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Design and synthesis of sulfoximine based inhibitors for HIV-1 protease

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ABSTRACT

A new class of potent sulfoximine inhibitors for HIV-1 protease has been designed and synthesized. Substitution of the sulfoximine moiety into different parent compounds yields different inhibition effects. While our previously studied sulfoximine-based inhibitors display potency of 2.5 nM (IC_{50}) against HIV-1 protease, introduction of the sulfoximine moiety into the asymmetric Indinavir yielded only micromolar inhibition. Docking studies showed structural variations in their modes of binding which explains this unexpected observation. The implication of these observations in the development of other sulfoximine inhibitors is discussed.

Human immunodeficiency virus (HIV) infection can lead to acquired immunodeficiency syndrome (AIDS).[1,2] It appears in two forms as HIV-1 and HIV-2. Currently, there are over 30 US Food and Drug Administration (FDA) approved antiviral drugs available[1] for the highly active antiretroviral regimen treatment (HAART) for AIDS.[2–4] The treatment targets simultaneously various viral protein enzymes found only in HIV, thereby, preventing full maturation during viral replication within the host cells. Due to the persistence of the viral reservoir within tissues, AIDS remains an incurable disease.[5] As HIV with drug resistant viral strains continues to emerge, extensive efforts are being spent on the development of novel therapeutic agents that can be used as alternative drug substitutes to augment HAART antiviral activities.[6]

One of the vulnerable viral protein targets most studied is the HIV protease.[7] It is a homo dimeric aspartyl enzyme that catalyzes the proteolytic cleavage of a large polyprotein precursor during viral assembly and maturation.[8,9] Since the characterization of its structure[6] and mechanistic function,[10,11] innumerous rationally designed inhibitors have been chemically synthesized and clinically tested.[12–15] To-date, there are only 10 commercially available protease inhibitors (PIs) approved by the FDA.[1] Nine of these inhibitors contain a free hydroxyl group that acts as a transition state mimic (TSM) for the tetrahedral hybridized amide linkage during catalytic cleavage. Structurally, this secondary alcohol is shown to interact directly with the catalytic Asp25 and Asp25′ residues in the active site and has been shown to be crucial in the design of highly potent HIV PIs. In addition to the TSM, many of the HIV PIs include a hydrogen bond acceptor motif, which interact directly with a crystallographic conserved water molecule that is hydrogen bonded to the carbonyl backbone of Ile50 and Ile50′ residues in the active site. Compounds designed to include this conserved water mimic have also been shown to improve on binding affinity.[16] Inclusion of multiple hydrophobic substituents, which project into the various hydrophobic binding pockets (S_1, S_2, S_1', and S_2') of HIV protease as well as compounds which take advantage of the C_2 symmetry of the homodimeric HIV protease[16,17] are also desirable (Fig. 1).

Sulfoximines, which are often referred to as 'chemical chameleons', have been well studied for their bioactivity as antibiotics, antithrombotics and tumor metastasis inhibitors. Its tetrahedral structural feature can act as an alternative substitute for the TSM of the secondary alcohol. In addition, the sulfoximine has the potential to act simultaneously as a hydrogen bond donor as well as an acceptor. Recently,[18] we have designed and synthesized a new set of sulfoximine inhibitors for HIV-1 protease based on the Merck compound, L700,417.[19] The most active sulfoximine stereoisomer (2S,2′S) (**1**) displays nanomolar inhibition potency of 2.5 nM against HIV-1 protease, similar to that of L700,417 (IC_{50} of 0.6 nM).

Encouraged by these results, we proceeded to substitute the same sulfoximine moiety into an FDA approved HIV PI with the expectations of retaining the inhibition potency by replacing the secondary alcohol (TSM). Thus, a novel class of inhibitors similar to one of the most potent commercially available HIV PI was

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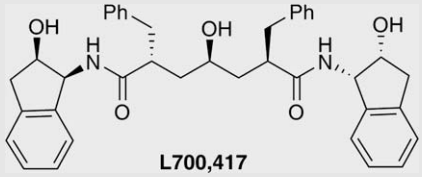
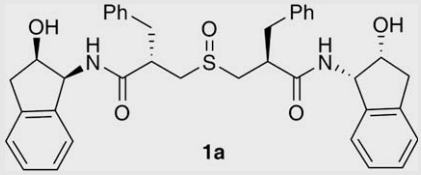
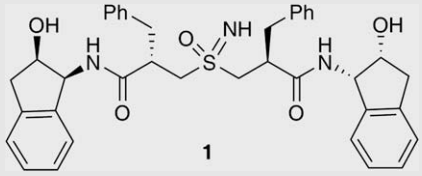
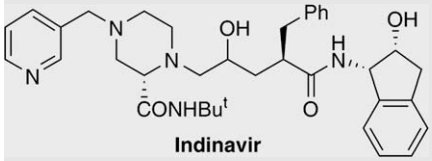
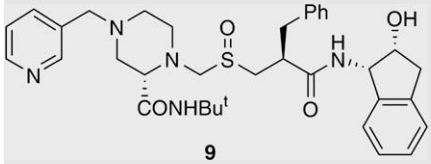
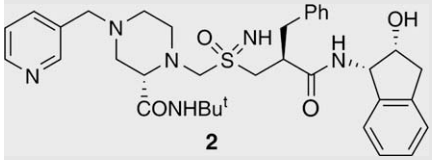
Table 1
HIV-1 protease inhibition assay results (in vitro) for compounds **2** and **9**

Compound	IC_{50}
 L700,417	0.6 nM
 1a	21 nM
 1	2.5 nM
 Indinavir	0.4 nM
 9	100 μM
 2	100 μM

expected. The parent compound selected was Indinavir,[20] which is commercially known as Crixivan^R (Fig. 2). It is an asymmetric PI that is closely related to the previously studied compound of L700,417.[21] The strategy to synthesize Indinavir with a sulfoximine moiety **2** was to first make optically pure thioester **3**, the desired isomer. The absolute stereochemistry of the isomer **3** was assigned based on 2D NMR correlation experiments and the X-ray crystal structure. The synthesis of the compound **3** was also carried out as reported (**CCDC 65535**).[18]

A protecting group was appended to compound **3** using 2,2′-DMP to generate the isopropylidine derivative **4** (Scheme 1). The thioester of **4** was hydrolyzed using K_2CO_3 in methanol, giving almost quantitative conversion to thiol **5** which was used directly in the next step without further purification. The key intermediate **6** (Scheme 2) was conveniently prepared in optically pure form according to the reported procedure.[22]

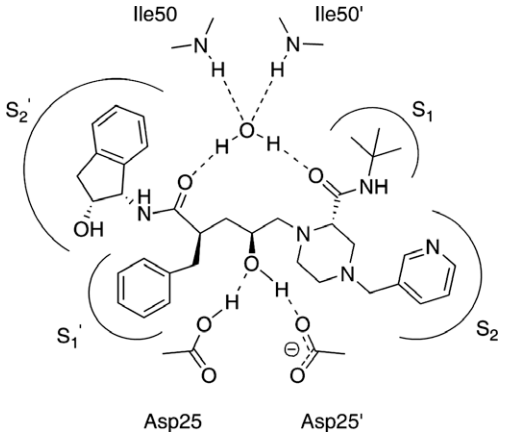


Figure 1. Binding of Indinavir to HIV-1 protease.

Sequential reaction of amine **6** with *p*-formaldehyde under reflux conditions generated the Schiff base in situ to which the thiol **5** in DMF was added to give the sulfide **7**. The sulfide derivative **8** was accessed by the removal of Boc and hydrolysis of the isopropylidine of sulfide 7, followed by the alkylation with 3-picolyl chloride in the presence of Et_3N. Subsequent oxidation of sulfide **8** with $NaIO_4$ produced sulfoxide **9**. Finally, the reaction of sulfoxide **9** with *O*-mesitylsulfonylhydroxylamine (MSH)[23] afforded the corresponding sulfoximine **2**.[24]

We anticipated that incorporating the sulfoximine moiety into an existing drug molecule such as Indinavir would enhance the potency through a better binding mode of the sulfoximine as proposed in our earlier report. However, the in vitro assay of **2** and **9** in the recombinant HIV-1 protease enzyme provided only micromolar inhibition (Table 1). This suggested a major deviation from the sulfoximine group binding into the active site of the enzyme resulting in a significant loss of activity.

To further understand the effect of replacing the secondary alcohol with sulfoximine on inhibition potency, subsequent docking calculations were carried out using Glide.[25] The starting X-ray crystal structure of HIV-1 protease in complex with the inhibitor Indinavir (PDB ID:1SDT)[26] and with L700,417 (PDB ID:4PHV)[27] were used. Both complexes are structurally similar with a RMSD of 0.41 Å with the TSM secondary alcohol positioned near equidistance to each of the hydrogens binding to Asp25 and Asp25′. The sulfoximine analogues for each of the parent inhibitors were generated by the replacement of the secondary alcohol moiety. Since Indinavir is an asymmetric compound consisting of a stereogenic *S* chiral center at the secondary alcohol carbon atom, both the *R* and *S* forms of the sulfoximine analogue were considered. All compounds were energy minimized using OPLS 2005 forcefield in the presence of implicit water solvent. The calculations were carried out with the van der Waals radii scaled by a factor of 0.8 to compensate for the non-native binding conformation of the HIV protease binding site for the sulfoximine analogues. All compounds were docked and scored using the Glide extra-precision (XP) mode in the presence of the conserved crystallographic water. To account for protein flexibility, conformations with the best G-score were refined by energy minimization within a 5 Å radius of the ligand inside the protein active site while restraining the external second outer shell of 3 Å by a force constant of 50 kcal/mol Å^2. The remaining residues were held fixed.[28]

Based on the best scoring conformation poses, the overall interactions of the hydrophobic groups and the hydrogen bonding interactions to the crystallographic water were conserved in both of the sulfoximine analogues from their parent compounds. In compound **1** (Fig. 3 C), the relative distances between the heavy atoms of the sulfoximine group to the nearest carboxylate atom

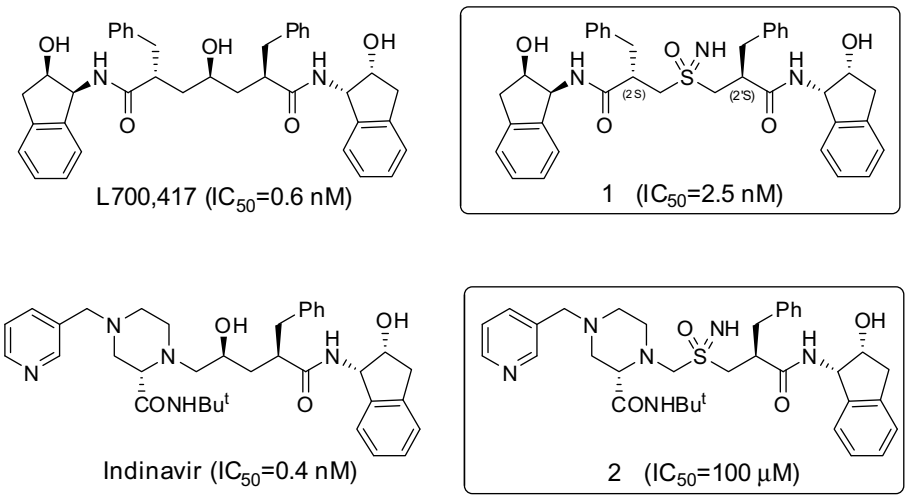
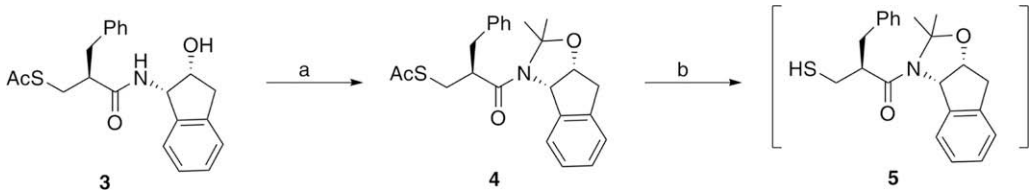
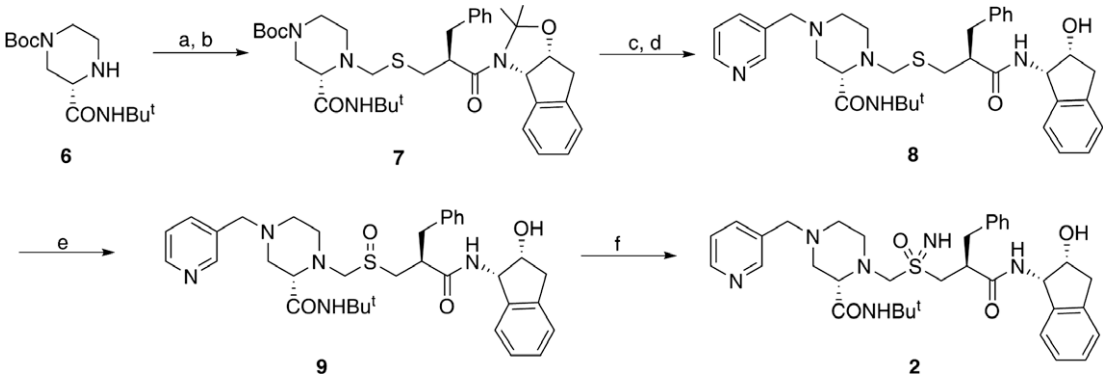


Figure 2. Known inhibitors of HIV protease and the designed inhibitors.



Scheme 1. Reagents and conditions: (a) 2,2′-dimethoxy propane, PTSA, DCM, 85%; (b) K_2CO_3, MeOH.



Scheme 2. Reagents and conditions: (a) *p*-formaldehyde, toluene, reflux, 2 h; (b) 5 in DMF, rt, 65%; (c) 4 N HCl/dioxane, MeOH, 0 °C; (d) 3-picolyl chloride·HCl, Et_3N, DMF, 70 °C, 4 h, 61%; (e) *m*-CPBA (1.1 equiv), DCM, 60%; (f) MSH, DMF, 65%.

of the Asp25 and Asp25′ were within 3.3 Å, similar to that of L700,417 in the original X-ray structure. Conformation of the pseudo symmetric compound **1** (2S,2S′) bound in 180 degrees rotated along the C2 axis of the HIV protease was also observed as an alternative pose with similar G-score. The binding of compound **1** did show interactions of the sulfoximine group interacting both as a hydrogen bond donor and acceptor as proposed in our earlier work. This mode of binding was accompanied by small changes in the dihedral angles along the carbon backbone of the L700,417 (Fig. 3 A) with minor changes in the binding of the hydrophobic groups in each of the hydrophobic sites. This suggested, the incorporation of the sulfoximine moiety requires the parent compound to possess sufficient degree of conformation flexibility. While it is desirable to maximize the hydrophobic interactions within the hydrophobic binding pockets and to retain the crystallographic water interaction in the design of potent PI, conformational flexibility is essential in order for the sulfoximine moiety to adapt the desired bound conformation with the dual role of hydrogen bond donor and acceptor property.

For compound **2**, both sulfur stereoiosomers (*R* and *S*) bind similarly to that of Indinavir (Fig. 3 B) in the active site of HIV protease except in the overall orientation of the sulfoximine moiety. In compound *S*-**2**, the oxygen atom of the sulfoximine group is at near equidistance to the nearest carboxylate atom of the Asp25 and Asp25′ residues while the nitrogen atom is positioned within 3.5 Å from the conserved crystallographic water (Fig. 3 D). For compound *R*-**2**, its vice versa (not shown). No significant change in the overall dihedral angles of scaffold backbone was observed as compared to the original X-ray structure. While Indinavir does possess a degree of ligand flexibility, steric restriction by the piperazine moiety prevents the sulfoximine to adapt the proper conformation as a dual hydrogen bond donor/acceptor.

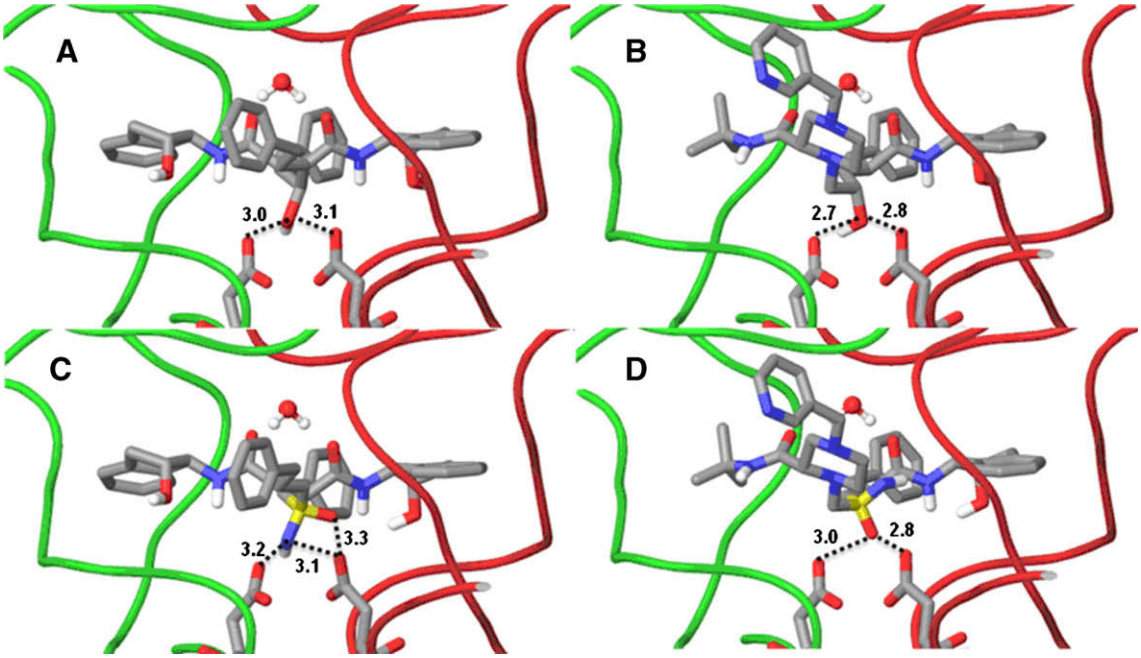


Figure 3. Binding of L700,417 (A), Indinavir (B), 1 (C), S-2 (D) to HIV-1 protease.

Both of the two conformations showed hydrogen bonding interactions involving only one of the heavy atoms of the sulfoximine group to both of the Asp25 and Asp25′, contrary to our earlier expectation. For compound *S*-**2**, such orientation would result in a non-TSM binding, which could greatly diminish its overall binding affinity. Interestingly, both compound **2** and its sulfoxide precursor **9** exhibit similar inhibition at 100 μM, supporting the view that both compounds may bind similarly in the HIV protease active site.

For successful incorporation of the sulfoximine moiety, the parent compound should possess sufficient conformation flexibility to accommodate the proper mode of binding. The incorporation of the sulfoximine moiety into Indinavir most likely resulted in the loss of key interactions for the binding of compound **2** to HIV protease, resulting in the observed drastic loss in inhibition. Based on the predicted binding conformation of the two diastereomers of compound **2**, the close proximity of the nitrogen or the oxygen atom of the sulfoximine group within the crystallographic water may potentially disrupt the pre-existing hydrogen bonding network of the crystallographic water since both heavy atoms may act as either a hydrogen bond donor or acceptor. Alternatively, it is possible that due to the orientation of the bound conformation, the sulfoximine moiety displaces the conserved crystallographic water from the active site. As interactions with the conserved crystallographic water molecule are crucial in binding, such loss of interaction could drastically diminish the binding affinity.

In conclusion, the introduction of a sulfoximine group to Indinavir afforded less potency with in vitro activity against HIV-1 protease. The docking studies have revealed the potential disruption of the crystallographic water network of interactions as the cause for the loss of potency. Further work, to design and synthesize new classes of sulfoximine inhibitors for HIV-1 protease and understanding their mechanisms of action in the active site of the enzyme is currently underway.

Acknowledgments

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(m, 1H), 2.41–2.58 (m, 7H), 2.31 (m, 2H), 1.32 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.69, 169.49, 150.51, 149.10, 140.63, 140.46, 139.12, 136.84, 132.42, 129.02, 128.60, 127.81, 126.67, 126.45, 124.98, 124.20, 123.43, 72.79, 76.83, 67.51, 63.90, 60.13, 59.91, 57.48, 54.68, 52.71, 51.25, 50.77, 50.70, 47.93, 39.57, 38.73, 37.92, 35.44, 28.97; ESI-HRMS (m/z): $[M+H]^+$ calcd for $C_{35}H_{46}N_6O_4S$ 647.3379; found, 646.3364. Optical rotation $[\alpha]_D^{20} + 7.5^\circ$ (c 1.0, $CHCl_3$).

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