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SYNTHESIS OF IRREVERSIBLE HIV-1 PROTEASE INHIBITORS CONTAINING SULFONAMIDE AND SULFONE AS AMIDE BOND ISOSTERES

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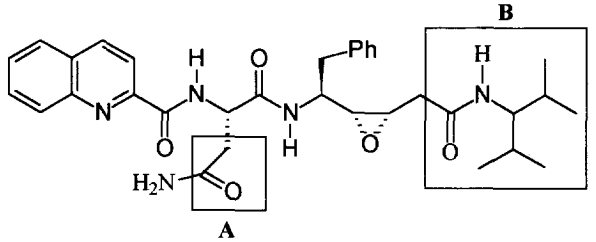
Abstract. Novel irreversible HIV-1 protease inhibitors containing sulfonamide and sulfone as amide bond isosteres were designed, synthesized, and kinetically characterized. A representative compound **2** displayed rapid, time-dependent inactivation of HIV-1 protease and high potency in cell culture with IC_{50} of 6.6 nM.

The human immunodeficiency virus encodes a protease which is responsible for the processing of polyprotein products of the *gag* and *pol* genes into their mature forms.[1] Noninfectious viral particles with immature morphology and dramatically reduced reverse transcriptase activity are formed when the retroviral protease is mutated.[2] Also, cell culture studies using inhibitors of HIV protease have established that this enzyme is essential for viral replication and infectivity.[3] Therefore, chemical inhibition of this critical viral enzyme is regarded as a promising approach for the treatment of AIDS and related diseases.[4]



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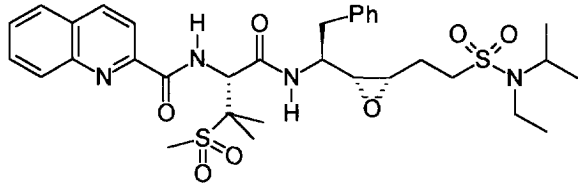
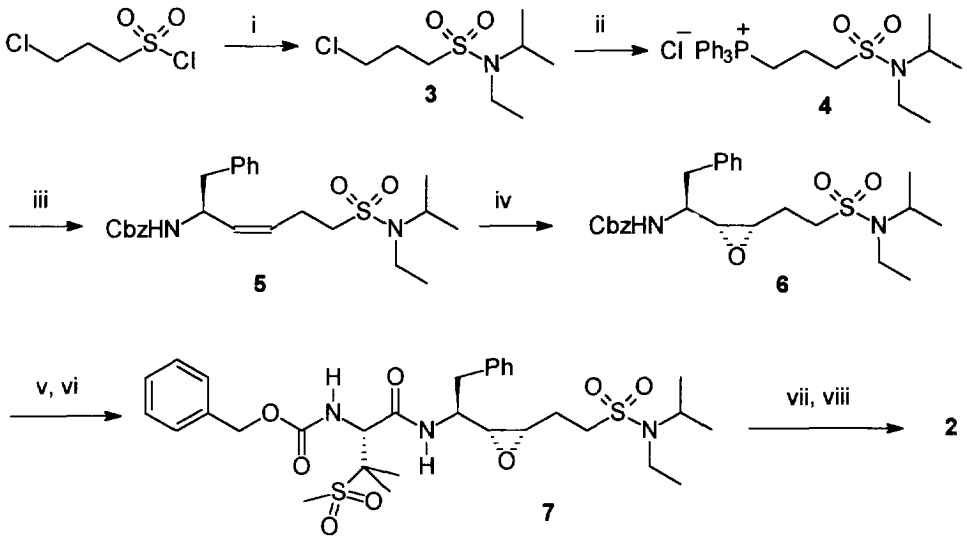


2

Recently, Lee and his coworkers reported an irreversible inhibitor **1**, which contained epoxide[5] as an active site isosteres with IC_{50} of 20 nM in cell culture. The object of this work was to design and synthesize inhibitors with less peptide bonds to improve the potency in cell culture. With this in mind, two additional factors were taken into account for the design of inhibitors. First, the hydrogen bonding between inhibitor and water in enzyme was reported[6] as one of the major factors in determining the binding affinity, so the replacement of the amide carbonyl group of C-terminal in compound **1** with sulfonamide would maintain the hydrogen bonding with water and improve the stability of inhibitors against proteolytic enzymes causing amide bond hydrolysis. Second, amphiphilic character of P_2 site was taken into account for the design of inhibitors. It was reported that the pocket size of P_2 are rather big to accommodate asparagine and valine in a number of potent transition state mimetic inhibitors[7], the replacement of asparagine with the proper amphiphilic group would increase the potency of inhibitors. To apply these concepts, sulfonamide for P_1'-P_2' amide bond and sulfone for P_2 asparagine was designed as shown in compound **2**. The compound **2** was prepared from L-phenylalanine and Wittig salt **4** as shown in Scheme 1.



Scheme 1. Reagents: i) N-ethylisopropylamine, triethylamine, CH_2Cl_2, 93%; ii) Ph_3P, neat, 110 °C, 95%; iii) Cbz-L-phenylalanal, potassium bis(trimethylsilyl)amide, THF, -40 °C to rt, 86%; iv) 3-chloroperoxybenzoic acid, CH_2Cl_2, 77%; v) H_2, Pd/C, methanol; vi) cbz-β-methanesulfonyl-L-valine, iBuOCOCl, N-methylmorpholine, CH_2Cl_2, 67%; vii) H_2, Pd/C, methanol; ix) quinaldic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1-hydroxybenzotriazole hydrate, DMF, 75%.

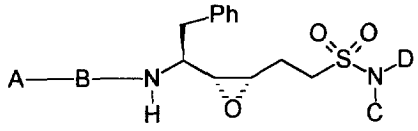
Addition of N-ethylisopropylamine to 3-chloropropanesulfonyl chloride afforded sulfonamide **3**, which was converted to phosphonium salt **4** by treatment with triphenylphosphine. The resulting salt **4** and cbz-L-phenylalanal were coupled at - 40 °C using potassium bis(trimethylsilyl)amide as a base to give olefin **5** in 86% yield. Epoxidation with 3-chloroperoxybenzoic acid of **5** gave the key intermediate **6** as predominantly one isomer. The stereochemistry of **6** was assigned in accordance with literature precedent.[8] Coupling of the deprotected amine with cbz-β-methanesulfonyl-L-valine furnished the key intermediate **7**, which itself was a

potent inhibitor of HIV-1 protease. Finally, deprotection and coupling with quinaldic acid of **7** afforded the target compound **2**.[9]

As expected, the target compound **2** displayed rapid, time-dependent inactivation of HIV-1 protease following pre-incubation of various concentrations of **2** with the enzyme at different time intervals. The calculated bimolecular rate constant[5] for the formation of HIV-1 protease-**2** complex (k_{ina}/K_i) was $2.9x10^7$ $M^{-1}min^{-1}$ compared with $2.0x10^8$ $M^{-1}min^{-1}$ for **1**. The enzyme activity was not recovered after exhaustive dialysis of the above inactivated enzyme, which indicates that the inactivation is irreversible. In addition, the active site titration studies using 0.1-1 molar equivalent of **2** over the concentration of the protease indicated 1:1 stoichiometric binding ratio of the inhibitor to the protease. Therefore, inactivation of HIV-1 protease by **2** involves enzyme-catalyzed alkylation of the unprotonated active site aspartyl residue, in analogy to the inactivation of HIV-1 protease by compound **1**.[5] Also, the covalent bond formation could be inferred from the X-ray crystallographic results of the HIV-1 proteinase with irreversible inhibitor **1**.[10] Compound **2** was tested for human renin and human cathepsin D. Human renin was not inactivated by compound **2** at 10 μM. Human cathepsin D was inhibited by compound **2** reversibly with IC_{50} of 8.2 μM. The results for human renin and human cathepsin D showed that compound **2** inactivated HIV-1 protease with good selectivity.[11]

The compounds in Table 1 were prepared by the similar method to Scheme 1.

Table 1. Structure and Potency of Novel HIV Protease Inhibitors.[a]



Compound	A	B	C	D	K_i (nM)	k_{ina} / K_i ($M^{-1}min^{-1}$) x 10^7	IC_{50} (nM) [12]
2	Qua	MSV	Et	^{i}Pr	7.0	2.9	6.6
7	Cbz	MSV	Et	^{i}Pr	0.8	9.8	10.9
8	Qua	MSV	Et	Ph	5.0	3.9	10.7
9	Qua	Asn	H	^{t}Bu	12.6	1.5	30.0
10	Cbz	MSV	Et	Ph	4.6	4.3	13.8
11	Cbz	MSV	H	^{t}Bu	0.7	8.0	20.2
12	Cbz	MSV	Et	^{i}Bu	2.9	6.9	10.7
13	Cbz	MSV	Bz	^{i}Pr	3.6	5.5	15.0
14	Thc	MSV	Et	^{i}Pr	3.1	6.3	18.2

[a] Cbz, benzyloxycarbonyl; Qua, quinoline-2-carbonyl; Thc, 2-thiophenecarbonyl; MSV, β-methanesulfonyl-L-valine; Asn, L-asparagine.

The 50% inhibition constant (IC_{50}) of **2** was 6.6 nM against HIV-1 in H9 and Sup T1 cell lines as assessed by syncytium formation and reverse transcriptase assay.[12] Most of the compounds listed in the table showed higher IC_{50} than compound **1** (IC_{50}=20 nM), which suggested that the sulfonamide group could be a good surrogate for the amide bond to improve the potency in cell culture. These data also demonstrated the sulfone group could be accommodated in P_2 for the binding of irreversible inhibitors with enzyme.

Thus, the design of **2** was successful in that it produced an inhibitor with high affinity, irreversibility and high potency in cell culture. Studies of partitioning ratio and exhaustive dialysis suggested that the inactivation of

HIV-1 protease by **2** should be due to covalent modification of an active-site residue. Studies are in progress to assess the potential of this novel compound as a chemotherapeutic agent for the treatment of AIDS.

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9. All new compounds gave satisfactory high-resolution FAB MS and ^{1}H NMR data and were homogeneous by TLC and/or HPLC.
10. A part of the results was presented at the *Tenth International Conference on AIDS Abstr. 323A* in Yokohama, Japan, August 10, 1994.
11. Human renin inhibition was studied by radioimmunoassay of released angiotensin I using human blood as renin source and RENIN-RIABEAD kit (Abbott Diagnotics). For human cathepsin D inhibition, cleavage reaction between (p-NO_2)-Phe and Phe of Boc-Phe-Ala-Ala-(p-NO_2)-Phe-Phe-Val-Leu-4-(hydroxymethyl)pyridine ester (BACHEM Chemical Co.) by human cathepsin D was monitored at pH 3.4 using HPLC according to the method of Marossy, K.; Rich, D. H. *Anal. Biochem.* **1983**, *130*, 158.
12. In the assay system used here, the IC_{50} for AZT and Ro-31-8959 were 50 nM and 20 nM, respectively. The 50% cytotoxic value (CT_{50}) of **2** to the host cell lines was higher than 100μM, indicating a high therapeutic index.

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