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Communications to the Editor

New Series of Potent, Orally Bioavailable, Non-Peptidic Cyclic Sulfones as HIV-1 Protease Inhibitors

Choung U. Kim,*,† Lawrence R. McGee,† Steven H. Krawczyk,† Erik Harwood,† Yoko Harada,† S. Swaminathan,† Norbert Bischofberger,† Ming S. Chen,† Julie M. Cherrington,† Shelly F. Xiong,† Linda Griffin,† Kenneth C. Cundy,† Angela Lee,‡ Betty Yu,‡ Sergli Gulnik,‡ and John W. Erickson‡

Gilead Sciences Inc., 353 Lakeside Drive, Foster City, California 94404, and Structural Biochemistry Program, SAIC Frederick, NCI-Frederick Cancer, Research and Development Center, Frederick, Maryland 21702

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Inhibitors of human immunodeficiency virus protease (HIV PR) prevent assembly of viral proteins and maturation of HIV and result in noninfective virions.[1] Despite intensive research effort and a great number of inhibitors reported in the literature, only a few have reached clinical trials mainly due to metabolic instability and low oral bioavailability.[2] Although some clinical results of HIV PR inhibitors for the treatment of AIDS are encouraging, emergence of viral resistance presents a major challenge for the clinical use of this class of drugs.[3] Therefore, there is still an urgent need for new classes of protease inhibitors possessing different binding interactions with protease, which could potentially lead to fewer and/or different resistance development.

HIV PR is an aspartic protease that functions as a homodimer.[4] Inhibitors incorporating C_2-symmetric diols as transition state analogues have been reported to impart high affinity toward the enzyme.[5] In addition to the inhibitory potency, good pharmacological properties (oral bioavailability, metabolic stability, and pharmacokinetics) are essential factors for protease inhibitors to be developed as clinical candidates. For-

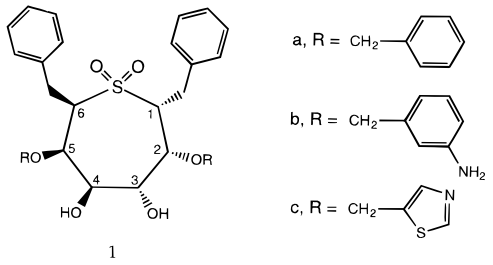

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Figure 1. Structures of protease inhibitors.

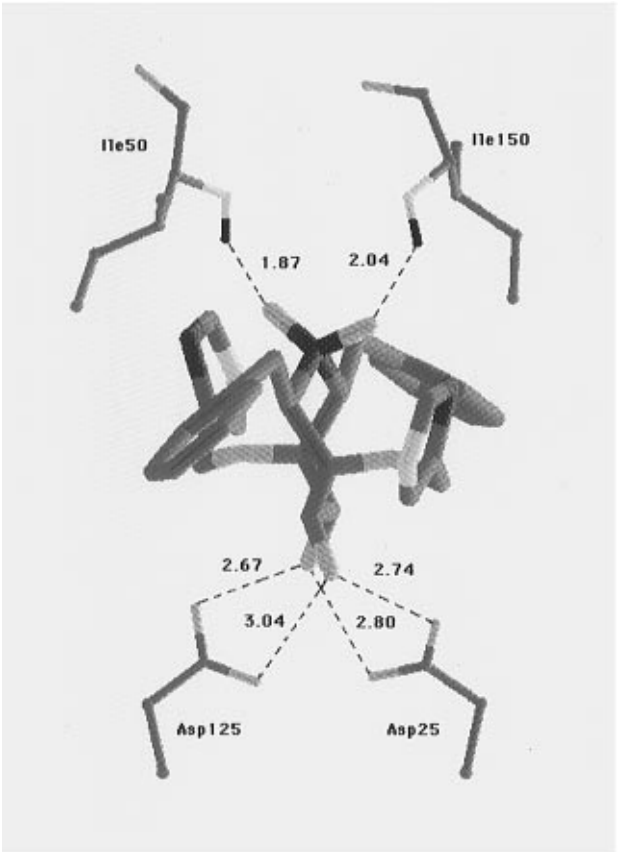

Figure 2. Hydrogen-bonding interaction between **1c** and HIV-1 protease.

mulating these goals, our design strategy had the following requirements: (1) low molecular weight (<600),

† Gilead Sciences Inc.
‡ NCI-Frederick Cancer, Research and Development Center.

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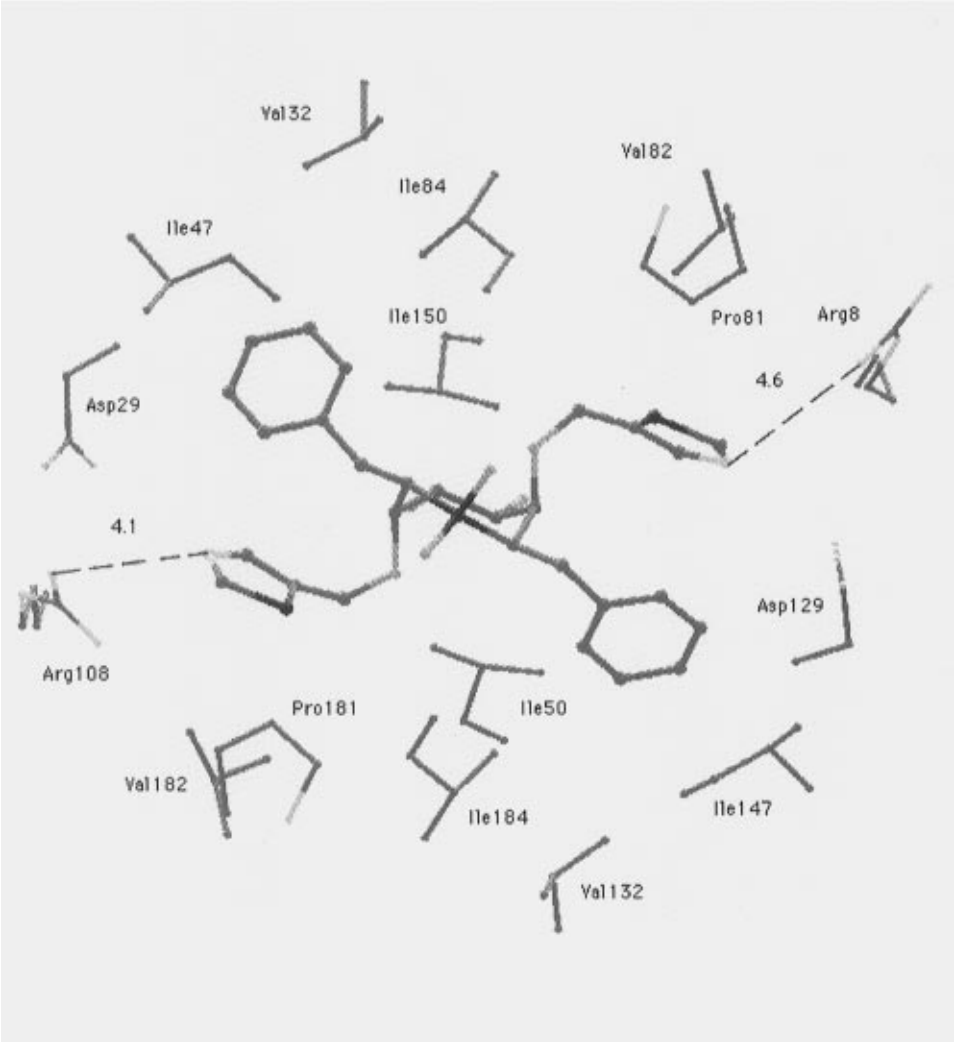

Figure 3. Stereoview of the X-ray structure of **1c** and HIV-1 protease complex.

(2) non-peptidic, (3) C_2-symmetric cyclic structure with 1,2-diol functionality, and (4) some water solubility.[6] Taking into account these factors, together with structural information available from X-ray crystallography of C_2-symmetric diol inhibitors complexed with HIV PR, our molecular modeling studies led to the discovery of the cyclic sulfones **1** as potent, non-peptidic inhibitors of HIV PR.[7] The absence of amide bonds in these structures prevents these molecules from proteolytic cleavage and should lead to pharmacologically superior antiviral agents compared to classical peptide-based inhibitors.

The X-ray crystallographic structure of the inhibitor **1c** complexed with HIV-1 PR at 2.2 Å resolution was obtained.[8] The inhibitor binds to the protease in a highly symmetric fashion revealing many of the stabilizing interactions (Figure 2). The sulfonyl and the two hydroxy groups of the inhibitor provide anchoring hydrogen bonds, while benzyl and thiazolyl side chains gain substantial hydrophobic interactions from the substrate pockets of the protease. The two oxygen atoms of the sulfonyl group form a hydrogen-bonding bridge to the backbone NH atoms (1.87 and 2.04 Å) of the residues Ile50 and Ile150 located at the tip of the flaps of the protease. These interactions replace the structural water 301 which is normally found in complexes with linear peptidomimetic inhibitors.[9] Such replacement and the hydrogen bond bridge may enhance the binding of the inhibitor in terms of both enthalpy and entropy. The replacement of the structural water in the enzyme with the sulfonyl group is a novel extension of DuPont Merck's previously published C_2-symmetric cyclic urea-based HIV PR inhibitors,[10] in which the carbonyl of the urea also replaced water 301.

The OH groups of the C_3 and C_4 atoms straddle the two active site aspartyl carboxylate groups symmetrically. The C_3 OH is 2.67 Å from OD_2 of Asp125 and 2.80 Å from OD_1 of Asp25. The C_4 OH is positioned 2.74 and 3.04 Å from OD_2 and OD_1, respectively, of Asp25 and Asp125. This geometry provides an extensive network of hydrogen bonds among all the oxygen atoms of the carboxylategroups of the protease and the hydroxyl groups of the inhibitor.

The side chain interactions of the inhibitor with the protease are described in Figure 3 in a view which is approximately perpendicular to the view in Figure 2. The C_1 and C_6 benzyl groups fill the S2 and S2′ pockets, while the C_2 and C_5 thiazoyl side chains occupy the S1 and S1′ pockets. All four rings make hydrophobic interactions with the side chains of the protease in these pockets. Ile50 and Ile184 line the S1 and S2′ pockets,

Table 1. Biological Activity of Cyclic Sulfones **1a**−**c** against HIV-1 Virus

compd	IC_{50} (nM)[a]	EC_{50} (nM)[b]/EC_{90} (nM)	CC_{50} (μM)[c]	SI
1a	0.6	40/70	6	150
1b	1.0	9/20	30	3333
1c	0.3	6.5/40	40	6153

[a] Concentration needed to inhibit HIV-1 protease activity by 50%. For the detailed assay, see ref 11. [b] Concentration needed to inhibit HIV-1 virus replication in MT2 cells by 50% as determined by an XTT assay. For the detailed assay, see ref 12. [c] Concentration needed to produce a 50% reduction in the number of viable cells as assayed by metabolism of a tetrazolium dye.

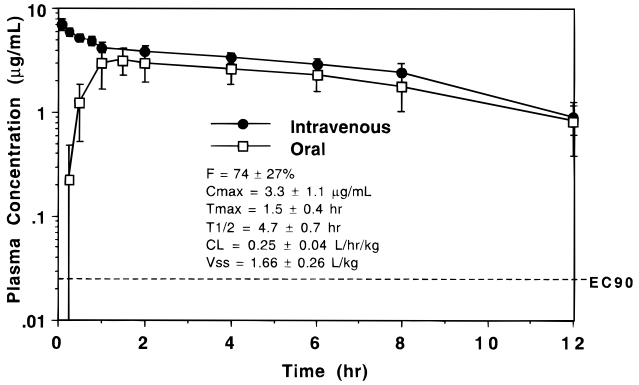

Figure 4. Mean ± SD concentrations of **1b** in plasma following intravenous or oral administration to male beagle dogs at 10 mg/kg.[13]

and Ile150 and Ile84 line the S1′ and S2 pockets. The other major hydrophobic interactions involve Val82 and Pro81 as well as Ile47 and Val32 along with the same groups from the other protease monomer. In addition, there are long range interactions (4.60 and 4.12 Å) between the N atoms of the thiazole rings and Arg8 and Arg108 of the protease. The absolute configuration of the C_1 and C_2 side chains (1*R*,2*R*) presented in structure **1** is critically important for potent antiviral activity. Thus, the 1*R*,2*S* diastereomer of **1a** was 20-fold less active compared to **1a**, and the 1*S*,2*S* isomer exhibited 500-fold reduced activity in the enzymatic assay. Modeling analysis of the 1*S*,2*S* isomer revealed that the (1*S*)-benzyl group is not well-accommodated in the active site where it collides with the flap.

In addition to the benzyl analogue **1a**, the meta aminobenzyl and thiazolyl analogues **1b**,**c** were also prepared to provide variations in lipophilic, electronic, and hydrogen-bonding properties. Both the meta aminobenzyl group in P2 (P2′) positions[16] and the thiazolyl group in P2′ position[17] in HIV PR inhibitors have been reported to impart high antiviral activity. Cyclic sulfones **1a**−**c** exhibited high antiviral potency against HIV-1 as assessed by inhibition of protease in an enzyme assay and viral replication in tissue culture (Table 1). The high level of antiviral activity (EC_{50}) and low cytotoxicity (CC_{50}) provided high selective indices (SI) for compounds **1b**,**c**. In our design goals, aqueous solubility was considered to be important in order to achieve good oral bioavailability. Without reducing antiviral activity, the amino group can be added on to the C_2 (C_5) benzyl phenyls at the meta position. The resulting **1b** had a water solubility of 20 μg/mL at pH 7.0. At a dose of 10 mg/kg in dogs ($n = 5$), compound **1b** had an oral bioavailability of 74% (Figure 4). The duration that the plasma drug concentration remained 40 times above EC_{90} exceeded 12 h. Conversely, compounds **1a**,**c**, which had almost no water solubility (< 1 μg/mL), showed virtually no oral absorption, confirming the importance of balancing water/lipid solubility for oral bioavailability.

In conclusion, we have demonstrated that the seven-membered ring sulfone (thiepane dioxide) served as a conformationally constrained scaffold for the rational drug design of potent HIV PR inhibitors. Certain members of this class represent some of the simplest, most potent HIV PR inhibitors reported to date. Studies are in progress to assess the potential of this novel class of compounds as chemotherapeutic agents for the treatment of AIDS.

Acknowledgment. We would like to thank Dr. John C. Martin for his many contributions and continuing support of this project.

Supporting Information Available: Physical and spectral data for compounds **1a**−**c** (2 pages). Ordering information is found on any current masthead page.

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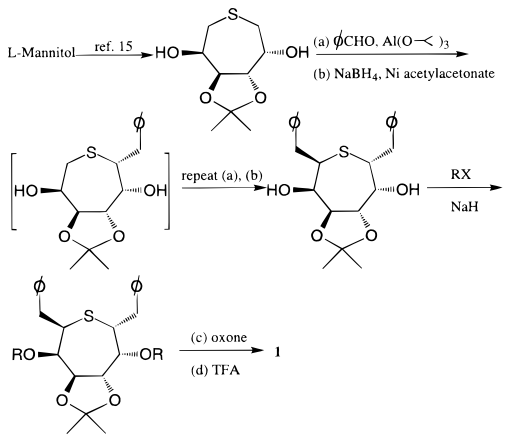
(13) Dog PK study and sample analysis of **1b**: The pharmacokinetics of **1b** were examined in five male beagle dogs (8−12 kg) following intravenous or oral administration. **1b** was formulated as an aqueous solution at pH 2.5 and administered at 10 mg/kg. Each dog received a single intravenous injection via a cephalic vein and a single oral administration by gavage with a 1 week washout period. Blood samples were obtained from a peripheral vein at intervals over 24 h and processed for plasma. Concentrations of **1b** in dog plasma were determined by reverse phase HPLC with UV detection. Plasma samples (100 μL) were mixed with 100 μL of acvetonitrile to precipitate proteins and centrifuged at 2500*g* and 4 °C for 10 min. Supernatant (170 μL) was added to 90 μL of 20 mM potassium phosphate buffer, pH 6.9, and injected onto the HPLC. Detection was by UV absorption at 250 nM. The limit of quantitation was 0.1 μg/mL.

(14) Compounds **1a**−**c** were prepared according to the synthetic sequence shown below. The detailed synthesis including alternative routes will be published separately.



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