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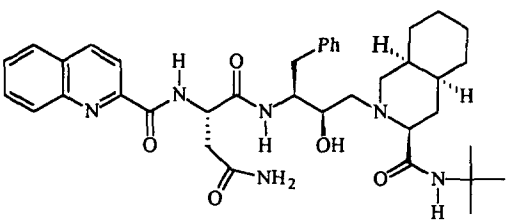
β-METHANESULFONYL-L-VALINE AS A NOVEL, UNNATURAL AMINO ACID SURROGATE FOR P_2 IN THE DESIGN OF HIV PROTEASE INHIBITORS.

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

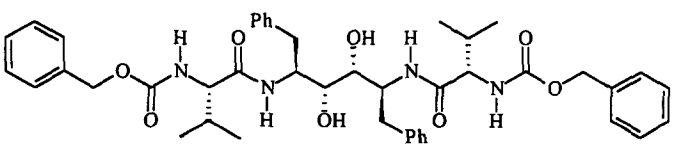
Biotech Research, LG Chemcal LTD./Research Park, P.O. Box 61 Yu Sung, Science Town, Taejon 305-380, Korea.

Abstract. β-Methanesulfonyl-L-valine as a surrogate for asparagine and valine was designed, synthesized, and characterized. A representative compound **1** showed IC_{50} 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]



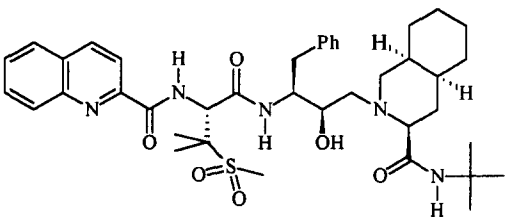
Ro 31-8959



A75925

Besides the C_2 symmetric character of **A75925**, there is a big difference between **Ro 31-8959** and **A75925** especially in the P_2 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 P_2 site have amphiphilic character and the pocket size of P_2 be rather big to accommodate the asparagine and valine

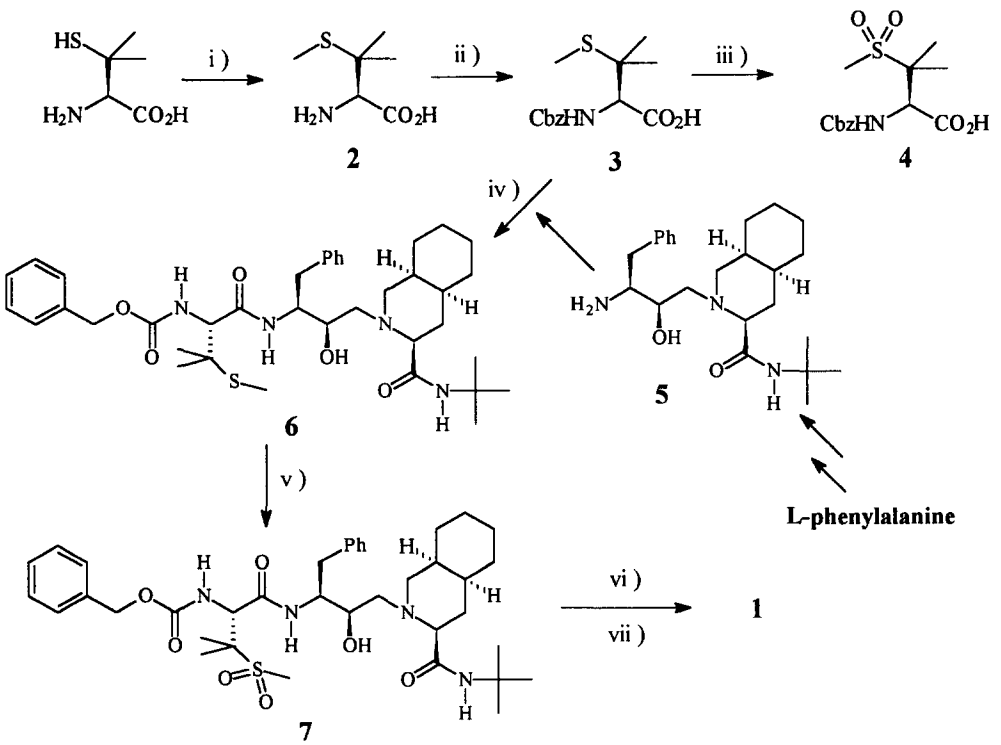
side chain at the same time. To test this concept, the following β-methanesulfonyl-L-valine was designed as shown in compound **1**. In this new surrogate, two oxygens of the sulfone were introduced for hydrogen bonding with Asp30 and dimethyl group at the β position was for hydrophobic interaction.



1

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

Scheme 1[a, b]



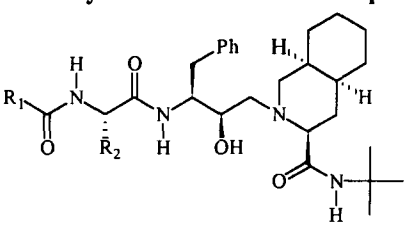
[a] Cbz, benzyloxycarbonyl.

[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 iodomethane[5] and subsequent N-protection with benzylchloroformate. Coupling of **3** with known amine **5** which was prepared from L-phenylalanine[6] 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 $I_t/(1-v_i/v_0) = E_t + K_I[(S + K_M)/K_M]v_i/v_0$, where I_t 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 P_2 showed higher potency than asparagine(**Ro 31-8959**) and valine at P_2. 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 S_2 binding domain of the HIV protease. The increase of inhibitory potency by introducing hydrogen boning 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 P_2 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 IC_{50} with 4.9 nM. In spite of the improved enzymatic potency, β-methanesulfonyl-L-alanine at P_2 (LB-71352) did not show the improved CIC IC_{50}. The lower CIC IC_{50}/K_I ratio of LB-71206 indicated that β-methanesulfonyl group at P_2 was more suitable for the penetration of cell membrane.

New classes of surrogates for P_2 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 P_2 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 P_2 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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