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SYNTHESIS AND PHARMACOKINETICS OF POTENT CARBAMATE HIV-1 PROTEASE INHIBITORS CONTAINING NOVEL HIGH AFFINITY HYDROXYETHYLAMINE ISOSTERES.

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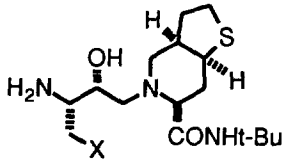
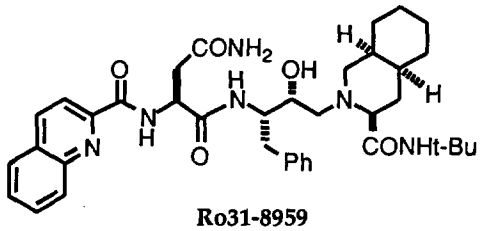
Abstract. Using the hydroxyethylamine isosteres **1** and **2** containing the novel *cis*-octahydrothienopyridine moiety, substantial enhancement of binding potencies for HIV-1 protease inhibitors which incorporate carbamate linked heterocyclic P_2 ligands has been realized. This increase in binding has led to a very potent antiviral compound (LY326188). The pharmacokinetics of selected derivatives are detailed in this report.

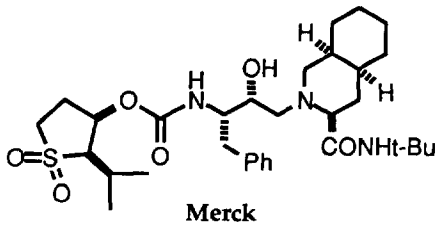
The search for new, potent, orally bioavailable chemotherapeutic agents for AIDS has been one of enormous proportion. HIV-1 protease, an essential enzyme in the life cycle of HIV, has become one of the most important targets.[1] The design of HIV-1 protease inhibitors has been aided by several reports of novel cyclic ligands that mimic natural amino acids.[2] The most notable perhaps is the *cis*-decahydroisoquinoline for the P_1' binding site in combination with the 2-quinolinoyl ligand for the P_3 region to produce the very potent, but modestly absorbed, Ro31-8959.[3] We have been engaged in an effort to prepare compounds that not only eliminate bulky P_3 ligands, but also increase the binding of ligands over the entire P_2/P_2' binding region. This work has yielded the discovery of a novel *cis*-octahydrothienopyridine as a ligand for P_1' and subsequently high affinity hydroxyethylamine isosteres **1** and **2**.[4] The subject of this report is the combination of these isosteres with small heterocyclic P_2 ligands, previously reported by the Merck group, as truncated versions of Ro31-8959.[5-7]



1 X = Ph
2 X = SPh



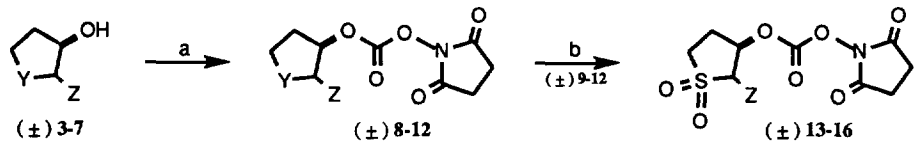
Ro31-8959



Merck

Previously it was observed that the Cbz carbamate derivatives of **1** and **2** demonstrated a much greater inhibition of HIV protease as compared to the corresponding *cis*-decahydroisoquinoline isostere.[4,8] Therefore, it was anticipated that the substitution of a relatively small, better binding P_2 ligand for Cbz might result in compounds with potent inhibitory activity and improved oral bioavailability. Thus, the desired cyclic sulfolane and tetrahydrofuran inhibitors were prepared starting from racemic alcohols **3** - **7**.[5-7,9] Alcohol **7** was obtained from the corresponding ketone[9] under reductive conditions (DIBAH / CH_2Cl_2) that gave a 15 : 1 *cis* / *trans* mixture. These same conditions provided **6** with much poorer stereoselectivity (2 : 1 *cis* / *trans*). These isomers were separated by normal phase HPLC.[10] However, we discovered that the use of the bulky boron reducing agent K-Selectride® increased selectivity, in the case of **6**, to > 20 : 1 *cis* / *trans*. The alcohols were activated with N,N'-disuccinimidyl carbonate, to afford carbonates **8** - **12** in quantitative yield. Carbonates **9** - **12** were then oxidized with m-CPBA, prior to coupling with amines **1** or **2** (Scheme 1), to yield sulfones **13** - **16** in 90% yield.

Scheme 1



3, 8: Y= O, Z= ▬ CH_3

4, 9: Y= S, Z= H

5, 10: Y= S, Z= ⋯ CH_3

6, 11: Y= S, Z= ▬ CH_3

7, 12: Y= S, Z= ⟙

13: Z= H

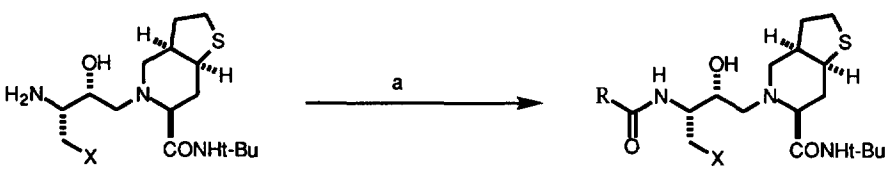
14: Z= ⋯ CH_3

15: Z= ▬ CH_3

16: Z= ⟙

REAGENTS: a. N,N'-disuccinimidyl carbonate / Et_3N / (quant.); b. 2 equiv. 85% m-CPBA / CH_2Cl_2

Scheme 2



1: X= CH_2Ph

2: X= CH_2SPh

28-31: X= CH_2Ph

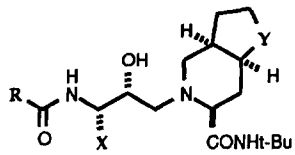
17-20, 22-25, 32, 33: X= CH_2SPh

REAGENTS: a. for **1**, carbonates **12** and **16** / CH_2Cl_2 / RT / 30 min.; for **2**, carbonates **8** and **13** - **16** / CH_2Cl_2 / RT/ 30 min. (75-85% for both amines).

Coupling (Scheme 2) of selected racemic carbonates to amines **1** and **2**, or the decahydroisoquinoline isostere, provided inhibitors **17** - **33** (Table I) in good yield. All diastereomers were separated by radial or flash

silica gel chromatography. Racemic alcohols **6** and **7** were kinetically resolved by the method of Kim[9] to provide optically active (2R,3R)-**6** and (2R,3R)-**7**. These enantiomerically pure alcohols were subjected to the previous coupling sequence with **2**, establishing the stereochemical assignments of **25** and **33**, respectively. The absolute stereochemistry of the sulfolane centers of compounds **19**, **20**, **22** and **23** were assigned arbitrarily. Comparison inhibitors **21**, **26**, and **27** were prepared[6,7] and evaluated for inhibition of HIV protease and anti-HIV activity. Ro31-8959 was used as a standard.

Table I. HIV-1 protease IC_{50}'s and HIV-1 antiviral IC_{95}'s (HXB2/CEM-SS) of carbamates **17-33**.



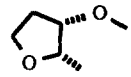
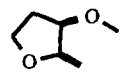
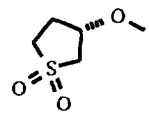
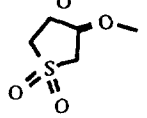
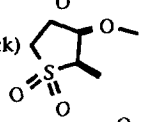
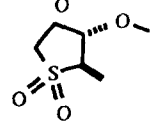
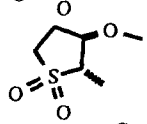
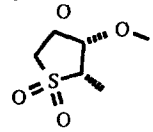
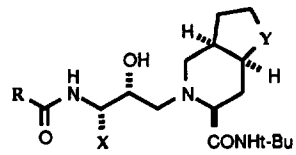
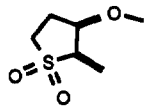
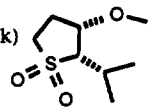
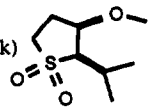
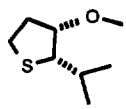
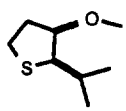
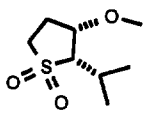
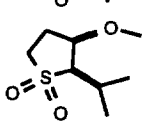
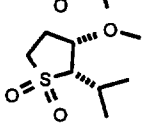
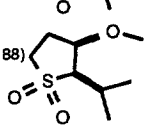
compd	R	X	Y	IC_{50}[a] (nM)	IC_{95}[b] (nM)
17		-CH_2SPh	-S-	0.28	162
18		-CH_2SPh	-S-	3.0	2562
19		-CH_2SPh	-S-	1.7	59
20		-CH_2SPh	-S-	2.0	59
21 (Merck)		-CH_2Ph	-CH_2CH_2-	10.0	171
22		-CH_2SPh	-S-	5.2	45
23		-CH_2SPh	-S-	1.4	44
24		-CH_2SPh	-S-	0.4	31

Table I (cont.)



compd	R	X	Y	IC_{50}[a] (nM)	IC_{95}[b] (nM)
25		$-CH_2SPh$	-S-	0.75	19
26 (Merck)		$-CH_2Ph$	$-CH_2CH_2-$	8.1	273
27 (Merck)		$-CH_2Ph$	$-CH_2CH_2-$	4.3	85
28		$-CH_2Ph$	-S-	2.6	686
29		$-CH_2Ph$	-S-	0.26	85
30		$-CH_2Ph$	-S-	2.6	77
31		$-CH_2Ph$	-S-	1.3	38
32		$-CH_2SPh$	-S-	0.95	89
33 (LY326188)		$-CH_2SPh$	-S-	0.42	9

[a] Ro 31-8959[3] displayed an IC_{50} value of 1.0 nM in this assay.[11] [b] The IC_{95} value (CEM cell line) for Ro 31-8959 was 21 nM (n=32) in this assay.[12]

As shown in Table I inhibitors **24, 25, and 30-33** show marked improvement in both HIV-1 protease inhibition (5-25 fold) and antiviral potency (5-10 fold) versus their *cis*-decahydroisoquinoline counterparts (**21, 26,** and **27**)[13a] in the same assay. It is surprising to note that the *trans* isomers **22** and **23** are nearly as potent as the *cis* compounds **24** and **25**. Compound **33** (LY326188) exhibits antiviral activity about 2-fold better (CEM-IC_{95}= 9nM) than Ro31-8959 when tested head to head. The tetrahydrofuran derivatives **17** and **18** also demonstrated superior enzyme (60-190 fold) inhibition; however, only the antiviral activity of **17** was superior to the previously reported inhibitors.[13b]

Inhibitors **33, 25** and **24** as well as their methanesulfonate salts, were evaluated in a preliminary oral absorption screen[14] in Sprague-Dawley rats (n=3) at a dose of 40 mg/kg. The compounds were formulated in either 10% acacia/1% tween 80, H_2O, or 25% EtOH/H_2O. Peak plasma levels were detected between 0.5 and 1.0 hour in a range of 1021 to 2551 ng/ml. Compound **27** was also evaluated in this screen displaying similar characteristics.

These inhibitors were further evaluated for oral bioavailability in fasted Fisher rats (n=3) at a dose of 20 mg/kg. The compounds were formulated in a 25% EtOH/H_2O vehicle. The plasma specimens were assayed for parent drug by HPLC. Cmax values for the three inhibitors ranged from about 200 to 500 ng/ml between 15 and 30 minutes post administration. The peak plasma levels achieved in this assay did not reach those of the preliminary screen. However, considering the potencies, the plasma half-life following oral administration of **33, 25,** and **24** was very favorable at 84, 152, and 93 minutes, respectively. Furthermore, the duration that the plasma drug concentrations remained above the IC_{95} was three hours for **33** (CEM-IC_{95} = 6.8 ng/ml = 9 nM), five hours for **25** (CEM-IC_{95} = 13.6 ng/ml = 19 nM), and six hours for **24** (CEM-IC_{95} = 22.2 ng/ml = 31 nM). The bioavailability was calculated to be 3.5% for **33**, 6.3% for **25**, and 7.4% for **24**.

In conclusion, the inhibitors evaluated have demonstrated modest bioavailability, comparable at least to Ro31-8959, while having equal or greater antiviral potency. The observed bioavailabilities may have been affected by poor absorption and/or high first pass metabolism based on the higher plasma levels observed in the preliminary oral absorption bioassay (HIVP activity of plasma samples is being measured[14]). However, since 40 mg/kg is a comparatively higher dose, it is possible that the discrepancies observed between the two assays could be due to non-linear pharmacokinetic behaviors of these inhibitors, even at a dose difference of only 20 to 40 mg/kg. It may be possible to improve the pharmacokinetic profile of these inhibitors considerably in fed rats or other species. Further work in this series is warranted in order to determine what factors may improve oral bioavailability.

Thus, the acylation of small carbamate linked heterocyclic P_2 ligands onto the high affinity hydroxyethylamine isosteres **1** and **2**, has provided another series of very potent potential HIV-1 drug candidates. Selected inhibitors have exhibited a reasonable pharmacokinetic profile and modest bioavailability in rats. Further sidechain modification may improve oral absorption while maintaining potency. Additional publications from our laboratory will highlight other such examples.

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7. Ghosh, A. K.; Thompson, W. J.; Lee, H. Y.; Culberson, C.; Holloway, M. K.; McKee, S. P.; Munson, P. M.; Duong, T. T.; Smith A. M.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif W. A.; Huff, J. R.; Anderson, P. S. *J. Med. Chem.* **1994**, *37*, 1177.

8. HIVP-IC_{50}'s for the Cbz derivatives of **1** and **2** were 6.3 nM and 3.3 nM, respectively. The HIVP-IC_{50} for the Cbz protected decahydroisoquinoline isostere was 127 nM in the same assay.

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13. (a) reference 6 reports the following values for the sulfolane inhibitors **21**, (IC_{50}= 22.3 nM, CIC_{95}= 200 nM), **26**, (IC_{50}= 11.0 nM, CIC_{95}= 200 nM), **27**, (IC_{50}= 3.0 nM, CIC_{95}= 50 nM). (b) reference 5 reports the following values for the corresponding tetrahydrofuran inhibitors compared to **17** and **18** IC_{50}'s= 52.4 and 169 nM, CIC_{95}'s= 400 and 1600 nM respectively. The antiviral assay protocol in these references monitors p^{24} production as an endpoint using a MT4 cell line. The present assay monitors the inhibition of the cytopathic effect of HIV-1 infected CEM cells by measuring the metabolic reduction of XTT (colorless) to XTT formazan (orange) in healthy cells.

14. Plasma concentrations of test compounds were determined by analysis of the plasma samples (which are composed of parent inhibitor as well as any metabolically derived HIV protease inhibitors) for anti-HIV protease activity with subsequent comparison to an anti-HIV protease activity - plasma concentration standard curve. Anti-HIV protease activity was readily quantified from plasma using a fluorescence-HPLC enzymatic assay[11] which was performed on a waters 660E with a Spectra Physics FL2000 fluorescence detector, using an APEX II C18 50 mm x 4.5 mm column.

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