Communications to the Editor

Cyclic Sulfolanes as Novel and High Affinity P_2 Ligands for HIV-1 Protease Inhibitors

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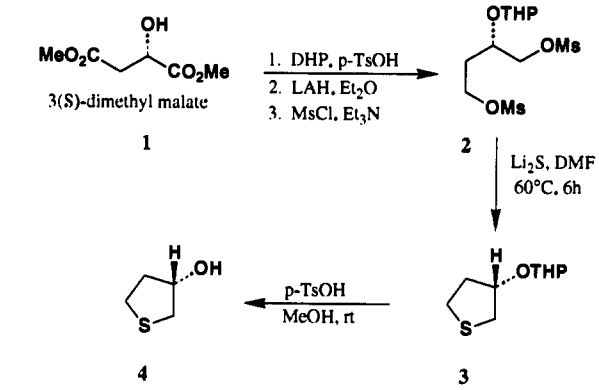
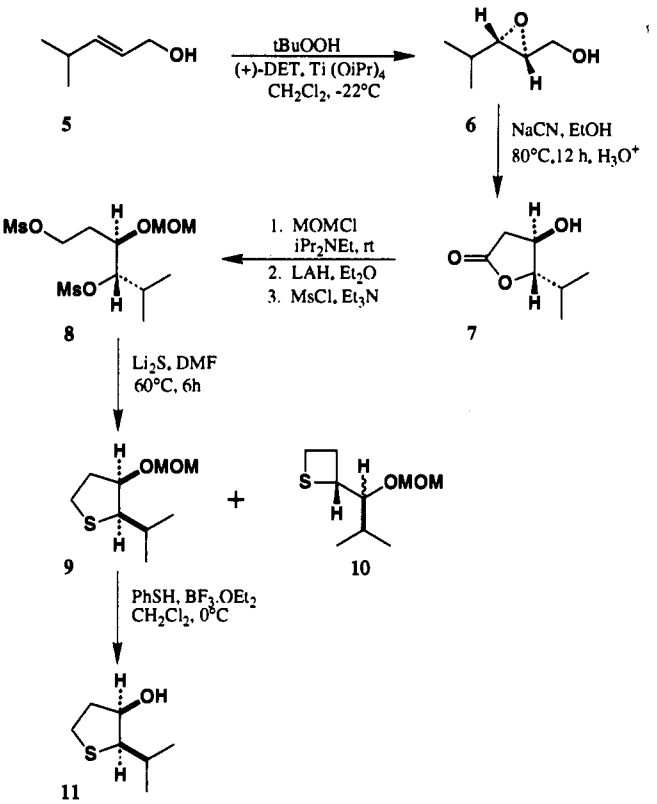
Recently we reported urethanes of 3-tetrahydrofuran as P_2 ligands for the HIV-1 protease inhibitor.[1] In our continuing effort to design novel and conformationally restricted cyclic ligands for the HIV protease substrate binding site, we subsequently found that urethanes of 3(*S*)-hydroxysulfolane substantially increased the in vitro potency of inhibitors relative to the heterocycle 3(*S*)-tetrahydrofuran. In this paper we report that introduction of a small 2-alkyl group cis to the 3-hydroxyl group of either heterocycle system further enhances enzyme affinity in a manner consistent with modeling studies using the X-ray crystal structure of the enzyme–inhibitor complex of tetrahydrofuran-derived inhibitor **16** with HIV-1 protease.[2] The *cis*-2-isopropyl group thus far offers optimum enhancement of the inhibitory properties of the 3-hydroxysulfolane providing an inhibitor of comparable in vitro antiviral potency to present clinical candidate (3*S*,4a*S*,8a*S*,2′*R*,3′*S*)-*N*-*tert*-butyl-2-(2′-hydroxy-4′-phenyl-3′-((*N*-(2-quinolinylcarbonyl)-L-asparaginyl)amino)butyl)-decahydroisoquinoline-3-carboxamide (Ro 31-8959), but of reduced molecular weight due to the exclusion of the P_3 quinoline ligand.[3]

The synthetic route leading to the 3(*S*)-hydroxytetrahydrothiophene **4** is outlined in Scheme I. As shown, enantiomerically pure 3(*S*)-dimethyl malate[4] was converted to bis-mesylate **2** by the following three-step sequence: (1) protection of the hydroxy group as the tetrahydropyranyl ether by treatment with dihydropyran and a catalytic amount of *p*-TsOH in diethyl ether, (2) reduction of the corresponding ester with lithium aluminum hydride (LAH) in diethyl ether to the diol, and (3) mesylation of the resulting diol with mesyl chloride and triethylamine in methylene chloride at −10 to 23 °C for 12 h to provide the bis-mesylate **2** (64% from **1**). Ring closure of the bis-mesylate **2** with an excess of lithium sulfide in DMF at 60 °C for 6 h furnished the protected tetrahydrothiophene **3** (76% yield). The removal of tetrahydropyranyl protecting group was effected by exposure to *p*-TsOH in methanol to afford the 3(*S*)-hydroxytetrahydrothiophene **4** (75% yield). Similarly, 3(*R*)-hydroxytetrahydrothiophene was prepared in good yield starting from optically pure 3(*R*)-dimethyl malate.

Scheme I



Scheme II



Various *cis*-2-alkyl-3-hydroxytetrahydrothiophene derivatives utilized for the preparation of target inhibitors **15** and **22–28** were synthesized following the general procedure for compound **11** (Scheme II). The allylic alcohol **5** was subjected to the Sharpless epoxidation[5] condition with (+)-diethyl L-tartrate to furnish the epoxide **6** in 78% isolated yield and 90% ee.[6] Reaction of epoxide **6** with sodium cyanide in refluxing ethanol for 12 h, followed by careful acidification with concentrated hydrochloric acid as described by Ganem and Wrobel,[7] afforded the corresponding Payne rearrangement product **7** (65%). Lactone **7** was then protected as the methoxymethyl ether by treatment with chloromethyl methyl ether and diisopropylethylamine in methylene chloride

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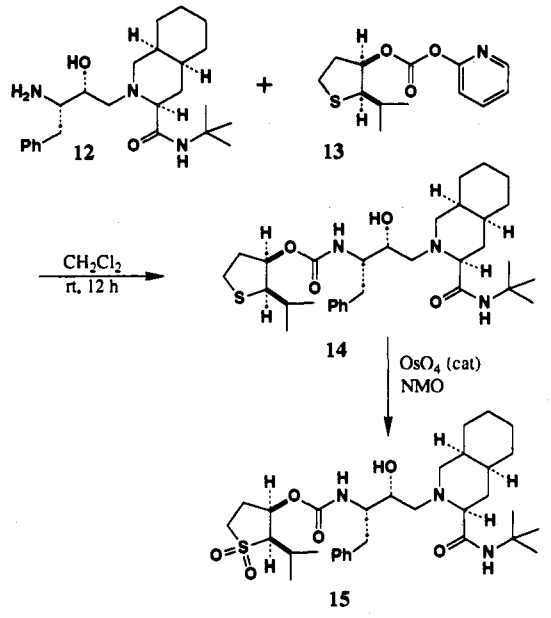
Scheme III

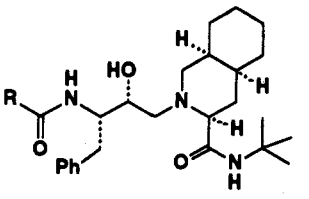


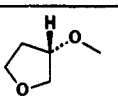
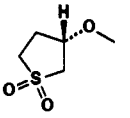
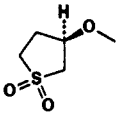
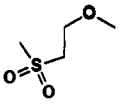
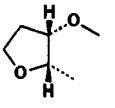
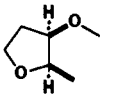
in the presence of a catalytic amount of 4-(dimethylamino)-pyridine. Reduction[8] of the protected lactone with LAH and subsequent mesylation of the resulting diol with mesyl chloride and triethylamine provided the bis-mesylate **8** in 61% yield in three steps. Ring closure of the bis-mesylate **8** with an excess of lithium sulfide in DMF as described for compound **3** provided a mixture (3:1) of desired tetrahydrothiophene derivative **9** and the thietane derivative **10**, resulting from the competitive solvolysis reaction.[9] The cyclization of the bis-mesylate with a C-2 methyl, ethyl, or isobutyl group afforded mainly the corresponding tetrahydrothiophene derivative (65–75%) and a small amount (5%) of thietane byproduct. Separation of the products by silica gel chromatography (5% ethyl acetate–hexane) followed by removal of the MOM protecting group with thiophenol and $BF_3 \cdot OEt_2$ furnished tetrahydrothiophene derivative **11** (43% from **8**). The corresponding 2(*S*)-alkyl-3(*S*)-hydroxytetrahydrothiophene derivatives were prepared utilizing (−)-diethyl D-tartrate in the Sharpless epoxidation step and then following a similar course of reaction as described in Scheme II.

Synthesis of various inhibitors with sulfolanes as the P_2 ligand was carried out as shown in Scheme III. Reaction of alcohol **11** with dipyridyl carbonate and triethylamine in methylene chloride afforded the active carbonate **13** after chromatography.[10] Treatment of the active carbonate with known[11] amine **12** in methylene chloride at 23 °C provided only the urethane **14** by HPLC analysis (yield 75%). Selective oxidation of the ring sulfur of compound **14** with a catalytic amount of osmium tetroxide and an excess of 4-methylmorpholine *N*-oxide in a mixture (3:1) of acetone and water furnished the sulfolane derivative **15** (mp 124–26 °C) in 85% isolated yield. The amine **12** was routinely converted to inhibitors **17–19** and **22–28** by following the procedure described above.[12]

As shown in Table I, 3(*S*)-sulfolane derivative **17** exhibited an inhibitory potency of 75 nM, a 2-fold increase over 3(*S*)-tetrahydrofuranylurethane **16**. Sulfolane derivative **18** with a 3*R* configuration showed an IC_{50} value of 140 nM. Interestingly, the preference for the 3*S* configuration by this S_2 binding region is consistent with our earlier observation with the 3-tetrahydrofuranylurethanes.[1] An examination of open-chain sulfone derivative

Table I. Structure and Inhibitory Potencies of Various Sulfone Derivatives

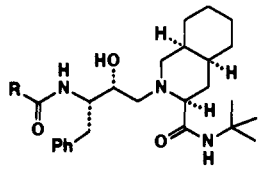


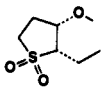
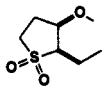
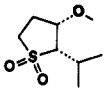
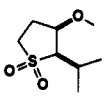
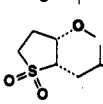
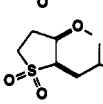
compd	R	IC_{50}[a] (nM)	CIC_{95}[b] (nM)
16		160	800
17		75	350
18		140	>800
19		1030	
20		52.4	400
21		169	1600

[a] Inhibitor Ro 31-8959[3] displayed an IC_{50} value of 0.23 nM (±0.1, n = 3) in this assay system.[16] [b] The CIC_{95} value for Ro 31-8959 was 22 nM (±7, n = 10) in this assay.[17]

19 established that the ring heterocycles are preferred for potency enhancement. The enhanced inhibitory potency of sulfolane **17** relative to compound **16** was also reflected in its antiviral potency. Compound **17** has prevented the spread of HIV-1 in MT4 human T-lymphoid cells infected with IIIb isolate[13] at an average concentration (n = 5) of 350 nM (CIC_{95}), again a 2-fold potency enhancement over compound **16**. Interestingly, however, attachment of a *cis*-2-methyl group[14] in compound **16** improved the enzyme affinity (compound **20**, IC_{50} 52.4 nM) as well as the antiviral potency (CIC_{95} 400 nM)[15] comparable to sulfolane derivative **17**. Furthermore, introduction of a *cis*-2-methyl substituent in the 3(*R*)-tetrahydrofuranylurethane resulted in compound **21** with an IC_{50} value of 169 nM. Although compound **21** is not quite as potent as compound **20**, nevertheless it has gained a greater than 4-fold potency enhancement compared to the 3(*R*)-tetrahydrofuranylurethane (IC_{50} 694 nM) reported previously.[1] The rationale for incorporation of the *cis*-2-methyl group came from examining the preliminary X-ray crystal structure of the enzyme-inhibitor complex of compound **16** and HIV-1 protease.[2] The presence of a *cis*-methyl group in compound **21**, appears to fill in the hydrophobic pocket effectively in the S_2 region of the substrate binding site. Based on this possible insight into the ligand binding site interaction, the effect of various *cis*-2-alkyl substitutions in sulfolane derivatives **17** and **18** were examined. From Table II, it can be seen that *cis*-2-alkylsulfolanes with 2*S*,3*S*- and 2*R*,3*R* configurations indeed yield inhibitors with enhanced enzyme affinity. Incorporation of a *cis*-

Table II. Structure and Inhibitory Potencies of Various Substituted Sulfone Derivatives



compd	R	IC_{50} (nM)	CIC_{95} (nM)
22		11.4	200
23		22.3	200
24		5.4	400
25		13.1	800
26		11.0	200
15		3.0	50
27		12	400
28		30	1500

methyl group in sulfolane **17** afforded the inhibitor **22** with an IC_{50} value of 11.4 nM, a greater than 6-fold potency enhancement over the corresponding unsubstituted derivative. Similarly, introduction of a *cis*-2-methyl substituent in sulfolane **18** resulted in (compound **23**; IC_{50} 22.3 nM) over a 6-fold increase in enzyme affinity compared to sulfolane **18**. Further increase in size of the alkyl group to ethyl group (compounds **24** and **25**) resulted in a further improvement (roughly 2-fold) in enzyme affinity (IC_{50} 5.4 and 13.1 nM, respectively). Unlike the methyl substitution, the ethyl homologues showed reduction in antiviral potencies. Further increase to propyl group indicated no further improvement in enzyme affinity or antiviral potency. However, going from ethyl to a branched chain isopropyl group resulted in a significant effect on the enzyme affinity as well as antiviral potency for 2*R*,3*R* isomer (compound **15**). As shown, sulfolane derivative **15** has an IC_{50} value of 3 nM and more importantly, an antiviral activity of 50 nM (n = 3). Further increase to an isobutyl group did not increase enzyme inhibitory activity. For example, isobutyl group with 2*S*,3*S* or 2*R*,3*R* configurations (compounds **27** and **28**) exhibited IC_{50} values of 12 and 30 nM, respectively. It should be noted that the IC_{50} values of the sulfolane derivatives were in general 3–10 times greater than the corresponding sulfides. Thus compound **14** displayed an IC_{50} of 9 nM. Furthermore, the corresponding cyclic sulfides of compounds **27** and **28** have exhibited IC_{50} values of 69 and 117 nM, respectively. One possible explanation for these results is that the sulfolane oxygens are making specific interactions in the S_2 binding domain of the HIV-1 protease.[18]

In summary, urethanes of *cis*-3-hydroxy-2-alkylsulfolanes are a novel class of high affinity ligands for the S_2 substrate binding site of HIV-1 protease. For easy access to this class of ligands in optically pure form, a general and convenient synthetic route has been developed. Of particular interest, *cis*-2-isopropylsulfolane containing inhibitor **15** is a potent inhibitor of HIV protease (for HIV-1, IC_{50} 3 nM; for HIV-2,[19] IC_{50} 17 nM). In compound **15**, both the P_2 asparagine and the P_2 quinoline moieties of present clinical candidate Ro 31-8959 have been effectively replaced with a novel sulfolane ligand, providing an inhibitor of comparable in vitro antiviral potency. Further investigations, particularly the effects of substitution at other positions of the sulfolane ring and incorporation of heteroatoms in the alkyl side chain, are currently in progress.

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Supplementary Material Available: Experimental procedures and spectral data for compounds **2**–**15** and melting point, elemental analysis and mass spectral data for compounds **17**–**28** (13 pages). Ordering information is given on any current masthead page.

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(2) Modeling studies were performed by visual inspection using a preliminary model of the X-ray crystal structure of the inhibitor **16** complexed with the HIV-1 protease at 2.5-Å resolution provided by Dr. Paula Fitzgerald, Department of Biophysical Chemistry. The final coordinates of the fully refined structure will be published in due course. To date, no small *cis*-2-alkyl substituent offers the same level of potency in the 3-hydroxytetrahydrofuran series, presumably as a result of the inherent decreased affinity of the parent heterocycle.

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(9) Interestingly, the mixture ratio depends on the nature of the protecting group. When the hydroxyl group was protected as tetrahydropyranyl ether, the ratio of compound **9** and **10** was 1:1 ratio (76% isolated yield).

(10) (a) Ghosh, A. K.; Duong, T. T.; McKee, S. P. Di(2-Pyridyl) Carbonate Promoted Alkoxycarbonylation of Amines: A Convenient Synthesis of Functionalized Carbamates. *Tetrahedron Lett.* **1991**, *32*, 4251–54. (b) Ghosh, A. K.; Duong, T. T.; McKee, S. P. N,N'-Disuccinimidyl Carbonate: A Useful Reagent for Alkoxycarbonylation of Amines. *Tetrahedron Lett.* **1992**, *33*, 2781–84.

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(12) Proton NMR and infra red spectra are consistent with assigned structures. Satisfactory (±0.4%) elemental analysis were obtained for all compounds and all melting points were determined on a Thomas-Hoover capillary melting apparatus and are uncorrected.
(13) For assay protocol, see: Thompson, W. J.; Fitzgerald, P. M. D.; Holloway, M. K.; Emini, E. A.; Darke, P. L.; McKeever, B. M.; Schleif, W. A.; Quintero, J. C.; Zugay, J. A.; Tucker, T. J.; Schwering, J. E.; Homnick, C. F.; Nunberg, J.; Springer, J. P.; Huff, J. R. Synthesis and Antiviral Activity of a Series of HIV-1 Protease Inhibitors with Functionality Tethered to the P_1 or P_1' Phenyl Substituents: X-ray Crystal Structure Assisted Design. *J. Med. Chem.* **1992**, *35*, 1685–01 and references cited therein.
(14) Commercially available methyltetrahydrofuran-3-one was reduced by DIBAL-H in THF at −78 °C. The resulting racemic cis-3-hydroxy-2-methyltetrahydrofuran was resolved and utilized in the preparation of compounds **20** and **21**.
(15) Unless otherwise indicated all determinations were $n = 1$.
(16) Heimbach, J. C.; Garsky, V. M.; Michelson, S. R.; Dixon, R. A.; Sigal, I. S.; Darke, P. L. Affinity Purification of the HIV-1 Protease. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 955–60.
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(18) X-ray crystal structure of a protein–ligand complex of compound **15** and HIV-1 protease in progress.
(19) (a) The HIV-2 PR (ROD) was expressed with the same system as the HIV-1 PR which was previously described: Darke, P. L.; Leu, C.-T.; Davis, L.; Heimbach, J. C.; Diehl, R. E.; Hill, W. S.; Dixon, R. A. F.; Sigal, I. S. Human Immunodeficiency Virus Protease: Bacterial Expression and Characterization of the Purified Aspartic Protease. *J. Biol. Chem.* **1989**, *264*, 2307-12. (b) The HIV-2 PR (ROD) was purified with the same methods as the HIV-1 PR which was previously described: see ref 16.