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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5614–5619

Discovery of potent HIV-1 protease inhibitors incorporating sulfoximine functionality

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Received 9 May 2007; revised 25 July 2007; accepted 26 July 2007
Available online 23 August 2007

Abstract—Based on the unique property of sulfoximine and the homodimeric C_2 structural symmetry of HIV-1 protease, a novel class of sulfoximine-based pseudosymmetric HIV-1 protease inhibitors was designed and synthesized. The sulfoximine moiety was demonstrated to be important for HIV-1 protease inhibitor potency. The most active stereoisomer (2*S*,2′*S*) displays a potency of 2.5 nM (IC_{50}) against HIV-1 protease and an anti-HIV-1 activity of 408 nM (IC_{50}). A possible mode of action is proposed.

Human immunodeficiency virus type 1 (HIV-1) is the etiological agent of acquired immunodeficiency syndrome (AIDS).[1] HIV-1 protease plays a vital role in the HIV-1 life cycle and its inhibition results in immature virons which are incapable of replication. The combination of HIV-1 protease inhibitors (PIs) and reverse transcriptase inhibitors (RTIs), known as highly affective antiretroviral therapy (HAART), has dramatically improved the life quality of HIV infected patients.[2] However, since HIV can easily generate mutants with diminished sensitivity to the known PIs,[3] there is an urgent need to identify new structural types of inhibitors.

The sulfoximine functional group has the potential for useful incorporation into enzyme inhibitors as it is chemically stable and tetrahedrally hybridized.[4] It was first discovered in late 1940s as a key structure of methionine sulfoximine, a toxic substance found in wheat flour treated with nitrogen trichloride.[5] Although this compound was identified as a potent mechanism-based inhibitor of glutamine synthetase, most early work focused on the utilization of sulfoximines and their derivatives as valuable reagents for asymmetric synthesis.[6] Recently, more attention has been directed toward exploring this functionality as an amide replacement in peptide chemistry. Sulfoximine has been successfully incorporated in designing inhibitors of metalloproteinase such as carboxypeptidase A[7] and ATP-dependent ligases such as asparagine synthetase A.[8] It has also been used in the design of pseudopeptides to alter conformation, polarity, and metabolic stability in drug discovery.[9] To our knowledge, there has been no report on applying sulfoximine in HIV-1 protease inhibitor design.

During our continuous efforts to develop potent HIV-1 PIs, we have designed and synthesized sulfoximine-based symmetric molecules as possible scaffolds for HIV-1 PIs. We reasoned that the tetrahedron structural feature of sulfoximine and its role as a potential hydrogen bond donor/acceptor would render it an ideal candidate for a transition state mimic (TSM) for HIV-1 PI. Currently there are 10 HIV-1 PIs approved by the US FDA.[10] All carry a free hydroxyl group that interacts with the catalytic aspartic acids in the active site to mimic the hydrated amide of the substrate. Since HIV-1 protease functions as a homodimer which has a C_2-symmetric active site, it may be desirable to include approximate C_2 symmetry through the center of the inhibitor. In fact, the Merck compound, L700,417 (**1**, Fig. 1)[11] which is a carbinol analog, is a very potent pseudosymmetric HIV-1 PI with an IC_{50} value of 0.6 nM against the enzyme. Other functional groups that have been studied as effective isosteres of TSMs include silanediol[12] and phosphinate.[13] To assess the effect of sulfoximine in HIV-1 PIs, we constructed our target molecule **4** shown

Keywords: HIV-1 protease inhibitor; C_2 symmetry; Sulfoximine; Transition state mimic.
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doi:10.1016/j.bmcl.2007.07.095

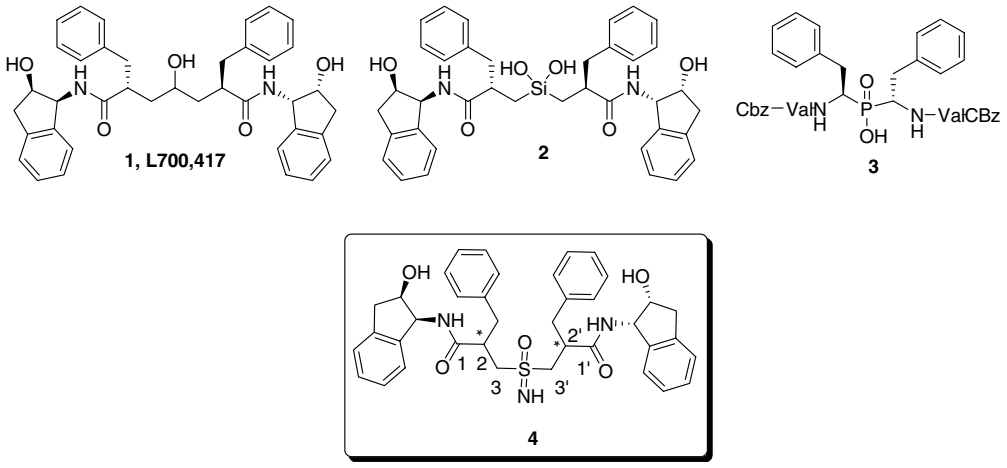
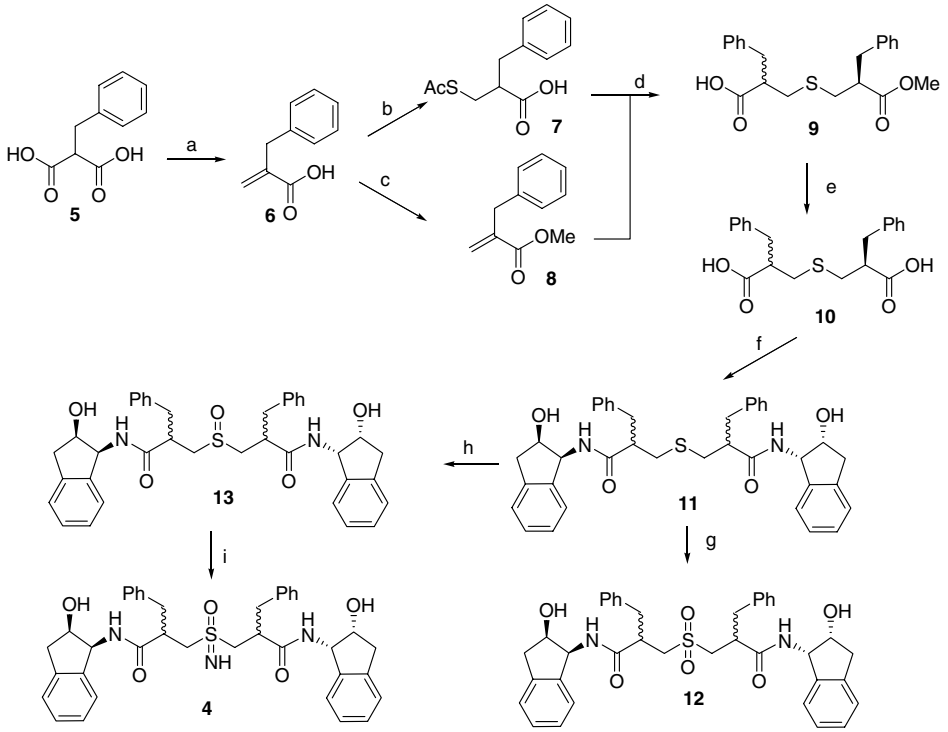


Figure 1. Examples of symmetric HIV-1 PIs and designed target molecule.

in Figure. 1. In this structure, there are two unassigned stereogenic carbons (C2/C2′). Our initial approach was to find a convenient and quick way to obtain the target molecules and verify our hypothesis, thus we synthesized compound **4** as a mixture of diastereomers and tested them for HIV-1 protease activity. Since the resulting sulfoximine mixture was shown to be potent against HIV-1 protease, we subsequently prepared each stereoisomer separately and identified the most potent isomer to be (2*S*,2′*S*) in configuration (Fig. 1).

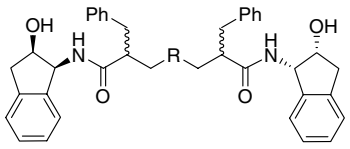
We pursued our initial strategy to obtain sulfoximine compounds as a mixture of four diastereomers (Scheme 1). Briefly, Acrylic acid **6**, obtained from benzylmalonic acid **5** via a Mannich reaction,[14] was converted to intermediate **7** by refluxing with thioacetic acid and methyl acrylate **8** by reacting with $TMSCHN_2$. Michael addition between **7** and **8** afforded intermediate **9** as a mixture of diastereomers. Hydrolysis of the resulting esters with LiOH in aqueous THF afforded the corresponding diacid **10**, which was then coupled with (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol using BOP reagent[15] to generate diamide compound **11**. Subsequent oxidation of **11** by *m*CPBA produced sulfoxide **13**, while the sulfone analog **12** was obtained with oxone.[16] Finally, sulfoxide **13** was converted into the desired sulfoximine **4** using *O*-mesitysulfonylhydroxylamine (MSH).[17]



Scheme 1. Reagents and conditions: (a) formalin, Et_2NH, reflux, 82%; (b) AcSH, benzene, reflux, 77%; (c) $TMSCHN_2$, 89%; (d) NaOMe, 70%; (e) LiOH, THF/H_2O, 86%; (f) (1*S*,2*R*)-(−)-*cis*-1-amino 2-indaol, BOP reagent, Et_3N, 50%; (g) oxone, 80%; (h) *m*CPBA, quantitative; (i) MSH, 35%.

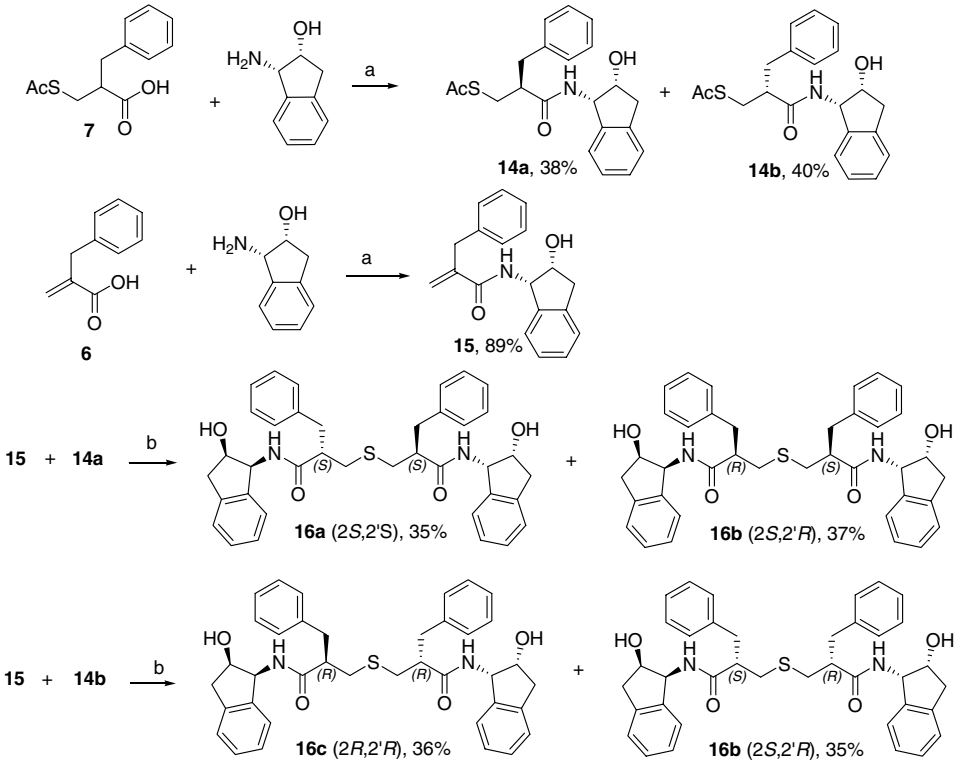
Evaluation of these sulfoximine compounds and their precursors in the recombinant HIV-1 protease enzyme assay indicated that both sulfoximine and sulfoxide compounds are potent inhibitors while the sulfone and sulfide compounds exhibit much less activity (Table 1). As a diastereomeric mixture, **13** showed 75% inhibition at 10 μM, while sulfoxide containing C_2-symmetric peptide analogs[18] were previously shown to be inactive against HIV-1 protease within the limit of detection. On the other hand, the target sulfoximine compound **4** showed 87% inhibition at 10 μM. These preliminary results suggest that the sulfoxide/sulfoximine moiety could serve as an effective isostere of TSM for HIV-1 PIs. The activity data from sulfoxide and sulfoximine compounds indicate that sulfur containing C_2-symmetric analogs could be used in designing potent HIV-1 protease inhibitors, which is contrary to the previous report that sulfide and its oxidative derivatives are not good TSM in C_2-symmetric HIV-1 PIs. The major difference between the literature[18] molecules and ours is the insertion of one methylene unit between the sulfur and benzyl groups. This extra carbon boosts the activity of **13** and **4** significantly against the HIV-1 protease suggesting that the proper length between P_1/P_1' (as defined by Schechter and Berger's nomenclature)[19] is an important requirement for high inhibitory potency. This promising result encouraged us to determine the relationship between stereochemistry and bioactivity of the diastereomeric sulfoximine compounds.

Table 1. Evaluation of compounds **11**, **12**, **13**, and **4** as HIV-1 protease inhibitors

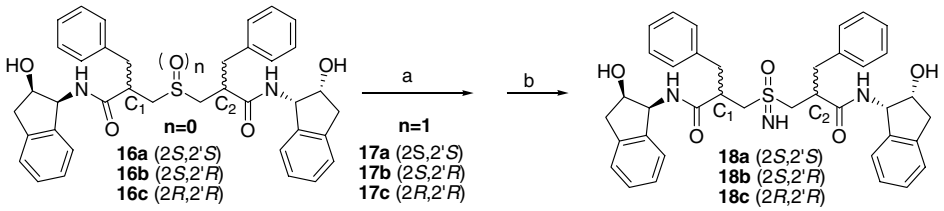


Compound	R	% Inhibition at 10 μM
11		15
12		30
13		75
4		87

The synthetic strategy leading to each stereoisomer of **4** is outlined in Scheme 2. A modified approach to control the stereochemistry of the central diacid unit was evaluated. Thus, optically pure amides from each side of the target molecules were prepared. The number of diastereomers produced by the subsequent Michael Addition was minimized. Amide coupling of **7** with (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol using BOP reagent afforded **14** as a mixture of two diastereomers which were well separated in an equal ratio by flash chromatography. Coupling between acrylic acid **6** and (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol provided **15** in good yield. Michael addition between **15** and **14a** afforded a mixture of two diastereomers **16a/16b**. The two isomers were separated by reverse phase HPLC[20] in equal amount indicat-



Scheme 2. Reagents: (a) BOP reagent, Et_3N; (b) NaOMe/MeOH.



Scheme 3. Reagents: (a) *m*CPBA; (b) MSH.

ing lack of diastereoselectivity in this reaction. The same procedure was applied to **15** and **14b** to yield another two diastereomers **16b/16c**. Because of the structural symmetry, three diastereomers of sulfide compounds (**16a**, **16b**, and **16c)** were obtained. All isomers were individually oxidized to sulfoxide by *m*CPBA. While **16a**(2*S*,2′*S*) and **16c**(2*R*,2′*R*) afforded corresponding sulfoxide as a single diastereomer, **16b**(2*R*,2′*S*) yielded sulfoxide **17b** as a mixture of two epimers. Since HPLC separation was not satisfactory for **17b**, further reaction was carried out without separation. Each sulfoxide was then treated with MSH to produce the corresponding sulfoximines **18a/18b/18c** (Scheme 3).

The absolute stereochemistry was examined in detail by means of 2D NMR correlation experiments and X-ray crystallography studies. COSY spectra of **14a**(2*S*) and **14b**(2*R*) revealed that the chemical shifts from benzyl and indanol groups are within 6.9–7.4 ppm for **14a**, while for **14b** an extra peak at 6.4 ppm was observed. It was identified from HMQC that the extra peak corresponds to an aromatic proton. This chemical shift pattern reflects an *anti*-(2*S*) or *syn*-(2*R*) configuration between benzyl and indanol groups. In the X-ray crystal structure of **14b**,[21] one H from amino indanol is situated in close proximity to the benzyl group and well within the range of the shielding zone. The corresponding H of diastereomer **14a** is presumably positioned away from the benzyl group. Given the fact that the absolute stereochemistry of amino indanol is known, this observation could be used to determine the absolute stereochemistry of pseudosymmetric sulfur-containing targets. Similar COSY and HMQC experiments were carried out on each diastereomer of **16** and the chemical shift variations associated with changes in absolute configurations of these groups were easily observed. Thus, in the ^{1}H NMR spectrum of **16b**(2*S*,2′*R*), one H corresponding to an aromatic group with a shielding of about 0.8 ppm from the aromatic region was observed when compared to **16a**(2*S*,2′*S*). In **16c**(2*R*,2′*R*) there are two such peaks (Table 2). By counting the number of proton peaks close to 6.3 ppm, the absolute configuration of the pseudosymmetric sulfide compound could be determined. The oxidation of **16** to **17** and the imination[17]

Table 2. Key chemical shift

Compound	H integration in aromatic region	Chemical shift (shielding from aromatic region) ppm	H integration
14a(2*S*)	9	—	0
14b(2*R*)	8	6.43 (d,*J* = 7.8 Hz)	1
16a(2*S*,2′*S*)	18	—	0
16b(2*S*,2′*R*)	17	6.27 (d, *J* = 7.5 Hz)	1
16c(2*R*,2′*R*)	16	6.20 (d, *J* = 7.5 Hz)	2

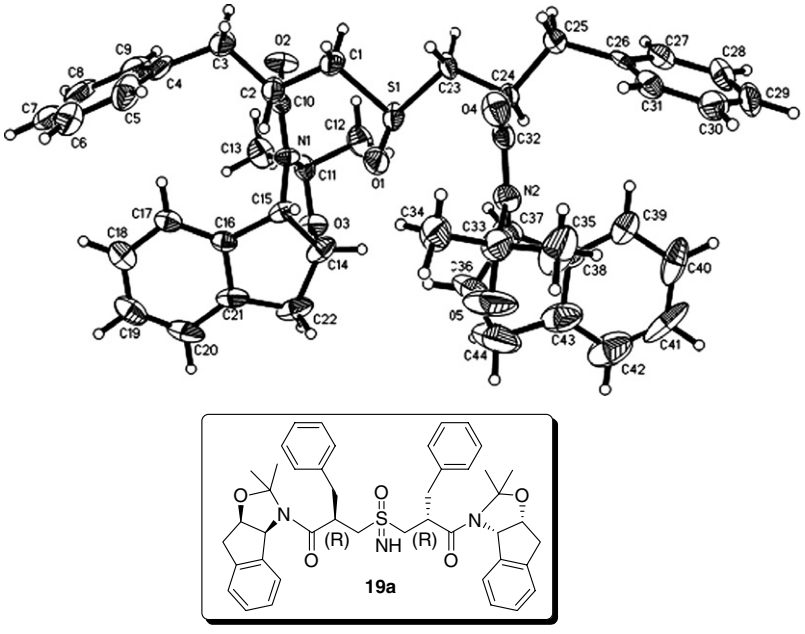


Figure 2. X-ray structure of **19a**.

of **17** to **18** should all occur with retention of the chirality of intermediate **16**. Similar chemical shift scattering patterns were indeed observed for all the described molecules, which led to the assignment of the stereochemistry of each isomer as shown in Schemes 2 and 3. Acetonide derivative **19a** was prepared from **17a** and the crystal structure[22] was obtained (Fig. 2) which confirms the stereochemical assignment.

Each sulfoximine as well as sulfoxide compound was evaluated against the HIV-1 protease (Table 3). In each class, the order of activity is (2*S*,2′*S*) > (2*R*,2′*S*) > (2*R*,2′*R*). Configuration switching of either C2 or C2′ from *S* to *R* resulted in a loss of potency, while switching of both carbons led to a significant drop in activity. This is exemplified in the case of sulfoxide analogs, where the IC_{50} of **17a**(2*S*,2′*S*) is 21.1 nM while **17b**(2*R*,2′*S*) is 53.1 nM, and **17c** (2R,2′R) has significantly less activity (IC_{50} > 10 μM). It can be concluded that the stereochemistry at C2 and C2′ appears to be crucial for HIV-1 protease inhibition. The importance of the stereochemistry for inhibitory activity observed in this study is in agreement with that of Merck L-700,417 in which an *anti*-relationship between benzyl and amino indanol moiety is observed. The protease activity data also indicate that sulfoximine is more potent than sulfoxide as exemplified by **17a** and **18a**. They both have the same configuration at C2 and C2′, but the sulfoximine analog **18a** is 8-fold more potent than the sulfoxide analog **17a**. The same relationship can also be identified between **17b** and **18b** as well as **17c** and **18c**. The most active compound **18a**(2*S*,2′*S*) was determined to have an IC_{50} value of 2.5 nM. These results indicate that the sulfoximine group plays a significant role in the binding to the enzyme.

The X-ray structure of Merck L700,417 complexed with the HIV-1 protease indicated that the benzyl groups occupy S_1/S_1' pockets while amino indanol occupies S_2/S_2' pockets in the active site.[23] We anticipated that the sulfoximine moiety would form hydrogen bonds with the catalytic aspartic acids 25 and 25′, while the carbonyl groups would interact with backbone amide groups of ILe 50 and 50′ on the flaps of the enzyme via an H_2O molecule (Fig. 3). The presence of a hydrogen bond donor is essential for the inhibitory potency where the NH might be engaged in similar hydrogen bond interaction as an OH. It has been shown that in the active pH range one aspartic acid in the active site is deprotonated[24] to serve as a good hydrogen bond acceptor. In the sulfoxide case, oxygen might function as a hydrogen bond acceptor to accommodate one aspartic acid which is intact. For the sulfone derivative, two oxygen atoms may cause some negative impact to abolish or drastically decrease the preferred interactions with either aspartic acid residue in the active site.

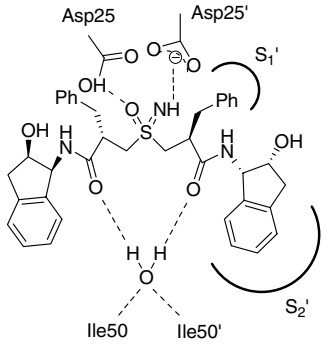
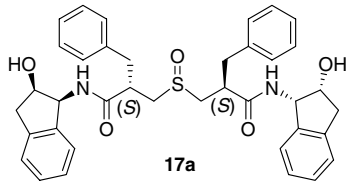
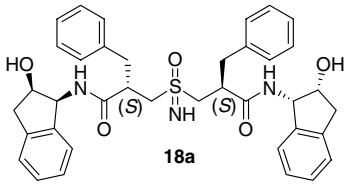
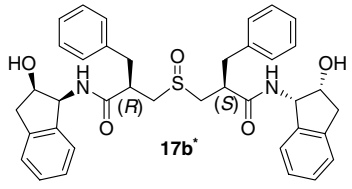
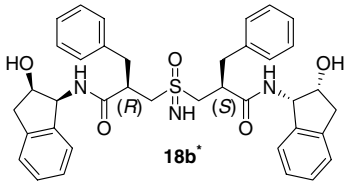
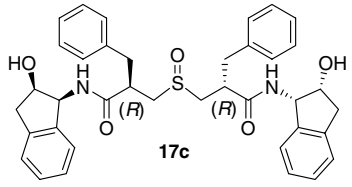
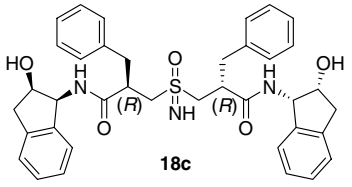


Figure 3. Proposed mode of action of **18a**.

Table 3. Evaluation of sulfoxide and sulfoximine analogs against the HIV-1 protease

Compound	IC_{50} (nM)	Compound	IC_{50} (nM)
 **17a**	21.1	 **18a**	2.5
 **17b***	53.1	 **18b***	37.3
 **17c**	>10,000	 **18c**	153.9

*Mixture of 2 diastereomers.

The in vitro antiviral activity of the most potent inhibitor **18a** was assessed using a cell viability assay. It displayed a clear dose response with an antiviral IC_{50} value of 408 nM and no toxicity was observed at 10 μM, the highest concentration tested.

In summary, we have demonstrated for the first time that sulfoximine is a novel moiety potentially functioning as a transition state mimic in HIV-1 PIs. We also confirmed the importance of proper stereochemistry and identified **18a**(2*S*,2′*S*) to be the most potent stereoisomer with activity comparable to indinavir against HIV-1 protease. The sulfoximine-based compounds are of great interest in designing more potent HIV-1 protease inhibitors. Further studies on asymmetric synthesis, SAR, and mechanism of inhibition are underway and will be reported in future accounts.

Acknowledgments

The authors gratefully acknowledge Dr. Steve Patterson for assistance in HPLC separation, Dr. Victor Young from X-ray Crystallographic Laboratory, Department of Chemistry, University of Minnesota for X-ray analysis. We acknowledge Ms. Christine Dreis for performing the HIV protease assays, Roger Ptak at Southern Research Institute for the antiviral testing data. We thank Dr. Abbas Raza for providing X-ray crystal structure of compound **14b**. We also thank Dr. William M. Shannon for his advice as a consultant for the antiviral work on this project.

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20. Reverse-phase HPLC was run on Varian Microsorb column (8μ, C_{18}, 250X41.4 mm) using two solvent systems with 40 mL/min flow rate and detected at 220 and 230 nm. Solvent system A: 0.02 M TEAB (triethylammonium bicarbonate) in water, B: 70% acetonitrile in water, 75–100% B linear in 40 min.
21. Crystallographic data (excluding structure factors) for the structures of **14b1** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 655535. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.
22. Cambridge Crystallographic Data Centre deposition number for the structure of **19a** is CCDC 645798.
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