheme I[a]



[a] (a) Reference 5. (b) Trifluoroacetic acid, CH_2Cl_2, 0 °C, 30 min. (c) Tetrahydrofuranylsuccinimidyl carbonate, CH_2Cl_2, Et_3N.

Table I. Structure and Inhibitory Potencies of Urethanes in Hydroxyethylene Series



compd	R	mp (°C)	IC_{50}[a] (nM)	CIC_{95}[b] (nM)
2			0.3	400
4		231–33	<0.03	3
5		217–19	0.03	100
6		237–38	0.33	400
7		228–30	<0.03	50
8		223–24	<0.03	25
9		215–17	1.22	

[a] The HIV protease inhibitor Ro-31-8959[4] displaced an IC_{50} value of 0.23 nM (±0.1, n = 3) in this assay system.[11] [b] The CIC_{95} value for Ro-31-8959 was 22 nM (±7, n = 10) in this assay.[16] No overt cytotoxicity was observed for any compounds tested under the conditions used for the cell-based assay.[17]

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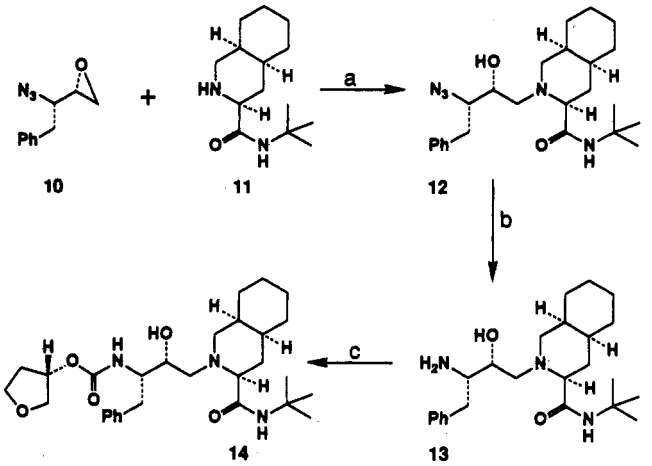
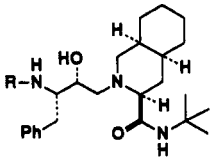
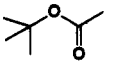
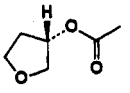
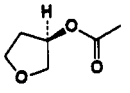
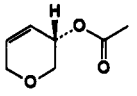
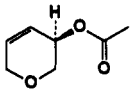
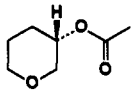
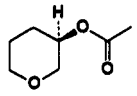
Scheme II[a]



[a] (a) 2-Propanol, 80 °C, 12 h. (b) H_2, 10% Pd–C, acetic acid, MeOH–THF, 12 h. (c) Tetrahydrofuranylsuccinimidyl carbonate, CH_2Cl_2, Et_3N, 23 °C, 12 h.

Table II. Structure and Inhibitory Potencies of Urethanes in Hydroxyethylamine Series



compd	R	mp (°C)	IC_{50} (nM)
15			>3000
14		80–82	160
16		65–68	694
17		87–88	314
18		89–90	48.9
19		95–96	392
20		92–93	308

in its inhibitory potency.[11] Tetrahydrofuranylurethane with 3(*R*) configuration (compound **5**) also exhibited similar potency-enhancing effect. The potency-enhancing effect of 3-tetrahydrofuranylurethanes was also successfully extended to sterically more demanding 3-tetrahydropyranylurethanes. As is evident, irrespective of ring stereochemistry, both 3(*S*) and 3(*R*) configurations[12] have shown a potency-enhancing effect by a factor of greater than 10-fold relative to BOC derivative **2**. Examination of cyclopentylurethane **6** established that the presence of ring oxygen was essential for potency enhancement. As is evident from the lower activity (IC_{50} = 1.22 nM) of the 4-tetrahydropyranylurethane **9**, the position of pyranyl oxygen (3-position) is critical. The reason for this high affinity could be due to hydrophobic binding along with some specific interaction of ring oxygen as well as urethane oxygen in the S_2 binding domain of the HIV-1 protease. However, the proper understanding of this effect should await the solution of the X-ray crystal structure of a protein–ligand complex.[13]

Incorporation of various 3-tetrahydrofuranyl- or tetrahydropyranylurethanes as the P_2 ligand in the hydroxyethylamine series has resulted in similar potency enhancement. As shown in Table II, the tetrahydrofuranylurethane with 3(*S*) configuration (compound **14**, IC_{50} = 160 nM) has exhibited more than 18-fold potency increase relative to BOC derivative **15**.[14] The ring stereochemistry and conformational rigidity associated with various urethane moieties have a major effect on the in vitro potencies in this series. Tetrahydrofuranylurethane with 3(*R*) configuration (compound **16**) showed a 4-fold loss in its inhibitory potency. Dihydropyranylurethane **17** has an IC_{50} value of 314 nM, and saturation of the dihydropyran ring resulted in slight loss in potency (compound **19**, IC_{50} 392 nM). Incorporation of dihydropyranylurethane at P_2 subsite with 3(*R*) configuration (compound **18**) afforded the most potent compound in this series with an IC_{50} value of 49 nM, a greater than 61-fold potency enhancement over BOC urethane **15**. The conformational rigidity as well as the ring stereochemistry of this urethane moiety was presumably making favorable interactions with the residues in the S_2 subsite.[13] In accord with earlier observation, saturation of the dihydropyran ring of **19** resulted in loss of potency (compound **20**, IC_{50} = 308 nM).

Several compounds in the hydroxyethylene series have been tested for their ability to inhibit the spread of HIV-1 in MT4 human T-lymphoid cells infected with the IIIb isolate.[5a] As evident in Table I, substitution of BOC urethane either with 3-tetrahydrofuranyl or 3-tetrahydropyranylurethane not only increases intrinsic potency against the enzyme but generally leads to significant enhancement of antiviral potency as well. Compound **4** has prevented the spread of HIV-1 at a concentration of 3 nM (CIC_{95}), a greater than 133-fold potency enhancement over BOC derivative **2**. Furthermore, introduction of 3(*R*)-tetrahydropyranylurethane as P_2 ligand (compound **8**) attenuated the CIC_{95} by a factor of 15 compared to **2**. In the hydroxyethylamine series, antiviral potencies of compound **14** and **18** have been determined and their CIC_{95} values were 800 (n = 3)[15] and 1600 nM, respectively.

In conclusion, urethanes of 3-tetrahydrofurans and pyrans are high-affinity ligands for the S_2 binding domains of the HIV-1 protease. Incorporation of these ligands provided potent inhibitors in the hydroxyethylene and hydroxyethylamine series with picomolar and nanomolar in vitro potencies. Of particular interest, substitution of the BOC urethane in compound **2** with urethane of commercially available 3-(*S*)-hydroxytetrahydrofuran led to a significant enhancement of antiviral potency (CIC_{95}, 3 nM) in compound **4**. Further investigations of these ligands are in progress.

Acknowledgment. The authors gratefully acknowledge the assistance of Dr. H. Ramjit for mass spectra, J. P. Moreau for elemental analysis, and Ms. Jean Kaysen for help in manuscript preparation.

Supplementary Material Available: Experimental procedures and spectral data for compounds **3**, **4**, and **12–14** and elemental analysis and mass spectral data for compounds **4–9** and **14–20** (6 pages). Ordering information is given on any current masthead page.

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(17) Direct assay of cytotoxicity was found to be highly dependent on culture medium and cell type. Unless overt toxicity was noted during the spread assay, no further measure of cytotoxicity was performed.