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NOVEL HIV-1 PROTEASE INHIBITORS CONTAINING A β-HYDROXY SULFIDE ISOSTERE.

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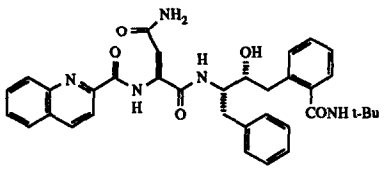
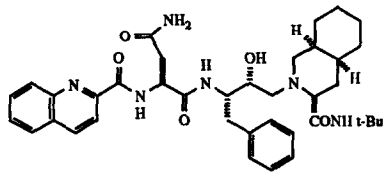
Abstract: A series of analogues of **LY289612** were prepared as HIV-1 protease inhibitors and evaluated *in vitro* for their enzyme inhibitory and antiviral activities. Compound **1**, a potent HIV-1 protease inhibitor, and its analogues were synthesized from key intermediate **9**, which was prepared by alkylation of mercaptan **4** with α-bromo ketone **7** followed by stereoselective reduction of the ketone intermediate **8**.

HIV-1 protease has been shown to be an essential enzyme in the life cycle of the Human Immunodeficiency Virus (HIV), and thus is an attractive target for AIDS therapy.[1] With several HIV-1 protease inhibitors already in clinical trials, e.g., **Ro-31-8959**,[2] researchers are continuing efforts to identify more potent compounds with enhanced pharmacological properties. The discovery of **LY289612**, a potent HIV-1 protease inhibitor, has prompted us to pursue analogues of this compound.[3] X-ray crystallographic studies[4] of **LY289612** established that the disubstituted phenyl moiety fits in the S1' subsite, the hydroxyl moiety hydrogen bonds to the active site aspartate residues, and the t-butyl carboxamide moiety makes an important hydrogen bond to the flap water present in the active site.[5] Furthermore, it was evident that the S1' subsite was not completely filled, as this region of the enzyme is able to accommodate the tetrahydroisoquinoline moiety found on **Ro-31-8969**. Another noteworthy feature of **Ro-31-8969** is the nitrogen heteroatom adjacent to the hydroxy ethylene isostere. We thought it would be interesting to replace the nitrogen heteroatom with sulfur, giving rise to a β-hydroxy sulfide isostere. Inspection of Drieding models suggested that a compound like **1**[6] would perhaps better fill the S1' subsite than **LY289612**, yet maintain the ability of the t-butylcarboxamide to hydrogen bond the flap water molecule and incorporate a heteroatom near the hydroxyl moiety. Herein we report on the synthesis and HIV-1 protease inhibition and antiviral activity of **1** as well as several analogues.



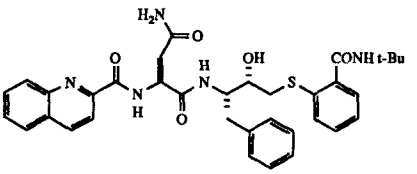
LY289612



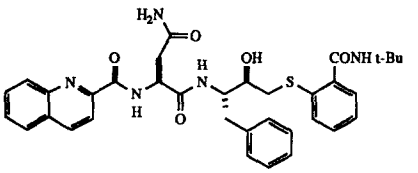
Ro-31-8959

We desired a synthetic approach which would allow ready access to analogue production. Retrosynthetic analysis suggested the molecule be dissected into three parts: quinaldic acid,

asparagine, and the β-hydroxy sulfide isostere **9**. Furthermore, we could not *a priori* predict the optimal stereochemistry of the hydroxyl moiety for enzyme inhibition,[7] thus we opted to access both isomers via reduction of a single ketone intermediate **8**. We expected, however, that the *S* isomer at the hydroxyl center would be the more active isomer based on the activity of **LY289612** and its hydroxyl isomer.



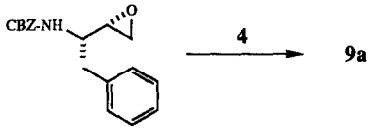
1



15

The mercaptan **4** was prepared in two steps from 2,2'-dithiosalycylic acid, **2** (Scheme 1). Treatment of **2** with oxalyl chloride and a catalytic amount of DMF followed by addition of t-butyl amine and triethylamine afforded the bisamide **3** in 55% yield.[8] This was reduced quantitatively to mercaptan **4** by treatment with zinc dust, acetic acid, HCl, and mild heating for 1 hr.

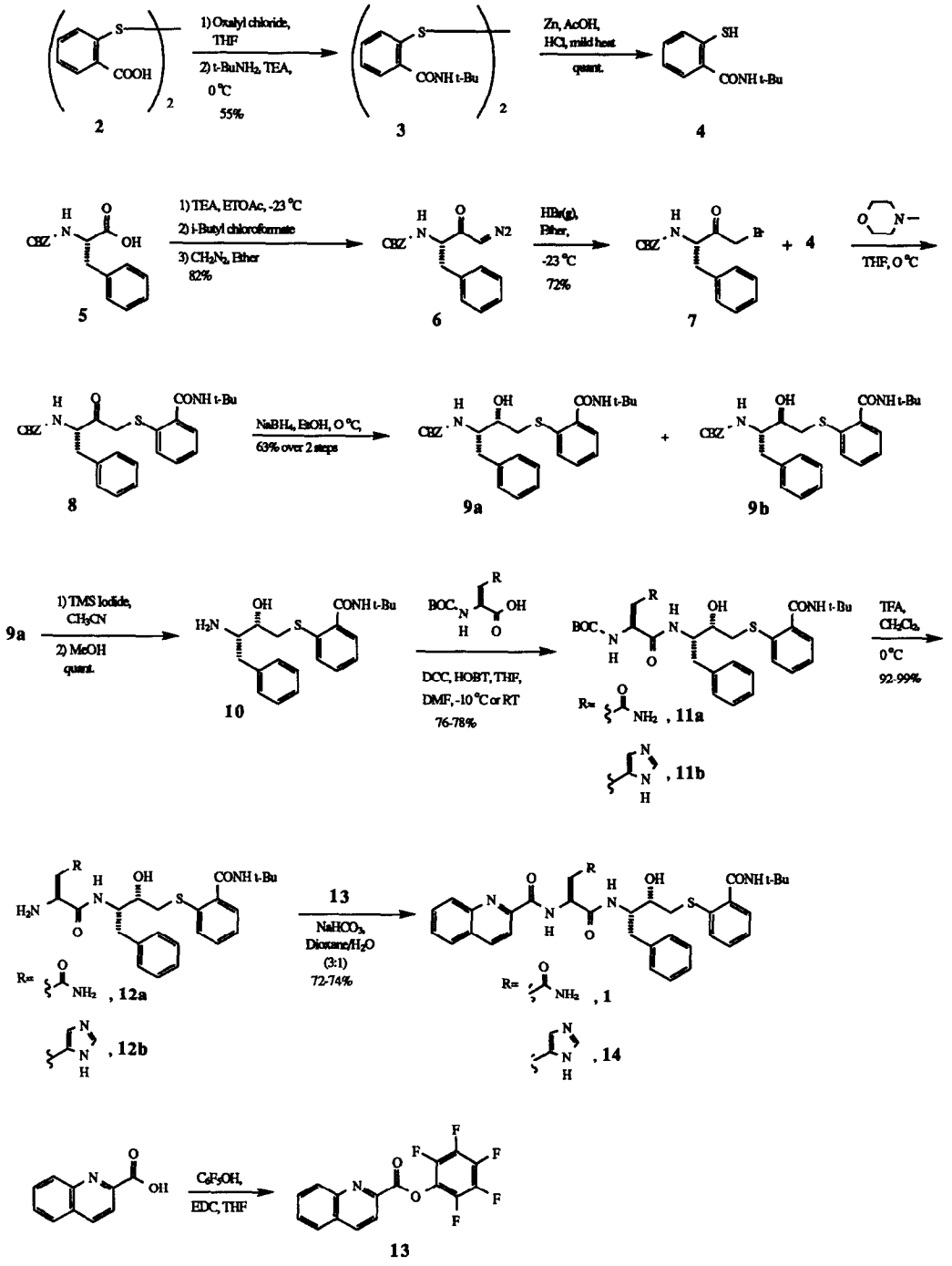
CBZ-L-phenylalanine, **5**, was converted to the mixed anhydride (Et_3N, isobutyl chloroformate) then alkylated with diazomethane to give the diazoketone **6** as a yellow solid in 82% yield after crystallization. Treatment of **6** with anhydrous HBr afforded crystalline bromoketone **7** in 72% yield.[9] Alkylation of **7** with mercaptan **4** gave sulfide **8** which was directly reduced without purification with $NaBH_4$ to give two diastereomeric alcohols **9a** and **9b** (approx. 3:1, 63% yield over 2 steps). The stereochemical assignment of **9a** was initially based upon the similarity of its proton NMR spectrum with that of **LY289612**, particularly the region between δ2.40-δ3.40 which contains the resonance for the methine proton on the hydroxyl bearing carbon as well as the adjacent methylene protons.[3] The stereochemistry was unambiguously confirmed by reacting the known epoxide **18** with mercaptan **4** to give **9a**.[6,10]



18

We were unable to remove the CBZ protecting group via catalytic hydrogenation due to the poisoning of the catalyst by the sulfur atom. Thus, **9a** was treated with excess TMS iodide to provide amine **10** in essentially quantitative yield. Amine **10** was coupled with either t-BOC-L-asparagine or t-BOC-L-histidine (DCC, HOBT) to give **11a** and **11b**, respectively (76-78% yield). The BOC protecting group was then removed with TFA to provide amines **12a,b** in high yield. Amines **12a** and **12b** were acylated with the pentafluoro ester of quinaldic acid, **13**, to give 1 and 14, respectively, in 72-74% yield. The activated ester, **13**, was prepared by coupling quinaldic acid with pentafluorophenol with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

Scheme 1


1) Oxalyl chloride, THF
2) t-BuNH2, TEA, 0 °C
55%
Zn, AcOH, HCl, mild heat
quant.
1) TEA, EtOAc, -23 °C
2) i-Butyl chloroformate
3) CH2N2, Ether
82%
HBr(g), Ether, -23 °C
72%
THF, 0 °C
NaBH4, EtOH, 0 °C,
63% over 2 steps
1) TMS Iodide, CH3CN
2) MeOH
quant.
DCC, HOBT, THF, DMF, -10 °C or RT
76-78%
TFA, CH2Cl2, 0 °C
92-99%
NaHCO3, Dioxane/H2O (3:1)
72-74%
C6F5OH, EDC, THF

Scheme 2

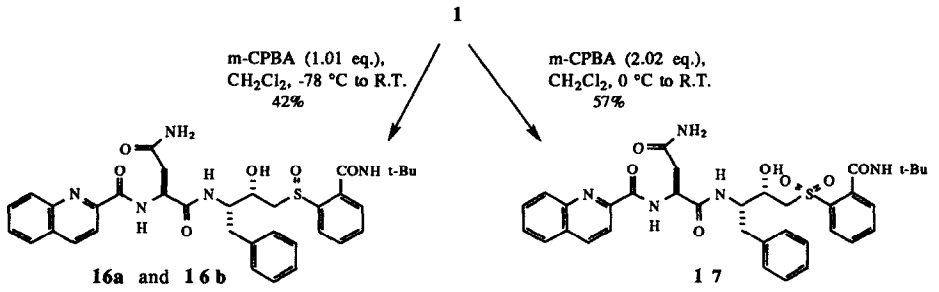
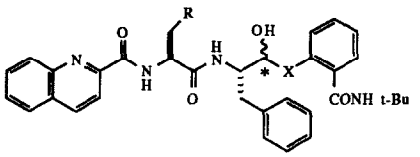


Table 1



	R	X	*	Enzyme Inhib. IC_{50} (ng/ml)	Antiviral Activity CEM IC_{50} (ng/ml)	MT2
LY289612	$-CONH_2$	$-CH_2-$	R	1.40±1.26(n=5)	13.67±9.47(n=42)	31.70±14.52(n=34)
1	$-CONH_2$	$-CH_2S-$	S	8.57±2.97(n=3)	480	410
14	imidazole	$-CH_2S-$	S	138	NT	NT
15	$-CONH_2$	$-CH_2S-$	R	36	4990	4380
16a	$-CONH_2$	$-CH_2SO-$	S	69% inhib. @ 1 μg/ml	NT	NT
16b	$-CONH_2$	$-CH_2SO-$	S	28% inhib. @ 1 μg/ml	NT	NT
17	$-CONH_2$	$-CH_2SO_2-$	S	343	NT	NT

Results are a single determination unless otherwise noted. NT=Not Tested.

The *R* hydroxyl isomer, **15**, was synthesized from **9b** via the same route. The sulfoxides **16a** and **16b** and sulfone **17** were readily prepared by the oxidation of analogue **1** with m-CPBA (Scheme 2). Although the two sulfoxide isomers were separated by preparative reverse-phase HPLC,[11] no attempt was made to assign stereochemistry.

The six compounds (**1**, **14**, **15**, **16a,b**, and **17**) were evaluated for their ability to inhibit the isolated HIV-1 protease enzyme,[12] and the most potent enzyme inhibitors, **1** and **15**, were tested for whole cell antiviral activity.[13] The data for **LY289612** are reported to serve as a comparison (Table 1). Overall, the sulfide isosteres were significantly less active enzyme inhibitors relative to **LY289612**. Compounds **1** and **15** demonstrated the best activity in the enzyme inhibition assay,

IC_{50} of 8.57 ng/ml and 36 ng/ml, respectively. As expected, **1** was more active than **15**, a result consistent with the trend shown by **LY289612** and its *S* hydroxyl isomer. Noteworthy is the fact that **1** is only four-fold more potent than **15** as an enzyme inhibitor, as many other HIV protease inhibitors exhibit a much greater difference in activity between hydroxyl epimers.[7] It is interesting to note that both sulfoxide isomers **16a,b** were only weakly active against the isolated protease enzyme while the corresponding sulfone **17** was a somewhat better inhibitor than either sulfoxide isomer. Furthermore, the imidazole analogue **14** was 16 fold less active than **1**. Similarly, **1** was a more potent antiviral agent than its isomer **15**. However, both β-hydroxy sulfide isostere compounds were less potent than **LY289612**.

Unfortunately, we have not been able to obtain an X-ray crystal structure of **1** bound to the active site of the HIV-1 protease enzyme in order to critically evaluate its interaction with the enzyme's active site compared to **LY289612**, thus making it difficult to predict how one might improve the enzyme inhibition activity of these novel β-hydroxy sulfide isosteres. While these prototypical analogues of **LY289612** demonstrate somewhat reduced anti-HIV activity, we continue to pursue this series of compounds and additional results will be reported in due course.

Acknowledgments:

We wish to recognize the efforts of Theresa Gygi, Joe Manetta and Joe Colacino for assistance in obtaining enzyme inhibition data.

References and Notes:

1. Debouck, C. *AIDS Res Hum. Retrov.*, **1992**, 8(2), 153-164.

2. (a) Martin, J. A. *Drugs of the Future*, **1991**, 16(3), 210-212. (b) Martin, J. A. *Drugs of the Future*, **1993**, 18(3), 286-7. (c) Enzyme inhibitory IC_{50}<0.4 nM, antiviral activity IC_{50}=2 nM as reported by Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Kröhn, A.; Lambert, R. W.; Merrett, J. H.; Mills, J. S.; Parker, K. E. B.; Redshaw, S.; Ritchie, A. J.; Taylor, D. L., Thomas, G. J.; Machin, P. J. *Science*, **1990**, 248, 358-361.

3. Kaldor, S. W.; Hammond, M.; Dressman B.; Schevitz, R. W.; Wery, J. P.; Clawson D. A.; Appelt, K. *Abstracts of Papers*, 205th National Meeting of the American Chemical Society, Chicago, IL; American Chemical Society: Washington, D. C., **1993**; MED 138.

4. Kaldor, S. W.; Hammond, M.; Dressman B.; Fritz, J. E.; Crowell, T. A.; Schevitz, R. W.; Wery, J. P.; Clawson D. A.; Appelt, K. *Abstracts of Papers*, 205th National Meeting of the American Chemical Society, Chicago, IL; American Chemical Society: Washington, D. C., **1993**; MED 144.

5. For a more detailed description of the active site of HIV-1 protease, see: Miller, M.; Schneider, J.; Sathyanarayana, B. K.; Toth, M. V.; Marshall, G. R.; Clawson, L.; Selk, L. M.; Kent, S. B. H.; Wlodawer, A. *Science*, **1989**, 246, 1149-1152.

6. After this work was completed, a World Patent application, WO 93/04043, was published by the Schering Plough Corp. which described an alternate approach to this compound and related analogues.

7. Rich, D. H.; Sun, C.; Prasad, J. V. N. V.; Pathiasseril, A.; Toth, M. V.; Marshall, G. R.; Clare, M. M Mueller, R. A.; Houseman, K. *J. Med. Chem.*, **1991**, 34, 1222-1225.

8. Satisfactory spectral data were obtained for all new compounds. Structure and purity determinations were based upon TLC, HPLC, proton NMR, mass spectra, IR, and either elemental analysis or FAB exact mass spectra.
1: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9 H), 2.64-2.81 (m, 2 H), 2.85-2.94 (m, 2 H), 3.07 (dd, 1 H, J=4.4, 14.0 Hz), 3.16 (dd, 1 H, J=2.4, 14.0 Hz), 3.56 (m, 1 H), 4.26 (m, 1 H), 4.92 (m, 1 H), 5.32 (br s, 1 H), 6.00 (br s, 2 H), 6.80 (d, 1 H, J=9.0 Hz), 6.91 (d, 1 H, J=7.2 Hz), 6.99 (t, 2 H, J=7.2 HZ), 7.13 (d, 2 H, J=7.0 Hz), 7.29-7.39 (m, 4 H), 7.58 (d, 1 H, J=7.4 Hz), 7.68 (m, 1 H), 7.82 (m, 1 H), 7.92 (d, 1 H, J=7.9 Hz), 8.21 (d, 1 H, J=8.6 Hz), 8.30 (ABq, 2 H, J=8.6, 32.4 Hz), 9.14 (d, 1 H, J=7.9 Hz). FD-MS (m/e): 642 (MH^+, 100). IR (KBr): 3323, 1657, 1517, 1500 cm^{-1}. Analysis calculated for $C_{35}H_{39}N_5O_5S_1$: C, 65.50; H, 6.12; N, 10.91; found: C, 65.29; H, 6.18; N, 10.77.
15: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (s, 9 H), 2.71-3.03 (m, 6 H), 3.58 (d, 1 H, J=9.0 Hz), 4.17 (m, 1 H), 4.99 (m, 1 H), 5.41 (s, 1 H), 5.98 (s, 1 H), 6.13 (s, 1 H), 7.00 (d, 1 H, J=7.0 Hz), 7.05-7.15 (m, 5 H), 7.25-7.36 (m, 4 H), 7.47 (d, 1 H, J=6.4 Hz), 7.66 (t, 1 H, J=7.4 Hz), 7.81 (t, 1 H, J=7.4 Hz), 7.90 (d, 1 H, J=8.1 Hz), 8.21 (d, 1 H, J=8.5 Hz), 8.29 (AB q, 2 H, J=8.5, 28.0 Hz), 9.16 (d, 1 H, J=8.1 Hz). FD-MS (m/e): 641 (M^+, 100). IR (KBr): 3335, 2968, 2927, 1657, 1517, 1500, 1455, 1428 cm^{-1}. Analysis calculated for $C_{35}H_{39}N_5O_5S_1$: C, 65.50; H, 6.12; N, 10.91; found: C, 65.61; H, 6.02; N, 10.66.

9. Close monitoring of the reaction via TLC was essential. Over-addition of HBr was found to cleave the CBZ protecting group.

10. Getman, D. P.; DeCrescenzo, G. A.; Heintz, R. M.; Reed, K. L.; Talley, J. J.; Bryant, M. L.; Clare, M.; Houseman, K. A.; Marr, J. J.; Mueller, R. A.; Vazquez, M. L.; Shieh, H.; Stallings, W. C.; Stegman, R. A. *J. Med. Chem.*, **1993**, 36, 288-291.

11. Separation of the sulfoxide isomers **16a** and **16b** was accomplished by preparative reverse-phase HPLC using a Waters LC4000 system with NovaPak C_{18} radial compression cartridge system (25mm X 200mm) running 60% methanol/40% H_2O at 40 ml/min. All fractions were assayed for purity using an analytical HPLC system with a NovaPak C_{18} steel column (3.9mm X 150mm) running 63% methanol/37% H_2O at 1.0 ml/min. Retention times are reported as observed in the analytical trace before separation: **16a** R_T=6.56 min, **16b** R_T=8.03 min.

12. Manetta, J. V.; Lai, M.-H. P.; Osborne, A. D.; *et al. Anal. Biochem.*, **1992**, 202, 10-15.

13. Testing done at the Southern Research Institute, Birmingham, Alabama according to the method of Weislow, O. S.; Kiser, R.; Fine, D. L.; *et al. J. Natl. Cancer Inst.*, **1989**, 81(8), 577-586.

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