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Design, asymmetric synthesis, and evaluation of pseudosymmetric sulfoximine inhibitors against HIV-1 protease

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ABSTRACT

The HIV-1 protease is a validated drug target for the design of antiretroviral drugs to combat AIDS. We previously established the sulfoximine functionality as a valid transition state mimetic (TSM) in the HIV-1 protease inhibitors (PI) design and have identified a lead pseudosymmetric compound with nanomolar enzymatic inhibitory activity. Here, we report the asymmetric synthesis of this compound and its application in the synthesis of sulfoximine-based peptidomimetic HIV-1 protease inhibitors. Molecular modeling revealed the potential mode of binding of the sulfoximine inhibitor as a TSM. The predicted absolute binding free energies suggested similar inhibitory effect as observed in our enzymatic inhibitory studies.

1. Introduction

Acquired Immunodeficiency Syndrome (AIDS) remains one of the world's greatest public health challenges with over 25 million mortalities estimated since it was first recognized in 1981.[1] The highly active antiretroviral therapy (HAART) used in the treatment of AIDS utilizes a combination of antiviral drugs with at least two mechanisms of action to effectively inhibit the replication cycle of HIV.[2] Due to the persistence of HIV in the infected host cell reservoir within the tissues, AIDS remains an incurable disease.[3] With the rapid emergence of drug resistance strains worldwide, the urgency to expand the drug repertoire for maintaining the efficacy of HAART continues to be a priority in antiviral research.

The HIV protease is a C_2 symmetric homodimeric aspartyl protease[4,5] that catalyzes the proteolysis of the polyprotein precursor, *gag-pol*, and is responsible for producing functional and structural proteins required for the assembly and maturation of infectious HIV.[6] The HIV-1 protease has been one of the primary targets for antiviral drug development.[7] Currently, there are nine HIV PI's approved by the US Food and Drug Administration (FDA) for AIDS treatment. These drugs feature a secondary alcohol as the transition state mimetic (TSM) of the tetrahedral intermediate during amide bond hydrolysis. This secondary alcohol specifically targets the catalytic D25/D25′ residues as a hydrogen bond donor and an acceptor within the enzymatic active sites.[8]

Previously, we have characterized the sulfoximine moiety as a valid TSM in HIV-1 PI design and have identified a potent pseudosymmetric lead compound **1**. This compound exhibits an IC_{50} of 2.5 nM against the HIV-1 protease and IC_{50} of 408 nM against the virus.[9] We are continuing our efforts towards the development of a novel HIV PI featuring the sulfoximine moiety to exploit its dual functionality as a hydrogen donor and acceptor. We have recently demonstrated that the non-symmetric sulfoximine-based inhibitors are far less potent than their pseudosymmetric analogues.[10] This report presents a novel asymmetric synthetic method specifically developed to construct the pseudosymmetric sulfoximine core inhibitors. Conformationally relaxed analogues at the P_2/P_2' 2 substituents were also synthesized to explore stereochemical preferences of the S_2/S_2' subsites (Fig. 1). The application of this methodology to construct sulfoximine-based peptidomimetic HIV-1 PI's was also demonstrated. To access the role of the sulfoxime moiety at the active site of HIV-1 protease, an *N*-methyl substituted sulfoxime compound **23** was synthesized to remove the hydrogen bonding donor ability of **1**. The resulting compound displayed a decreased potency validating the importance of the dual functionality of sulfoximine both as a hydrogen donor and an acceptor. A molecular modeling study was carried out to evaluate the mode of binding of the sulfoximine compounds. The observed binding modes further support the rationality of the TSM design. Additionally, the absolute binding free energies of these inhibitors were evaluated and were consistent with the observed enzymatic inhibitory activities.

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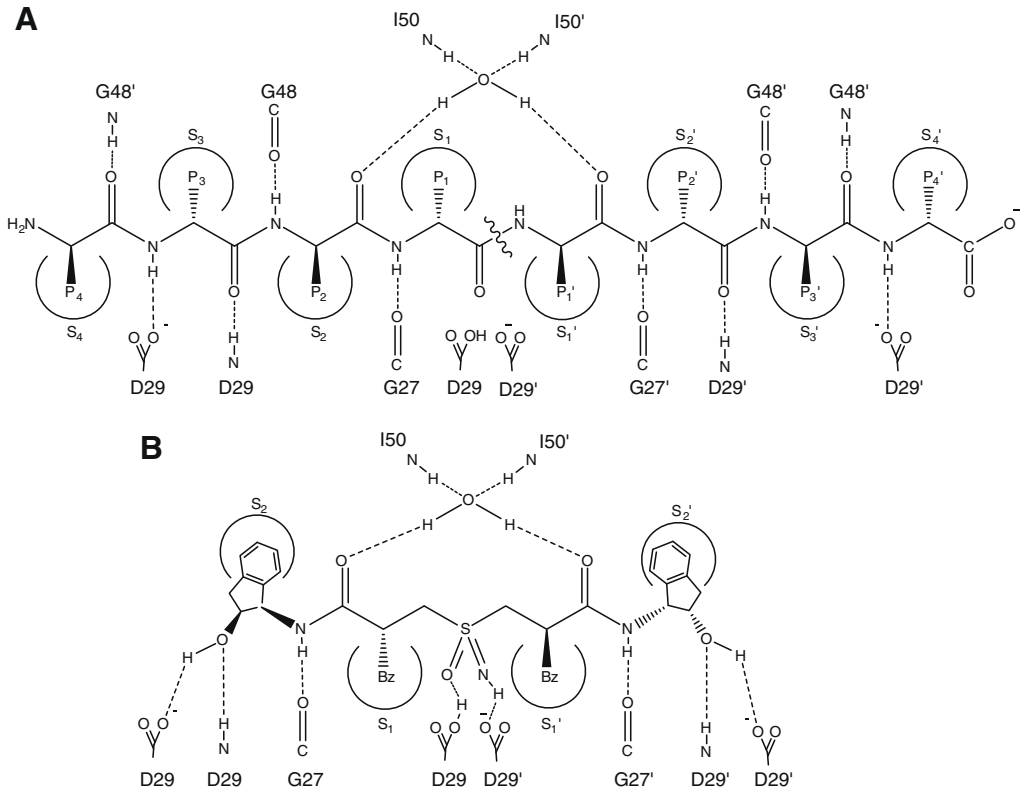


Figure 1. General hydrogen bonding scheme for HIV-1 protease binding to (A) a putative peptide substrate and (B) the symmetric sulfoximine inhibitor, **1**. While HIV protease is a C2 symmetric homodimeric protein in the apo form, the binding of native peptide substrates involves asymmetric hydrogen bonding in each monomeric unit.[30] Symmetric inhibitors involve the same hydrogen bonding network on both monomeric units.

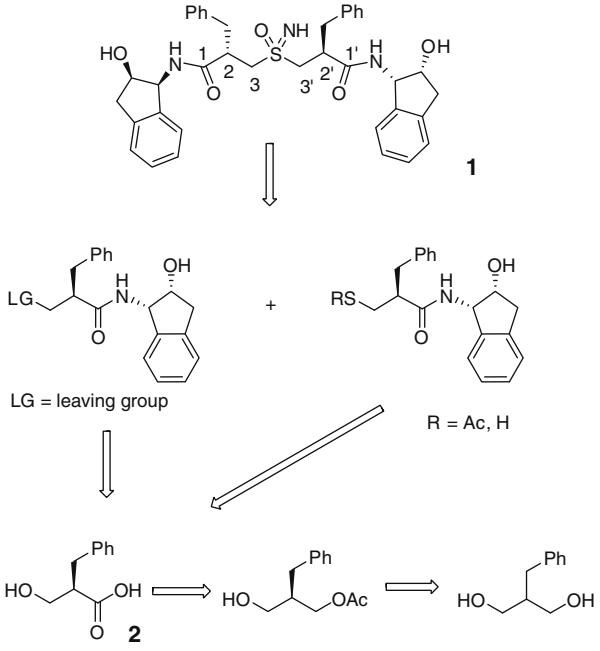
2. Results and discussion

2.1. Chemistry

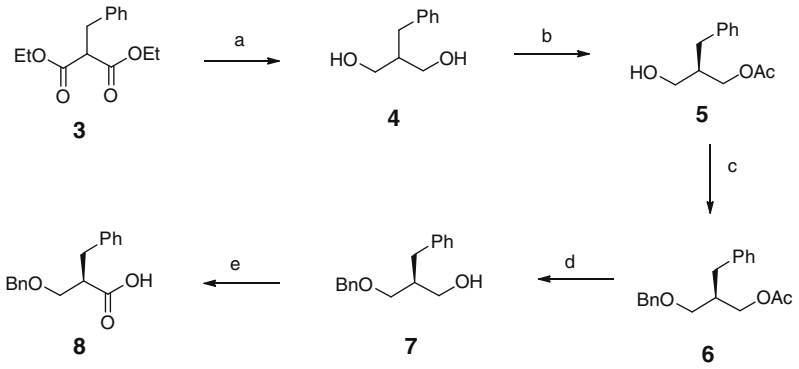
The identification of the sulfoximine-based compound **1** (2*S*,2′*S*) as a potent HIV-1 PI has prompted us to further explore the potential of this new molecular scaffold in the design of HIV PIs. The previous racemic synthesis generates several diastereomers for the key sulfide precursor and requires excessive HPLC separations.[9] Therefore, sufficient supply of the key intermediate requires a more scalable synthetic route. We envision that an asymmetric synthesis with enantiomerically enriched early intermediates would significantly alleviate the complication of generating and separating diastereomeric mixtures, hence, greatly increase the overall synthetic efficiency.

The symmetry of the target sulfoximine molecule allows the adoption of a divergent–convergent synthetic strategy, which relies on an enzyme catalyzed desymmetrization reaction of prochiral diols to construct key chiral building blocks (Scheme 1). Creating stereogenic carbon centers with high enantioselectivity has proven valuable in the study of reaction mechanisms and in the practical syntheses of optically active compounds. Specifically, the lipase catalyzed enantiotopic differentiation of two primary hydroxyl groups of a prochiral diol molecule has been shown to be an efficient method for the preparation of important chiral building blocks with defined stereochemistry.[11] Thus, we anticipated that the lipase catalyzed desymmetrization reaction would provide an efficient synthetic approach to optically pure intermediate **2**.

The execution of this strategy started with diethyl benzylmalonate **3** which was reduced to 2-benzyl-1,3-propanediol **4** in high yield with lithium aluminum hydride (LAH) in ether. A lipase PS catalyzed desymmetrization reaction was carried out by treating diol **4** with lipase PS (50% wt/wt) in the presence of excess vinyl acetate as an acyl donor and diisopropyl ether and water (1000:1) as a mixed solvent (Scheme 2). This reaction successfully produced optically active monoester (*R*)-(+)-**5** in excellent yield.



Scheme 1. Retrosynthetic strategy for pseudosymmetric lead compound.



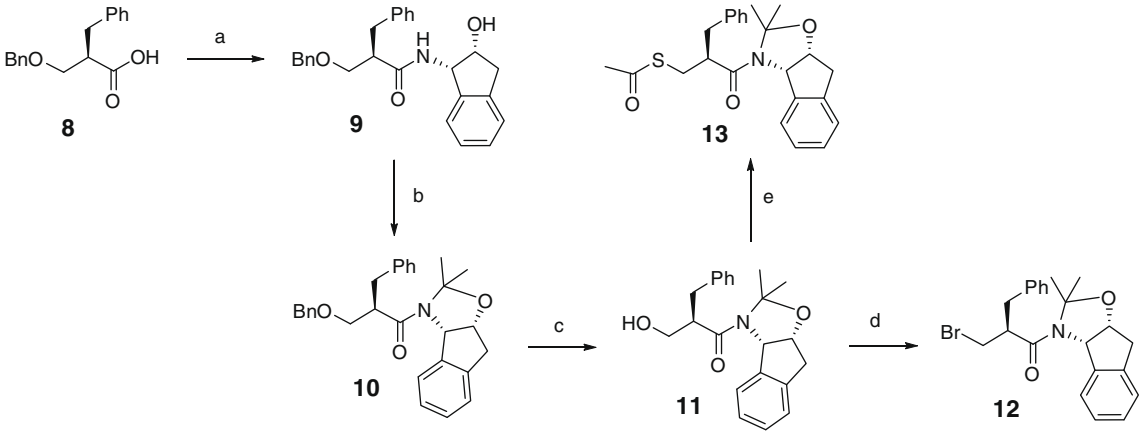
Scheme 2. Reagents and conditions: (a) LAH in ether, quantitative; (b) lipase PS, vinyl acetate, 92%; (c) benzyl 2,2,2-trichloroacetimidate, CF_3SO_3H (cat.), 70%; (d) K_2CO_3, MeOH, 87%; (e) Jones reagent, 99%.

The assignment of (*R*)-configuration to the predominant isomer was based on literature precedent[12] and later confirmed by NMR and single crystal X-ray analysis of its derivatives.[9] The enantiomeric composition of **5** was measured by NMR analysis of the corresponding (+)-α-methoxy-α-trifluoromethyl-α-phenyl acetate (Mosher's ester) and the enantiomeric ratio was determined to be 96:4.

This lipase PS catalyzed monoacylation of prochiral diol was found to be applicable to a variety of diols with both electron withdrawing and electron donating groups tolerated on the aromatic ring.[13] Thus, this strategy could be applied to control electron density of P_1/P_1' substituents of the designed HIV-1 PIs. It is noticed that chemical manipulation of a monoacetate such as **5** may be complicated by the vulnerability of the acyl moiety to migrate to the vicinal hydroxyl group under basic conditions.[14] Therefore, typical alkaline benzylation conditions (benzyl bromide, sodium hydride in DMF) must be avoided. A solution was to use the acid-catalyzed conditions[15] in which the enantiomerically enriched monoester (*R*)-(+)-**5** was treated with the commercially available benzyl 2,2,2-trichloroacetimidate in the presence of a catalytic amount of trifluoromethanesulfonic acid (CF_3SO_3H) to furnish the benzyl ether **6** in 70% yield. The enantiomeric purity was subsequently checked by derivatizing with Mosher's reagent and no racemization was observed. The acetate group in **6** was removed by treatment with K_2CO_3 in MeOH[16] to afford intermediate **7**. The primary alcohol in **7** was subsequently oxidized by Jones' reagent[17] to afford the carboxylic acid compound **8** with the desired stereochemistry (Scheme 2).

Coupling of **8** with (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol in the presence of BOP reagent afforded amide (2*R*)-**9** in good yield. A small amount of (2*S*)-**9** generated from (*S*)-**8** was isolated by flash chromatography. Therefore, (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol also functions as a chiral resolving agent, which leads to an enantiomeric enrichment of the starting material. We planned to carry out a coupling reaction between a nucleophile and an electrophile to construct the C–S bond in the target skeleton (Scheme 1), and both parts could be derived from **9** after unmasking the benzyl ether functional group. Consequently, the hydroxyl group of the amino indanol moiety was protected to avoid potential interference with the new primary hydroxyl group formed from debenzylation at the next step. Therefore, **9** was reacted with excess 2,2-dimethoxypropane using camphorsulfonic acid (CSA) as a catalyst to afford acetonide **10**, which was then subjected to hydrogenolysis to remove benzyl protection and provide intermediate **11** with a free hydroxyl functionality for the subsequent coupling reactions. Compound **11** served as the precursor for the bromide compound **12**, which is an important intermediate in our divergent–convergent synthetic scheme. The hydroxyl group in **11** was successfully converted to bromide **12** with *N*-bromosuccinimide (NBS) and triphenylphosphine (PPh_3) in good yield. The free hydroxyl group in **11** was converted to thioacetate in **13** via a Mitsunobu reaction (Scheme 3).

A phase-transfer catalysis (PTC) was applied to construct the C–S coupling product. This strategy was successfully demonstrated in a stereoselective synthesis of various glycosyl derivatives, including *S*-aryl glycosides, from glycosyl halides with complete



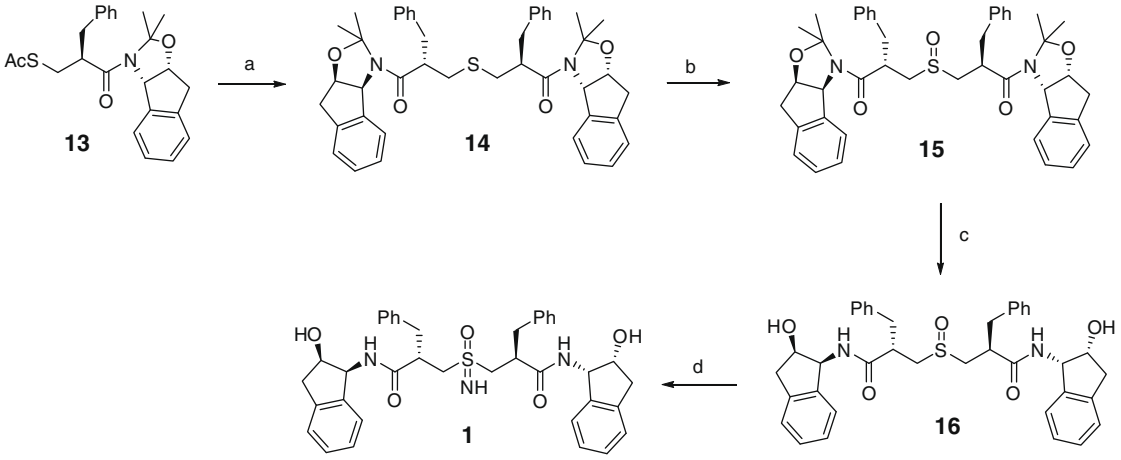
Scheme 3. Reagents and conditions: (a) (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol, BOP reagents, TEA, 94%; (b) DMP, CSA, 92%; (c) H_2, Pd/C, 88%; (d) NBS, PPh_3, 88%; (e) DIAD, PPh_3, 84%.

anomeric inversion[18] (Scheme 4). The acetate group in **13** was removed by NaOH under dithiothreitol (DTT) protection to afford the free thiol compound, which was directly coupled with **12** in a two-phase solvent system of ethyl acetate and saturated aqueous $NaHCO_3$ solution in the presence of tetrabutylammonium hydrogen sulfate (TBAHS) as the phase-transfer catalyst. The desired symmetric sulfide **14** was obtained in 70% yield. No racemization of the product or elimination of the starting materials was observed. Having obtained intermediate **14** in sufficient quantity, we proceeded to synthesize the sulfoxide derivative **15** via direct *meta*-chloroperoxybenzoic acid (*m*CPBA) oxidation. The subsequent deprotection of isopropylidene in **15** using *para*-toluenesulfonic acid (PTSA) as catalyst in methanol provided optically pure intermediate **16**, which was finally converted to the target sulfoximine derivative **1** by MSH (Scheme 4).

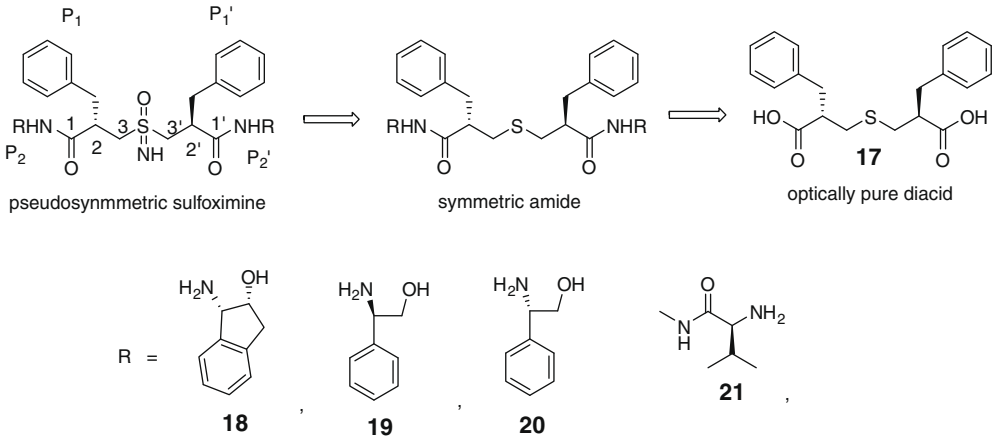
The development of an asymmetric synthesis method to make stereochemically controlled core structure prompted us to investigate its application. Previous results revealed the importance of the sulfoximine functional group and the stereochemical preference of the $P_1/P_{1'}$ substituents in our HIV-1 PI design.[9] Therefore, the sulfoximine group and the (2*S*,2′*S*)-benzyl substituents were retained as a core structure. Based on the retrosynthetic analysis shown in Scheme 5, the key intermediate is optically pure (2*S*,2′*S*)-diacid **17**. Compound **17** was subsequently obtained by refluxing diamide intermediate **14** in 4 N HCl, and verified by NMR as a single diastereomer.

It has been shown that conformationally constrained P_2/P_2' substituents, such as (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol moiety **18**, effectively occupy the lipophilic S_2/S_2' pockets of the HIV-1 protease and also form hydrogen bonds with D29/D29′ in the backbone of the enzyme.[19] This observation has led to the discovery of numerous highly potent inhibitors, such as Merck L700,417[20] and the subsequent clinically approved drug, Indinavir. However, the amino indanol group is susceptible to CYP450 metabolism,[21] which limits its oral bioavailability. To find a replacement for the metabolically labile (aromatic and benzylic CYP450 oxidation) amino indanol moiety and to investigate contributions of binding affinity from hydrogen bond interactions with the HIV-1 protease, we incorporated more conformationally relaxed amino indanol analogs such as **19** and **20** into our pseudosymmetric sulfide core structure **17**. The L-valine *N*-methylamide has been previously reported to improve ligand binding to the S_2/S_2' pockets of the HIV-1 protease.[22] It has been featured in various peptidomimetic HIV-1 PI's with different TSMs to maintain a high degree of enzymatic inhibition activities.[23] Therefore, we incorporated the L-valine *N*-methylamide substituent into the pseudosymmetric sulfide core structure **17** to construct the corresponding peptidomimetic inhibitor **21**.

It is reasonable to believe that a sulfoximine group may function as a TSM, which presumably forms hydrogen bonds with the two aspartic acid residues in the active site. To further demonstrate the importance of the sulfoximine core hydrogen bonding ability,



Scheme 4. Reagents and conditions: (a) (i) NaOH, DTT, $MeOH/H_2O$; (ii) **12**, $NaHCO_3$, TBAHS, ethyl acetate/H_2O, 70%; (b) *m*CPBA, 91%; (c) PTSA, 97%; (d) MSH, 60%.



Scheme 5. Retrosynthetic analysis of pseudosymmetric sulfoximine compounds and selections of P2/P2′ substitution.

the N-methylated sulfoximine analogue **23** was synthesized and evaluated to determine the biological effect of this chemical modification.

Several methods have been developed for the preparation of N-substituted sulfoximine from NH-sulfoximines, such as Eschweiler–Clark conditions[24] and Meerwein's salt (trimethyloxonium tetrafluroborate)[25] enforced N-methylation, and base-promoted N-alkylation.[26] We chose the powerful Meerwein's salt as the methylating agent in our synthesis because it allows methylation of sensitive or weakly nucleophilic functional groups under very mild reaction conditions. However, direct N-methylation of **1** proved to be challenging in the presence of nearby hydroxyl groups. Therefore the amino indanol groups were protected as acetonides to afford **22** (Scheme 6). On the other hand, it was observed that direct imination of **15** by MSH failed to provide compound **22**. Instead, deprotection of acetonide was observed, which is presumably due to the mesitylsulfonic acid produced in the reaction media. Non-nucleophilic base of *N*,*N*-diisopropyl-ethylamine (DIPEA)[27] was employed in the N-methylation reaction using the Meerwein's salt to prevent the deprotection of the acid-labile acetonide groups. As expected, the crude reaction mixture of the N-methylation reaction was directly reacted with PTSA in methanol to remove the isopropylidene and afforded the desired product **23** (Scheme 6).

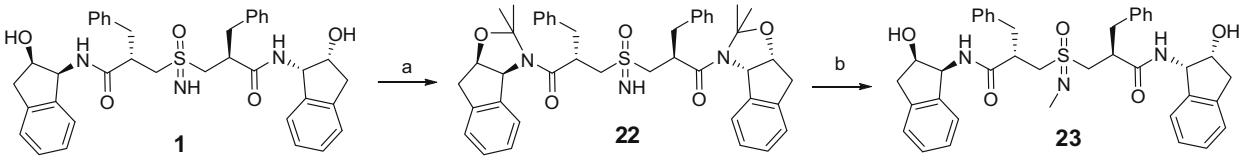
2.2. Biological activities

The enzymatic inhibitory effects of synthesized compounds were evaluated with purified HIV-1 protease. Enzymatic activity was determined based on substrate conversion quantified by a HPLC method. All compounds were also tested for their ability to inhibit the spread of viral infection in a cell based assay. The results are presented as percent inhibition against the HIV-1 protease/HIV-1 at 10 μM concentration. For those compounds that exhibited significant inhibitory effects, IC_{50} values were also obtained.

The amino indanol residue in **1** was replaced with phenylglycinols, which can be considered as acyclic unconstrained versions of amino indanol, to afford a series of HIV-1 PIs in the sulfide, sulfoxide, and sulfoximine forms. With the intention to evaluate the stereochemical preference of the hydroxyl amine, *S*- and *R*-phenylglycinol derivatives were prepared. All compounds show moderate activity against HIV-1 protease (Table 1), albeit less potent than (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol substituted lead compound **1**. Apparently, the conformational constraint inherent in the amino indanol system did not allow placement of even smaller, flexible substituents at maximum binding position. The observed trend of enzymatic activity of sulfoximine > sulfoxide > sulfide is in agreement with previous conclusions. The stereochemical preference of phenylglycinol is *S*, as in the most potent compound **31**, which is similar to that of (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol group in **1**.

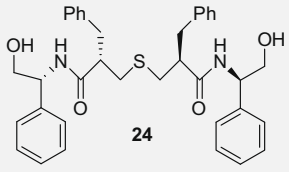
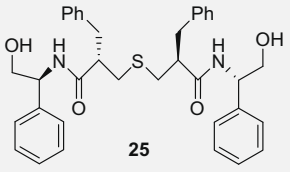
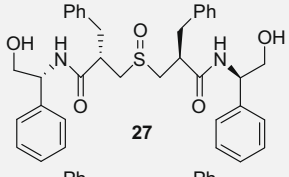
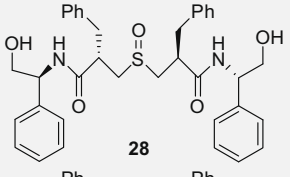
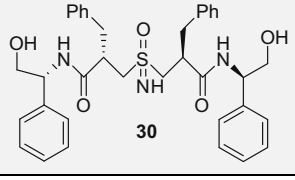
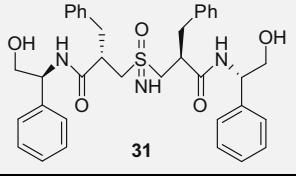
The antiviral activities of these compounds were evaluated in a cell based assay. Surprisingly, these compounds with opposite configurationally stereogenic centers at the P_2/P_2' sites are not very active against virus and exhibit marginal differences in potency. Thus, we conclude that the stereochemistry of phenylglycinols appears to have little effect on antiviral potency. It is observed that sulfide compounds **24** and **25** have the best antiviral activity in the two series suggesting that sulfide compounds have better cell permeability than the sulfoxide compounds.[28]

Replacement of amino indanol with L-valine-*N*-methylamide resulted in peptidomimetic analogues **26**, **29**, and **32** displaying



Scheme 6. Reagents and conditions: (a) 2,2-dimethoxypropane, CSA, 76%; (b) (i) $Me_3{\cdot}BF_3$, DIPEA; (ii) PTSA, MeOH, 56% in two steps.

Table 1
Biological activities against the HIV-1 protease and virus

Compound Structure	% Inhibition @ 10 μM against enzyme	% Inhibition @ 10 μM against virus	Compound structure	% Inhibition @ 10 μM against enzyme	% Inhibition @ 10 μM against virus
 **24**	51	15.4	 **25**	41	1.4
 **27**	50	2.5	 **28**	47	0
 **30**	54	0	 **31**	87	0

very potent activity in the enzymatic assay, with the sulfoximine derivative **32** exhibiting the highest enzyme activity (Table 2). This suggests that pseudosymmetric sulfoximine is an efficient core structure with higher activity than sulfide and sulfoxide cores. However, it is noticed that **32**, with L-valine *N*-methylamide as P_2/P_2' substitution, exhibits lower antiviral activity than **1**, which has an amino indanol group in the same position. The difference in antiviral activities may be attributed to a comparably lower cell permeability of **32** and higher peptide character, that is, four amide bonds rather than two.

From the preliminary biological activity data of the new compounds, we determined that incorporating the selected P_2/P_2' substituents into the central sulfoximine core structure resulted in compounds with moderate to good enzymatic inhibitory activity. However, this modification failed to improve enzymatic binding affinity. Apparently, different P_2/P_2' substituents are well tolerated in the enzymatic binding pockets. It is also concluded that the binding of phenyl residues at S_1/S_1' pockets and their stereochemical orientation within the enzyme binding sites are critical to the enzymatic inhibition as the relative potencies of our newly synthesized compounds differ slightly for different P_2/P_2' substituents. Nevertheless, the activities of these compounds are clearly dependent on the sulfoximine-centered core structure.

By introducing the *N*-methyl group into the sulfoximine structure, we observed decreased enzymatic and antiviral activities compared with the parent compound. *N*-Methylsulfoximine compound **23** exhibits an IC_{50} value of 0.46 μM against the HIV-1 protease, which is about 180-fold less potent than the sulfoximine compound **1** (IC_{50} of 2.5 nM). Additionally, this compound exhibits poor activity against virus (Table 3). These results reveal that introducing an *N*-methyl group into the lead compound significantly reduces the hydrogen bonding ability of the sulfoximine group.

2.3. Computational evaluation

The substrate binding site is characterized by the presence of the catalytic D25/D25′ residues with extended S_n/S_n' binding subsites for each of the corresponding P_n/P_n' substituents on the ligand (Schechter and Berger notation[29]) (Fig. 1). While binding of the natural peptide substrates involves a network of asymmetric hydrogen bonding on each of the two monomeric units,[30] symmetric inhibitor binding involves the same hydrogen bonding network on both monomeric units. The nearest S_1/S_1' subsites from the catalytic D25/D25′ residues are considered hydrophobic subsites consisting of the sidechains of L23, V80, P81, V82, and I84. The S_2/S_2' sites are mostly hydrophobic, consisting of the sidechains of A28, V32, I47, I50, L76 as well as residues 28–32 of the opposing chain. A conserved crystallographic water, which mediates ligand binding, is commonly found hydrogen bonded to the backbone amide hydrogen of I50/I50′ of the 'flap' regions at residues 45–55. Replacement of this crystallographic water has been shown previously to enhance inhibition potency.[31] Compounds which also take advantage of the C_2 symmetry of the homodimeric protein have also been previously reported.[32] All of the published X-ray structures of HIV-1 protease in complex with each of the known FDA approved HIV PI consist of the hydroxyl group positioned within hydrogen bonding distance between the catalytic D25/D25′ carboxyl groups.

Table 2
Biological data of peptidomimetic inhibitors

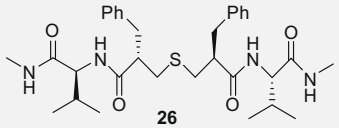
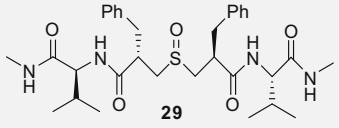
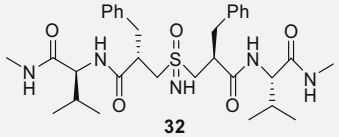
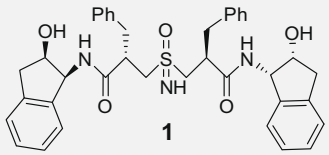
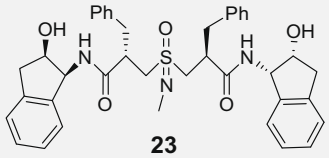
Compound structure	% Inhibition @ 10 μM against enzyme	% Inhibition @ 10 μM against virus
 **26**	87	12.6
 **29**	90	2.1
 **32**	99	5.5

Table 3
Biological activity of lead compounds

Compound structure	IC_{50} against enzyme (nM)	IC_{50} against virus (μM)
 **1**	2.5	0.41
 **23**	460	>10

In the final docked models of **1**, **30**, **31**, and **32**, several conserved intermolecular hydrogen bonds were observed. The main chain amide oxygens were found to hydrogen bond to the conserved crystallographic water (Fig. 2) while its amide donated hydrogen to the carbonyl group of G27/G27′ backbone. The hydroxyl groups of **1** and **31** acted as hydrogen acceptors to the D29/D29′ amide hydrogens as well as hydrogen donors to the charged carboxylate oxygens of D29/D29′. The S_1/S_1' pockets were occupied by the P_1/P_1' benzyl substituents, forming favorable hydrophobic interactions. The hydrophobic rings of the indanol groups occupied the S_2/S_2' binding sites. The central sulfoximine moiety of **1**, **30**, **31**, and **32** were found to be within the hydrogen bonding distances of both D25/D25′ carboxylate sidechain, similar to that conformation found in the binding of the parent compound, L700,417. Previous studies have demonstrated that the pK_a in one of the two aspartates is perturbed when the other is ionized.[33] As a result, the central sulfoximine group of the inhibitor, when hydrogen bonded to the two catalytic aspartic groups, simultaneously functioned as a hydrogen donor to the charged D25 and as a hydrogen acceptor to the protonated D25′. The replacement of the central hydroxyl group to the sulfoximine moiety in L700,417 has minimum effect on the mode of binding as observed in **1**. With the exception of the central sulfoximine, the hydrogen bonding network of the main chain and P_2/P_2' substituents for all four sulfoximine inhibitors to each of the HIV-1 protease were symmetric in each of the two HIV protease monomeric units. Compound **30** showed a relative slight decrease in enzymatic inhibitory activity. While the change in the pseudosymmetric chiral center did not affect the placement of the indanol group into the S_2/S_2' pockets, it resulted in the indanol hydroxyl bonding with the backbone carbonyl oxygen of G48/G48′. Overall, the incorporation of the more conformationally flexible indanol analogues did not have a major effect in the overall binding affinity relative to compound **1**. Replacement of the indanol group with the L-valine-*N*-methylamide group in

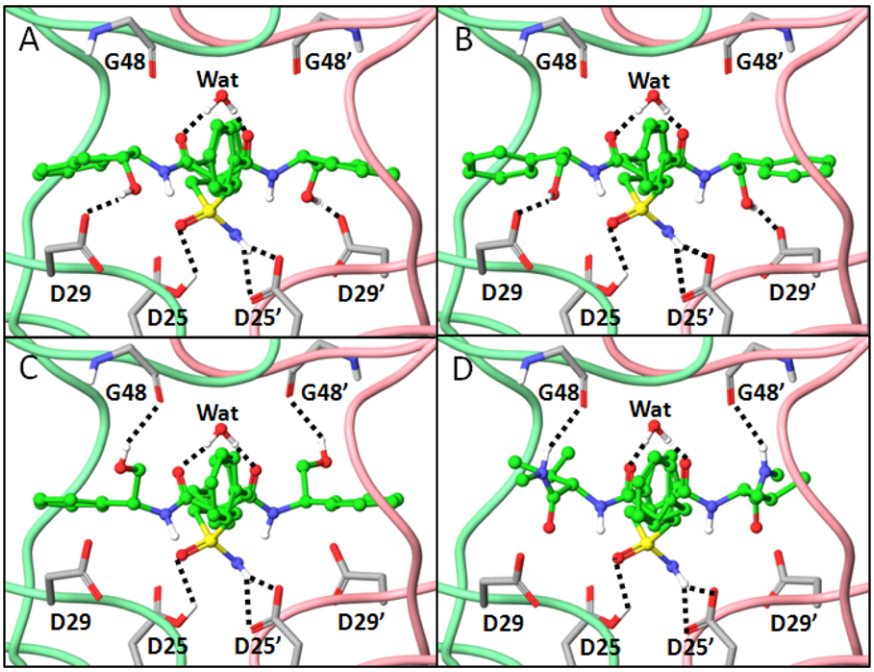


Figure 2. Binding of compounds **1**, **30**, **31**, and **32** (a–d) to HIV-1 protease. Chains A and B are shown in ribbons and are color in pink and green, respectively. The consistently observed modes of binding support the validity of the sulfoximine moiety as a TSM, participating both as a hydrogen donor and an acceptor. The incorporation of the less restrained stereospecific P2/P2′ substituents resulted in a change in the hydrogen bonding network from the carboxylate sidechain of D29/D29′ for compound **31** to the backbone carbonyl oxygen of G48/G48′ for **30**.

the pseudosymmetric peptidomimetic compound **32** resulted in the placement of the isopropyl group in the P_2/P_2' subsites as well as the *N*-methyl groups into the P_3/P_3' subsites. The indanol hydroxyl groups that hydrogen bonded to D29/D29′ carboxylates in **1** were replaced by the terminal amides in **32**, which bonded directly to D29/D29′ backbone amide oxygen and the G48/G48′ amide hydrogen.

The calculated binding free energy of all the sulfoximine inhibitors are shown (see Table 4). As expected, the calculated binding free energies of all synthesized sulfoximine inhibitors were higher than all of the FDA approved inhibitors, as observed in the lower inhibitory enzymatic activities. The replacement of the hydroxyl in L700,419 to the sulfoximine group resulted in a net loss of 0.9 kcal/mol in binding free energy in **1.** The major contribution to the change was due to the overall desolvation effect during binding with an insufficient compensation by the protein-ligand coulombic energy in the bound state. The opening of the five-member ring in the indanol resulted in the chiral species of **30** and **31** did not entropically affect their overall binding free energies. Both compounds were able to bind in a similar mode of binding within the HIV protease binding site by re-establishing alternative hydrogen bonding to D29/D29′ and G48/G48′, respectively (Fig. 2), resulting in a relative difference of 0.4 kcal/mol change in their binding free energy. The most potent sulfoxime inhibitor observed by the enzymatic inhibition study was the pseudosymmetric peptidomimetic compound **32**, which was evaluated to have the lowest binding energy at −12.5 kcal/mol, similar to that of L700,417.

Table 4
Calculated absolute binding free energies are in kcal/mol

Compound	$\langle \Delta U^{f\to b}_{elec} \rangle_{\bar{q}}$	$\langle \Delta U^{f\to b}_{vdw} \rangle_{\bar{q}}$	$\langle \Delta U^{f\to b}_{cav} \rangle_{\bar{q}}$	ΔG^{exp}_{bind}	ΔG^{calc}_{bind}
SQR	−11.9	−82.4	11.1	−13.0	−12.5
IDV	−32.1	−81.2	8.4	−12.4	−13.4
RTV	−38.5	−82.9	10.7	−13.7	−13.9
APV	−29.6	−64.1	5.5	−13.2	−13.9
LPV	−43.6	−76.7	7.5	−15.1	−14.1
NFV	−31.9	−72.6	7.7	−12.8	−13.8
DRV	−32.8	−66.2	6.5	−15.2	−14.1
L700,417	−20.4	−81.1	5.7	−12.7	−12.4
1	−13.4	−91.9	6.8	N/A	−11.5
30	−13.7	−86.1	5.9	N/A	−11.8
31	−19.7	−82.4	4.8	N/A	−12.2
32	−14.1	−77.5	9.1	N/A	−12.5

3. Conclusions

HIV-1 PIs based on the pseudosymmetric sulfoximine-centered core structure were synthesized. All prepared compounds exhibit micromolar to nanomolar enzymatic inhibitory activities; however, most of the compounds show moderate or poor antiviral activity against the HIV-1 strain in cells. Nevertheless, it is observed that enzymatic activity is marginally affected by changing the P_2/P_2' substituents, which argues for the importance of the central sulfoximine core structure as well as the preferred stereochemistry of the P_1/P_1' sites.

Pseudosymmetric sulfoximine core structure was identified as a valid TSM in the design of potent anti-HIV drugs with novel structures and properties. The asymmetric synthesis we developed will allow the design of structurally diversified inhibitors featuring the stereochemically controlled sulfoximine functionality. The divergent–convergent synthesis of **1** could be readily modified to generate analogs with different P_1/P_1' and P_2/P_2' substituents which may better match the enzyme's binding pocket and lead to improved antiviral activity.

The sulfoximine group may function as a TSM by providing NH and O as potential hydrogen bond donor and acceptor to the catalytic aspartates. N-Methylation of the sulfoximine derivative exhibits decreased potency against both the enzyme and virus. Docking studies further support the sulfoximine as a TSM and pro-

vide insight into the symmetric hydrogen bonding network during binding. Further confirmation of this hypothesis by X-ray crystallography study is currently underway.

4. Experimental

4.1. Chemistry

Lipase PS and chemicals were purchased from Sigma Chemicals (St. Louis, MO). All commercial reagents were used as provided unless otherwise indicated. An anhydrous solvent dispensing system (J. C. Meyer) using two columns packed with neutral alumina was utilized for drying THF, Et_2O and CH_2Cl_2, while two columns packed with molecular sieves were used to dry DMF. For all compounds, ^1H NMR and ^{13}C NMR spectra were acquired using a Mercury 300 or Varian 600 spectrometer in deuterium solvent with Me_4Si as internal standard unless otherwise stated. Optical rotations were recorded on Rudolph autopol III polarimeter. Mass spectra were recorded using Bruker BioTOF II or Agilent LC-TOF 1100 mass spectrometers equipped with either an ESI or APCI source. All melting points were uncorrected and obtained from Mel-Temp II melting point apparatus. All moisture and/or air sensitive experiments were performed under a positive pressure of nitrogen or argon atmosphere in oven dried glassware. Solvent and liquid reagents were transferred by a syringe or cannula. Solvents were removed in vacuum using a rotary evaporator equipped with a condenser. Yields were not optimized.

4.1.1. 2*R*-Hydroxymethyl 3-phenylpropyl acetate (5)

A suspension of **4** (1.000 g, 6.0 mmol) and lipase PS (0.500 g) in vinyl acetate (830 μL) and H_2O (30 μL) was stirred at room temperature. After being stirred for 5 h, the mixture was filtered through a pad of Celite. The filtrate was evaporated and purified by flash chromatography on silica gel using 30% ethyl acetate in hexane. Compound **5** was isolated as an oil (1.153 g, 92%). ^1H NMR ($CDCl_3$, 600 MHz) δ 7.29–7.17 (m, 5H), 4.15 (dd, J = 4.8, 11.4 Hz, 1H), 4.07 (dd, J = 6, 10.8 Hz, 1H), 3.58 (dd, J = 4.8, 11.4 Hz, 1H), 3.50 (dd, J = 6.6, 11.4 Hz, 1H), 2.68 (dd, J = 7.2, 13.8 Hz, 1H), 2.61 (dd, J = 7.8, 13.8 Hz, 1H), 2.54 (br, 1H), 2.14–2.09 (m, 1H), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.2, 138.6, 129.3, 128.6, 126.4, 63.4, 62.1, 42.3, 34.1, 21.2; $[\alpha]_D^{20}$ +26.3° (c 1.0 $CHCl_3$); ESI MS $C_{12}H_{16}O_3$ 208.1, found 209.1 $(M+H)^+$.

4.1.2. 2*R*-Phenyl 3-benzyloxy propyl acetate (6)

To a stirred solution of **5** (0.200 g, 0.96 mmol) and benzyl 2,2,2-trichloroacetimidate (0.290 g, 1.15 mmol) in a mixed solvent of DCM (3 mL) and cyclohexane (6 mL) was added trifluoromethanesulfonic acid (10 μL) under argon. The reaction mixture was stirred at room temperature overnight. The crystalline trichloroacetamide was removed by filtration and the filtrate was washed with aqueous saturated solution of $NaHCO_3$ (15 mL) and H_2O (15 mL). The organic layer was dried over Na_2SO_4, evaporated and purified by flash chromatography on silica gel using 5% ethyl acetate in hexane. Compound **6** was isolated as an oil (0.201 g, 70%). ^1H NMR ($CDCl_3$, 600 MHz) δ 7.33–7.13 (m, 10H), 4.49–4.44 (m, 2H), 4.12–4.06 (m, 2H), 3.43–3.37 (m, 2H), (dd, J = 6.6, 9 Hz, 1H), 2.74 (dd, J = 7.8, 13.8 Hz, 1H), 2.67 (dd, J = 7.2, 13.8 Hz, 2H), 2.27–2.23 (m, 1H), 1.99 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.3, 140.4, 137.5, 129.8, 128.5, 127.8, 126.2, 125.7, 75.2, 72.3, 67.8, 45.5, 40.3, 23.9; $[\alpha]_D^{20}$ −2.6° (c 1.0 $CHCl_3$); ESI MS $C_{19}H_{22}O_3$ 298.2, found 321.2 $(M+Na)^+$.

4.1.3. 2*R*-Phenyl 3-benzyloxy propan-1-ol (7)

To a solution of **6** (0.200 g, 0.67 mmol) in methanol (40 mL) was added K_2CO_3 (0.185 g, 1.34 mmol) and the mixture was stirred at room temperature for 3 h. The reaction mixture was then filtered through Celite. After evaporation of methanol, the remaining solid was taken up in H_2O, extracted with DCM, washed with water and brine. The organic layer was dried over Na_2SO_4, evaporated and purified by flash chromatography on silica gel using 15% ethyl acetate in hexane. Compound **7** was isolated as an oil (0.148 g, 87%). ^1H NMR ($CDCl_3$, 600 MHz) δ 7.35–7.13 (m, 10H), 4.34–4.29 (m, 2H), 3.70 (dd, J = 4.2, 11.4 Hz, 1H), 3.61 (dd, J = 6.6, 11.4 Hz, 1H), 3.56 (dd, J = 4.2, 9 Hz, 1H), 3.46 (dd, J = 6.6, 9 Hz, 1H), 2.68–2.57 (m, 3H), 2.15–2.09 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 140.3, 138.2, 129.3, 128.7, 128.0, 127.9, 126.3, 73.8, 72.9, 65.4, 42.9, 34.8; $[\alpha]_D^{20}$ −34.0° (c 1.0 $CHCl_3$); ESI MS $C_{17}H_{20}O_2$ 256.1, found 279.1 $(M+Na)^+$.

4.1.4. 2*R*-Benzyl 3-benzyloxy propanoic acid (8)

Compound **7** (0.049 g, 0.19 mmol) was dissolved in 2 mL of acetone and cooled to 0 °C. Jones reagent (0.38 mmol, 2.67 M, prepared as follows: mix 2.67 g CrO_3 with 23 mL of concentrated H_2SO_4, slowly diluted with distilled H_2O to a total volume of 10 mL) was added slowly and the mixture was stirred at 0 °C for 2 h. 2-Propanol was added until the solution turned greenish blue and the solvent was then evaporated. Ethyl acetate and H_2O were added to the residue and the separated organic layer was washed with brine and concentrated under reduced pressure. Flash chromatography eluting with 30% ethyl acetate in hexane afforded compound **8** as an oil (0.051 g, 99%). ^1H NMR ($CDCl_3$, 600 MHz) δ 7.34–7.15 (m, 10H), 4.54–4.48 (m, 2H), 3.64–3.58 (m, 2H), 3.05–2.97 (m, 2H), 2.90–2.86 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 179.4, 138.6, 137.9, 129.2, 128.7, 128.6, 128.4, 127.9, 126.7, 73.5, 69.6, 47.8, 24.6; $[\alpha]_D^{20}$ −90.0° (c 1.0 $CHCl_3$); ESI MS $C_{17}H_{18}O_3$ 270.1, found 293.1 $(M+Na)^+$, 541.1 $(2\,M+H)^+$.

4.1.5. *N*-[(1*S*,2*R*)-*cis*-Aminoindan-2-ol] 2*R*-benzyl-3-benzyloxy propionamide (9)

A solution of **8** (0.050 g, 0.19 mmol) and (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol (0.033 g, 0.22 mmol) in DCM (3 mL) was cooled in an ice bath and treated with triethyl amine (51.5 μL, 0.37 mmol) followed by adding (benzotrizol-1-yloxy) tris (dimethylamino) phosphonium hexafluorophosphate (BOP reagent, 0.098 g, 0.22 mmol). The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate and washed successively with 0.5 N HCl, H_2O, saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel using 30% ethyl acetate in hexane. Compound **9** was isolated as a white solid (0.070 g, 94%). Mp: 128–130 °C; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.32–7.09 (m, 14H), 6.14 (d, J = 7.8 Hz, 1H), 5.32 (dd, J = 4.8, 7.8 Hz, 1H), 4.56–4.51 (m, 2H), 4.35 (br, 1H), 3.77 (t, J = 8.4 Hz, 1H), 3.68–3.66 (m, 1H), 3.07–2.95 (m, 2H), 2.91–2.77 (m, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.0, 140.6, 140.4, 139.6, 138.0, 128.8, 128.6, 128.3, 128.0, 127.2, 126.8, 125.4, 124.5, 73.7, 73.5, 7.09, 57.7, 50.6, 39.5, 35.6; $[\alpha]_D^{20}$ +29.2° (c 1.0 $CHCl_3$); ESI MS $C_{26}H_{27}NO_3$ 401.2, found 424.2 $(M+Na)^+$.

4.1.6. 1-[2,2-Dimethyl-8,8a-dihydro-3aH-indeneo[(1*S*,2*R*)-1,2]-oxazol-3-yl] (2*R*)-benzyl-3-benzyloxy propanone (10)

To a suspension of **9** (0.062 g, 0.16 mmol) in toluene (4 mL) was added 2,2-dimethoxypropane (0.59 mL, 6.4 mmol) followed by (±)-camphor-10-sulfonic acid (0.008 g, 0.03 mmol). The mixture was stirred at room temperature for 4 h and the reaction was quenched by adding ethyl acetate and 10% $NaHCO_3$. The organic layer was separated and dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography eluting with 15% ethyl acetate in hexane afforded **10** as a solid (0.063 g, 92%). Mp: 109–110 °C; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.38–7.10 (m, 12H), 6.86–6.80 (m, 1H),

6.12 (d, J = 15 Hz, 1H), 5.54 (d, J = 9 Hz, 1H), 4.51–4.44 (m, 3H), 3.80–3.66 (m, 2H), 3.52–3.43 (m, 1H), 3.38–3.31 (m, 1H), 3.03–2.92 (m, 2H), 2.74–2.69 (m, 1H), 1.62 (s, 3H), 1.32 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.7, 141.1, 140.5, 139.9, 138.3, 129.7, 128.9, 128.6, 128.2, 127.9, 127.7, 127.2, 126.9, 125.7, 124.2, 96.9, 79.1, 73.9, 73.7, 65.8, 48.6, 36.3, 35.3, 26.7, 24.1; $[\alpha]_D^{20}$ +120.2° (c 1.0 $CHCl_3$); ESI MS $C_{29}H_{31}NO_3$ 441.2, found 442.2 $(M+H)^+$.

4.1.7. 1-[2,2-Dimethyl-8,8a-dihydro-3aH-indeneo[(1*S*,2*R*)-1,2]-oxazol-3-yl] (2*R*)-benzyl-3-hydroxyl propanone (11)

A mixture of ethanol (4 mL) solution of **10** (0.062 g, 0.14 mmol) and Pd/C (10 wt%, 0.012 g) was hydrogenated at room temperature under hydrogen atmosphere for 12 h. The catalyst was filtered through Celite, the cake was rinsed with ethanol and ethyl acetate. The filtrate was evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel using 50% ethyl acetate in hexane to afford compound **11** as a white solid (0.043 g, 88%). Mp: 136–138 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.34–7.16 (m, 7H), 6.90 (t, J = 7.2 Hz, 1H), 6.28 (d, J = 7.8 Hz, 1H), 5.48 (d, J = 4.2 Hz, 1H), 4.85–4.83 (m, 1H), 3.88–3.83 (m, 2H), 3.39–3.16 (m, 2H), 3.14–3.02 (m, 3H), 2.87–2.82 (m, 1H), 1.68 (s, 3H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 171.1, 140.7, 139.7, 129.7, 128.9, 128.9, 128.4, 127.3, 126.9, 125.8, 124.2, 97.1, 79.3, 65.9, 65.2, 49.7, 36.3, 35.0, 26.6, 24.3; $[\alpha]_D^{20}$ −21.4° (c 1.0 $CHCl_3$); ESI MS $C_{22}H_{25}NO_3$ 351.2, found 374.5 $(M+Na)^+$.

4.1.8. 1-[2,2-Dimethyl-8,8a-dihydro-3aH-indeneo[(1*S*,2*R*)-1,2]-oxazol-3-yl] (2*R*)-benzyl-3-bromo propanone (12)

To a solution of **11** (0.810 g, 2.3 mmol) and triphenylphosphine (0.725 g, 2.9 mmol) in CH_2Cl_2 (40 mL) in an ice bath, was added crystalline *N*-bromosuccinimide (0.492 g, 2.8 mmol, recrystallized from hot H_2O) slowly. After addition was complete, the mixture was stirred at room temperature for 12 h. The reaction was quenched by adding saturated $NaHCO_3$, the organic layer was then washed by brine, dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by flash chromatography on silica gel using 15% ethyl acetate in hexane to afford compound **12** as a white solid (0.844 g, 88%). Mp: 139–141 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.36–7.18 (m, 7H), 6.92 (t, J = 6.6 Hz, 1H), 6.24 (d, J = 7.2 Hz, 1H), 5.59 (d, J = 4.8 Hz, 1H), 4.87 (m, 1H), 3.68 (m, 1H), 3.53–3.49 (m, 2H). 3.45–3.41 (m, 1H), 3.08 (m, 2H), 2.89 (dd, J = 5.4, 13.2 Hz, 1H), 1.72 (s, 3H), 1.37 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.4, 140.7, 140.6, 138.5, 139.7, 129.2, 128.5, 127.4, 125.9, 124.1, 97.5, 79.2, 66.4, 49.3, 38.7, 36.3, 34.6, 26.7, 24.1; $[\alpha]_D^{20}$ +100.2° (c 1.0 $CHCl_3$); ESI MS $C_{22}H_{24}BrNO_2$ 413.1, found 414.1 $(M+H)^+$.

4.1.9. 1-[2,2-Dimethyl-8,8a-dihydro-3aH-indeneo[(1*S*,2*R*)-1,2]-oxazol-3-yl] (2*R*)-benzyl-3-acetylthio propanone (13)

A mixture of **11** (0.090 g, 0.26 mmol) and thioacetic acid (28.6 μL, 0.38 mmol) in THF (1 mL) was added dropwise at −10 °C to a stirred suspension of preformed adduct of triphenylphosphine (0.101 g, 0.26 mmol) and diisopropyl azocarboxylate (97 μL, 0.38 mmol). The mixture was stirred at −10 °C for 1 h then room temperature for 2 h. The reaction was quenched by addition of ether (5 mL) and washed twice with saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel using 15% ethyl acetate in hexane. Compound **13** was isolated as a pale yellow oil (0.087 g, 84%); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.34–7.11 (m, 6H), 6.74 (t, J = 7.8 Hz, 1H), 5.80 (d, J = 7.2 Hz, 1H), 5.51 (d, J = 3.6 Hz, 1H), 4.89–4.87 (m, 1H), 3.44 (m, 1H), 3.28 (dd, J = 5.4, 13.2 Hz, 1H), 3.18–3.16 (m, 1H), 3.12–3.00 (m, 3H), 2.86 (dd, J = 3.6, 13.2 Hz, 1H), 2.34 (s, 3H), 1.67 (s, 3H), 1.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 195.8, 169.6, 140.6, 139.7, 129.9, 129.0, 128.3, 127.3, 127.1, 125.7, 124.2, 97.0, 79.4, 65.8, 48.4, 37.6, 36.4, 33.1, 30.9, 26.7, 24.3; $[\alpha]_D^{20}$ +50.5° (c 1.0 $CHCl_3$); ESI MS $C_{24}H_{27}NO_3S$ 409.2, found 410.2 $(M+H)^+$.

4.1.10. Bis-[*N*-(2,2-dimethyl-8,8a-dihydro-3a*H*-indeno[(*1S*,*2R*)-1,2]-oxazol-3-yl) (2*R*)-benzyl-3-yl propianamide] sulfide (14)

DL-Dithiothreitol (0.083 g, 0.54 mmol) was added to a solution of **13** (1.100 g, 2.6 mmol) in methanol (45 mL). The solution was sparged with N_2 for 15 min, cooled to 0 °C and kept under N_2 atmosphere. In a separate flask, 1 N NaOH (10 mL, 10.4 mmol) was sparged with N_2 for 20 min and then added to the reaction mixture slowly. After 1 h, the ice bath was removed to allow the mixture to warm to room temperature. The reaction was quenched by adding saturated NH_4Cl and then diluted with $CHCl_3$. The organic layer was washed by brine, dried over Na_2SO_4. All the solvent was evaporated to afford 1.2 g oil, which was used directly for the next step. To ethyl acetate (70 mL) and saturated $NaHCO_3$ (60 mL) solution, which were sparged with N_2 for 20 min, of **12** (1.100 g, 2.6 mmol) and the above crude product was added tetrabutylammonium hydrogen sulfate (TBAHS, 4.5 g, 5 equiv). The two-phase reaction mixture was vigorously stirred at room temperature under N_2 atmosphere. The reaction was quenched after seven days and the organic layer was separated, washed with brine and dried over Na_2SO_4. The crude product was purified by flash chromatography on silica gel using 10% ethyl acetate in hexane to afford compound **14** as a white solid (1.200 g, 70%). Mp: 80–81 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.37–7.01 (m, 14H), 6.92–6.89 (m, 2H), 6.21 (d, J = 7.8 Hz, 2H), 5.49 (d, J = 4.2 Hz, 2H), 4.87–4.85 (m, 2H), 3.38 (dd, J = 7.8, 12.6 Hz, 2H), 3.28 –3.23 (m, 2H), 3.08–3.02 (m, 4H), 2.97–2.94 (m, 2H), 2.86 (dd, J = 5.4, 13.2 Hz, 2H), 2.72 (dd, J = 6.0, 12.6 Hz, 2H), 1.70 (s, 6H), 1.35 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.4, 140.8, 140.6, 139.4, 129.7, 129.0, 128.5, 127.4, 125.9, 97.2, 90.3, 66.3, 47.9, 38.6, 37.5, 36.3, 26.7, 24.4; $[\alpha]_D^{20}$ +90.0° (c 1.0 $CHCl_3$); HRMS (ESI) $C_{44}H_{48}N_2O_4S$ 700.3335, found 723.3235 $(M+Na)^+$ 1.1 ppm error.

4.1.11. Bis-[*N*-(2,2-dimethyl-8,8a-dihydro-3a*H*-indeno[(*1S*,*2R*)-1,2]-oxazol-3-yl) (2*R*)-benzyl-3-yl propianamide] sulfoxide (15)

To a solution of **14** (0.586 g, 0.84 mmol) in DCM (15 mL) at −78 °C under N_2 was added a solution of *m*CPBA (0.187 g, 0.84 mmol) in DCM (3 mL). The mixture was stirred for 1 h then warmed to room temperature for 1 h. The reaction was quenched by diluting with DCM and extracted with saturated $NaHCO_3$. The organic layer was washed by brine, dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by flash chromatography on silica gel using 50% ethyl acetate in hexane to afford compound **15** as a white solid (0.545 g, 91%). Mp: 198–199 °C; ^{1}H NMR (acetone-d_6, 600 MHz) δ 7.48–7.09 (m, 14H), 6.85 (t, J = 7.8 Hz, 1H), 6.75 (t, J = 7.2 Hz, 1H), 6.11 (d, J = 7.2 Hz, 1H), 5.94 (d, J = 7.8 Hz, 1H), 5.83 (br, 1H), 5.44 (br, 1H), 4.84–4.79 (m, 2H), 3.63–3.55 (m, 2H), 3.52–3.47 (m, 2H), 3.23–2.88 (m, 10H), 1.64 (s, 3H), 1.59 (s, 3H), 1.26 (s, 3H), 1.21 (s, 3H); ^{13}C NMR (acetone-d_6, 150 MHz) δ 169.4, 168.8, 141.2, 141.1, 141.0, 139.9, 139.4, 129.9, 129.9, 129.0, 128.9, 128.1, 128.0, 127.2, 127.1, 127.1, 127.0, 125.6, 125.5, 124.1, 96.6, 96.2, 79.4, 79.3, 66.1, 65.2, 57.4, 56.9, 43.0, 42.9, 38.9, 36.7, 35.9, 26.3, 26.2, 23.5, 23.3; $[\alpha]_D^{20}$ +103.4° (c 1.0 $CHCl_3$); HRMS (ESI) $C_{44}H_{48}N_2O_5S$ 716.3284, found 717.3339 $(M+H)^+$ 2.5 ppm error.

4.1.12. Bis-[[*N*-(1*S*,2*R*)-2,3-dihydro-2-hydroxy-1*H*-inden-1-yl]-2*S*-phenyl-3-yl-propionamide] sulfoxide (16)

To a stirred and cooled solution of sulfide **15** (1 equiv) in DCM (2 mL) and MeOH (1 mL) was added *m*CPBA (1.1 equiv in 1 mL of DCM) slowly. The reaction was kept stirring under N_2 for 3 h, and the solution was concentrated and diluted with $CHCl_3$. Organic layer was washed by saturated $NaHCO_3$, brine and dried over Na_2SO_4. The solvent was evaporated to afford **16** as white solids

(29 mg, 97%). Mp: 200–201 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.15 (d, J = 9.0 Hz, 1H), 8.09 (d, J = 9.0 Hz, 1H), 7.28–7.05 (m, 18H), 5.18 (t, J = 4.8 Hz), 5.12 (t, J = 4.8 Hz, 1H), 4.76 (d, J = 3.0 Hz, 1H), 4.60 (d, J = 3.0 Hz, 1H), 4.34 (d, J = 3.6 Hz, 1H), 4.29 (d, J = 3.6 Hz, 1H), 3.18–2.96 (m, 7H), 2.79–2.66 (m, 5H), 2.41 (d, J = 10.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 173.1, 173.1, 142.7, 142.6, 141.4, 141.4, 139.3, 139.2, 129.9, 129.8, 128.9, 127.9, 127.8, 127.0, 126.9, 126.8, 125.5, 125.3, 125.0, 124.9, 72.9, 57.7, 57.6, 54.6, 54.2, 42.6, 42.2, 39.8, 39.2, 38.6; $[\alpha]_D^{20}$ −4.4° (*c* 0.26 MeOH/$CHCl_3$ = 1:1); HRMS (ESI) $C_{38}H_{40}N_2O_5S$ 636.2658, found 637.2715 $(M+H)^+$, 2.5 ppm error.

4.1.13. Bis-{[*N*-(1*S*,2*R*)-2,3-dihydro-2-hydroxy-1*H*-inden-1-yl]-2*S*-phenyl-3-yl-propionamide} sulfoximine (1)

Sulfoxide **16** (1 equiv) was dissolved in DMF (2.5 mL) in an ice bath. MSH (4 equiv) was added to the flask all in once. The reaction was stirred at room temperature for 20 h and quenched by adding pre-cooled $NaHCO_3$ solution and kept stirring for additional 10 min. The reaction mixture was concentrated and partitioned between $CHCl_3$ and brine. Combined organic layers were dried over Na_2SO_4 and purified by HPLC to afford **1** as white solids (7 mg, 45%). Mp: 208–209 °C; ^{1}H NMR ($CDCl_3$ + MeOH-d_4, 300 MHz) δ 7.38–6.93 (m, 18H), 5.24 (br, 2H), 4.31 (dd, J = 2.7, 2.7 Hz, 2H), 3.77 (dd, J = 5.4, 5.4 Hz, 2H), 3.33–3.28 (m, 2H), 3.14–2.97 (m, 6H), 2.88–2.80 (m, 4H); ^{13}C NMR ($CDCl_3$ + MeOH-d_4, 75 MHz) δ 177.8, 177.7, 144.3, 144.2, 144.1, 141.9, 141.7, 133.3, 133.3, 133.8, 132.7, 131.9, 131.8, 131.1, 130.9, 130.8, 128.9, 128.3, 128.6, 77.1, 77.0, 61.8, 61.7, 60.4, 59.9, 58.7, 47.5, 46.7, 43.3, 43.3, 43.2, 43.0; ESI *m*/*e* $C_{38}H_{41}N_3O_5S$ 651.2767, found 652.2849 $(M+H)^+$, 0.61 ppm error; $[\alpha]_D^{20}$ = +60.4° (*c* 0.25 MeOH/$CHCl_3$ = 1:1).

4.1.14. Bis-(2*S*-benzyl-3-yl propanoic acid) sulfide (17)

Compound **14** (100 mg, 0.14 mmol) was suspended in 4 N HCl (20 mL, H_2O/dioxane = 1:1). The reaction was refluxed in oil bath for 8 h and quenched by diluting with DCM followed by washing with brine. The combined organic layers, after being dried over Na_2SO_4, were evaporated to give the crude product which was purified by flash chromatography on silica gel using 2% MeOH in DCM with addition of 0.3% acetic acid. Compound **17** was isolated as an oil (39 mg, 77%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.27–7.13 (m, 10H), 2.93 (dd, J = 6.6, 13.2 Hz, 2H), 2.82–2.73 (m, 4H), 2.64 (dd, J = 8.4, 13.2 Hz, 2H), 2.51 (dd, J = 4.8, 13.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 180.5, 138.1, 129.1, 128.8, 127.0, 47.4, 38.0, 32.7; $[\alpha]_D^{20}$ −14.8° (*c* 1.0 $CHCl_3$); HRMS (ESI) $C_{22}H_{26}O_4S$ 358.1239, found 358.1299, 3.7 ppm error.

4.1.15. Bis-[*N*-(2,2-dimethyl-8,8a-dihydro-3a*H*-indeno[(1*S*,2*R*)-1,2]-oxazol-3-yl) 2*R*-benzyl-3-yl propianamide] sulfoximine (22)

To a suspension of **1** (50 mg, 0.07 mmol) in toluene (4 mL) was added 2,2-dimethoxypropane (4 mL) followed by 10-camphorsulfonic acid (17.6 mg, 0.07 mmol). The reaction was stirred at 50 °C for 12 h. The reaction was quenched by adding ethyl acetate and 10% $NaHCO_3$. Organic layer was separated, dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography eluting with 20% ethyl acetate in hexane afforded **22** as an oil (42 mg, 76%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.34–6.70 (m, 16H), 6.76 (d, J = 5.4 Hz, 1H), 6.70 (d, J = 5.4 Hz, 1H), 5.84–5.73 (m, 2H), 4.79–4.74 (m, 2H), 3.76–3.68 (m, 2H), 3.57–3.46 (m, 4H), 3.06–2.87 (m, 8H), 1.35 (s, 6H), 1.26 (s, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.9, 140.9, 140.8, 140.8, 137.4, 137.4, 129.3, 129.3, 129.1, 129.1, 128.3, 128.3, 127.4, 127.2, 127.2, 126.0, 125.9, 123.9, 123.9, 97.0, 79.2, 66.6, 66.4, 58.8, 58.3, 41.3, 40.5, 39.1, 39.0, 36.0, 29.7, 26.5; $[\alpha]_D^{20}$ +102.0° (*c* 0.5 $CHCl_3$); HRMS (ESI) $C_{44}H_{49}N_3O_5S$ 731.3393, found 732.3430 $(M+H)^+$ 4.9 ppm error.

4.1.16. *N*-Methyl bis-[*N*-(2,2-dimethyl-8,8a-dihydro-3a*H*-indeno[(1*S*,2*R*)-1,2]-oxazol-3-yl) 2*R*-benzyl-3-yl propianamide] sulfoximine (23)

To a solution of **22** (20 mg, 0.027 mmol) in 2 mL of DCM was added DIPEA (3.5 mg, 0.03 mmol) followed by Meerwein's salt (4.0 mg, 0.03 mmol) in an ice bath. The mixture was stirred under N_2 till no starting material left. The reaction was quenched by adding H_2O and MeOH. To this crude reaction mixture was added PTSA (5 mg, 0.03 mmol) and the reaction was stirred for additional 12 h. All the solvent was removed under reduced pressure and the residue was partitioned between $CHCl_3$ and saturated $NaHCO_3$. The combined organic layers were washed by brine and dried over Na_2SO_4. The crude product was purified by flash chromatography using 1% MeOH in $CHCl_3$ to afford **23** as a solid (10 mg, 56%). Mp: 132–133 °C; ^{1}H NMR ($CDCl_3$/MeOH-d_4, 600 MHz) δ 7.64 (br, 2H), 7.38–6.94 (m, 18H), 5.24 (d, J = 4.2 Hz, 1H), 5.22 (d, J = 4.2 Hz, 1H), 4.35 (t, J = 4.8 Hz, 1H), 4.32 (t, J = 4.8 Hz, 1H), 3.89 (dd, J = 10.8, 15 Hz, 1H), 3.69 (dd, J = 10.8, 14.4 Hz, 1H), 3.28–3.22 (m, 2H), 3.08–3.05 (m, 4H), 3.02–2.98 (m, 2H), 2.93–2.85 (m, 2H), 2.82–2.77 (m, 2H), 2.63 (s, 3H); ^{13}C NMR ($CDCl_3$/MeOH-d_4, 150 MHz) δ 172.0, 171.8, 140.4, 140.4, 136.7, 136.6, 129.4, 129.4, 129.0, 129.0, 128.6, 128.1, 128.0, 127.6, 127.6, 127.0, 126.7, 125.2, 125.1, 124.3, 124.2, 73.0, 72.8, 57.8, 57.8, 52.7, 51.8, 42.3, 42.3, 39.6, 38.7, 38.7, 26.2; $[\alpha]_D^{20}$ +14.0° (*c* 0.3 MeOH/$CHCl_3$ = 1:1); HRMS (ESI) $C_{39}H_{44}N_3O_5S$ 665.2923, found 666.2969 $(M+H)^+$ 4.0 ppm error.

4.2. General procedure for the preparation of diamide sulfide (24, 25, 26)

A solution of **17** (1 equiv) and **19**, **20**, or **21** (3 equiv) in DCM (4 mL) was cooled in an ice bath and treated with triethylamine (6 equiv) followed by adding (benzotrizol-1-yloxy) tris (dimethylamino) phosphonium hexafluorophosphate (BOP reagent, 3 equiv). The mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate and washed successively with 0.5 N HCl, H_2O, saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel using 60% ethyl acetate in hexane.

4.2.1. Bis-[[*N*-(2*R*)-phenylglycinol]-2-phenyl-3-yl-propionamide] sulfide (24)

Compound **24** was isolated as a white solid (145 mg, 62%). Mp: 207–208 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.18–6.81 (m, 20H), 4.85 (dd, J = 4.2, 7.2 Hz, 2H), 3.66 (dd, J = 4.8, 11.4 Hz, 2H), 3.54 (dd, J = 4.8, 11.4 Hz, 2H), 2.87 (dd, J = 6.6, 12.0 Hz, 2H), 2.78–2.72 (m, 6H), 2.56–2.53 (m, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.9, 139.0, 139.0, 129.0, 128.5, 128.4, 127.2, 126.6, 126.4, 65.2, 55.5, 49.2, 38.5, 34.0; $[\alpha]_D^{20}$ −52.7° (*c* 0.22 MeOH/$CHCl_3$ = 1:1); ESI MS $C_{36}H_{40}N_2O_4S$ 596.3, found 597.3 $(M+H)^+$.

4.2.2. Bis-[[*N*-(2*S*)-phenylglycinol]-2-phenyl-3-yl-propionamide] sulfide (25)

Compound **25** was isolated as a white solid (160 mg, 64%). Mp: 188–189 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.13 (d, J = 8.4 Hz, 2H), 7.26–7.12 (m, 20H), 4.74 (dd, J = 6.6, 13.8 Hz, 2H), 4.61 (t, J = 5.4 Hz, 2H), 3.35–3.32 (m, 4H), 2.77–2.57 (m, 8H), 2.40–2.38 (m, 2H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 173.0, 141.7, 140.0, 129.5, 128.8, 128.5, 127.7, 127.2, 126.7, 65.1, 55.3, 48.0, 38.5, 34.5; $[\alpha]_D^{20}$ +52.0° (*c* 0.2 MeOH/$CHCl_3$ = 1:1); ESI MS $C_{36}H_{40}N_2O_4S$ 596.3, found 597.2 $(M+H)^+$.

4.2.3. Bis-[(L-valine-*N*-methylamide)-2-phenyl-3-yl-propionamide] sulfide (26)

The desired product was crystallized from ethyl acetate as a white solid (198 mg, 81%). Mp: 267–268 °C; ^{1}H NMR (DMSO-d_6,

600 MHz) δ 7.87 (d, J = 9.0 Hz, 2H), 7.40 (d, J = 4.2 Hz, 2H), 7.21–7.10 (m, 10H), 4.01 (t, J = 8.4 Hz, 2H), 2.88–2.51 (m, 10H), 2.47 (s, 6H), 1.88 (dd, J = 6.6, 13.2 Hz, 2H), 0.77 (t, J = 6.0 Hz, 12H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 173.4, 171.8, 139.9, 129.5, 126.7, 47.5, 38.5, 34.2, 30.9, 26.1, 19.9, 19.1; $[\alpha]_D^{20}$ −46.7° (c 0.24 MeOH/$CHCl_3$ = 1:1); ESI MS $C_{32}H_{46}N_4O_4S$ 582.3, found 583.3 $(M+H)^+$.

4.3. General procedure for the preparation of diamide sulfoxide (27, 28, 29)

To a solution of **24**, **25**, or **26** (1 equiv) in DCM (12 mL) and methanol (12 mL) at −78 °C under N_2 was added *m*CPBA (1 equiv). The mixture was stirred at −78 °C for 1 h then at room temperature for another 1 h. The reaction was quenched by diluting with DCM and extracted with saturated $NaHCO_3$. The organic layer was then washed by brine, dried over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel using 4% MeOH in $CHCl_3$ to afford compound **27**, **28**, or **29**.

4.3.1. Bis-[[*N*-(2*R*)-phenylglycinol]-2-phenyl-3-yl-propionamide] sulfoxide (27)

Compound **27** was isolated as a white solid (95 mg, 92%). Mp: 208–209 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.53 (d, J = 7.8 Hz, 1H), 8.34 (d, J = 8.4 Hz, 1H), 7.26–6.93 (m, 20H), 4.79–4.75 (m, 2H), 3.53–3.44 (m, 4H), 3.20–3.12 (m, 2H), 3.08–3.04 (m, 1H), 2.93 (dd, J = 7.2, 13.2 Hz, 1H), 2.88 (dd, J = 7.8, 13.8 Hz, 1H), 2.83–2.75 (m, 3H), 2.68 (dd, J = 7.8, 13.2 Hz, 1H), 2.57–2.55 (m, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 172.4, 172.3, 141.6, 141.1, 139.2, 139.0, 129.8, 129.7, 128.9, 128.8, 128.6, 128.5, 127.5, 127.3, 127.2, 127.2, 127.0, 126.9, 65.3, 55.6, 55.4, 55.3, 54.5, 42.5, 39.2, 38.2; $[\alpha]_D^{20}$ −60.0° (c 0.13 MeOH/$CHCl_3$ = 1:1); ESI MS $C_{36}H_{40}N_2O_5S$ 612.3, found 613.2 $(M+H)^+$.

4.3.2. Bis-[[*N*-(2*S*)-phenylglycinol]-2-phenyl-3-yl-propionamide] sulfoxide (28)

Compound **28** was isolated as a white solid (147 mg, quantitative). Mp: 205–206 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.39 (d, J = 7.8 Hz, 1H), 8.28 (d, J = 7.8 Hz, 1H), 7.28–7.12 (m, 20H), 4.79–4.77 (m, 1H), 4.63 (t, J = 6.0 Hz, 1H), 3.39 (t, J = 6.0 Hz, 2H), 3.35–3.29 (m, 2H), 3.13–3.12 (m, 1H), 3.02 (t, J = 6.6 Hz, 1H), 2.96–2.88 (m, 2H), 2.84–2.72 (m, 3H), 2.67 (dd, J = 7.2, 13.2 Hz, 1H), 2.62 (dd, J = 7.2, 13.2 Hz, 1H), 2.44–2.41 (m, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 172.2, 172.2, 141.6, 141.5, 139.2, 139.1, 129.8, 129.7, 128.9, 128.8, 128.6, 128.6, 127.7, 127.3, 127.3, 127.0, 126.9, 65.1, 64.9, 55.5, 55.5, 54.9, 54.4, 42.4, 41.8, 39.3, 38.0; $[\alpha]_D^{20}$ +15.5° (c 0.22 MeOH/$CHCl_3$ = 1:1); ESI MS $C_{36}H_{40}N_2O_5S$ 612.3, found 613.2 $(M+H)^+$.

4.3.3. Bis-[(L-valine-*N*-methylamide)-2-phenyl-3-yl-propionamide] sulfoxide (29)

Compound **29** was isolated as a white solid (85 mg, 92%). Mp: 228–229 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.06 (d, J = 8.4 Hz, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.46 (br, 2H), 7.23–7.11 (m, 10H), 4.01–3.96 (m, 2H), 3.17–2.63 (m, 10H), 2.49 (s, 6H), 1.88–1.86 (m, 2H), 0.77 (s, 6H), 0.75 (s, 6H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 172.6, 172.6, 171.7, 171.7, 139.0, 129.8, 129.7, 128.9, 128.8, 127.0, 126.9, 58.7, 58.7, 42.0, 41.7, 39.0, 38.2, 31.0, 31.0, 26.1, 26.1, 19.9, 19.1, 18.9; $[\alpha]_D^{20}$ −79.2° (c 0.24 MeOH/$CHCl_3$ = 1:1); ESI MS $C_{32}H_{46}N_4O_5S$ 598.3, found 599.3 $(M+H)^+$.

4.4. General procedure for the preparation of diamide sulfoximine (30, 31, 32)

To a solution of **27**, **28**, or **29** (1 equiv) in DMF (10 mL) was added MSH (5 equiv). The mixture was stirred at room temperature for 12 h and was quenched by adding saturated $NaHCO_3$ solution. The mixture was stirred for 30 min in an ice bath and the crude product was purified by flash chromatography using 4% MeOH in DCM to afford compound **30**, **31**, or **32**.

4.4.1. Bis-[[*N*-(2*R*)-phenylglycinol]-2-phenyl-3-yl-propionamide] sulfoximine (30)

Compound **30** was obtained as a white solid (60 mg, 74%). Mp: 218–219 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.38 (d, J = 8.4 Hz, 1H), 8.34 (d, J = 7.8 Hz, 1H), 7.19–6.95 (m, 20H), 4.81–4.78 (m, 2H), 4.70–4.60 (m, 2H), 3.53–3.47 (m, 4H), 3.41–3.38 (m, 2H), 3.29–3.23 (m, 2H), 2.96–2.92 (m, 2H), 2.79–2.68 (m, 4H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 172.4, 172.3, 141.1, 141.0, 138.9, 138.8, 129.8, 129.7, 128.9, 128.8, 128.7, 128.5, 127.8, 127.4, 127.3, 127.2, 127.1, 127.0, 126.9, 65.4, 65.2, 57.1, 56.6, 55.4, 55.3, 42.4, 42.2, 38.9, 38.8; $[\alpha]_D^{20}$ −23.8° (c 0.26 MeOH/$CHCl_3$ = 1:1); ESI MS $C_{36}H_{41}N_3O_5S$ 627.3, found 628.3 $(M+H)^+$.

4.4.2. Bis-[[*N*-(2*S*)-phenylglycinol]-2-phenyl-3-yl-propionamide] sulfoximine (31)

Compound **31** was obtained as a white solid (50 mg, 54%). Mp: 185–186 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.25 (d, J = 7.8 Hz, 1H), 8.22 (d, J = 7.8 Hz, 1H), 7.28–7.15 (m, 20H), 4.70–4.63 (m, 2H), 3.38–3.12 (m, 10H), 2.78–2.64 (m, 6H), 2.63–2.57 (m, 2H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 175.1, 141.4, 141.3, 129.9, 129.8, 128.8, 128.7, 128.6, 128.6, 128.5, 127.9, 127.8, 127.3, 127.3, 127.3, 127.0, 127.0, 64.9, 64.9, 56.6, 56.4, 55.5, 55.4, 42.3, 42.2, 39.0, 38.9; $[\alpha]_D^{20}$ +82.0° (c 0.2 MeOH/$CHCl_3$ = 1:1); ESI MS $C_{36}H_{41}N_3O_5S$ 627.3, found 628.2 $(M+H)^+$.

4.4.3. Bis-[(L-valine-*N*-methylamide)-2-phenyl-3-yl-propionamide] sulfoximine (32)

Compound **32** was obtained as a white solid (19 mg, 41%). Mp: 195–196 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.97 (d, J = 6.6 Hz, 2H), 7.20 (br, 2H), 7.22–7.14 (m, 10H), 3.99–3.91 (m, 2H), 3.29–2.55 (m, 10H), 2.49 (s, 6H), 1.53–1.48 (m, 2H), 0.77 (s, 12H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 172.6, 172.4, 171.7, 163.6, 138.9, 138.8, 129.8, 129.7, 128.8, 127.0, 73.2, 58.8, 56.7, 56.2, 41.9, 41.7, 38.9, 38.7, 31.1, 26.1, 19.9, 19.1, 19.0; $[\alpha]_D^{20}$ −11.7° (c 0.24 MeOH/$CHCl_3$ = 1:1); HRMS (ESI) $C_{32}H_{47}N_5O_5S$ 613.3298, found 636.3158 $(M+Na)^+$ 5.0 ppm error.

4.5. Biology

All the synthesized compounds were tested against recombined HIV-1 protease (Bachem Biosciences, Philadelphia PA) by a HPLC assay using HIV-1 Protease Substrate III (Bachem Biosciences, Philadelphia PA) or a fluorescence assay using EnzoLyte 520 HIV-1 Protease Assay Kit (AnaSpec, San Jose CA).

4.6. HPLC assay

The reactions were carried out in a buffer containing 50 mM sodium acetate (pH 4.9), 200 mM NaCl, 5 mM DTT, and 10% glycerol. The HIV-1 Substrate III was added to the buffer followed by inhibitors. The inhibitors were dissolved in DMSO, with a final concentration of 5% (v/v). Baseline values were determined from enzymatic reactions containing 5% DMSO in the absence of inhibitor. The reactions were initiated by the addition of the HIV-1 protease enzyme for 3.5 min at 37 °C. The reactions were quenched with 1/10 volume of 10% TFA and stored at 4 °C until analyzed. The samples were analyzed on a Beckman Coulter HPLC system, using a reverse-phase C_{18} column with a gradient of 5–50% acetonitrile in the presence of 0.1% TFA at a rate of 1 mL/min.

4.7. Fluorescence assay

These reactions were incubated in Costar 3915 plates (96 well, non-treated, flat bottom, black) and followed the manufacturer recommended protocol for screening HIV-1 protease inhibitors. The fluorescent signal was measured every 2–3 min (Ex/Em = 490/520) in a BioTek Synergy HT plate reader. The data were collected using KC4 and further analyzed for IC_{50} values with Prism Graph 4.

4.8. Molecular modeling

All modeling was carried out using the Schrödinger modeling suite package.[34] Compounds **1**, **30**, **31**, and **32** were built and energy minimized using the OPLS-AA force field[35] with implicit generalized born solvent. Two previously reported X-ray crystallographic structures of HIV-1 protease complexes were used for the docking of the pseudosymmetric sulfoximine inhibitors of **1**, **30**, and **31** (PDB: 4PHV)[36] and the sulfoximine peptidomimetic **32** (PDB: 1EBW).[37] The conserved crystallographic water, commonly observed to hydrogen bond to the amide hydrogen atom of G49 and G49′, was kept as part of the catalytic binding site for the protein for the docking calculation. To account for the previously observed ionization state of D25/D25′, each of the four oxygen atoms in D25/D25′ carboxylate groups was systematically protonated and allowed to be energy minimized in implicit solvent while all heavy atoms were held fixed. D25′ OD1 which was found with the optimal hydrogen bonding network was selected as our reference protonated state for docking of all our sulfoximine analogues. The docking calculations were carried using GLIDE v5.5 with the extra precision protocol. The van der Waals radii of non-polar atoms for each of the ligands were scaled by a factor of 0.8. To account for the protein flexibility, each of the final docked complexes was further refined by 100 ps equilibration followed by 1 ns molecular dynamics simulation using spherical boundary condition with explicit TIP3P solvent. A two layer spherical shell systems were employed with the nearest 18 Å sphere from the center of the ligand restrained by force constant of 0.3 kcal/mol Å^2 while fixing the residues in the outer 4 Å region shell. A constant dielectric of 1.0 with a 14 Å non-bond cutoff was used. All TIP3P water, except for the conserved crystallographic water bound to the ligand and G49/G49′, was removed from the final structure.

The absolute binding energy, ΔG_{bind}, was evaluated based on the linear response method[38–41] implemented within Liason.[34]

$$\Delta G_{\text{bind}} = \alpha\langle \Delta U^{f\to b}_{\text{elec}}\rangle_{\overline{q}} + \beta\langle \Delta U^{f\to b}_{\text{vdw}}\rangle_{\overline{q}} + \gamma\langle \Delta U^{f\to b}_{\text{cav}}\rangle_{\overline{q}} \quad (1)$$

where $\langle \Delta U^{f\to b}_{\text{elec}}\rangle_{\overline{q}}$, $\langle \Delta U^{f\to b}_{\text{vdw}}\rangle_{\overline{q}}$, $\langle \Delta U^{f\to b}_{\text{cav}}\rangle_{\overline{q}}$ is the change in the van der Waals, electrostatic and cavity energy from the free (f) to bound (b) state during binding at the average charged state, $\overline{q}$, as evaluated by the Surface Generalized Born (SGB) continuum solvent model. α, β, and γ are empirical fitting coefficients evaluated by multivariable regression analysis. The absolute binding energies of the HIV PI used for the training set was based on the previous studies[42] and the fitted equation was used to predict the absolute binding affinities of the sulfoximine inhibitors.

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