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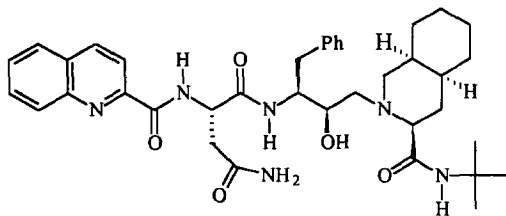
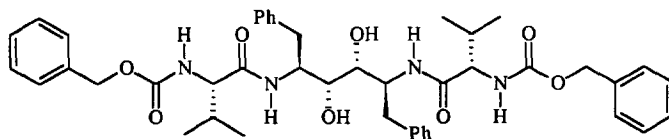
β -METHANESULFONYL-L-VALINE AS A NOVEL, UNNATURAL AMINO ACID SURROGATE FOR P₂ IN THE DESIGN OF HIV PROTEASE INHIBITORS.

Chihyo Park, Hoil Choi, Young-Chan Son, Chang Sun Lee, Nakyeon Choy,
Jong Sung Koh, Tae Gyu Lee, Young Do Kwon, Sung Chun Kim*, Heungsik Yoon*

*Biotech Research, LG Chemical LTD./Research Park, P.O. Box 61 Yu Sung,
Science Town, Taejon 305-380, Korea.*

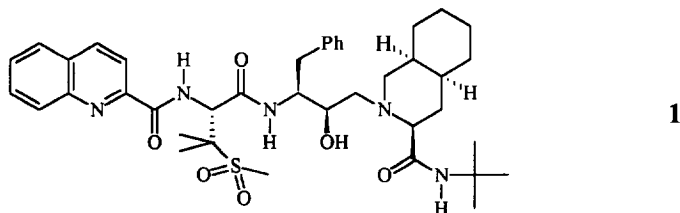
Abstract. β -Methanesulfonyl-L-valine as a surrogate for asparagine and valine was designed, synthesized, and characterized. A representative compound **1** showed IC₅₀ of 4.9 nM.

The human immunodeficiency virus encodes a proteinase which is responsible for the processing of polyprotein products of the *gag* and *pol* genes into their mature forms.¹ Genetic inactivation of the protease produced noninfectious viral particles with immature morphology and dramatically reduced reverse transcriptase activity.² Accordingly, chemical inhibition of this critical enzyme is regarded as a promising approach for the treatment of AIDS and related diseases.³ Starting from the modification of the substrate cleavage site, a number of potent transition state analogues such as **Ro 31-8959** and **A75925** have been developed with success.⁴

**Ro 31-8959****A75925**

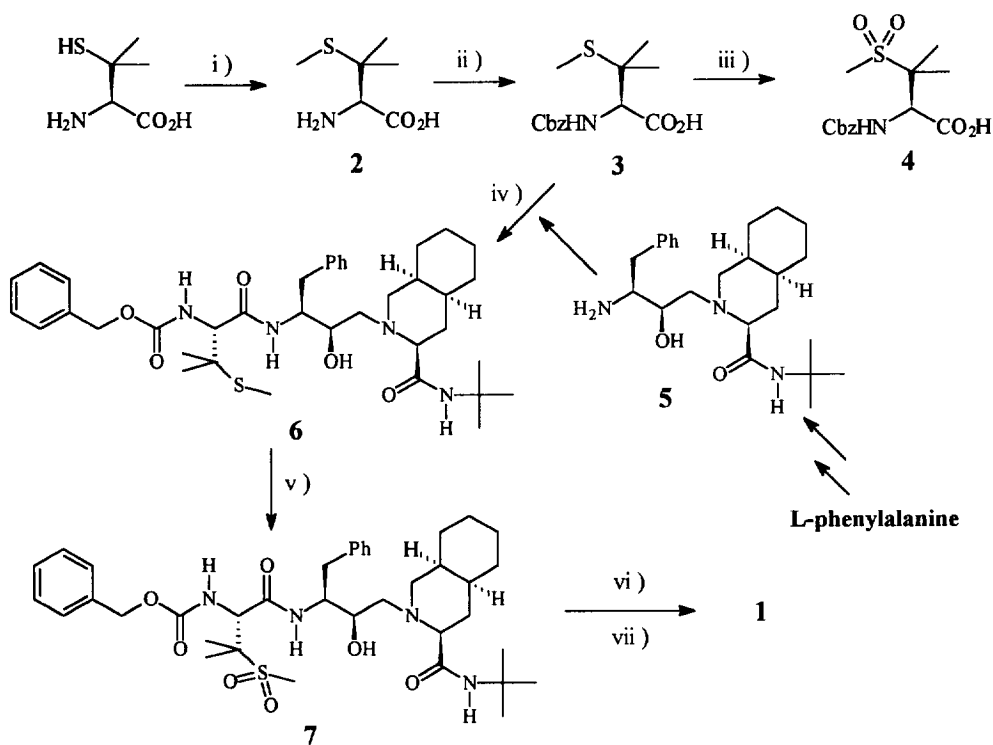
Besides the C₂ symmetric character of **A75925**, there is a big difference between **Ro 31-8959** and **A75925** especially in the P₂ site: while the carbonyl oxygen of the asparagine side chain in **Ro 31-8959** made hydrogen bonding to the NH of the Asp30 residue of the enzyme (hydrophilic), the valine side chain of **A75925** had no hydrogen bonding sources (hydrophobic). This difference prompted us to postulate that P₂ site have amphiphilic character and the pocket size of P₂ be rather big to accommodate the asparagine and valine

side chain at the same time. To test this concept, the following β -methanesulfonyl-L-valine was designed as shown in compound 1. In this new surrogate, two oxygens of the sulfone were introduced for hydrogen bonding with Asp30 and dimethyl group at the β position was for hydrophobic interaction.



The synthetic route leading to compound 1 (LB-71206) is outlined in Scheme 1.

Scheme 1^{a, b}



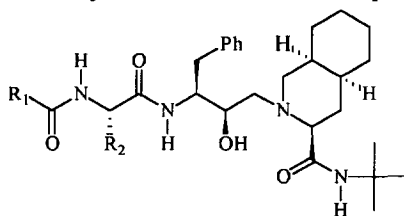
^a Cbz, benzyloxycarbonyl.

^b Reaction condition: (i) CH_3I , NaOMe, (ii) CbzCl, (iii) mCPBA, CH_2Cl_2 , (iv) EDC, HOBT, DMF, (v) OsO_4 , 4-methylmorpholine N-oxide, (vi) Pd/C, H_2 , (vii) quinaldic acid, EDC, HOBT, DMF.

As shown, N-benzyloxycarbonyl-S-methyl-L-penicillamine **3** was prepared from L-penicillamine by S-methylation with iodomethane⁵ and subsequent N-protection with benzylchloroformate. Coupling of **3** with known amine **5** which was prepared from L-phenylalanine⁶ provided **6** in high yield. Selective oxidation of the sulfur of **6** 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 sulfone **7** in 75% isolated yield. Compound **7** not only served as a key intermediate for the preparation of other inhibitors, but also worked as a potent HIV protease inhibitor itself. Another attempted route to prepare **7**, coupling of **4** with **5** by the EDC/HOBT method resulted in the elimination of the sulfone to give dihydrovaline derivatives only. Coupling of the deprotected amine from **7** with quinaldic acid afforded the target compound **1** (LB-71206) as a white solid.⁷

The binding properties (K_i) of the inhibitors were determined by tight binding inhibition assays according to the equation $I_i/(1-v_i/v_0) = E_t + K_i[(S + K_M)/K_M]v_i/v_0$, where I_i was initial inhibitor concentration, v_i was velocity at inhibitor concentration, v_0 was velocity in the absence of inhibitor, E_t was total enzyme concentration, and S was substrate concentration.⁸

Table: Structure and Inhibitory Potencies of Various Compounds.



LB #	R ₁	R ₂	K _i (nM)	CIC IC ₅₀ (nM)	CIC IC ₅₀ /K _i
Ro-31-8959	2-quinolinecarbonyl	Asn	0.452	23	51
LB-71351	2-quinolinecarbonyl	Val	0.550	54	98
LB-71206	2-quinolinecarbonyl	β -methylsulfonyl-L-valine	0.172	4.9	28
LB-71332	2-quinolinecarbonyl	β -thiomethoxy-L-valine	2.59	27	10
LB-71207	benzyloxycarbonyl	β -methylsulfonyl-L-valine	0.162	11	68
LB-71208	5-hydroxyisoquinolinol-methylenecarbonyl	β -methylsulfonyl-L-valine	0.151	19	126
LB-71352	2-quinolinecarbonyl	β -methylsulfonyl-L-alanine	0.113	22	195

As can be seen in the table, β -methanesulfonyl-L-valine and β -methanesulfonyl-L-alanine at P₂ showed higher potency than asparagine(**Ro 31-8959**) and valine at P₂. The introduction of β -thiomethoxy-L-valine instead of β -methanesulfonyl-L-valine in LB-71206 resulted in 5-6 fold decrease in the inhibitory potency, which suggested that the sulfone oxygens make specific interactions in the S₂ binding domain of the HIV protease. The increase of inhibitory potency by introducing hydrogen bonding acceptor such as cyclic sulfolane and tetrahydrofuran was already reported.^{6,9,11} The increase of inhibitory potency was explained by the hydrogen bonding with the NH of the Asp30 amide bond at P₂ site. The increase in size of the alkyl group from β -methanesulfonyl to β -ethanesulfonyl resulted in total loss of its inhibitory potency, which suggested that the

size of the methyl group was optimum for this surrogate. In antiviral effects, the spread of HIV-1 was measured in PBMC infected with NL4-3 isolate. LB-71206, especially, have shown the impressive antiviral activity with CIC IC₅₀ with 4.9 nM. In spite of the improved enzymatic potency, β -methanesulfonyl-L-alanine at P₂ (LB-71352) did not show the improved CIC IC₅₀. The lower CIC IC₅₀/K_i ratio of LB-71206 indicated that β -methanesulfonyl group at P₂ was more suitable for the penetration of cell membrane.

New classes of surrogates for P₂ which have hydrophobic and hydrophylic character at the same time were reported previously.^{6,9,10,11} Although the potencies of the inhibitors containing these amphiphilic surrogates for P₂ were very high, the synthetic route leading to them was very long and tedious compared to our new surrogate.

Thus, a new class of unnatural amino acid surrogate for P₂ of HIV protease inhibitor was rationally designed and synthesized and their structure-activity relationship was studied. This class of inhibitors competitively inhibits HIV protease at subnanomolar concentrations. Studies are in progress to further improve antiviral potency and pharmacokinetic profile in this class of inhibitors.

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References and Notes:

1. Kay, J.; Dunn, B. M. *Biochim. Biophys. Acta.* **1990**, *1048*, 1.
2. (a) Kohl, N. E.; Emini, E. A.; Schleif, W. A.; Davis, L. J.; Heimbach, J. C.; Dixon, R. A.; Scolnick, E. M.; Sigal, I. S. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4686. (b) Peng, C.; Ho, B. K.; Chang, T. W.; Chang, N. T. *J. Virol.* **1989**, *63*, 2550. (c) Adachi, A.; Ono, N.; Sasaki, H.; Okawa, K.; Shibata, R.; Kiyomasu, T.; Masuike, H.; Ueda, S. *Arch. Virol.* **1991**, *117*, 45.
3. (a) Huff, J. R. *J. Med. Chem.* **1991**, *34*, 2305. (b) Debouck, C. *AIDS Res. and Human Retroviruses* **1992**, *8*, 153.
4. Meek, T. D.; Lambert, D. M.; Metcalf, B. W.; Petteway, Jr., S. R.; Dreyer, G. B.; in E. De Clercq "Design of anti-AIDS Drugs", Elsevier, Amsterdam, 1990; pp225-255.
5. Hwang, D.-R.; Helquist, P.; Shekhani, M. S. *J. Org. Chem.* **1985**, *50*, 1264.
6. Thompson, W. J.; Ghosh, A. K.; Holloway, M. K.; Lee, H. Y.; Munson, P. M.; Schwering, J. E.; Wai, J.; Darke, P. L.; Zugay, J.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. *J. Am. Chem. Soc.* **1993**, *115*, 801.
7. All new compounds gave satisfactory FAB MS and ¹H NMR data and were homogeneous by TLC and/or HPLC.
8. Williams, J. W.; Morrison, J. F. *Methods Enzymol.* **1979**, *63*, 563.
9. Ghosh, A. K.; Thompson, W. J.; Lee, H. Y.; McKee, S. P.; Munson, P. M.; Duong, T. T.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. A. *J. Med. Chem.* **1993**, *36*, 924.
10. Ghosh, A. K.; Thompson, W. J.; Fitzgerald, P. M. D.; Culbertson, J. C.; Axel, M. G.; McKee, S. P.; Huff, J. R.; Anderson, P. S. *J. Med. Chem.* **1994**, *37*, 2506.
11. Ghosh, A. K.; Thompson, W. J.; Munson, P. M.; Liu, W.; Huff, J. R. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 83.