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Structure-based Design of Potent HIV-1 Protease Inhibitors with Modified P1 - Biphenyl Ligands: Synthesis, Biological Evaluation, and Enzyme-inhibitor X-ray Structural studies

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Abstract

We report the design, synthesis, X-ray structural studies, and biological evaluation of a novel series of HIV-1 protease inhibitors. We designed a variety of functionalized biphenyl derivatives to make enhanced van der Waals interactions in the S1 subsite of HIV-1 protease. These biphenyl derivatives were conveniently synthesized using a Suzuki-Miyaura cross-coupling reaction as the key step. We examined the potential of these functionalized biphenyl-derived P1 ligands in combination with 3-(*S*)-tetrahydrofuranyl urethane and bis-tetrahydrofuranyl urethane as the P2 ligands. Inhibitor **21e**, with a 2-methoxy-1, 1'-biphenyl derivative as P1 ligand and bis-THF as the P2 ligand, displayed the most potent enzyme inhibitory and antiviral activity. This inhibitor also exhibited potent activity against a panel of multidrug-resistant HIV-1 variants. A high resolution X-ray crystal structure of related Boc-derivative **17a**-bound HIV-1 protease provided important molecular insight into the ligand-binding site interactions of the biphenyl core in the S1 subsite of HIV-1 protease.

Introduction

Human immunodeficiency virus 1 (HIV-1) protease inhibitors are important components of current antiretroviral treatment regimens.^{1,2} The advances in HIV/AIDS therapy over the past decade markedly reduced the mortality and morbidity of HIV/AIDS patients. Despite

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this improvement, the emergence of drug resistance has raised serious questions about the long-term prospects of HIV/AIDS treatments.^{3,4} Over the years, our structure-based design of inhibitors targeting the protein-backbone led to the design and development of a variety of novel HIV-1 protease inhibitors (PIs) exhibiting broad-spectrum activity against multidrug-resistant HIV-1 variants.^{5,6} To combat drug-resistance, one of our inhibitor design strategies has been to maximize active site interactions with the protease, particularly to promote extensive hydrogen bonding interactions with backbone atoms throughout the active site.^{5,6} We have extensively utilized this ‘backbone binding’ molecular design strategy to develop a variety of unique nonpeptide ligands and scaffolds for the HIV-1 protease active site. Darunavir (**1**, Figure 1) emerged from our structure-based design efforts and was approved by the FDA for the treatment of HIV/AIDS.^{7–9}

Based upon the overlay of X-ray structures of darunavir-bound HIV-1 protease and saquinavir-bound HIV-1 protease, we recently reported the design and synthesis of a stereochemically defined tris-tetrahydrofuran (*tris*-THF) as the P2-ligand.^{10–12} The resulting inhibitor **3** with a *syn-anti-syn*-fused tris-THF ligand exhibited nearly 10-fold improvement of antiviral activity against a panel of highly resistant clinical HIV-1 strains compared to darunavir.^{10,11} A protein-ligand X-ray structure of inhibitor **3**-bound HIV-1 protease revealed that the A and B-rings of the tricyclic ligand in inhibitor **3** maintained key backbone interactions similar to the bis-THF P2 ligand of darunavir. Interestingly, the ring C of the tricyclic ligand appeared to fill in the hydrophobic site corresponding to saquinavir P3 quinaldic amide substituent. We then speculated that the additional hydrophobic interaction due to the ring C tetrahydrofuran moiety may be responsible for enhanced activity compared to darunavir.

Based upon this molecular insight, we have now investigated the possibilities of attaching substituent of the P1 phenyl ring of inhibitor **1** or **2** to fill in the hydrophobic site corresponding to quinaldic amide of saquinavir or the C-ring of inhibitor **3**. Such molecular modification may be less convoluted in terms of stereochemical complexity, as the tris-THF-derived ligand contains multiple stereocenters. One of our other design objectives is to incorporate hydrophobic groups to improve lipophilicity which may help improve the inhibitor’s CNS properties.^{13,14} In particular, we envisioned that introduction of a substituted phenyl ring at the *meta*-position of the P1 phenyl ring may not only lead to additional interactions in the S1 subsite, also an appropriately positioned polar substituent such as methoxy or methyl amine functionality may form hydrogen bonds with backbone residues in the S1 and S2 subsites. Herein, we report the design, synthesis, and biological evaluation of a series of protease inhibitors that incorporated a number of substituted biphenyl-based P1 ligand in darunavir-like HIV-1 protease inhibitors. The protein-ligand X-ray structure of **17a**-bound HIV-1 protease revealed important insights into the ligand-binding site interactions of the biphenyl structural core in the S1 subsite of HIV-1 protease active site.

To obtain further molecular insight into the possible ligand-binding site interactions, we created a molecular model based up on the X-ray structure of **2**-bound-HIV-1 protease.¹⁵ As shown in Figure 2, a *meta*-substituted phenyl ring appears to make van der Waals interactions with residues in the S1 subsite. In particular, a phenyl ring can be

accommodated in the hydrophobic pocket surrounding Leu23', Pro81' and Val82' residues. Such molecular design may maximize hydrophobic interactions. Furthermore, introduction of a polar substituent such as methoxy or dioxolane may be able to form hydrogen bonding interactions with polar hydrophilic residues such as Arg8' in the S1 subsite.

Chemistry

For introduction of various polar and nonpolar functionalities on the biphenyl ring, we planned to carry out Suzuki-Miyaura cross-coupling reactions with a triflate derivative **6** and functionalized boronic acid **7** as shown in Scheme 1. This synthetic strategy is particularly attractive as the synthesis of such biphenyl derivatives using Suzuki coupling has been extensively reported in the literature.^{16,17} Suzuki-Miyaura coupling has also been utilized in the synthesis of unrelated HIV-1 protease inhibitors.^{18,19} As depicted, coupling product **5** will provide access to the amine which can be converted to various P2 carbamate derivatives as developed by us previously.^{8,9}

The synthesis of tyrosine-derived hydroxyethylamine isostere is outlined in Scheme 2. Reaction of commercially available butadiene monoxide **8** with 3-benzyloxyphenylmagnesium bromide in the presence of a catalytic amount of CuCN provided (*E*)-allylic alcohol **9**.²⁰ Sharpless asymmetric epoxidation of **9** using (–)-diethyl D-tartrate under standard conditions provided epoxide **10** in excellent yield.²¹ Regioselective opening of epoxide with TMSN₃ and Ti(O-*i*Pr)₄ in benzene at 80° C for 2 h afforded azidodiol **11** in 55% yield.²² This was treated with 2-acetoxyisobutryl chloride in chloroform to form the corresponding chloroacetate which was reacted with sodium methoxide to provide epoxide **12** in excellent yield.²³ This epoxide was converted to sulfonamide **13** in a two-step sequence involving (1) opening the epoxide with isobutylamine in 2-propanol at reflux, and (2) reaction of the resulting amine with *p*-methoxybenzenesulfonyl chloride in the presence of aqueous NaHCO₃ solution to give **13** in 87% yield over 2 steps. Catalytic hydrogenation of azide using 10% Pd/C in the presence of (Boc)₂O followed by protection of Boc-aminoalcohol with dimethoxypropane and catalytic amount of *p*-TsOH furnished phenol derivative **14** in 65% yield.²⁴ Reaction of **14** with triflic anhydride in CH₂Cl₂ provided Suzuki-Miyaura precursor triflate **15** in 92% yield.

Suzuki-Miyaura coupling of triflate **15** with a variety of commercially available boronic acids is shown in Scheme 3. Initially, we optimized this coupling of **15** with 3,5-dimethoxyboronic acid with a number of commercially available Pd reagents. For this reaction, a catalytic amount of Pd(Ph₃P)₄ (5 mol %) in the presence of K₃PO₄ in dioxane at reflux provided the best results. Biphenyl derivative **5a** was obtained in 78% yield. A series of functionalized biphenyl derivatives were then conveniently prepared using this reaction condition. As shown, the yields of coupling products were good to excellent (58–92 %).

The synthesis of various inhibitors containing functionalized biphenyl derivatives on the P1 ligand is shown in Scheme 4. As shown, oxazolidines **5a–g** were treated with 1.5 equivalents 50% aqueous trifluoroacetic acid (TFA) in CH₂Cl₂ at 23° C for 5–8 h to provide Boc-derivatives **17a–d** in good to excellent yields (78–94%). For the synthesis of inhibitors with 3(*S*)-tetrahydrofuranyl urethane and bis-tetrahydrofuranyl urethane on the P2-ligand,

oxazolidine derivatives **5a–g** were treated with 25% TFA in CH₂Cl₂ at 23°C for 1 h. This condition resulted in the deprotection of the Boc- and isopropylidene groups to provide the corresponding amines. Reactions of the resulting amines with 3-(*S*)-THF and bis-THF-derived activated carbonates **18** and **19** respectively furnished the target inhibitors **20a–c** and **21a–e** in good yields (62–84%).²⁵ The structure of these inhibitors are shown in Table 1.

All inhibitors in Table 1 were first evaluated in an enzyme-inhibitory assay using methods reported by Toth and Marshall.²⁶ Inhibitors that showed potent K_i values were then further evaluated in antiviral assays. The results are shown in Table 1. As can be seen, Boc-derivative **17a** showed most potent enzymatic inhibitory activity, however its antiviral activity was greater than 1 μM. Other Boc-derivatives **17b–d** were less potent in enzyme inhibition assay and showed no appreciable antiviral activity. We then examined the potency enhancing effect of 3-(*S*)-THF as the P2 ligand on inhibitors **20a–c**. Introduction of 3-(*S*)-THF urethane in place of the Boc-group resulted in both improvement in enzyme inhibitory and antiviral activity. Inhibitor **20a** exhibited an antiviral activity of 180 nM. Inhibitor **20b** with a 3-methoxybiphenyl P1 ligand and 3-(*S*)-THF P2 ligand showed loss of potency in both enzyme and antiviral assays compared to inhibitors **20a**. This indicates the importance of both methoxy substituents on the biphenyl rings. Inhibitor **20c** with a 3,5-dimethylbiphenyl P1 ligand and 3(*S*)-THF as P2 ligand improved only enzyme activity. Introduction of a bis-THF as the P2 ligand in combination with P1 biphenyl ligand, resulted in significant improvement of enzyme and antiviral potency. Inhibitor **21a** showed HIV-1 protease inhibitory K_i of 14 pM and antiviral activity of 5 nM. The corresponding 3,5-dimethyl derivative **21b** is significantly less potent than the 3,5-dimethoxy derivative **21a**. Inhibitor **21c** with a 3-methoxy biphenyl derivative as the P1 ligand showed similar activity as inhibitor **21a**.

We have determined an X-ray crystal structure of **17a**-bound HIV-1 protease to obtain insight into the ligand-binding site interactions. The structure revealed that 3,5-dimethoxy groups on the biphenyl ring do not form any polar interaction in the active site. Based upon this structure, we then examined 2,6-dimethoxy biphenyl ligand shown in inhibitor **21d**. This inhibitor showed reduced activity compared to 3,5-dimethoxy derivative **21a**. Inhibitor **21e** with a 2-methoxy biphenyl P1 ligand showed the best results, showing enzyme K_i and antiviral activity similar to inhibitors **1** and **2**.²⁷

Because of the potent enzyme inhibitory and antiviral proprieties of inhibitor **21e**, we selected this inhibitor for further evaluation against a panel of multidrug resistant (MDR) HIV-1 variants. The antiviral activities of these inhibitors were compared to clinically available PIs, darunavir (DRV) and amprenavir (APV).^{7, 27} The results are shown in Table 2. Inhibitor **21e** exhibited low nanomolar EC₅₀ values against the wild-type HIV-1ERS_{104pre} laboratory strain, isolated from a drug-naïve patient.²⁷ It displayed EC₅₀ value similar to that of DRV and nearly 10-fold better than APV. It was then tested against a panel of multidrug-resistant HIV-1 strains. The EC₅₀ of **21e** remained in the low nanomolar value ranging from 2.9 nM to 36 nM. Its fold-change in activity against viral strain B was similar to that observed with DRV.^{7, 27} In contrast, inhibitor **21e** displayed superior antiviral activities against viral strains C and G compared to DRV. It essentially maintained full antiviral

activity against these viral strains. Inhibitor **21e** exhibited a superior profile compared to another approved PI, APV. Overall, inhibitor **21e** maintained impressive potency against all tested multidrug-resistant HIV-1 strains and it compared favorably with DRV, a leading PI for the treatment of multidrug resistant HIV infection.⁹

The crystal structure of wild type HIV-1 protease with the inhibitor **17a** was determined and refined at 1.53 Å resolution. The crystal structure contains the protease dimer and a single orientation of inhibitor. The overall structure is comparable to the structure with HIV-1 protease and darunavir²⁸ with root mean square difference of 0.32 Å for Ca atoms. Larger differences between corresponding Ca atoms occur in one subunit of the dimer, where differences of 2.5 Å are seen for residues 77' to 80' in the 80's loop and 0.7 Å for the flap residues 51' and 52'. The interactions of the inhibitor with HIV-1 protease atoms in the active cavity resemble those in the HIV-1 protease darunavir complex²⁸. As shown in Figure 3A, the inhibitor formed strong hydrogen bonding interactions with the catalytic aspartates of HIV-1 protease. The major changes in inhibitor occur at the P1 and P2 groups. The large 3,5-dimethoxy-biphenyl group replaces the P1 phenyl group of darunavir, and the *tert*-butyl group substitutes for P2 *bis*-THF. To accommodate the larger 3,5-dimethoxy-biphenyl group in P1, the first phenyl group shifts towards Arg8', Leu23', and Gly27 to form additional van der Waals interactions relative to those of the P1 phenyl of darunavir. As shown in Figure 3B, the outer 3,5-dimethoxy-phenyl group rotates about 34° to sandwich between Gly48, Gly49, and Ile50 in the flap region and Thr80' to Pro81' in the 80's loop, which stabilizes these flexible regions by forming new hydrophobic contacts, including C-H... π interactions with Gly49 and Pro81'.

Conclusion

We have designed and synthesized a series of functionalized biphenyl derivatives on the P1 ligand to improve van der Waals interactions in the S1 subsite. The synthesis of biphenyl derivatives was carried out using Suzuki-Miyaura cross-coupling as the key step. We have investigated a range of functionalities. Also, we have incorporated 3-(*S*)-THF and *bis*-THF urethanes as the P2 ligands in combination with the P1-biphenyl derivatives. In general, inhibitors with *bis*-THF ligand showed the best results. Inhibitor **21e** displayed enzyme inhibitory and antiviral activity comparable to darunavir and TMC-126. Inhibitor **21e** was evaluated against a panel of multidrug-resistant HIV-1 variants and it exhibited excellent antiviral activity. To obtain molecular insight into the ligand-binding site interactions in the S1 subsite, an X-ray structure of **17a**-bound HIV-1 protease structure was determined. The structure revealed that the biphenyl ring nestled in the S1 subsite nicely. The 3,5-disubstituted phenyl ring is surrounded by hydrophobic residues such as Leu23', Arg8', Pro81' and Val82'. Both methoxy groups do not form any polar interaction in this region. The enhanced van der Waals interactions of the biphenyl derivative may be responsible for its potent antiviral activity and its excellent drug resistance profiles. Further design of inhibitors utilizing this molecular insight is in progress.

Experimental Section

General Experimental Details

Those reactions which required anhydrous conditions were carried out under an argon atmosphere using oven-dried glassware. All chemicals and reagents were purchased from commercial suppliers and used without further purification. Anhydrous solvents were obtained as follows: anhydrous tetrahydrofuran and diethyl ether were distilled from sodium metal under argon, anhydrous dichloromethane was dried via distillation from CaH_2 immediately prior to use under argon, and anhydrous methanol and ethanol were distilled from activated magnesium under argon. All other solvents were reagent grade. TLC analysis was conducted using glass-backed Thin-Layer Silica Gel Chromatography Plates (60 Å, 250 µm thickness, F-254 indicator). Flash chromatography was performed using 230–400 mesh, 60 Å pore diameter silica gel. ^1H NMR spectra were recorded at 400 or 500 MHz. ^{13}C NMR spectra were recorded at 100 or 125 MHz. Chemical shifts are reported in parts per million and are referenced to the deuterated residual solvent peak. NMR data is reported as: δ value (chemical shift, J -value (Hz), integration, where s = singlet, d = doublet, t = triplet, q = quartet, brs = broad singlet). High and low resolution mass spectra were carried out by the Mass Spectroscopy Center at Purdue University. The purity of all test compounds was determined by HRMS and HPLC analysis. All test compounds showed 95% purity.

(*E*)-4-(3-(benzyloxy)phenyl)but-2-en-1-ol (**9**)

Magnesium turnings (219 mg, 9.1 mmol) and a catalytic amount of iodine were dissolved in a solution of 3-benzyloxybromobenzene (1.6 g, 6.1 mmol) in anhydrous tetrahydrofuran (15 mL) under argon. The resulting mixture was heated for 5 h until magnesium disappeared. Then it was cooled to room temperature and transferred into a solution of butadiene monoxide **8** (267 mg, 3.8 mmol) in THF (5 mL) with 10 mol% of copper cyanide as catalyst via cannular at -78°C . The reaction was stirred under these conditions for 1 h, then it was warmed to room temperature slowly. Sat. NH_4Cl solution (20 mL) was added to quench the reaction. The aqueous phase was extracted with EtOAc (3×20 mL). The combined organic extracts were dried over MgSO_4 , filtered, and concentrated. The residue was purified via silica gel chromatography (3:1 hexane/ethyl acetate) to afford butanol **9** (645 mg, 66%) as a colorless oil; ^1H NMR (400 MHz, CDCl_3) δ 7.45–7.33 (m, 5H), 7.22–7.20 (m, 1H), 6.84–6.79 (m, 3H), 5.83–5.73 (m, 2H), 5.11 (s, 2H), 4.12 (s, 2H), 3.34 (d, $J = 6.5$ MHz, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 159.1, 141.8, 137.2, 131.4, 130.6, 129.6, 128.7, 128.1, 127.7, 121.4, 115.5, 112.5, 70.1, 63.7, 38.8; LRMS-ESI (m/z) 255.2 (M+H).

((2*R*,3*R*)-3-(3-(benzyloxy)benzyl)oxiran-2-yl)methanol (**10**)

Flame dried 4 Å molecular sieves (7 g) were activated by adding dichloromethane (100 mL). Titanium isopropoxide (256 mL, 0.88 mmol) and (–)-diethyl tartrate (113 mL, 0.67 mmol) were added to the reaction, which was cooled to -20°C . *Tert*-butyl hydroperoxide (4.44 mL, 24.4 mmol) was added dropwise and the resulting mixture was stirred for 30 min at -20°C . A solution of (*E*)-4-(3-(benzyloxy)phenyl)but-2-en-1-ol **9** (2.81 g, 11.1 mmol) in dichloromethane (60 mL) was transferred to above mixture dropwise by cannular, then the reaction was keeping stirring overnight at -20°C . The molecular sieves were removed by filtration and the solvent was evaporated under reduced pressure. The crude residue was

purified by flash chromatography (3:1 to 1:1 hexane/ethyl acetate) to obtain the Sharpless epoxidation product **10** (2.56 g, 86%) as an oil. ¹H NMR (400 MHz, CDCl₃) δ 7.45-7.22 (m, 6H), 6.88-6.84 (m, 3H), 5.07 (s, 2H), 3.90 (d, *J* = 8.4 MHz, 1H), 3.62 (d, *J* = 8.4 Hz, 1H), 3.22-3.19 (m, 1H), 2.99-2.98 (m, 1H), 2.90-2.86 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 159.0, 138.6, 137.0, 129.7, 128.6, 128.0, 127.6, 121.6, 115.7, 113.0, 69.9, 61.5, 58.3, 55.9, 37.9; LRMS-ESI (*m/z*) 271.3 (M+H).

(2S,3S)-3-azido-4-(3-(benzyloxy)phenyl)butane-1,2-diol (11)

A solution of titanium isopropoxide (4.35 g, 15.3 mmol) and trimethylsilyl azide (3.52 g, 30.6 mmol) in benzene (50 mL) was heated to 80 °C for 5 h. To the resulting yellow solution was added a solution of epoxidation product **10** (2.56 g, 9.44 mmol) in benzene (50 mL) via cannular under argon. The reaction was continued at 80°C for 15 min, and then cooled to room temperature. Subsequently, it was treated by addition of 5% of sulfuric acid (120 mL) over 1 h. The aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated. The residue was purified via flash chromatography (2:3 hexane/ethyl acetate) to afford diol **11** (1.62 g, 55%) as a colorless oil; ¹H NMR (400 MHz, CDCl₃) δ 7.45-7.33 (m, 5H), 7.25-7.23 (m, 1H), 6.90-6.86 (m, 3H), 5.07 (s, 2H), 3.77-3.65 (m, 4H), 3.11-3.02 (m, 1H), 2.80-2.70 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 158.9, 138.7, 136.9, 129.6, 128.5, 127.9, 127.4, 121.9, 116.0, 113.2, 72.8, 69.9, 65.3, 63.1, 36.9; LRMS-ESI (*m/z*) 336.3 (M+Na).

(S)-2-((S)-1-azido-2-(3-(benzyloxy)phenyl)ethyl)oxirane (12)

To a solution of diol **11** (200 mg, 0.64 mmol) in chloroform (10 mL) was added 2-acetoxyisobutyryl chloride (128 μL, 0.9 mmol). After stirring for 5 h, the reaction was treated with saturated aqueous NaHCO₃ solution. The aqueous phase was extracted with chloroform (3 × 10 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated. The residue was dissolved in anhydrous tetrahydrofuran (10 mL), then sodium methoxide (52 mg, 0.96 mmol) was added. The reaction was stirred for 4 h and quenched with sat. NH₄Cl solution. The aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated. The residue was purified via flash chromatography (5:1 hexane/ethyl acetate) to afford compound **12** (184 mg, 97%) as a white amorphous solid; ¹H NMR (400 MHz, CDCl₃) δ 7.45-7.33 (m, 5H), 7.27-7.23 (m, 1H), 6.90-6.85 (m, 3H), 5.08 (s, 2H), 3.60-3.58 (m, 2H), 3.06-3.04 (m, 1H), 2.96 (dd, *J* = 4.8 and 14.0 Hz, 1H), 2.83-2.77 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 159.1, 138.3, 137.1, 129.7, 128.7, 128.1, 127.6, 122.1, 116.3, 113.5, 70.1, 63.6, 53.1, 45.3, 38.4; LRMS-ESI (*m/z*) 318.7 (M+Na).

N-((2R,3S)-3-azido-4-(3-(benzyloxy)phenyl)-2-hydroxybutyl)-N-isobutyl-4-methoxybenzenesulfonamide (13)

To a solution of epoxide **12** (180 mg, 0.61 mmol) in isopropyl alcohol (10 mL) was added isobutyl amine (178 mg, 2.44 mmol). Reaction was heated to reflux and stirred for 4–6 h. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (10 mL). Another 10 mL of saturated aqueous NaHCO₃ and 4-methoxybenzenesulfonyl chloride was added and then the reaction was stirred for 8 h. Water

was added and the aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated. The residue was purified via flash chromatography (2:3 hexane/ethyl acetate) to afford sulfonamide **13** (286 mg, 87%) as a colorless oil; ¹H NMR (400 MHz, CDCl₃) δ 7.75 (d, *J* = 8.8 Hz, 2H), 7.45-7.33 (m, 5H), 7.25 (t, *J* = 7.2 Hz, 1H), 7.02-6.99 (m, 2H), 6.92-6.87 (m, 3H), 5.29 (s, 2H), 3.87 (s, 3H), 3.77 (s, br, 1H), 3.61-3.56 (m, 2H), 3.24-3.20 (m, 1H), 3.09-3.01 (m, 3H), 2.84-2.77 (m, 2H), 1.85-1.81 (m, 1H), 0.95-0.86 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 163.2, 159.1, 138.9, 137.0, 129.6, 128.7, 128.0, 127.8, 127.6, 123.5, 122.0, 116.1, 114.5, 113.4, 71.9, 70.0, 66.5, 58.9, 55.7, 52.9, 37.0, 27.3, 20.3, 19.9; LRMS-ESI (*m/z*) 539.3 (M + H).

(4*S*,5*R*)-tert-butyl-4-(3-hydroxybenzyl)-5-((*N*-isobutyl-4-methoxyphenylsulfonamido)-methyl)-2,2-dimethyloxazolidine-3-carboxylate (14**)**

To a solution of sulfonamide **13** (1.32g, 2.45 mmol) in methanol (20 mL) was added palladium on active carbon (10%) (52 mg, 0.20 mmol) and di-*tert*-butyl dicarbonate (1.07 g, 4.9 mmol) under argon. Then the reaction was stirred under 1 atm hydrogen overnight at 23 °C. The Pd/C was filtered through celite and the filtrate was evaporated. The residue was dissolved in dry dichloromethane (15 mL) and 2,2-dimethoxypropane (1.0 g, 9.8 mmol) with catalytic amount *p*-toluenesulfonic acid (10 mol%, 46 mg) was added. After stirring for 12 h, saturated aqueous NaHCO₃ was added and the aqueous phase was extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated. The residue was purified via flash chromatography (2:1 to 1:1 hexane/ethyl acetate) to afford the isopropylidene derivative **14** (895 mg, 65% over two steps) as white amorphous solid. ¹H NMR (400 MHz, CDCl₃) δ 7.48 (d, *J* = 8.4 Hz, 2H), 7.20-7.14 (m, 1H), 6.90 (d, *J* = 8.4 Hz, 2H), 6.81-6.67 (m, 3H), 5.11 (s, br, 1H), 4.25-4.24 (m, 2H), 3.86 (s, 3H), 3.33-3.30 (m, 1H), 3.00-2.95 (m, 3H), 2.70-2.64 (m, 2H), 2.07-1.90 (m, 1H), 1.61-1.38 (m, 1.5 H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.84 (d, *J* = 8.4 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 162.6, 162.5, 156.2, 151.9, 151.6, 140.1, 139.9, 137.3, 133.3, 132.8, 131.4, 130.6, 129.9, 129.7, 129.2, 121.2, 121.0, 116.1, 115.8, 114.1, 113.5, 93.2, 92.7, 80.5, 79.9, 59.8, 59.6, 57.1, 56.9, 55.6, 49.2, 36.2, 35.5, 29.7, 28.4, 28.3, 27.9, 27.4, 26.9, 24.5, 23.4, 21.2, 20.0, 19.9; LRMS-ESI (*m/z*) 585.5 (M+Na).

(4*S*,5*R*)-tert-butyl 5-((*N*-isobutyl-4-methoxyphenylsulfonamido)methyl)-2,2-dimethyl-4-(3-(((trifluoromethyl)sulfonyl)oxy)benzyl)oxazolidine-3-carboxylate (15**)**

Compound **14** (895 mg, 1.59 mmol) was dissolved in anhydrous dichloromethane (10 mL) and pyridine (138 mg, 1.75 mmol) was added. The reaction was cooled to -78 °C and trifluoromethanesulfonic anhydride (538 mg, 1.9 mmol) was added dropwise. Then the reaction was warmed to 23 °C slowly. Diethyl ether (10 mL) and 5 % HCl solution (10 mL) were added to quench reaction. The mixture was extracted with dichloromethane (3 × 10 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated. The residue was purified via flash chromatography (4:1 hexane/ethyl acetate) to afford the triflate **15** (704 mg, 92%). [α]_D²⁰ +18.6 (*c* 1.18, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 7.56-7.51 (m, 2H), 7.42-7.35 (m, 1.5H), 7.24 (s, 0.5H), 7.18-7.10 (m, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 4.29-4.28 (m, 1H), 4.23-4.22 (m, 1H), 3.86 (s, 3H), 3.30-3.25 (m, 1H), 3.06-3.03 (m, 1H), 2.95-2.70 (m, 4H), 2.04-1.98 (m, 1H), 1.58 (s, 2H), 1.50-1.47 (m, 3H), 1.40 (d, *J* =

5.0 Hz, 5H), 1.35 (s, 5H), 0.92 (d, $J = 6.5$ Hz, 3H), 0.85 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (125 MHz, CDCl_3) δ 162.6, 151.7, 151.2, 149.6, 149.4, 141.5, 141.4, 130.8, 130.6, 130.3, 130.0, 129.3, 129.0, 122.2, 121.9, 119.9, 119.2, 118.9, 117.3, 113.9, 93.3, 92.7, 80.2, 79.9, 77.3, 76.2, 59.4, 57.1, 56.8, 55.4, 48.7, 48.5, 35.9, 35.3, 28.1, 27.9, 27.2, 26.6, 24.4, 23.3, 19.9, 19.7; LRMS-ESI (m/z) 717.5 ($\text{M}+\text{Na}$).

General procedure for Suzuki-Miyaura cross coupling reaction for the synthesis of 5a–g—To a solution of triflate **15** (34.7 mg, 0.05 mmol) in dioxane (2 mL) was added potassium phosphate tribasic (21.2 mg, 0.1 mmol). The tetrakis(triphenylphosphine)palladium (0) (5.1 mg, 10 mol%) was added as a catalyst under argon. The resulting mixture was stirred at 23 °C for 20 min. Substituted phenylboronic acids **16** (0.3 mmol) were added, then the reaction was refluxed for 1–4 h until the triflate was completely consumed. This reaction was treated with saturated aqueous NH_4Cl and the aqueous phase was extracted with EtOAc (3×10 mL). The combined organic extracts were dried over MgSO_4 , filtered, and concentrated. The residue was purified via flash chromatography (3:1 hexane/ethyl acetate) to afford cross coupling compounds **5a–g** (58–92% yields).

General procedure for preparation of Boc-substituted inhibitors (17a–d)—To a solution of compounds **5a–d** in dichloromethane (3 mL) was added TFA/ H_2O (1:1) (1.5 equiv) at 23 °C. The reaction was stirring for 4–8 h and treated with saturated aqueous NaHCO_3 solution. The aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried over MgSO_4 , filtered, and concentrated. The residue was purified via flash chromatography (3:1 to 2:1 hexane/ethyl acetate) to afford Boc-substituted inhibitors **17a–d** (78–94% yields).

***tert*-butyl-((2*S*,3*R*)-1-(3',5'-dimethoxy-[1,1'-biphenyl]-3-yl)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)butan-2-yl)carbamate (**17a**)**

$[\alpha]_{\text{D}}^{20} +7.6$ (c 0.88, CH_2Cl_2); ^1H NMR (500 MHz, CDCl_3) δ 7.70 (d, $J = 8.5$ Hz, 2H), 7.48–7.44 (m, 2H), 7.36 (t, $J = 7.5$ Hz, 1H), 7.25–7.24 (m, 1H), 6.94 (d, $J = 8.5$ Hz, 2H), 6.74 (d, $J = 2.0$ Hz, 2H), 6.46 (t, $J = 2.5$ Hz, 1H), 4.70 (d, $J = 8.0$ Hz, 1H), 4.02 (s, 1H), 3.86 (s, 3H), 3.82 (s, 6H), 3.81–3.78 (m, 2H), 3.10–3.06 (m, 3H), 2.99–2.92 (m, 2H), 2.83–2.79 (m, 1H), 1.88–1.82 (m, 1H), 1.32 (s, 9H), 0.89 (d, $J = 6.5$ Hz, 3H), 0.86 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (125 MHz, CDCl_3) δ 162.9, 161.0, 156.1, 143.3, 141.3, 138.4, 129.9, 129.4, 128.8, 128.7, 128.5, 125.3, 114.3, 105.4, 99.4, 79.8, 72.8, 58.7, 55.6, 55.4, 54.7, 53.8, 35.4, 29.7, 28.2, 27.2, 20.1, 19.9; HRMS-ESI (m/z) $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{34}\text{H}_{47}\text{N}_2\text{O}_8\text{S}$ 643.3054; found, 643.3053.

***tert*-butyl-((2*S*,3*R*)-1-(3-(benzo[d][1,3]dioxol-5-yl)phenyl)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)butan-2-yl)carbamate (**17b**)**

$[\alpha]_{\text{D}}^{20} +5.6$ (c 1.02, CH_2Cl_2); ^1H NMR (500 MHz, CDCl_3) δ 7.70 (d, $J = 9.0$ Hz, 2H), 7.36–7.32 (m, 3H), 7.20 (d, $J = 7.0$ Hz, 1H), 7.07–7.05 (m, 2H), 6.95 (d, $J = 8.0$ Hz, 2H), 6.87 (d, $J = 8.0$ Hz, 1H), 5.99 (s, 2H), 4.69 (d, $J = 9.0$ Hz, 1H), 3.99 (s, 1H), 3.86 (s, 3H), 3.84–3.79 (m, 2H), 3.14–3.04 (m, 3H), 2.97–2.93 (m, 2H), 2.82–2.78 (m, 1H), 1.87–1.82 (m, 1H), 1.35–1.23 (m, 9H), 0.90 (d, $J = 6.5$ Hz, 3H), 0.86 (d, $J = 6.5$ Hz, 3H); ^{13}C NMR (125

MHz, CDCl₃) δ 162.9, 156.0, 148.1, 147.0, 140.9, 138.3, 135.4, 129.9, 129.4, 128.9, 128.1, 124.9, 120.6, 114.3, 108.5, 107.6, 101.1, 79.7, 72.7, 58.7, 55.6, 54.6, 53.8, 35.4, 28.2, 27.2, 20.1, 19.9; HRMS-ESI (m/z) [M+H]⁺ calcd for C₃₃H₄₅N₂O₈S 627.2740; found, 627.2742.

***tert*-butyl-((2*S*,3*R*)-1-(3',5'-bis(trifluoromethoxy)-[1,1'-biphenyl]-3-yl)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)butan-2-yl)carbamate (17c)**

[α]_D²⁰ +8.8 (*c* 0.86, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 8.01 (s, 2H), 7.85 (s, 1H), 7.74-7.72 (m, 2H), 7.48-7.42 (m, 3H), 7.36 (d, *J* = 7.0 Hz, 1H), 6.97 (dd, *J* = 2.0 and 7.0 Hz, 2H), 4.69 (d, *J* = 10.5 Hz, 1H), 4.04 (s, 1H), 3.87 (s, 3H), 3.86-3.80 (m, 2H), 3.14-3.12 (m, 3H), 2.99-2.92 (m, 2H), 2.86-2.82 (m, 1H), 1.89-1.83 (m, 1H), 1.28 (s, 9H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.0, 155.9, 143.3, 139.3, 138.3, 132.4, 132.2, 131.9, 131.6, 130.2, 129.8, 129.5, 129.4, 128.5, 127.2, 125.3, 124.4, 122.3, 120.9, 114.3, 79.8, 72.9, 58.8, 55.6, 54.6, 53.8, 35.3, 28.1, 27.2, 20.1, 19.9; HRMS-ESI (m/z) [M+H]⁺ calcd for C₃₄H₄₁F₆N₂O₈S 751.2448; found, 751.2445.

***tert*-butyl-((2*S*,3*R*)-1-(2',6'-dimethyl-[1,1'-biphenyl]-3-yl)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)butan-2-yl)carbamate (17d)**

[α]_D²⁰ +8.9 (*c* 0.78, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 7.70 (d, *J* = 9.0 Hz, 2H), 7.45-7.43 (m, 2H), 7.34 (t, *J* = 8.0 Hz, 2H), 7.24-7.21 (m, 3H), 7.02-6.90 (m, 3H), 4.69 (d, *J* = 8.5 Hz, 1H), 3.97 (s, 3H), 3.87-3.82 (m, 2H), 3.15-3.05 (m, 3H), 2.98-2.94 (m, 2H), 2.82-2.78 (m, 1H), 2.38 (s, 6H), 1.87-1.82 (m, 1H), 1.33 (s, 9H), 0.90 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 162.9, 156.0, 141.5, 141.0, 138.2, 129.9, 129.6, 129.5, 128.9, 128.8, 128.4, 128.3, 125.3, 125.0, 114.3, 79.7, 72.8, 58.7, 55.6, 54.7, 53.8, 35.6, 29.7, 28.2, 27.2, 21.4, 20.1, 19.9; HRMS-ESI (m/z) [M+H]⁺ calcd for C₃₄H₄₇N₂O₆S 611.3155; found, 611.3157.

General procedure for preparation of mono-THF Ligand-derived inhibitors (20a–c)—A solution of compounds **5a–c** in TFA/CH₂Cl₂ (1:3) (2 mL) was stirring for 1 h at 23 °C. The solvent was removed under vacuum and the resulting residue was dissolved in acetonitrile (1 mL). Activated *mono*-THF ligand **18** (1.2 equiv.) was added to the above solution, then the reaction was continuing stirring for 2–3 days. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (2:1 to 1:1 hexane/ethyl acetate) to afford *mono*-THF ligand-derived inhibitors **20a–c** (67–84% yields).

(*S*)-tetrahydrofuran-3-yl ((2*S*,3*R*)-1-(3',5'-dimethoxy-[1,1'-biphenyl]-3-yl)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)butan-2-yl)carbamate (20a)

[α]_D²⁰ +9.6 (*c* 0.59, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 7.69 (d, *J* = 8.5 Hz, 2H), 7.47-7.44 (m, 2H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 8.5 Hz, 1H), 6.95 (d, *J* = 9.0 Hz, 2H), 6.45 (t, *J* = 2.5 Hz, 2H), 5.12 (s, 1H), 4.91 (d, *J* = 8.5 Hz, 1H), 3.90-3.85 (m, 2H), 3.86 (s, 3H), 3.85 (s, 6H), 3.81-3.74 (m, 4H), 3.60 (d, *J* = 10.5 Hz, 1H), 3.12-2.92 (m, 5H), 2.81-2.77 (m, 1H), 2.07-2.06 (m, 1H), 1.91-1.81 (m, 2H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.0, 161.0, 156.1, 143.1, 141.4, 138.1, 129.7, 129.4, 128.9, 128.6, 128.3, 125.4, 114.3, 105.4, 99.3, 75.4, 73.1, 72.6, 66.8, 58.8, 55.6, 55.4, 55.1, 53.7, 35.2, 32.8, 27.3, 20.1, 19.9; HRMS-ESI (m/z) [M+H]⁺ calcd for C₃₄H₄₅N₂O₉S 657.2846; found, 657.2842.

(S)-tetrahydrofuran-3-yl ((2S,3R)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)-1-(3'-methoxy-[1,1'-biphenyl]-3-yl)butan-2-yl)carbamate (20b)

$[\alpha]_D^{20} +11.8$ (c 0.56, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 7.69 (d, *J* = 9.0 Hz, 2H), 7.47-7.46 (m, 2H), 7.38-7.35 (m, 2H), 7.24-7.13 (m, 3H), 6.96-6.88 (m, 3H), 5.12 (s, 1H), 4.92 (d, *J* = 8.0 Hz, 1H), 3.92-3.89 (m, 3H), 3.87 (s, 3H), 3.86 (s, 3H), 3.77-3.74 (m, 3H), 3.59 (d, *J* = 10.5 Hz, 1H), 3.14-2.81 (m, 5H), 2.79-2.77 (m, 1H), 2.11-2.05 (m, 1H), 1.91-1.81 (m, 2H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.0, 159.9, 156.1, 142.4, 141.2, 138.1, 129.8, 129.4, 128.9, 128.5, 128.4, 125.4, 119.6, 114.3, 112.8, 75.4, 73.1, 72.6, 66.8, 58.8, 55.6, 55.3, 55.1, 53.7, 35.3, 32.8, 27.3, 20.1, 19.9; HRMS-ESI (*m/z*) [*M*+Na]⁺ calcd for C₃₃H₄₂N₂O₈SNa 649.2560; found, 649.2562.

(R)-tetrahydrofuran-3-yl ((2S,3R)-1-(2',6'-dimethyl-[1,1'-biphenyl]-3-yl)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)butan-2-yl)carbamate (20c)

$[\alpha]_D^{20} +12.6$ (c 0.86, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 7.69 (d, *J* = 9.0 Hz, 2H), 7.45-7.43 (m, 2H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.23-7.20 (m, 3H), 6.99-6.94 (m, 3H), 5.11 (s, br, 1H), 4.89 (d, *J* = 8.0 Hz, 1H), 3.89-3.85 (m, 5H), 3.83-3.75 (m, 4H), 3.60 (d, *J* = 10.5 Hz, 1H), 3.18-2.94 (m, 5H), 2.81-2.77 (m, 1H), 2.38 (s, 6H), 2.07-2.04 (m, 1H), 1.89-1.82 (m, 2H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.1, 156.1, 141.7, 140.9, 138.3, 137.9, 129.5, 129.0, 128.9, 128.3, 128.2, 125.5, 125.0, 114.4, 75.4, 73.1, 72.6, 66.8, 58.8, 55.6, 55.2, 53.8, 35.5, 32.8, 29.7, 27.3, 21.4, 20.1, 19.9; HRMS-ESI (*m/z*) [*M*+H]⁺ calcd for C₃₄H₄₅N₂O₇S 625.2947; found, 625.2949.

General procedure for preparation of bis-THF Ligand-derived inhibitors (21a–e)—A solution of compounds **5a–e** in TFA/CH₂Cl₂ (1:3) (2 mL) was stirred for 1 h at 23°C. The solvent was removed under vacuum and the resulting residue was dissolved in acetonitrile (1 mL). Activated bis-THF ligand **19** (1.2 equiv.) was added to the above solution, and the reaction was allowed to continue stirring for 2–3 days. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (2:1 to 1:1 hexane/ethyl acetate) to afford *bis*-THF ligand-derived inhibitors **21a–e** (62– 80% yields).

(3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ((2S,3R)-1-(3',5'-dimethoxy-[1,1'-biphenyl]-3-yl)-3-hydroxy-4-(N-isobutyl-4-methoxyphenylsulfonamido)butan-2-yl)carbamate (21a)

$[\alpha]_D^{20} +20.6$ (c 0.60, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 7.70 (d, *J* = 8.5 Hz, 2H), 7.44-7.43 (m, 2H), 7.35 (t, *J* = 8.5 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 6.97 (d, *J* = 9.0 Hz, 2H), 6.71 (d, *J* = 2.5 Hz, 2H), 6.46 (t, *J* = 2.5 Hz, 1H), 5.61 (d, *J* = 5.0 Hz, 1H), 5.03-4.94 (m, 2H), 4.00-3.90 (m, 3H), 3.88 (s, 3H), 3.85 (s, 6H), 3.72-3.62 (m, 4H), 3.25-3.10 (m, 2H), 3.03-2.96 (m, 2H), 2.88-2.78 (m, 3H), 1.88-1.82 (m, 1H), 1.56-1.52 (m, 2H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.2, 161.2, 155.5, 143.0, 141.4, 138.1, 129.8, 129.5, 129.0, 128.6, 128.2, 125.4, 114.4, 109.6, 109.3, 105.5, 73.4, 72.8, 70.6, 69.5, 58.9, 55.6, 55.5, 55.0, 53.8, 45.3, 35.7, 27.3, 25.7, 21.2, 19.9; HRMS-ESI (*m/z*) [*M*+H]⁺ calcd for C₃₆H₄₇N₂O₁₀S 699.2952; found, 699.2952.

(3*R*,3*aS*,6*aR*)-hexahydrofuro[2,3-*b*]furan-3-yl-((2*S*,3*R*)-1-(2',6'-dimethyl-[1,1'-biphenyl]-3-yl)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)butan-2-yl)carbamate (21b)

$[\alpha]_D^{20} +24.8$ (*c* 0.68, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 7.71 (d, *J* = 9.0 Hz, 2H), 7.44-7.42 (m, 2H), 7.33 (t, *J* = 7.5 Hz, 1H), 7.19-7.18 (m, 3H), 6.97 (t, *J* = 9.0 Hz, 3H), 5.59 (d, *J* = 5.5 Hz, 1H), 5.00-4.94 (m, 2H), 3.95-3.90 (m, 3H), 3.86 (s, 3H), 3.67-3.59 (m, 4H), 3.20-3.12 (m, 2H), 3.02-2.97 (m, 2H), 2.89-2.79 (m, 3H), 2.37 (s, 6H), 1.84-1.80 (m, 1H), 1.53-1.44 (m, 2H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.88 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.0, 155.3, 141.5, 140.6, 138.2, 137.8, 129.7, 129.4, 128.9, 128.8, 127.9, 125.3, 124.8, 114.3, 109.1, 73.3, 72.7, 70.5, 69.4, 58.7, 55.5, 55.0, 53.7, 45.2, 35.7, 29.6, 27.2, 25.5, 21.3, 20.0, 19.8; HRMS-ESI (*m/z*) [M+H]⁺ calcd for C₃₆H₄₇N₂O₈S 667.3053; found, 667.3050.

(3*R*,3*aS*,6*aR*)-hexahydrofuro[2,3-*b*]furan-3-yl-((2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-(3'-methoxy-[1,1'-biphenyl]-3-yl)butan-2-yl)carbamate (21c)

$[\alpha]_D^{20} +19.4$ (*c* 0.68, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.70 (d, *J* = 9.0 Hz, 2H), 7.42-7.37 (m, 2H), 7.34-7.29 (m, 2H), 7.18-7.15 (m, 3H), 7.03-6.96 (m, 3H), 5.61 (d, *J* = 6.0 Hz, 1H), 5.00 (t, *J* = 8.0 Hz, 2H), 3.95-3.92 (m, 3H), 3.88 (s, 3H), 3.86 (s, 3H), 3.73-3.65 (m, 4H), 3.21-3.09 (m, 2H), 3.03-2.96 (m, 2H), 2.90-2.77 (m, 3H), 1.86-1.80 (m, 1H), 1.56-1.44 (m, 2H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 163.0, 156.1, 155.5, 138.7, 137.0, 130.7, 130.5, 130.2, 129.6, 129.5, 128.8, 128.1, 127.9, 120.8, 114.4, 111.2, 109.2, 73.4, 72.7, 70.6, 69.6, 58.8, 55.6, 55.4, 54.9, 53.8, 45.3, 35.6, 27.3, 25.7, 20.1, 19.9; HRMS-ESI (*m/z*) [M+Na]⁺ calcd for C₃₅H₄₄N₂O₉SNa 691.2665; found, 691.2664.

(3*R*,3*aS*,6*aR*)-hexahydrofuro[2,3-*b*]furan-3-yl-((2*S*,3*R*)-1-(2',6'-dimethoxy-[1,1'-biphenyl]-3-yl)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)butan-2-yl)carbamate (21d)

$[\alpha]_D^{20} +22.4$ (*c* 0.78, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 7.70 (d, *J* = 8.5 Hz, 2H), 7.44-7.43 (m, 2H), 7.35 (t, *J* = 8.5 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 6.97 (d, *J* = 9.0 Hz, 2H), 6.71 (d, *J* = 2.5 Hz, 2H), 6.46 (t, *J* = 2.5 Hz, 1H), 5.61 (d, *J* = 5.0 Hz, 1H), 5.03-4.94 (m, 2H), 4.00-3.90 (m, 3H), 3.88 (s, 3H), 3.85 (s, 6H), 3.72-3.62 (m, 4H), 3.25-3.10 (m, 2H), 3.03-2.96 (m, 2H), 2.88-2.78 (m, 3H), 1.88-1.82 (m, 1H), 1.56-1.52 (m, 2H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.2, 161.2, 155.5, 143.0, 141.4, 138.1, 129.8, 129.5, 129.0, 128.6, 128.2, 125.4, 114.4, 109.6, 109.3, 105.5, 73.4, 72.8, 70.6, 69.5, 58.9, 55.6, 55.5, 55.0, 53.8, 45.3, 35.7, 27.3, 25.7, 21.2, 19.9; HRMS-ESI (*m/z*) [M+Na]⁺ calcd for C₃₆H₄₆N₂O₁₀SNa 721.2771; found, 721.2765.

(3*R*,3*aS*,6*aR*)-hexahydrofuro[2,3-*b*]furan-3-yl-((2*S*,3*R*)-3-hydroxy-4-(*N*-isobutyl-4-methoxyphenylsulfonamido)-1-(2'-methoxy-[1,1'-biphenyl]-3-yl)butan-2-yl)carbamate (21e)

$[\alpha]_D^{20} +21.8$ (*c* 0.74, CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 7.73-7.70 (m, 2H), 7.42-7.27 (m, 5H), 7.18-7.17 (m, 1H), 7.03-6.96 (m, 4H), 5.61 (d, *J* = 5.0 Hz, 1H), 5.00 (t, *J* = 8.0 Hz, 2H), 3.95-3.92 (m, 3H), 3.86 (s, 3H), 3.73 (s, 3H), 3.71-3.65 (m, 4H), 3.18-3.11 (m, 2H), 3.09-2.96 (m, 2H), 2.90-2.77 (m, 3H), 1.88-1.80 (m, 1H), 1.60-1.40 (m, 2H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 163.1, 156.3, 155.5, 138.7, 137.0, 130.7, 130.5, 130.2, 129.6, 129.5, 128.8, 128.1, 127.9, 120.8, 114.4, 111.2,

109.2, 73.4, 72.7, 70.6, 69.6, 58.8, 55.6, 55.5, 55.0, 53.8, 45.3, 35.6, 27.3, 25.7, 20.1, 19.9;
HRMS-ESI (m/z) $[M+H]^+$ calcd for $C_{35}H_{45}N_2O_9S$ 669.2846; found, 669.2849.

Methods: Determination of X-ray structures of HIV-1 protease-inhibitor complexes

The optimized HIV-1 protease was expressed and purified as described.²⁹ The protease-inhibitor complex was crystallized by the hanging drop vapor diffusion method with well solutions of 1.4 M NaCl, 0.1 M Sodium acetate buffer (pH 4.8). X-ray diffraction data were collected on a single crystal cooled to 90 K at SER-CAT (22-BM beamline), Advanced Photon Source, Argonne National Laboratory (Chicago, USA) with X-ray wavelength of 1.0 Å, and processed by HKL-2000 with Rmerge of 6.3%.³⁰ Using one of the previous isomorphous structures³¹, the crystal structure was solved by PHASER³² in CCP4i Suite^{33,34} and refined by SHELX-97³⁵ to 1.53 Å resolution. COOT³⁶ was used for visual modification. PRODRG-2³⁷ was used to construct the inhibitor and the restraints for refinement. Alternative conformations were modeled, isotropic atomic displacement parameters (B factors) were applied for all atoms including solvent molecules. The final solvent structure comprised one Na⁺ ion, three Cl⁻ ions, one acetate and 146 water molecules. The crystallographic statistics are listed in the supporting information section. The coordinates and structure factors of the PR with GRL-096-13A structure have been deposited in Protein Data Bank with code 4ZLS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

THF	tetrahydrofuran
bis-THF	bis-tetrahydrofuran
tris-THF	tris-tetrahydrofuran
PI	protease inhibitor
CNS	central nervous system
TFA	trifluoroacetic acid
DRV	darunavir

APV amprenavir

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Figure 1.
Structures of HIV-1 protease inhibitors **1–4**.

Figure 2.
Model of inhibitor 4 (green, X = H) created based upon the X-ray structure of inhibitor 2-bound HIV-1 protease active site (PDB code: 317E).

Figure 3.

A. X-ray structure of inhibitor **17a** -bound HIV-1 protease (PDB code:4ZLS). Hydrogen bonding interactions are shown as dotted lines. **B.** The main van der Waals interactions of the outer 3,5-dimethoxy-phenyl group interactions of with Gly49 Thr80' and Pro81' are shown.

Scheme 1.

Synthetic strategy of inhibitors with biphenyl P1-ligands

Scheme 2.
Synthesis of Triflate **9**

Scheme 3.

Synthesis of functionalized biphenyl by Suzuki-Miyaura cross-coupling

Scheme 4.
Synthesis of P2-Inhibitors **17**, **20**, and **21**

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Table 1

Enzymatic inhibitory and antiviral activity of inhibitors

Entry	Inhibitor	K _i (nM)	IC ₅₀ (μM) ^{a,b}	Entry	Inhibitor	K _i (nM)	IC ₅₀ (μM) ^{a,b}
1.		0.82	>1	7.		0.63	>1
2.		18.0	>1	8.		0.014	0.005
3.		92.8	>1	9.		0.45	0.056
4.		48.8	>1	10.		0.014	0.005
5.		0.58	0.18	11.		0.025	0.024
6.		1.53	0.381	12.		0.012	0.003

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Human T-lymphoid (MT-2) cells were exposed to 100 TCID₅₀ values of HIV-1 LAI and cultured in the presence of each PI, and IC₅₀ values were determined using the MTT assay. Darunavir exhibited $K_i = 16$ pM, IC₅₀ = 0.003 μ M.

_p
nt = not tested.

Table 2

Comparison of the Antiviral Activity of 21e, APV and DRV against Multidrug Resistant HIV-1 Variants.

virus	<u>EC₅₀ ± SD, (μM) (fold change)^{a,b}</u>		
	APV	DRV	Inhibitor 21e
HIV-1 _{104pre} (wt)	0.037 ± 0.0003	0.0035 ± 0.0004	0.0048 ± 0.0002
HIV-1 _{MDR/B}	0.044 ± 0.13 (12)	0.028 ± 0.006 (8)	0.036 ± 0.003 (8)
HIV-1 _{MDR/C}	0.38 ± 0.11 (10)	0.019 ± 0.009 (5)	0.0029 ± 0.0002(1)
HIV-1 _{MDR/G}	0.398 ± 0.009 (11)	0.023 ± 0.001 (7)	0.0047 ± 0.0007(1)

^aThe amino acid substitutions identified in the protease-encoding region of HIV-1_{ERS104pre} (wild type), HIV-1_{MDR/B}, HIV-1_{MDR/C}, and HIV-1_{MDR/G} compared to the consensus type B sequence cited from the Los Alamos database include L63P; L10I, K14R, L33I, M36I, M46I, F53I, K55R, I62V, L63P, A71V, G73S, V82A, L90M, I93L; L10I, I15V, K20R, L24I, M36I, M46L, I54V, I62V, L63P, K70Q, V82A, L89M; and L10I, V11I, T12E, I15V, L19I, R41K, M46L, L63P, A71T, V82A, L90M, respectively. HIV-1_{ERS104pre} served as a source of wild-type HIV-1.

^bThe EC₅₀ values were determined by using PHA-PBMs as target cells and the inhibition of p24 Gag protein production by each drug was used as an endpoint. The numbers in parentheses represent the fold changes of EC₅₀ values for each isolate compared to the EC₅₀ values for wild-type HIV-1_{ERS104pre}. All assays were conducted in duplicate, and the data shown represent mean values (± 1 standard deviations) derived from the results of two independent experiments.