Published in final edited form as:

Bioorg Med Chem. 2017 October 01; 25(19): 5114–5127. doi:10.1016/j.bmc.2017.04.005.

Design of novel HIV-1 protease inhibitors incorporating isophthalamide-derived P2-P3 ligands: synthesis, biological evaluation and X-ray structural studies of inhibitor-HIV-1 protease complex

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Abstract

Based upon molecular insights from the X-ray structures of inhibitor-bound HIV-1 protease complexes, we have designed a series of isophthalamide-derived inhibitors incorporating substituted pyrrolidines, piperidines and thiazolidines as P2-P3 ligands for specific interactions in the S2–S3 extended site. Compound **4b** has shown an enzyme K_i of 0.025 nM and antiviral IC_{50} of 69 nM. An X-ray crystal structure of inhibitor **4b**-HIV-1 protease complex was determined at 1.33 Å resolution. We have also determined X-ray structure of **3b**-bound HIV-1 protease at 1.27 Å resolution. These structures revealed important molecular insight into the inhibitor–HIV-1 protease interactions in the active site.

Graphical abstract

Supplementary data

Supplementary data associated with this article can be found in the online version.

Keywords

HIV-1 protease; Drug-resistance; X-ray structure; Synthesis; Isophthalamide

1. Introduction

Protein structure-based molecular design has had tremendous impact in modern drug design and medicinal chemistry efforts.^{1,2} The design and development of HIV-1 protease inhibitors and their combination with reverse transcriptase inhibitors, marked the beginning of a new era of treatment of HIV infection and AIDS.^{3,4} The combination antiretroviral therapies (ART) significantly improved life expectancy and dramatically improved the course of HIV management in developing countries.^{5,6} Therapeutic protease inhibitors (PIs) block HIV-1 replication by inhibiting viral HIV-1 protease and generate morphologically immature and noninfectious virions. However, selective drug pressure blocks replication of viruses that are susceptible to the drug and generates drug-resistant HIV-1 variants.^{7,8} A growing number of HIV/AIDS patients are harboring multidrug-resistant HIV-1 variants that can be readily transmitted. These drug-resistant viruses are difficult to treat and raise a serious concern for the long-term treatment options of HIV management.^{9,10} Therefore, one of the major current therapeutic objectives is to design and develop novel PIs that display broad-spectrum activity against drug-resistant HIV-1 variants and show improved drug-like properties.^{3,11}

In our continuing studies towards the design and development of PIs to combat drug-resistance, we reported a series of novel nonpeptide PIs that showed very potent enzyme inhibitory and antiviral activity. Also, these PIs exhibited potent antiviral activity^{3,12,13} against a panel of multidrug-resistant HIV-1 variants.

One of our major design strategies is to incorporate cyclic ether or polyether-like templates where ether oxygens are positioned to form specific hydrogen bonds with the active site backbone atoms.^{14,15} In essence, these ether oxygens mimic the peptide carbonyl group in the active site. This design concept is inspired by the occurrence of numerous bioactive natural products with polyether subunits.^{16,17} Our laboratory reported a range of exceptionally potent PIs with intriguing molecular features such as TMC-114, TMC126, GRL-06579, GRL-02031, and GRL-519.^{18,19,20} These inhibitors show broad-spectrum antiviral activity against multidrug-resistant HIV-1 variants and show high genetic barrier to resistance. Darunavir (DRV, **1**) has emerged as front-line treatment of HIV/AIDS patients.^{21,22} In DRV, we have incorporated a stereochemically defined *bis*-tetrahydrofuran (*bis*-THF) template to promote hydrogen bonding interactions with the backbone atoms at S2-subsites.^{17,21} Our structure-based design efforts created numerous intriguing ligands such as 3*S*-THF, *bis*-THF, *Cp*-THF, *Tp*-THF, and *tris*-THF.¹² All these ligands are involved in interactions in the protease active site, especially extensive hydrogen bonding with the protease backbone atoms.

Recently, we have explored a number of substituted benzoic acid-based acyclic and macrocyclic inhibitors with good to excellent potency.^{23,24} These inhibitors were designed based upon the P2-ligand of FDA approved nelfinavir and P1' and P2' ligands inherent to DRV (**1**) and TMC-126 (**2**).^{17,25} In particular, a series of PIs containing isophthalamide-

based P2-P3 ligands showed very potent enzyme inhibitory and antiviral activity.²⁶ One of the important features of these PIs is that they contain only two chiral centers and structural variants of these PIs can be synthesized readily for structure-activity studies. Furthermore, some of these ligand moieties have been utilized in the design and synthesis of inhibitors of β -secretase (BACE-1), an aspartic acid protease target for the treatment of Alzheimer's disease.^{27–30} Our initial efforts led us to determine X-ray structures of HIV-1 protease complexes with a number of isophthalamide based inhibitors.²⁶ These X-ray structural studies provided molecular insights into the ligand-binding site interactions in the HIV-1 protease active site. We have now further explored these structural insights and herein we report the design, synthesis, and biological evaluation of a series of PIs incorporating isophthalamide-based P2-P3 ligands with substituted pyrrolidines, piperidines and thiazolidines for specific interactions in the S2-S3 extended site.

2. Results and discussion

2.1. Synthesis

Our general strategy for the synthesis of HIV-1 protease inhibitors **3a–h** and **4a–l** is illustrated in Scheme 1. We planned to synthesize these inhibitors by amide coupling of the appropriate isophthalic, phthalic or terephthalic acid derivatives **5** with the amines belonging to hydroxyethylamine sulfonamide isosteres (**6a–c**).^{19,28} The required acid derivatives were obtained by coupling of readily available isophthalic (**7a–d**), phthalic (**8**) or terephthalic (**9**) monoacids with amines **10a–d** or cyclic amines **11a–h**.^{28,31,32}

The synthesis of oxazole- and thiazole-based P2-P3 ligands **12a–g** is shown in Scheme 2. The known²⁸ amine **10a** was coupled with isophthalic acid derivatives **7c,d** using EDC, HOBt in the presence of diisopropylethylamine to provide the corresponding amide derivatives. The resulting amides were *N*-methylated with NaH and MeI in THF. Saponification of the resulting methyl ester with aqueous lithium hydroxide afforded isophthalic acid derivatives **12a,b**. Acid derivatives **12c–f** were prepared using similar reaction sequences using suitable commercially available amines **10b,c** and oxazolylmethylamine **10d**²⁶ and carboxylic acids **7a**, **8** or **9**. The resulting amides were *N*-methylated and subsequent saponification of the methyl ester functionality afforded acids **12c–f**.

The synthesis of various methylthiazolylpyrrolidine-derived isophthalic acids is shown in Scheme 3. As shown, amines **11a,b** and isophthalic acid monomethyl esters **7a** or **7b** were coupled together using standard EDC and HOBt coupling. The resulting esters were saponified with aqueous lithium hydroxide to provide carboxylic acids **13a–c**. Compound **13a** was also employed for the synthesis of P2-P3 ligand **15** in which a carbonyl group was converted to a methylene linker by reducing the isophthalate carbonyl group. Thus, treatment of **13a** with LiAlH₄ in dry THF at reflux provided aminoalcohol **14**. The hydroxymethyl group of **14** was oxidized with manganese dioxide to aldehyde. Oxidation³³ of this aldehyde with NaClO₂ provided acid **15**. Acid derivatives **16a–c** were prepared by following similar sequence of reactions as compound **13a**.

The synthesis of pyrrolidin-3-yl-thiazole amine-derived P2-P3 ligands **11g,h** is shown in Scheme 4. (3*R*)- and (3*S*)-pyrrolidines were prepared by Michael addition of (*R*)- α -methylbenzylamine with dimethyl itaconate (**17**) followed by cyclization of the resulting amino-ester to give an equal mixture of lactams as described previously.³⁴ This mixture was separated by chromatography. Treatment of the resulting esters with benzylalcohol in the presence of PPTS provided benzyl ester derivatives **18a,b**. These lactams were treated with Lawesson's reagent to provide thioamides **19a,b**. Reduction of the thioamide functionality by treatment with MeI followed by NaBH₄ in EtOH led to derivatives **20a,b**. Simultaneous removal of benzyl and α -methylbenzyl groups by catalytic hydrogenation was followed by *N*-Cbz protection and conversion of the carboxylic functionality to the corresponding amide to furnish carboxamide derivatives **21a,b**. Treatment of these carboxamides with Lawesson's reagent afforded the corresponding thioamides, which underwent cyclization upon treatment with chloroacetone in EtOH. Removal of the Cbz group by exposure to HBr in AcOH, provided the desired amine derivatives **11g,h**. These ligands were coupled with the isophthalic acid methyl ester **7a** to furnish the corresponding amide derivatives. Saponification of the methyl ester with aqueous lithium hydroxide afforded acids **22a,b**.

The synthesis of isophthalic acid derivative **25** is reported in Scheme 5. Ester **23** was treated with LiBH₄ in THF at 23 °C to provide alcohol derivative **24**. Alkylation of alcohol **24** with MeI and NaH in THF provided the corresponding methyl ether. Removal of the Cbz-group with HBr in acetic acid afforded amine **11f**. This amine was coupled with isophthalic acid monomethyl ester **7a** using EDC and HOBt in the presence of *i*-Pr₂NEt to provide the corresponding amide derivative. Saponification of the methyl ester with aqueous LiOH afforded acid **25**.

Our syntheses of the final inhibitors are shown in Scheme 6. Synthesis of inhibitors **3a–h** and **4a–l** were carried out by coupling of acids **12a–g**, **13a–c**, **15**, **16a–c**, **22a,b** and **25** with hydroxyethylamine sulfonamide isosteres (**6a–c**) using standard EDC and HOBt coupling. Various isophthalamide-based inhibitors were obtained in good to excellent yields (49–95%).

2.2. Biological evaluation, structure-activity relationships, and X-ray studies

On the basis of the X-ray studies reported for compounds **3a**²⁶ and darunavir-bound HIV-1 protease,³⁵ we first sought to investigate the outcome of a focused series of derivatives bearing isophthalamide as the P2-ligand and various cyclic and acyclic heterocycles as the P3-ligand. An overlay of the X-ray crystal structures of inhibitors **1** (DRV) and **3a** in the HIV-1 protease active site is shown in Figure 2. As can be seen, the key interactions in the S1' and S2' subsites are very similar. The major differences are in the S2-S3 subsites. The *bis*-THF of DRV forms two strong hydrogen bonds with Asp-29 and Asp-30 backbone amide NH's in the S2-subsite. Inhibitor **3a** forms a strong hydrogen bond with Asp29 backbone amide NH and forms strong van der Waals interactions with oxazolylmethyl ligand. Based upon this molecular insight, we speculated that cyclic and acyclic heterocycles could form hydrogen bonds with backbone atoms as well as form stronger van der Waals interactions in the active site. In particular, we first sought to investigate the outcome of a focused series of derivatives bearing oxazolylmethyl- or thiazolylmethyl-derived P3-ligands

(compounds **3b-h**, Table 1). HIV-1 protease inhibitory potency of all inhibitors was first evaluated using the assay protocol reported by Toth and Marshall.⁶ The antiviral activity of the new inhibitors was determined based on a previously published assay protocol using MT-2 cells exposed to HIV-1 LAI.³⁷ These results are shown in Tables 1 and 2. In compound **3b**, we incorporated a 4-methyl oxazole as P3 to promote hydrogen bond through the oxazole nitrogen. However, this compound lost significant potency over compound **3a**. Compounds **3c** (entry 3) and **3d** (entry 4) contained a 2-methyloxazole and a 2-methylthiazole ring, respectively. Derivative **3d** displayed 5-fold enhanced protease inhibitory activity over **3c**. Nevertheless, the compounds similarly performed in antiviral assay, displaying an over 20-fold improvement with respect to their forerunner **3b** (entry 2). The dimethyloxazole moiety was also explored in combination with polar substituents placed at the 3-position of the isophthalamide moiety (entries 5 and 6). As it turned out, phenol derivative **3f** overtook its methylamino analogue **3e** by 10-fold in terms of protease inhibitory potency. However, the presence of a 3-substituted isophthalamide portion was found detrimental for the antiviral outcome of both compounds. Finally, the exploration of the dimethyloxazole moiety linked to phthalamide- or terephthalamide-based ligands (compounds **3g** and **3h**, entries 7 and 8, respectively) led to a strong drop in both the enzyme inhibitory and the antiviral activities of the compounds, clearly validating the key role of the isophthalamide moiety for inhibitors' efficacy. Taking into account the information gained with the first set of compounds we sought to explore a novel series isophthalamide-based extended P2-P3 ligands incorporating substituted pyrrolidines, piperidines and thiazolidines as the suitable support for thiazole or oxazole rings (inhibitors **4a-l**, Table 2). The incorporation of a 2-thiazolyl pyrrolidine in both (*S*) or (*R*) configuration (compounds **4a** and **4b**, entries 1 and 2, respectively) clearly highlighted the key role of stereochemistry for this series of compounds. Inhibitor **4b** with (*R*)-configuration displayed over 100-fold improved potency over its (*S*) counterpart **4a**. The same trend was also shown for the antiviral outcome of the compounds. The combination of the (*R*)-2-thiazolyl pyrrolidine moiety with different hydroxyethylamine sulfonamide isosteres (compounds **4c** and **4d**, entries 3 and 4, respectively) substantially led to a significant drop in both the enzyme inhibitory and the antiviral activities. The increase of the ring size in piperidine-based compound **4e** (entry 5) significantly reduced enzyme affinity and antiviral efficacy. Compound **4f** (entry 6) bearing a 2-thiazolyl thiazolidine moiety, although characterized by a reduced enzyme inhibitory potency with respect to **4a**, displayed the best antiviral activity of this series of compounds ($IC_{50} = 32$ nM). Compounds **4g** and **4h** (entries 7 and 8) containing a 2-oxazolyl pyrrolidine moiety were also explored. In particular, methyloxazole derivative **4h** resulted in a 10-fold improved antiviral profile with respect to its methoxymethyloxazole analogue **4g**. The exploration of compounds **4i** (entry 9) with a methylene replacing one of the isophthalate carbonyl groups, and **4j** (entry 10) bearing a pyridyl ring in the isophthalic moiety led to a substantial loss of protease inhibitory activity. The shift of the thiazole ring in 3-position of the pyrrolidine was also investigated for both (*R*) and (*S*) configurations (inhibitors **4k** and **4l**, entries 11 and 12, respectively). Both compounds exhibited comparable protease inhibitory profile and a moderate antiviral activity, indicating in this case a negligible contribution of stereochemistry. To obtain molecular insight into the inhibitor-HIV-1 protease interactions, we have determined the X-ray crystal structures of the inhibitors **3b**- and **4b**-bound HIV-1 protease complexes.

The crystal structures of wild type HIV-1 protease in complex with the inhibitors **3b** (GRL-0518A) and **4b** (GRL-1118A) were determined and refined at 1.27 Å and 1.33 Å resolution, respectively. Both crystal structures contain the protease dimer and inhibitor bound in a single orientation. The overall structures are comparable to the structure of HIV-1 protease and darunavir³⁵ with low root mean square differences (RMSD) of 0.18 Å and 0.21 Å, respectively, for C α atoms. Most differences between corresponding C α atoms are less than 0.4 Å. Only residues 47'–51' exhibit root mean square differences of about 0.5–0.6 Å, likely due to displacement by the P2 group. For residues 78'–81', the larger RMSD of about 0.6–0.9 Å occurs due to the opposite effect since the 80's loop moves in to generate more contacts with the single smaller isobutyl group in P1' position of these two complexes. The third larger RMSD of about 0.7–0.8 Å in residue 4 may be due to the rotation of its side chain. Both inhibitors form hydrogen bonding interactions of their urethane NH with the carbonyl oxygen of Gly27. In addition, water-mediated interactions connect the inhibitor carbonyl oxygen and sulfonamide oxygen with the amides of Ile50 and 50' in the flaps. Similar interactions are shared by the protease-darunavir complex and are considered responsible for the high affinity of darunavir for HIV protease and its potency against drug resistant HIV-1 variants.³⁵

Inhibitor **3b** contains a 4-methyloxazolyl-2-methylamide as the P3-ligand and a stereoview of the active site interactions is shown in Figure 3. The P2-isophthalamide carbonyl group of the inhibitor maintains a strong hydrogen bonding with backbone amide and side chain of Asp29. However, the oxazole ring flipped toward the S2-extended site and was unable to form any hydrogen bond in the active site. The isophthalamide group in P2 is sandwiched in a cavity formed by the hydrophobic side chains of Ile50', Ala28, Val32, Ile47 and Ile84. The extended P3 group (4-methyl-2-oxazole) in inhibitor **3b** forms hydrophobic interactions with the side chains of Val82'. In comparison, the 2,5-dimethyl-4-oxazole in inhibitor **3a** fits in two conformations with occupancy ratio of 50:50.²⁶ The ligand-binding site interactions of inhibitor **3b** in the S1, S1' and S2' subsites are similar to darunavir. The hydroxymethyl group on the P2'-ligand form a strong hydrogen bond with the backbone amide NH of Asp30' in the S2'-site.

Inhibitor **4b** contains a thiazolylpyrrolidine moiety as the P3-liagand and a stereoview of the active site interactions is shown in Figure 4. Like compound **3b**, the carbonyl group of inhibitor **4b** in the P2 position maintains polar hydrogen bonding interactions with the amide and side chain of Asp29' with interatomic distances of 2.9 Å. The pyrrolidine group and the larger sulfur atom in thiazoline of inhibitor **4b** may constrain the conformation of the extended P3 group (methyl-thiazole) to form π - π stacking interactions with the guanidine group of Arg8'. These extended interactions are expected to stabilize the inhibitor in the binding site. Furthermore, the P2 isophthalamide phenyl ring forms van der Waals contacts with Ile50', Val32, Ile47 and Ile84.³⁸

3. Conclusion

In summary, we have designed and synthesized potent HIV-1 protease inhibitors incorporating isophthalamide derivatives as the P2-P3 ligands for the HIV-1 protease active site. These inhibitors were specifically designed to interact with the extended S2 subsite,

particularly to form hydrogen bonds with the backbone atoms as well as to promote van der Waals interactions with residues in the S3 subsite. In this context, we have synthesized and evaluated a range of heterocyclic derivatives of isophthalamides to occupy the extended S2-subsite. In particular, we have investigated the suitability of oxazolyl and thiazolyl isophthalamides as well as pyrrolidinyl thiazolyl and pyrrolidinyl oxazole amide derivatives of isophthalamides with donor and acceptor groups to serve as P2-P3 ligands. A number of compounds showed very potent enzyme inhibitory and antiviral activity. Inhibitor **4b** with a pyrrolidinyl thiazole isophthalamide derivative showed very potent enzyme inhibitory activity ($K_i = 25$ pM) and antiviral IC_{50} value of 69 nM in MT-2 cells exposed to HIV-1 LAI. Further optimization efforts led to inhibitor **4h** with a pyrrolidinyl oxazole isophthalamide derivative which showed an improved antiviral IC_{50} value of 33 nM. We have determined X-ray structures of inhibitor **3b**-bound HIV-1 protease and inhibitor **4b**-bound HIV-1 protease. These structures provided molecular insights into the key ligand-binding site interactions responsible for inhibitors potency. The isophthalamide P2-P3 ligand amide carbonyl formed very strong hydrogen bonds with Asp29 backbone amide NH and the pyrrolidinylthiazole heterocycles are involved in van der Waals interactions with Leu23' and Val82' in the S2 extended site. These ligand-binding site interactions can be further exploited to design more potent and less complex protease inhibitors.

4. Experimental

4.1 General

All moisture-sensitive reactions were carried out in oven-dried glassware under an argon atmosphere unless otherwise stated. Anhydrous solvents were obtained as follows: Diethyl ether and tetrahydrofuran were distilled from sodium metal/benzophenone under argon. Toluene and dichloromethane were distilled from calcium hydride under argon. All other solvents were reagent grade. Column chromatography was performed using Silicycle SiliFlash F60 230–400 mesh silica gel. Thin-layer chromatography was carried out using EMD Millipore TLC silica gel 60 F254 plates. 1H NMR and ^{13}C NMR spectra were recorded on a Varian INOVA300 or a Bruker ARX400. Low-resolution mass spectra were collected on a Waters 600 LCMS or by the Purdue University Campus-Wide Mass Spectrometry Center. High-resolution mass spectra were collected by the Purdue University Campus-Wide Mass Spectrometry Center.

4.2 Experimental procedures

4.2.1. 3-((*tert*-Butoxycarbonyl)(methyl)amino)-5-(((2,5-dimethyloxazol-4-yl)methyl)(methyl)carbamoyl)benzoic acid (12a**)**—To a solution of **7c** (45 mg, 0.215 mmol) in dry CH_2Cl_2 (2 mL), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI) (62 mg, 0.322 mmol), and 1-hydroxybenzotriazole hydrate (HOBt) (50 mg, 0.322 mmol) were added followed by amine **10a** (27 mg, 0.215 mmol) and diisopropylethylamine (225 μ L, 1.290 mmol). The reaction mixture was stirred at 25 °C for 16 h then CH_2Cl_2 was added and the resulting solution was washed with saturated aqueous NH_4Cl and water. The organic layers were dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/*n*-hexane 1:4) to furnish *methyl 3-((tert-butoxycarbonyl)(methyl)amino)-5-(((2,5-*

dimethyloxazol-4-yl) methyl)carbamoyl)benzoate (80%). ¹H NMR (400 MHz, CDCl₃) δ 8.13 (t, 1H, *J* = 1.5 Hz), 8.03–8.00 (m, 1H), 7.93 (t, 1H, *J* = 1.8 Hz), 7.32 (t, 1H, *J* = 5.2 Hz), 4.38 (d, 2H, *J* = 5.3 Hz), 3.87 (s, 3H), 3.24 (s, 3H), 2.32 (s, 3H), 2.30 (s, 3H), 1.42 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 166.0, 165.9, 159.8, 154.3, 145.2, 144.5, 135.2, 130.8, 129.2, 128.5, 124.1, 81.2, 52.5, 37.0, 35.4, 28.3, 13.8, 10.1. To a solution of this latter (35 mg, 0.084 mmol) in dry THF (2 mL), NaH (60% suspension in mineral oil, 7 mg, 0.168 mmol) was added and the reaction mixture was stirred at 25 °C for 1 h. The reaction was quenched by adding water at 0 °C, diluted with CH₂Cl₂ and washed twice with water. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/*n*-hexane 1:5) providing methyl *methyl 3-((tert-butoxycarbonyl)(methyl)amino)-5-((2,5-dimethyloxazol-4-yl) methyl)(methyl)carbamoyl)benzoate* (68%). ¹H NMR (400 MHz, CDCl₃) δ 8.14–7.90 (m, 1H), 7.88–7.72 (m, 1H), 7.53 (s, 1H), 4.50 (s, 1H), 4.22 (s, 1H), 3.90 (s, 3H), 3.27 (s, 3H), 3.04 (s, 3H), 2.46–2.22 (m, 6H), 1.43 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 166.1, 159.4, 154.3, 146.3, 144.3, 137.0, 130.8, 130.4, 128.6, 124.7, 81.1, 52.5, 42.4, 37.8, 37.1, 28.4, 14.0, 10.2.

To a solution of the methyl ester (17 mg, 0.039 mmol) in a in THF/MeOH/H₂O mixture (1.5 mL: 0.5 mL: 0.5 mL) at 0 °C was added LiOH·H₂O (3 mg, 0.059 mmol). The mixture was stirred at 25 °C for 16 h. THF and MeOH were evaporated under reduced pressure and the residue was diluted with water, acidified with 1N HCl to pH = 2 and extracted with EtOAc. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure providing pure **12a** (99%). ¹H NMR (400 MHz, CDCl₃) δ 8.07–7.92 (m, 1H), 7.80 (m, 1H), 7.53 (s, 1H), 4.50 (s, 1H), 4.22 (s, 1H), 3.27 (s, 3H), 3.04 (s, 3H), 2.44–2.25 (m, 6H), 1.43 (s, 9H).

4.2.2. 3-(((2,5-Dimethyloxazol-4-yl)methyl)(methyl) carbamoyl)-5-hydroxybenzoic acid (**12b**)

—Compound **12b** was prepared from **10a** and **7d** following the procedure described for **12a**. ¹H NMR (400 MHz, CDCl₃) δ 7.61–7.34 (m, 2H), 7.06–6.80 (m, 1H), 4.42 (s, 1H), 4.19 (s, 1H), 2.95–2.84 (m, 3H), 2.41–2.16 (m, 6H).

4.2.3. 3-(Methyl((2-methyloxazol-4-yl)methyl)carbamoyl) benzoic acid (**12c**)

—Compound **12c** was prepared from **10b** and **7a** following the procedure described for **12a**. ¹H NMR (400 MHz, CDCl₃) δ 8.54 (t, 1H, *J* = 1.5 Hz), 8.07 (dt, 1H, *J* = 7.7 and 1.4 Hz), 8.02–7.97 (m, 1H), 7.52 (s, 1H), 7.40 (q, *J* = 7.9 Hz, 1H), 4.46 (s, 1H), 4.37 (s, 1H), 2.95 (m, 3H), 2.34 (s, 3H).

4.2.4. 3-(Methyl((2-methylthiazol-4-yl)methyl)carbamoyl) benzoic acid (**12d**)

—Compound **12d** was prepared from **10c** and **7a** following the procedure described for **12a**. ¹H NMR (300 MHz, CDCl₃) δ 11.83 (br, 1H), 8.20–8.06 (m, 2H), 7.64 (d, 1H, *J* = 6.8 Hz), 7.47–7.42 (m, 1H), 6.87 (s, 1H), 5.04 (s, 1H), 4.74 (s, 1H), 3.11–2.94 (m, 3H), 2.40–2.31 (m, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 170.7, 168.6, 166.1, 151.6, 135.3, 131.7, 131.4, 130.6, 128.7, 128.5, 115.2, 48.3, 37.7, 16.4. LRMS-ESI (*m/z*): 313.0 [M+Na].

4.2.5. 4-(((2,5-Dimethyloxazol-4-yl)methyl)(methyl) carbamoyl)benzoic acid (**12e**)

—Compound **12e** was prepared from **10a** and **9** following the procedure described for

12a. ^1H NMR (400 MHz, CDCl_3) δ 8.09 (d, 2H, $J = 8.2$ Hz), 7.69–7.63 (m, 1H), 7.49 (d, 1H, $J = 7.2$ Hz), 4.56 (s, 1H), 4.27 (s, 1H), 3.01–2.97 (m, 3H), 2.51–2.27 (m, 6H).

4.2.6. 2-(((2,5-Dimethyloxazol-4-yl)methyl)(methyl) carbamoyl)benzoic acid (12f)—Compound **12f** was prepared from **10a** and **8** following the procedure described for **12a**. ^1H NMR (400 MHz, CDCl_3) δ 8.08–8.01 (m, 1H), 7.54 (q, 1H, $J = 7.9$ Hz), 7.46–7.36 (m, 1H), 7.28–7.22 (m, 1H), 4.61 (s, 1H), 4.06 (s, 1H), 2.79–2.72 (m, 3H), 2.45–2.34 (m, 6H).

4.2.7. 3-(Methyl((4-methyloxazol-2-yl)methyl)carbamoyl) benzoic acid (12g)—Compound **12g** was prepared from **10d** and **7a** following the procedure described for **12a**. ^1H NMR (400 MHz, CDCl_3) δ 9.82 (br, 1H), 8.23 (s, 1H), 8.14–8.09 (m, 1H), 7.72–7.67 (m, 1H), 7.49 (t, 1H, $J = 7.7$ Hz), 7.40 (s, 1H), 4.89 (s, 1H), 4.55 (s, 1H), 3.18–3.03 (m, 3H), 2.19 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 170.9, 159.6, 136.2, 135.4, 135.2, 131.9, 131.4, 130.3 (2), 128.8, 128.6, 60.4, 37.9, 14.1.

4.2.8. 3-[(*R*)-2-(4-Methylthiazol-2-yl)pyrrolidine-1-carbonyl]benzoic acid (13a)—To a solution of isophthalic acid mono methyl ester **7a** (133 mg, 0.74 mmol), EDCI (283 mg, 1.48 mmol) and HOBT (200 mg, 1.48 mmol) in anhydrous CH_2Cl_2 (8 mL) was added a solution of (*R*)-2-(4-methylthiazol-2-yl)pyrrolidine **11a** (124 mg, 0.74 mmol) and diisopropylethylamine (0.77 mL, 4.44 mmol) in anhydrous CH_2Cl_2 (2 mL) at 0 °C under argon atmosphere and it was allowed to stir for 16 h at 23 °C. The reaction mixture was quenched with water and extracted with CH_2Cl_2 . The organic layers were dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1) to furnish 3-[(*R*)-2-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl]benzoic acid methyl ester (80%) as a colorless oil. ^1H NMR (300 MHz, CDCl_3) δ 8.26 (s, 1H), 8.13 (d, 1H, $J = 7.5$ Hz), 7.80 (d, 1H, $J = 7.5$ Hz), 7.53 (t, 1H, $J = 7.5$ Hz), 6.80 (s, 1H), 5.68 (dd, 1H, $J = 7.2$ and 5.4 Hz), 3.94 (s, 3H), 3.82–3.74 (m, 1H), 3.57–3.49 (m, 1H), 2.51–2.35 (m, 2H), 2.45 (s, 3H), 2.17–2.03 (m, 2H); ^{13}C NMR (75 MHz, CDCl_3) δ 171.5, 169.0, 166.1, 152.4, 136.6, 131.6, 131.0, 130.0, 128.5, 128.1, 112.7, 58.6, 52.1, 50.0, 32.1, 24.9, 17.0.

To a stirring solution of 3-[(*R*)-2-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl]benzoic acid methyl ester (190 mg, 0.58 mmol) in a THF/MeOH/ H_2O mixture (12 mL: 4 mL: 4 mL) at 0 °C was added solid $\text{LiOH}\cdot\text{H}_2\text{O}$ (36 mg, 0.86 mmol), and the resulting solution was stirred at 23 °C for 16 h. After this period, the reaction mixture was evaporated until 1 mL and the mixture was extracted with toluene to remove organic impurities. The aqueous layer was cooled to 0 °C, acidified with 1N HCl to pH = 3, extracted with EtOAc, and dried over anhydrous Na_2SO_4 . The solvent was evaporated to furnish title compound **13a** (99%). ^1H NMR (400 MHz, CDCl_3) δ 8.30 (s, 1H), 8.12 (d, 1H, $J = 7.6$ Hz), 7.78 (d, 1H, $J = 7.6$ Hz), 7.48 (t, 1H, $J = 7.6$ Hz), 6.77 (s, 1H), 5.65 (t, 1H, $J = 6.8$ Hz), 3.82–3.76 (m, 1H), 3.53–3.47 (m, 1H), 2.49–2.39 (m, 2H), 2.40 (s, 3H), 2.14–2.02 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 172.4, 169.6, 168.7, 152.3, 136.2, 132.0, 131.8, 130.9, 130.5, 128.6, 113.1, 58.7, 50.5, 32.8, 25.2, 16.6. LRMS-ESI (m/z): 338.9 [$\text{M}+\text{Na}$].

4.2.9. 3-[(S)-2-(4-Methylthiazol-2-yl)pyrrolidine-1-carbonyl] benzoic acid (13b)

—Compound **13b** was prepared from **11b** and **7a** following the procedure described for **13a**. ¹H NMR and ¹³C NMR data are identical to those reported for compound **13a**.

4.2.10. (R)-5-(2-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl) nicotinic acid (13c)

—Compound **13c** was prepared from **11a** and **7b** following the procedure described for **13a**. ¹H NMR (400 MHz, CD₃OD) δ 9.22 (s, 1H), 8.90 (s, 1H), 8.54 (s, 1H), 6.88 (s, 1H), 5.58–5.54 (m, 1H), 3.90–3.82 (m, 1H), 3.60–3.54 (m, 1H), 2.52–2.40 (m, 4H), 2.25–2.08 (m, 2H), 2.02–1.96 (m, 1H); ¹³C NMR (100 MHz, CD₃OD) δ 173.5, 172.9, 168.6, 153.8, 153.3, 152.3, 137.8, 137.1, 115.5, 114.6, 60.6, 51.7, 34.2, 26.3, 17.7. LRMS-ESI (*m/z*): 340.0 [M+Na].

4.2.11. (R)-3-((2-(4-methylthiazol-2-yl)pyrrolidin-1-yl) methyl)phenyl)methanol (14)

—To a solution of **13a** (146 mg, 0.442 mmol) in dry THF (8 mL) LiAlH₄ (42 mg, 1.106 mmol) was added portionwise at 0 °C and the reaction mixture was stirred at reflux temperature for 15 h. The reaction was quenched with MeOH and filtered through a Celite® pad. The filtrate was concentrated and the residue was purified by silica gel column chromatography (EtOAc/*n*-hexane 1:1) affording compound **14** (88%). ¹H NMR (300 MHz, CDCl₃) δ 7.38–7.27 (m, 4H), 6.83 (s, 1H), 4.72 (s, 2H), 4.08–3.97 (m, 2H), 3.41–3.36 (m, 1H), 3.10–3.05 (m, 1H), 2.44 (s, 3H), 2.38–2.28 (m, 2H), 1.95–1.79 (m, 4H); ¹³C NMR (75 MHz, CDCl₃) δ 177.5, 152.1, 141.2, 139.0, 128.2, 127.6, 127.1, 125.5, 113.2, 65.6, 64.7, 58.6, 53.2, 34.2, 22.9, 16.9.

4.2.12. (R)-3-((2-(4-methylthiazol-2-yl)pyrrolidin-1-yl)methyl) benzoic acid (15)

—To a solution of alcohol **14** (112 mg, 0.388 mmol) in dry CH₂Cl₂ (4 mL) MnO₂ (1.18 g, 12.86 mmol) was added at 0 °C and the reaction mixture was stirred for 5 h at 25 °C. The reaction mixture was then filtered through a Celite® pad. The filtrate was concentrated and the residue was purified by silica gel column chromatography (EtOAc/*n*-hexane 1:3) affording *(R)*-3-((2-(4-methylthiazol-2-yl)pyrrolidin-1-yl)methyl)benzaldehyde. ¹H NMR (300 MHz, CDCl₃) δ 9.97 (s, 1H), 7.84 (s, 1H), 7.71 (d, 1H, *J* = 7.6 Hz), 7.62 (d, 1H, *J* = 7.6 Hz), 7.43 (t, 1H, *J* = 7.6 Hz), 6.76 (s, 1H), 4.09–3.97 (m, 2H), 3.43–3.39 (m, 1H), 3.03–2.98 (m, 1H), 2.37 (s, 3H), 2.36–2.25 (m, 2H), 1.92–1.74 (m, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 192.4, 176.8, 152.4, 140.4, 136.4, 134.6, 129.6, 128.9, 128.4, 113.2, 66.1, 58.3, 53.4, 34.2, 23.1, 17.1.

To a solution of the aldehyde (72 mg, 0.251 mmol) in 3-methyl-2-butene (4 mL) and *t*-BuOH (2 mL), a solution of NaClO₂ (284 mg, 3.140 mmol) and NaH₂PO₄ (208 mg, 1.5 mmol) in water (1 mL) was added. The reaction mixture was stirred for 4 h at 25 °C then acidified to pH = 3 with 10% HCl and extracted with EtOAc. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/ *n*-hexane 1:1) providing compound **15** (45% over 2 steps). ¹H NMR (300 MHz, CDCl₃) δ 8.13 (s, 1H), 8.03 (d, 1H, *J* = 7.5 Hz), 7.67 (d, 1H, *J* = 7.5 Hz), 7.66–7.42 (m, 2H), 6.86 (s, 1H), 4.19–4.10 (m, 2H), 3.51 (d, 1H, *J* = 12.9 Hz), 3.16–3.09 (m, 1H), 2.49–2.32 (m, 5H), 1.96–1.87 (m, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 177.8, 170.7, 152.3, 139.4, 133.5, 130.6, 130.2, 128.9, 128.4, 113.6, 65.8, 58.6, 53.5, 34.4, 23.2, 17.0.

4.2.13. (*R*)-3-(4-(4-methylthiazol-2-yl)thiazolidine-3-carbonyl) benzoic acid

(16a)—Compound **16a** was prepared from **11d** and **7a** following the procedure described for **13a**. ¹H NMR (400 MHz, CDCl₃) δ 8.79 (br, 1H), 8.28–8.05 (m, 2H), 7.80–7.32 (m, 2H), 6.86 (s, 1H), 6.10 (br, 1H), 4.84 (d, 2H, *J* = 8.9 Hz), 3.66–3.56 (m, 2H), 2.59–2.30 (m, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 168.9, 166.0, 153.0, 135.8, 131.7, 130.5, 128.8, 128.3, 113.8, 52.3, 39.6, 17.0.

4.2.14. (*R*)-3-(2-(4-methyloxazol-2-yl)pyrrolidine-1-carbonyl) benzoic acid (16b)

—Compound **16b** was prepared from **11e** and **7a** following the procedure described for **13a**. ¹H NMR (400 MHz, CDCl₃) δ 8.38 (s, 1H), 8.13–8.00 (m, 1H), 7.77 (d, 1H, *J* = 7.5 Hz), 7.48 (t, 1H, *J* = 7.7 Hz), 7.34–7.20 (m, 2H), 5.36 (t, 1H, *J* = 6.5 Hz), 3.89–3.83 (m, 1H), 3.56–3.50 (m, 1H), 2.46–2.39 (m, 1H), 2.34–2.04 (m, 5H), 1.99–1.91 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 169.3, 168.4, 164.8, 136.2, 135.9, 131.7, 130.8, 130.0, 128.7, 128.4, 127.4, 54.8, 50.1, 31.2, 25.3, 10.9.

4.2.15. (*R*)-3-(2-(4-methylthiazol-2-yl)piperidine-1-carbonyl) benzoic acid (16c)

—Compound **16c** was prepared from **11c** and **7a** following the procedure described for **13a**. ¹H NMR (300 MHz, CDCl₃) δ 11.45 (br, 1H), 8.24 (s, 1H), 8.12 (d, 1H, *J* = 7.5 Hz), 7.57–7.48 (m, 1H), 6.90 (s, 1H), 6.27 (br, 1H), 5.16 (br, 0.5H), 4.69 (br, 0.5H), 3.73–3.61 (m, 1H), 3.39–3.25 (m, 1H), 2.66–2.60 (m, 1H), 2.44 (s, 3H), 2.06–2.00 (m, 1H), 1.78–1.57 (m, 4H); ¹³C NMR (75 MHz, CDCl₃) δ 170.2, 169.6, 168.6, 152.3, 135.5, 131.0 (2), 130.2, 128.5, 127.0, 113.9, 60.2, 51.3, 44.9, 28.0, 25.4, 19.6, 16.6. LRMS-ESI (*m/z*): 353.0 [*M* + Na].

4.2.16. Benzyl (*R*)-5-oxo-1-((*R*)-1-phenylethyl)pyrrolidine-3-carboxylate (18a) and benzyl (*S*)-5-oxo-1-((*R*)-1-phenylethyl)pyrrolidine-3-carboxylate (18b)—To a solution of dimethyl itaconate **17** (3 g, 18.97 mmol) in MeOH (4 mL) was added (*R*)-α-methylbenzylamine (3.14 mL, 26.66 mmol). The solution was stirred for 1 h at 80 °C and for 18 h at 115 °C. The reaction mixture was then concentrated under reduced pressure and water was added. The aqueous solution was adjusted to pH = 2 with 10% HCl and extracted with CH₂Cl₂. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/pentane 3:7 to 1:1) providing isolated diastereoisomers *methyl (R)-5-oxo-1-((R)-1-phenylethyl)pyrrolidine-3-carboxylate* (32%) and *methyl (S)-5-oxo-1-((R)-1-phenylethyl)pyrrolidine-3-carboxylate* (43%).

The isolated methyl esters (219 mg, 0.886 mmol) were dissolved in dry toluene (1.8 mL). Benzyl alcohol (140 μL, 1.328 mmol) and PPTS (45 mg, 0.179 mmol) were sequentially added and the reaction mixture was stirred under reflux for 24 h under argon atmosphere. The reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/*n*-hexane 1:2).

18a (73% yield): ¹H NMR (300 MHz, CDCl₃) δ 7.40–7.27 (m, 10H), 5.55 (q, 1H, *J* = 6.9 Hz), 5.12 (s, 2H), 3.64–3.55 (m, 1H), 3.33–3.22 (m, 2H), 2.87–2.69 (m, 2H), 1.58 (d, 3H, *J*

= 7.2 Hz); ^{13}C NMR (75 MHz, CDCl_3) δ 172.5, 172.0, 139.6, 135.3, 128.7 (2), 128.5 (2), 128.3 (2), 127.7 (2), 127.1 (2), 67.1, 49.2, 44.6, 36.2, 34.4, 16.3.

18b (81% yield): ^1H NMR (300 MHz, CDCl_3) δ 7.48–7.32 (m, 10H), 5.56 (q, 1H, J = 6.9 Hz), 5.22 (s, 2H), 3.65–3.57 (m, 1H), 3.26–3.15 (m, 2H), 2.89–2.70 (m, 2H), 1.58 (d, 3H, J = 7.2 Hz); ^{13}C NMR (75 MHz, CDCl_3) δ 172.7, 171.8, 139.8, 135.4, 128.7 (3), 128.4 (2), 127.7 (2), 127.2 (2), 67.2, 49.2, 44.4, 36.2, 34.6, 16.0.

4.2.17. Benzyl (*R*)-1-((*R*)-1-phenylethyl)-5-thioxopyrrolidine-3-carboxylate (**19a**)

—To a solution of lactam **18a** (2 g, 6.184 mmol) in dry toluene (39 mL) Lawesson's reagent (2.0 g, 4.945 mmol) was added and the reaction mixture was stirred for 3 h at 100 °C and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/*n*-hexane 1:6) providing **19a** (67%). ^1H NMR (400 MHz, CDCl_3) δ 7.39–7.22 (m, 8H), 7.07–6.99 (m, 2H), 6.34 (q, 1H, J = 7.0 Hz), 5.05 (m, 2H), 3.88–3.84 (m, 1H), 3.79–3.74 (m, 1H), 3.55–3.51 (m, 1H), 3.39–3.34 (m, 1H), 3.29–2.20 (m, 1H), 1.59 (d, 3H, J = 7.1 Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 198.1, 171.7, 138.0, 135.0, 128.8 (2), 128.4 (2), 128.3, 128.0, 127.2, 126.9, 67.1, 54.1, 53.9, 51.2, 47.5, 37.6, 37.4, 15.2.

4.2.18. Benzyl (*S*)-1-((*R*)-1-phenylethyl)-5-thioxopyrrolidine-3-carboxylate (**19b**)

—Compound **19b** was prepared following the procedure described for **19a** (99%). ^1H NMR (400 MHz, CDCl_3) δ 7.41–7.29 (m, 10H), 6.34 (q, 1H, J = 7.1 Hz), 5.14 (s, 2H), 3.90–3.85 (m, 1H), 3.43–3.32 (m, 3H), 3.19–3.10 (m, 1H), 1.57 (d, 3H, J = 7.0 Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 197.7, 171.9, 138.3, 135.1, 128.7 (2), 128.6 (2), 128.5 (2), 128.3 (2), 128.1, 127.1, 67.2, 54.0, 50.9, 47.6, 37.6, 14.7.

4.2.19. Benzyl (*R*)-1-((*R*)-1-phenylethyl)pyrrolidine-3-carboxylate (20a**)**—To a solution of thioamide **19a** (1.44 g, 4.242 mmol), in dry CH_2Cl_2 (21 mL) MeI (2.6 mL, 42.40 mmol) was added and the solution was stirred for 24 h at 25 °C. The reaction mixture was then concentrated and the residue dissolved in EtOH (7 mL). NaBH_4 (1.4 g, 37.00 mmol) was added portionwise at 0 °C and the reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched with water and extracted with CH_2Cl_2 . The organic layers were dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/pentane 1:6) providing **20a** (38%). ^1H NMR (300 MHz, CDCl_3) δ 7.58–7.32 (m, 10H), 5.27–5.18 (m, 2H), 3.38–3.31 (m, 1H), 3.20–3.09 (m, 1H), 2.97–2.86 (m, 2H), 2.77–2.72 (m, 1H), 2.29–2.17 (m, 2H), 1.50 (d, 3H, J = 7.2 Hz); ^{13}C NMR (75 MHz, CDCl_3) δ 174.9, 145.5, 136.2, 128.7 (2), 128.5 (2), 128.3, 128.2 (2), 127.2 (2), 127.1, 66.4, 65.4, 55.9, 52.4, 42.2, 27.7, 23.3.

4.2.20. Benzyl (*S*)-1-((*R*)-1-phenylethyl)pyrrolidine-3-carboxylate (**20b**)

—Compound **20b** was prepared following the procedure described for **20a** (50%). ^1H NMR (300 MHz, CDCl_3) δ 7.51–7.31 (m, 10H), 5.25 (s, 1H), 3.35 (q, 1H, J = 6.3 Hz), 3.25–3.15 (m, 1H), 3.11–3.05 (m, 1H), 2.81–2.76 (m, 1H), 2.72–2.54 (m, 2H), 2.27–2.12 (m, 2H), 1.50 (d, 3H, 6.3 Hz); ^{13}C NMR (75 MHz, CDCl_3) δ 175.0, 145.4, 136.2, 128.7 (2), 128.5 (2), 128.3, 128.2 (2), 127.2 (2), 127.1, 66.5, 65.5, 55.6, 52.7, 42.2, 27.7, 23.3.

4.2.21. Benzyl (*R*)-3-carbamoylpyrrolidine-1-carboxylate (21a)—To a solution of **20a** (500 mg, 1.616 mmol) in MeOH (13 mL) 10% Pd/C (179 mg) was added and the mixture was allowed to stir for 20 h at 25 °C under H₂ atmosphere. The reaction was filtered on Celite® and the filtrate was concentrated under reduced pressure quantitatively providing (*R*)-pyrrolidine-3-carboxylic acid submitted to the next step without further purification. To a solution of this latter (460 mg, 3.995 mmol) in 2N NaOH (2 mL) benzylchloroformate (740 µL, 5.194 mmol) and 4N NaOH (1.5 mL) were added and the mixture was stirred at 0 °C for 2 h. The reaction was extracted with diethyl ether. The aqueous layer was adjusted to pH = 2 with 10% HCl at 0 °C and extracted with EtOAc. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH₂Cl₂/MeOH 9:1) affording (*R*)-1-((benzyloxy)carbonyl)pyrrolidine-3-carboxylic acid (39%). ¹H NMR (300 MHz, CDCl₃) δ 9.17 (br, 1H), 7.44–7.32 (m, 5H), 5.18 (s, 2H), 4.73 (s, 1H), 3.73–3.47 (m, 3H), 3.19–3.09 (m, 1H), 2.24–2.13 (m, 2H); ¹³C NMR (75 MHz, CDCl₃) δ 177.0, 155.0, 136.6, 129.6, 128.6, 128.1, 127.6, 127.1, 67.2, 48.3/47.9, 45.7/45.3, 43.1/42.3, 28.8/28.2.

To a solution of this latter (307 mg, 1.231 mmol) in 1,4-dioxane (4 mL) pyridine (62 µL, 0.763 mmol), di-*tert*-butyldicarbonate (370 mg, 1.695 mmol) and NH₄HCO₃ (127 mg, 1.606 mmol) were sequentially added. The reaction mixture was stirred at 25 °C for 16 h and then concentrated under reduced pressure. The residue was diluted with EtOAc. The organic layer was washed with water, 5% H₂SO₄ and brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue containing **21a** was used for the next step without further purification. ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.43 (m, 1H), 7.34 (m, 4H), 7.30 (br, 1H), 6.94 (br, 1H), 5.04 (s, 1 H), 3.51–3.21 (m, 4 H), 2.93–2.87 (m, 1 H), 2.00–1.88 (m, 2 H).

4.2.22. Benzyl (*S*)-3-carbamoylpyrrolidine-1-carboxylate (21b)—Compound **21b** was prepared following the procedure described for **21a**. ¹H NMR data are identical to those reported for compound **21a**.

4.2.23. (*R*)-4-methyl-2-(pyrrolidin-3-yl)thiazole (11g)—To a solution of amide **21a** (254 mg, 1.023 mmol) in THF (5 mL) Lawesson's reagent (228 mg, 0.564 mmol) was added portionwise at 0 °C and the reaction mixture was allowed to stir at 25 °C for 16 h. The reaction was concentrated under reduced pressure, the residue was diluted with saturated aqueous NaHCO₃ and extracted with diethyl ether. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue containing (*R*)-benzyl 3-carbamothioylpyrrolidine-1-carboxylate was used for the next step without further purification. Accordingly, this latter (109 mg, 0.412 mmol) was dissolved in EtOH (2 mL), chloroacetone (49 µL, 0.618 mmol) was added at 25 °C and the resulting solution was allowed to stir at 80 °C for 18 h. The reaction mixture was cooled to 25 °C and saturated aqueous NaHCO₃ was added. EtOH was evaporated under reduced pressure and the aqueous layer was extracted with EtOAc. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/ *n*-hexane 1:3) affording benzyl (*R*)-3-(4-methylthiazol-2-yl)pyrrolidine-1-carboxylate. ¹H NMR (400 MHz, CDCl₃) δ 7.35–7.26 (m, 5H), 6.74 (s,

1H), 5.16–5.09 (m, 2H), 3.95–3.89 (m, 1H), 3.76–3.57 (m, 3H), 3.51–3.44 (m, 1H), 2.39 (s, 3H), 2.37–2.33 (m, 1H), 2.26–2.15 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 169.6, 154.5, 152.5, 136.7, 136.6, 128.4, 127.9, 127.8 (2), 112.6, 66.8, 51.9/51.4, 45.7/45.3, 42.5/41.7, 32.8/32.0, 17.0. This latter was submitted to Cbz deprotection as described for amine **11f** providing compound **11g**. ¹H NMR (400 MHz, CD₃OD) δ 6.96 (s, 1H), 3.82–3.73 (m, 1H), 3.49–3.44 (m, 1H), 3.32–3.26 (m, 1H), 3.19–3.11 (m, 2H), 2.53–2.44 (m, 4H), 2.19–2.06 (m, 1H); ¹³C NMR (100 MHz, CD₃OD) δ 173.3, 152.2, 112.7, 53.6, 46.5, 43.2, 33.9, 16.4. LRMS-ESI (*m/z*): 169.0 [M+H].

4.2.24. (S)-4-methyl-2-(pyrrolidin-3-yl)thiazole (11h)—¹H NMR and ¹³C NMR data are identical to those reported for compound **11g**.

4.2.25. (R)-3-(3-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl) benzoic acid (22a)—Compound **21a** was prepared from **11g** and **7a** following the procedure described for **13a**. ¹H NMR (400 MHz, CDCl₃) δ 12.0 (br, 1H), 8.20–8.03 (m, 2H), 7.72 (s, 1H), 7.46–7.42 (m, 1H), 6.90–6.83 (m, 1H), 4.20–3.63 (m, 5H), 2.46–2.22 (m, 5H); ¹³C NMR (100 MHz, CDCl₃) δ 169.4, 169.2, 168.9, 152.4, 136.5, 132.1, 131.9, 131.6, 130.1, 128.7, 113.0, 54.6, 45.7, 42.2, 31.3, 16.2. LRMS-ESI (*m/z*): 339.0 [M+Na].

4.2.26. (S)-3-(3-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl) benzoic acid (22b)—Compound **22b** was prepared from **11h** and **7a** following the procedure described for **13a**. ¹H NMR and ¹³C NMR data are identical to those reported for compound **22a**.

4.2.27. Benzyl (R)-2-(4-(hydroxymethyl)oxazol-2-yl) pyrrolidine-1-carboxylate (24)—To a solution of ester **23** (1.4 g, 4.238 mmol) in dry THF (10 mL) LiBH₄ (277 mg, 12.72 mmol) was added portionwise at 0 °C and the reaction mixture was allowed to stir at 25 °C for 4 h. EtOAc (5 mL) was added and the resulting solution was cooled to 0 °C. 1N HCl (10 mL) and water (20 mL) were then added and the reaction mixture was extracted with EtOAc. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH₂Cl₂/MeOH 97:3) providing compound **24** (42%). ¹H NMR (300 MHz, CDCl₃) δ 7.56–7.21 (m, 5H), 5.25–5.05 (m, 3H), 4.85–4.80 (m, 1H), 4.61–4.57 (m, 1H), 3.79–3.72 (m, 1H), 3.67–3.55 (m, 1H), 2.32–1.85 (m, 5H); ¹³C NMR (75 MHz, CDCl₃) δ 164.6, 154.5, 140.5, 136.5, 128.5, 128.4, 128.0, 127.9, 127.7, 127.2, 66.9, 55.1, 54.7, 46.9, 32.4, 24.3.

4.2.28. (R)-4-(methoxymethyl)-2-(pyrrolidin-2-yl)oxazole (11f)—To a solution of alcohol **24** (138 mg, 0.456 mmol) in dry THF (4 mL) NaH (60% suspension in mineral oil, 33 mg, 0.825 mmol) and MeI (34 μL, 0.547 mmol) were sequentially added at 0 °C and the reaction mixture was allowed to stir at 25 °C for 1 h. The reaction was quenched with saturated aqueous NH₄Cl at 0 °C and extracted with EtOAc. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/*n*-hexane 3:2) affording *benzyl (R)-2-(4-(methoxymethyl)oxazol-2-yl)pyrrolidine-1-carboxylate* (68%). ¹H NMR (400 MHz, CDCl₃) δ 7.50 (s, 1H), 7.41 (s, 1H), 7.40–7.29 (m, 3H), 7.23–7.13 (m, 1H), 5.16–4.94 (m, 3H), 4.31 (d, 2H, *J* = 17.4 Hz), 3.70–3.65 (m, 1H), 3.58–3.43 (m, 1H), 3.37 (s, 3H), 2.27–2.00 (m,

3H), 1.93–1.88 (m, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 164.7, 154.7/154.3, 137.7, 136.6/136.4, 136.0/135.7, 128.3, 128.2, 127.8, 127.6, 127.5, 66.9/66.7, 66.3/66.2, 58.4, 54.9/54.6, 46.8/46.4, 32.3/31.2, 24.1/23.3.

This latter (98 mg, 0.310 mmol) was dissolved in a solution of HBr in acetic acid (1.0 mL) and the resulting mixture was stirred at 25 °C for 1 h. The addition of ether (2 mL) caused the amine salt to precipitate. The solvent was drawn off and the resulting residue retained. To the ethereal solution was added petroleum ether (4 mL) to give a further precipitate. The solvent was evaporated under reduced pressure and the two batches of solid were combined and dried to provide amine **11f** (67%). ^1H NMR (400 MHz, CDCl_3) δ 7.48 (s, 1H), 4.28 (m, 3H), 3.35 (s, 3H), 3.09–3.03 (m, 1H), 2.96–2.90 (m, 1H), 2.54 (br, 1H), 2.16–2.07 (m, 1H), 2.02–1.94 (m, 1H), 1.89–1.71 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 166.7, 137.2, 136.0, 66.1, 58.3, 55.4, 46.7, 30.7, 25.3.

4.2.29. (*R*)-3-(2-(4-(methoxymethyl)oxazol-2-yl)pyrrolidine-1-carbonyl)benzoic acid (25**)**—Compound **25** was prepared from **11f** and **7a** following the procedure described for **13a**. ^1H NMR (400 MHz, CDCl_3) δ 9.39 (br, 1H), 8.13–8.08 (m, 1H), 8.00 (s, 1H), 7.79–7.75 (m, 1H), 7.57 (s, 1H), 7.49–7.45 (m, 1H), 5.40 (t, 1H, $J = 6.3$ Hz), 4.44 (s, 2H), 3.93–3.78 (m, 2H), 3.56–3.51 (m, 1H), 3.37 (s, 3H), 2.44–2.36 (m, 1H), 2.28–2.12 (m, 2H), 1.98–1.90 (m, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 169.2, 168.6, 164.8, 137.5, 136.5, 136.1, 132.0, 131.7, 131.0, 130.3, 128.7, 66.0, 58.4, 54.8, 50.1, 31.0, 25.2.

4.2.30. *N*¹-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl) sulfonamido)-1-phenylbutan-2-yl)-*N*³-methyl-*N*³-((2-methyloxazol-4-yl)methyl)isophthalamide (3c**)**— ^1H NMR (400 MHz, CDCl_3) δ 8.08 (m, 1H), 8.17–8.04 (m, 1H), 8.01–7.91 (m, 1H), 7.77 (d, 1H, $J = 7.8$ Hz), 7.73–7.61 (m, 3H), 7.43 (t, $J = 7.7$ Hz, 2H), 7.47–7.04 (m, 4H), 7.34–7.02 (m, 2H), 7.10–6.75 (m, 2H), 4.56 (s, 1H), 4.46 (d, $J = 5.4$ Hz, 1H), 4.42–4.21 (m, 2H), 4.07–3.94 (m, 1H), 3.89–3.78 (m, 3H), 3.21–2.70 (m, 5H), 2.49–2.35 (m, 2H), 2.10 (br, 1H), 1.92–1.78 (m, 2H), 1.66–1.50 (m, 1H), 0.84 (dd, $J = 6.4, 1.9$ Hz, 6H). LRMS-ESI (m/z): 685.5 [M+Na]. HRMS-ESI (m/z) [M+Na]⁺ calculated for $\text{C}_{35}\text{H}_{42}\text{N}_4\text{O}_7\text{SNa}$ 685.2672, found 685.2650.

4.2.31. *N*¹-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-*N*³-methyl-*N*³-((2-methylthiazol-4-yl)methyl)isophthalamide (3d**)**— ^1H NMR (300 MHz, CDCl_3) δ 8.07 (s, 1H), 7.99 (s, 1H), 7.96 (s, 1H), 7.77–7.59 (m, 4H), 7.49–7.40 (m, 1H), 7.27–7.16 (m, 3H), 6.97–6.82 (m, 3H), 6.54 (d, 1H, $J = 7.8$ Hz), 4.96 (s, 1H), 4.88 (d, 1H, $J = 5.4$ Hz), 4.38–4.18 (m, 2H), 4.03–3.97 (m, 1H), 3.86 (s, 3H), 3.17–2.98 (m, 4H), 2.87 (d, 2H, $J = 6.6$ Hz), 2.47–2.39 (m, 2H), 1.91–1.83 (m, 1H), 0.86 (dd, $J = 6.4, 1.9$ Hz, 6H). LRMS-ESI (m/z): 701.4 [M+Na]. HRMS-ESI (m/z) [M+Na]⁺ calculated for $\text{C}_{35}\text{H}_{42}\text{N}_4\text{O}_6\text{S}_2\text{Na}$ 701.2444, found 701.2438.

4.2.32. *N*¹-((2,5-dimethyloxazol-4-yl)methyl)-*N*³-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-*N*¹-methyl-5-(methylamino)isophthalamide (3e**)**— ^1H NMR (400 MHz, CDCl_3) δ 7.81–7.60 (m, 3H), 7.50 (s, 1H), 7.38–7.03 (m, 5H), 6.96 (d, 2H, $J = 8.7$ Hz), 6.85–6.61 (m, 1H), 6.38 (br, 1H), 4.93 (s, 1H), 4.49 (s, 1H), 4.42–4.15 (m, 2H), 4.16–3.90 (m, 3H), 3.86 (s, 3H), 3.41–

2.66 (m, 8H), 2.44–2.24 (m, 6H), 2.16 (s, 3H), 1.99–1.80 (m, 1H), 0.86 (dd, 6H, $J = 12.9$ and 6.8 Hz). LRMS-ESI (m/z): 728.5 [M+Na]. HRMS-ESI (m/z) [M+Na]⁺ calculated for C₃₇H₄₇N₅O₇SNa 728.3096, found 728.3088.

4.2.33. *N*¹-((2,5-dimethyloxazol-4-yl)methyl)-5-hydroxy-*N*³-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-*N*¹-methylisophthalamide (3f)—¹H NMR (400 MHz, CDCl₃) δ 8.01 (s, 1H), 7.77–7.61 (m, 3H), 7.36–6.85 (m, 8H), 6.72 (br, 1H), 4.64–3.92 (m, 2H), 3.84 (s, 3H), 3.36–2.71 (m, 12H), 2.52–2.24 (m, 3H), 2.20–1.68 (m, 2H), 0.97–0.72 (m, 6H). LRMS-ESI (m/z): 715.5 [M+Na]. HRMS-ESI (m/z) [M+Na]⁺ calculated for C₃₆H₄₄N₄O₈SNa 715.2778, found 715.2773.

4.2.34. *N*¹-((2,5-dimethyloxazol-4-yl)methyl)-*N*²-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-*N*¹-methylphthalamide (3g)—¹H NMR (400 MHz, CDCl₃) δ 7.72 (d, 2H, $J = 8.8$ Hz), 7.61–7.23 (m, 7H), 7.23–7.12 (m, 2H), 7.03 (d, 1H, $J = 8.3$ Hz), 6.94 (d, 2H, $J = 8.7$ Hz), 4.55 (br, 1H), 4.49–4.28 (m, 2H), 4.22 (br, 1H), 4.06 (m, 2H), 3.88–3.79 (m, 3H), 3.24–3.01 (m, 3H), 3.01–2.82 (m, 4H), 2.72 (s, 3H), 2.38 (s, 3H), 2.12–1.80 (m, 2H), 0.91–0.79 (m, 6H). LRMS-ESI (m/z): 699.6 [M+Na]. HRMS-ESI (m/z) [M+H]⁺ calculated for C₃₆H₄₅N₄O₇S 677.3009, found 677.2994.

4.2.35. *N*¹-((2,5-dimethyloxazol-4-yl)methyl)-*N*⁴-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-*N*¹-methylterephthalamide (3h)—¹H NMR (400 MHz, CDCl₃) δ 7.65 (d, 2H, $J = 8.9$ Hz), 7.62–7.48 (m, 2H), 7.35 (d, 1H, $J = 7.8$ Hz), 7.31–7.21 (m, 4H), 7.21–7.12 (m, 1H), 6.91 (d, 2H, $J = 8.9$ Hz), 6.75–6.57 (m, 1H), 4.49 (s, 1H), 4.42–4.25 (m, 2H), 4.13 (s, 1H), 3.98 (dd, 1H, $J = 7.5$ and 3.6 Hz), 3.83 (s, 3H), 3.23–2.89 (m, 6H), 2.84 (d, 2H, $J = 7.5$ Hz), 2.35 (m, 5H), 2.02 (s, 1H), 1.90–1.80 (m, 1H), 0.83 (dd, 6H, $J = 6.5$ and 3.3 Hz). LRMS-ESI (m/z): 699.6 [M+Na]. HRMS-ESI (m/z) [M+Na]⁺ calculated for C₃₆H₄₄N₄O₇SNa 699.2829, found 699.2820.

4.2.36. *N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-3-((*S*)-2-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl)benzamide (4a)—To a solution of 3-[(*S*)-2-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl]benzoic acid **13b** (40 mg, 0.13 mmol), EDCI (37.4 mg, 0.20 mmol) HOBt (26.4 mg, 0.20 mmol) in anhydrous CH₂Cl₂ (3 mL) a solution of amine **6a** (52 mg, 0.13 mmol) and diisopropylethylamine (0.14 mL, 0.78 mmol) in anhydrous CH₂Cl₂ (1 mL) were added at 0 °C under argon atmosphere and it was allowed to stir for 16 h at 23 °C. The reaction mixture was quenched with water and extracted with CH₂Cl₂. The organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH₂Cl₂/MeOH 9:1) to furnish inhibitor **4a** (95%) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.81 (s, 1H), 7.64–7.59 (m, 3H), 7.36 (t, 1H, $J = 7.7$ Hz), 7.29–7.09 (m, 7H), 6.91 (d, 2H, $J = 8.7$ Hz), 6.72 (s, 1H), 5.59 (dd, 1H, $J = 7.4$ and 4.8 Hz), 4.38–4.32 (m, 2H), 4.02–3.97 (m, 1H), 3.81 (m, 4H), 3.67–3.62 (m, 1H), 3.43–3.37 (m, 1H), 3.24–3.19 (m, 1H), 3.15–2.98 (m, 3H), 2.92–2.80

(m, 2H), 2.38 (s, 3H), 2.35–2.27 (m, 2H), 2.08–1.98 (m, 1H), 1.90–1.81 (m, 2H), 0.83 (dd, 6H, $J = 6.5$ and 3.2 Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 171.7, 171.1, 169.2, 167.1, 162.8, 152.5, 138.0, 136.7, 134.3, 130.2, 129.8, 129.3 (2), 129.2, 128.6, 128.4 (2), 126.4, 125.8, 114.3 (2), 112.9, 72.6, 58.8, 58.5, 55.5, 54.7, 53.2, 50.1, 34.8, 32.4, 27.0, 24.9, 20.0, 19.9, 17.1. LRMS-ESI (m/z): 727.6 $[\text{M}+\text{Na}]$. HRMS-ESI (m/z) $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{37}\text{H}_{45}\text{N}_4\text{O}_6\text{S}_2$ 705.2781, found 705.2784.

4.2.37. *N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-3-((*R*)-2-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl)benzamide (4b)— ^1H NMR (400 MHz, CDCl_3) δ 7.79 (s, 1H), 7.63 (d, 2H, $J = 8.7$ Hz), 7.60 (d, 1H, $J = 7.7$ Hz), 7.35 (t, 1H, $J = 7.7$ Hz), 7.30–7.11 (m, 6H), 6.90 (d, 2H, $J = 8.7$ Hz), 6.73 (s, 1H), 5.60 (dd, 1H, $J = 7.3$ and 4.9 Hz), 4.38–4.31 (m, 1H), 4.01–3.96 (m, 1H), 3.80 (s, 3H), 3.63–3.58 (m, 1H), 3.41–3.35 (m, 1H), 3.20 (dd, 1H, $J = 15.0$ and 3.0 Hz), 3.12–2.96 (m, 3H), 2.91–2.79 (m, 2H), 2.38 (s, 3H), 2.36–2.25 (m, 2H), 2.07–1.99 (m, 1H), 1.91–1.81 (m, 2H), 0.83 (dd, 6H, $J = 6.4$ and 2.8 Hz); ^{13}C NMR (100 MHz, CDCl_3) δ 171.7, 169.3, 167.1, 162.8, 152.6, 137.9, 136.6, 134.4, 130.1, 129.7, 129.3, 128.6, 128.4, 126.3, 125.7, 114.2, 112.8, 72.7, 58.7, 58.4, 55.5, 54.5, 53.3, 50.1, 35.0, 32.3, 27.0, 24.9, 20.0, 19.9, 17.0. LRMS-ESI (m/z): 705.7 $[\text{M}+\text{H}]$. HRMS-ESI (m/z) $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{37}\text{H}_{44}\text{N}_4\text{O}_6\text{S}_2\text{Na}$ 727.2600, found 727.2592.

4.2.38. *N*-((2*S*,3*R*)-4-((4-amino-*N*-isobutylphenyl) sulfonamido)-3-hydroxy-1-phenylbutan-2-yl)-3-((*R*)-2-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl)benzamide (4c)— ^1H NMR (400 MHz, CDCl_3) δ 7.79 (s, 1H), 7.62–7.53 (m, 2H), 7.39–7.32 (m, 2H), 7.27–7.12 (m, 6H), 6.88 (d, 1H, $J = 8.6$ Hz), 6.74 (s, 1H), 6.49 (d, 2H, $J = 8.6$ Hz), 5.61 (dd, 1H, $J = 7.5$ and 4.8 Hz), 4.35–4.30 (m, 3H), 4.03–3.97 (m, 1H), 3.63–3.57 (m, 1H), 3.42–3.36 (m, 1H), 3.17–3.08 (m, 2H), 3.00–2.84 (m, 3H), 2.76–2.71 (m, 1H), 2.40–2.24 (m, 5H), 2.06–1.99 (m, 1H), 1.92–1.82 (m, 1H), 0.85 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 171.7, 169.4, 167.0, 152.6, 151.1, 137.9, 136.7, 134.5, 130.0, 129.3 (3), 128.7 (3), 128.4 (2), 126.4, 125.9, 125.0, 113.9 (2), 112.9, 72.8, 58.8, 54.3, 53.6, 50.1, 35.4, 32.4, 29.6, 27.0, 24.9, 20.0 (2), 17.1. LRMS-ESI (m/z): 690.7 $[\text{M}+\text{H}]$. HRMS-ESI (m/z) $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{36}\text{H}_{43}\text{N}_5\text{O}_5\text{S}_2\text{Na}$ 712.2604, found 712.2601.

4.2.39. *N*-((2*S*,3*R*)-3-hydroxy-4-((4-(hydroxymethyl)-*N*-isobutylphenyl)sulfonamido)-1-phenylbutan-2-yl)-3-((*R*)-2-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl)benzamide (4d)— ^1H NMR (400 MHz, CDCl_3) δ 7.77 (s, 1H), 7.72–7.56 (m, 3H), 7.37–7.14 (m, 9H), 6.95 (d, 1H, $J = 8.6$ Hz), 6.75 (s, 1H), 5.57 (dd, 1H, $J = 7.5$ and 4.9 Hz), 4.68–4.58 (m, 2H), 4.31–4.23 (m, 1H), 4.02–3.95 (m, 1H), 3.64–3.57 (m, 1H), 3.42–3.36 (m, 1H), 3.21–2.72 (7H), 2.38–2.24 (m, 5H), 2.06–1.99 (m, 1H), 1.92–1.86 (m, 2H), 0.86 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 171.7, 169.6, 167.1, 152.6, 146.9, 137.9, 136.5, 136.4, 134.4, 129.9, 129.3 (2), 128.7 (2), 128.5 (2), 127.3 (2), 126.9 (2), 126.4, 125.9, 112.9, 72.8, 63.6, 58.8, 58.5, 54.4, 53.3, 50.2, 35.3, 32.5, 26.9, 24.9, 20.0 (2), 17.0. LRMS-ESI (m/z): 705.6 $[\text{M}+\text{H}]$. HRMS-ESI (m/z) $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{37}\text{H}_{44}\text{N}_4\text{O}_6\text{S}_2\text{Na}$ 727.2600, found 727.2595.

4.2.40. *N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-3-((*R*)-2-(4-methylthiazol-2-yl)piperidine-1-carbonyl)benzamide (4e)—¹H NMR (400 MHz, CDCl₃) δ 7.79 (s, 1H), 7.63 (d, 2H, *J* = 8.7 Hz), 7.60 (d, 1H, *J* = 7.7 Hz), 7.35 (t, 1H, *J* = 7.7 Hz), 7.30–7.11 (m, 6H), 6.90 (d, 2H, *J* = 8.7 Hz), 6.73 (s, 1H), 5.60 (dd, 1H, *J* = 7.3 and 4.9 Hz), 4.38–4.31 (m, 1H), 4.01–3.96 (m, 1H), 3.80 (s, 3H), 3.63–3.58 (m, 1H), 3.41–3.35 (m, 1H), 3.20 (dd, 1H, *J* = 3.0 and 15.0 Hz), 3.12–2.96 (m, 3H), 2.91–2.79 (m, 2H), 2.38 (s, 3H), 2.36–2.25 (m, 3H), 2.07–1.99 (m, 1H), 1.91–1.81 (m, 4H), 0.83 (dd, 6H, *J* = 6.3 and 2.8 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 171.7, 169.3, 167.1, 162.8, 152.6, 137.9, 136.6, 134.4, 130.1, 129.7, 129.3 (2), 128.6 (2), 128.4 (2), 126.3, 125.7, 114.2 (2), 112.8, 72.7, 58.7, 58.4, 55.5, 54.5, 53.3, 50.1, 35.0, 32.3, 27.0, 25.7, 24.9, 20.0, 19.9, 17.0. LRMS-ESI (*m/z*): 757.6 [M+K]. HRMS-ESI (*m/z*) [M+Na]⁺ calculated for C₃₈H₄₆N₄O₆S₂Na 741.2757, found 741.2749.

4.2.41. *N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-3-((*R*)-4-(4-methylthiazol-2-yl)thiazolidine-3-carbonyl)benzamide (4f)—¹H NMR (400 MHz, CDCl₃) δ 7.80 (s, 1H), 7.75–7.58 (m, 3H), 7.41 (br, 1H), 7.26–7.21 (m, 4H), 7.19–7.15 (m, 1H), 6.94 (d, 2H, *J* = 8.6 Hz), 6.82–6.76 (m, 2H), 6.13 (br, 1H), 4.63–4.58 (m, 1H), 4.38–4.24 (m, 2H), 4.05–3.98 (m, 1H), 3.83 (s, 3H), 3.49 (m, 2H), 3.19–3.03 (m, 5H), 2.86 (d, 2H, *J* = 7.4 Hz), 2.40 (s, 3H), 1.90–1.82 (m, 1H), 0.85 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 168.8, 166.8, 162.9, 153.0, 137.8, 135.8, 134.5, 129.7, 129.3 (3), 129.2 (3), 128.9, 128.5 (2), 126.5, 125.8, 114.3 (2), 113.0, 72.7, 60.3, 58.6, 55.6, 54.5, 53.4, 34.9, 27.1, 21.0, 20.0, 19.9, 17.0. LRMS-ESI (*m/z*): 745.5 [M+Na]. HRMS-ESI (*m/z*) [M+Na]⁺ calculated for C₃₆H₄₂N₄O₆S₃Na 745.2165, found 745.2161.

4.2.42. *N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-3-((*R*)-2-(4-(methoxymethyl)oxazol-2-yl)pyrrolidine-1-carbonyl)benzamide (4g)—¹H NMR (400 MHz, CDCl₃) δ 7.82 (s, 1H), 7.74–7.69 (m, 3H), 7.52 (s, 1H), 7.39–7.30 (m, 1H), 7.26–7.13 (m, 6H), 6.96–6.85 (m, 3H), 5.37 (dd, 1H, *J* = 7.3 and 4.9 Hz), 4.36–4.30 (m, 3H), 4.11 (m, 1H), 4.02–3.97 (m, 1H), 3.82 (s, 3H), 3.68–2.62 (m, 1H), 3.36–3.40 (m, 1H), 3.38 (s, 3H), 3.19–2.97 (m, 4H), 2.92–2.80 (m, 2H), 2.38–2.28 (m, 1H), 2.22–2.04 (m, 2H), 1.95–1.83 (m, 2H), 0.84 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 168.9, 167.0, 164.3, 162.9, 137.9, 137.7, 136.5, 136.0, 134.4, 134.2, 130.3, 129.7, 129.3 (3), 128.7, 128.6, 128.4 (2), 126.4, 125.8, 114.2 (2), 72.7, 66.2, 58.6, 58.4, 55.5, 54.6, 54.4, 53.4, 49.9, 35.0, 30.8, 27.0, 25.1, 20.0, 19.9. LRMS-ESI (*m/z*): 741.7 [M+Na]. HRMS-ESI (*m/z*) [M+Na]⁺ calculated for C₃₈H₄₆N₄O₈SNa 741.2935, found 741.2932.

4.2.43. *N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-3-((*R*)-2-(4-methyloxazol-2-yl)pyrrolidine-1-carbonyl)benzamide (4h)—¹H NMR (400 MHz, CDCl₃) δ 7.85 (s, 1H), 7.75–7.64 (m, 3H), 7.40–7.18 (m, 8H), 6.97–6.90 (m, 2H), 6.82 (d, 1H, *J* = 7.0 Hz), 5.36–5.33 (m, 1H), 4.39–4.32 (m, 1H), 4.02–3.97 (m, 1H), 3.84–3.82 (m, 3H), 3.68–3.61 (m, 1H), 3.49–3.44 (m, 1H), 3.24–2.95 (m, 4H), 2.86 (d, 2H, *J* = 7.1 Hz), 2.36–2.31 (m, 1H), 2.18–2.09 (m, 4H), 1.97 (br, 1H), 1.93–1.82 (m, 2H), 0.85 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 168.9, 167.0, 163.7, 162.9, 137.9, 136.6, 136.3, 134.1, 130.3, 129.7 (2), 129.3 (3), 128.7 (2), 128.5

(3), 126.4, 125.8, 114.2 (2), 72.7, 58.6, 55.5, 54.6, 54.4, 53.4, 49.9, 35.0, 30.9, 27.1, 25.1, 20.0, 19.9, 14.1. LRMS-ESI (m/z): 711.6 [M+Na]. HRMS-ESI (m/z) [M+Na]⁺ calculated for C₃₇H₄₄N₄O₇SNa 711.2829, found 711.2833.

4.2.44. *N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-3-(((*R*)-2-(4-methylthiazol-2-yl)pyrrolidin-1-yl)methyl)benzamide (4i)—¹H NMR (400 MHz, CDCl₃) δ 7.66 (d, 2H, J = 8.8 Hz), 7.60 (s, 1H), 7.51–7.45 (m, 2H), 7.34–7.25 (m, 5H), 7.21–7.17 (m, 1H), 6.92 (d, 2H, J = 8.8 Hz), 6.78 (s, 1H), 6.55 (d, 1H, J = 8.2 Hz), 4.41–4.34 (m, 1H), 4.04–3.96 (m, 3H), 3.84 (s, 3H), 3.36 (d, 1H, J = 13.0 Hz), 3.23–2.98 (m, 5H), 2.89–2.83 (m, 2H), 2.40 (s, 3H), 2.40–2.24 (m, 3H), 1.92–1.76 (m, 4H), 0.86 (dd, 6H, J = 6.5 and 1.8 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 176.9, 168.1, 162.9, 152.4, 139.8, 137.8, 133.9, 131.9, 129.7, 129.4 (4), 128.5 (3), 127.0, 126.5, 125.5, 114.2 (2), 113.3, 72.8, 66.0, 58.7, 58.4, 55.5, 54.5, 53.4, 34.9, 34.2, 29.6, 27.1, 23.1, 20.0, 19.9, 17.1. LRMS-ESI (m/z): 691.7 [M+H]. HRMS-ESI (m/z) [M+H]⁺ calculated for C₃₇H₄₇N₄O₅S₂ 691.2988, found 691.2983.

4.2.45. *N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-5-((*R*)-2-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl)nicotinamide (4j)—¹H NMR (400 MHz, CDCl₃) δ 8.82 (s, 1H), 8.78 (s, 1H), 8.09 (s, 1H), 7.65 (d, 2H, J = 8.7 Hz), 7.23–7.09 (m, 6H), 6.92 (d, 2H, J = 8.7 Hz), 6.76 (s, 1H), 5.64–5.58 (m, 1H), 4.39–4.33 (m, 1H), 4.10–4.06 (1H), 3.82 (s, 3H), 3.71–3.62 (m, 1H), 3.45–3.38 (m, 1H), 3.24–3.05 (m, 3H), 2.96–2.81 (m, 3H), 2.43–2.28 (m, 4H), 2.19–2.01 (m, 2H), 1.97–1.85 (m, 2H), 0.89–0.78 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 171.1, 166.9, 165.2, 162.9, 152.7, 150.1, 149.3, 138.0, 133.9, 131.9, 129.9, 129.7, 129.3 (2), 129.2, 128.6, 128.4 (2), 126.4, 114.3 (2), 113.0, 72.7, 59.0, 58.5, 55.6, 54.6, 53.3, 50.1, 32.4, 29.6, 27.0, 24.9, 20.0, 19.9, 17.1. LRMS-ESI (m/z): 728.6 [M+Na]. HRMS-ESI (m/z) [M+H]⁺ calculated for C₃₆H₄₄N₅O₆S₂ 706.2733, found 706.2723.

4.2.46. *N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-3-((*R*)-3-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl)benzamide (4k)—¹H NMR (400 MHz, CDCl₃) δ 7.76 (s, 1H), 7.67–7.58 (m, 3H), 7.43–7.38 (m, 1H), 7.26–7.14 (m, 5H), 6.8–6.88 (m, 3H), 6.79–6.70 (m, 2H), 4.40–4.33 (m, 1H), 4.13–4.09 (m, 1H), 4.03–3.97 (m, 1H), 3.85–3.64 (m, 6H), 3.58–3.48 (m, 2H), 3.19–3.03 (m, 4H), 2.87–2.79 (m, 2H), 2.42–2.23 (m, 3H), 1.92–1.84 (m, 2H), 0.93–0.85 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 168.7, 168.5, 167.1, 162.9, 152.7, 137.8, 136.9, 134.3, 130.1, 129.7, 129.3 (2), 129.2 (2), 128.7 (2), 128.5 (2), 126.5, 125.6, 114.3 (2), 112.9, 72.7, 58.7, 55.6, 54.6, 53.4, 45.7, 42.8, 41.2, 34.9, 29.6, 27.1, 20.0, 19.9, 16.9. LRMS-ESI (m/z): 743.6 [M+K]. HRMS-ESI (m/z) [M+Na]⁺ calculated for C₃₇H₄₄N₄O₆S₂Na 727.2600, found 727.2604.

4.2.47. *N*-((2*S*,3*R*)-3-hydroxy-4-((*N*-isobutyl-4-methoxyphenyl)sulfonamido)-1-phenylbutan-2-yl)-3-((*S*)-3-(4-methylthiazol-2-yl)pyrrolidine-1-carbonyl)benzamide (4l)—¹H NMR (400 MHz, CDCl₃) δ 7.77 (s, 1H), 7.67–7.59 (m, 4H), 7.42–7.37 (m, 1H), 7.27–7.14 (m, 5H), 6.93 (d, 2H, J = 8.8 Hz), 6.80–6.75 (m, 2H), 4.38–4.31 (m, 2H), 4.03–3.98 (m, 1H), 3.90–3.77 (m, 5H), 3.75–3.66 (m, 2H), 3.58–3.49

(m, 1H), 3.19–3.04 (m, 4H), 2.86 (d, 2H, $J = 7.4$ Hz), 2.47–2.20 (m, 4H), 1.90–1.84 (m, 2H), 0.91–0.84 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 168.7, 168.6, 167.1, 162.9, 152.6, 137.8, 136.9, 134.3, 130.1, 129.7, 129.3 (2), 129.2 (2), 128.7 (2), 128.5 (2), 126.5, 125.6, 114.2 (2), 112.8, 72.7, 58.7, 55.5, 54.6, 53.4, 45.7, 42.8, 41.2, 34.9, 29.6, 27.1, 20.0, 19.9, 16.9.

LRMS-ESI (m/z): 727.6 $[\text{M}+\text{Na}]$. HRMS-ESI (m/z) $[\text{M}+\text{Na}]^+$ calculated for

$\text{C}_{37}\text{H}_{44}\text{N}_4\text{O}_6\text{S}_2\text{Na}$ 727.2600, found 727.2596.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the National Institutes of Health (Grant GM53386, AKG and Grant GM62920, ITW). X-ray data were collected at the Southeast Regional Collaborative Access Team (SER-CAT) beamline 22BM at the Advanced Photon Source, Argonne National Laboratory. Use of the Advanced Photon Source was supported by the US Department of Energy, Basic Energy Sciences, Office of Science, under Contract No. W-31-109-Eng-38. This work was also supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health, and in part by a Grant-in-Aid for Scientific Research (Priority Areas) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Monbu Kagakusho), a Grant for Promotion of AIDS Research from the Ministry of Health, Welfare, and Labor of Japan, and the Grant to the Cooperative Research Project on Clinical and Epidemiological Studies of Emerging and Reemerging Infectious Diseases (Renkei Jigyo) of Monbu-Kagakusho. We thank Mr. Emilio Cardenas (Purdue University) for helpful discussions. The authors would like to thank the Purdue University Center for Cancer Research, which supports the shared NMR and mass spectrometry facilities.

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 38. The coordinates and structure factors for the structure of HIV-1 protease with inhibitors **3b** and **4b** have been deposited in Protein Data Bank with codes: 5UPZ and 5UOV.

39. For details of X-ray studies, please see Supplementary information.

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Figure 1.
Structures of inhibitors **1,2, 3a** and **4b**.

Figure 2.

Stereoview of the overlay X-ray structures of inhibitor **3a** (green) and darunavir (**1**, torquois)-bound HIV-1 protease. All strong active site hydrogen bonding interactions of darunavir with HIV-1 protease are shown as dotted lines.

Figure 3. Stereoview of the X-ray structure of inhibitor **3b** (torquoise)-bound HIV-1 protease (PDB code: 5UPZ). All strong active site hydrogen bonding interactions of inhibitor **3b** with HIV-1 protease are shown as dotted lines.

Figure 4.

Stereoview of the X-ray structure of inhibitor **4b** (green)-bound HIV-1 protease (PDB code: 5UOV). All strong active site hydrogen bonding interactions of inhibitor **4b** with HIV-1 protease are shown as dotted lines.

Scheme 1. General synthetic approach for inhibitors **3a-h** and **4a-l**.

Scheme 2.

Reagents and conditions: (a) **7c** or **7d**, EDCl, HOBt, *i*-Pr₂NEt, CH₂Cl₂, 25 °C, 16 h, 80%; (b) MeI, NaH, dry THF, 25 °C, 1 h, 68%; (c) LiOH·H₂O, THF/MeOH/H₂O (3:1:1), 25 °C, 16 h, 99%.

Scheme 3.

Reagents and conditions: (a) **7a** or **7b**, EDCI, HOBT, *i*-Pr₂NEt, CH₂Cl₂, 25 °C, 16 h, 80%; (b) LiOH·H₂O, THF/MeOH/H₂O (3:1:1), 25 °C, 16 h, 99%; (c) from **13a**, LiAlH₄, dry THF, reflux, 15 h, 88%; (d) MnO₂, CH₂Cl₂, 25 °C, 5 h; (e) NaClO₂, NaH₂PO₄, *t*-BuOH/H₂O, 2-methyl-2-butene, 25 °C, 4 h, 45% (over 2 steps).

Scheme 4.

Reagents and conditions: (a) (*R*)- α -methylbenzylamine MeOH, 115 °C, 18 h, 75%; (b) BnOH, PPTS, toluene, reflux, 24 h, (*S*) 81%, (*R*) 73%; (c) Lawesson's reagent, toluene, 100 °C, 2 h, (*S*) 99%, (*R*) 67%; (d) MeI, CH₂Cl₂, 25 °C, 24 h then NaBH₄, EtOH, 0 °C, 1 h, (*S*) 50%, (*R*) 38%; (e) H₂, 10% Pd/C, 25 °C, 20 h, (*S*) 99%, (*R*) 99%; (f) CbzCl, 4 N NaOH, 0 °C, 2 h, (*S*) 51%, (*R*) 39%; (g) Boc₂O, pyridine, NH₄HCO₃, 1,4-dioxane, 25 °C, 16 h; (h) Lawesson's reagent, THF, 25 °C, 16 h; (i) chloroacetone, EtOH, 80 °C, 18 h, (*S*) 42%, (*R*) 56% (over 3 steps); (j) HBr in AcOH, 25 °C, 1 h, (*S*) 89%, (*R*) 88%; (k) **7a**, EDCI, HOBt, *i*-Pr₂NEt, CH₂Cl₂, 25 °C, 16 h, (*S*) 55%, (*R*) 68%; (l) LiOH·H₂O, THF/MeOH/H₂O (3:1:1), 25 °C, 16 h, (*S*) 55%, (*R*) 54%.

Scheme 5.

Reagents and conditions: (a) LiBH₄, dry THF, 25 °C, 4 h, 42%; (b) MeI, NaH, dry THF, 25 °C, 1 h, 68%; (c) HBr in AcOH, 25 °C, 1 h, 67%; (d) **7a**, EDCI, HOBT, *i*-Pr₂NEt, CH₂Cl₂, 25 °C, 16 h, 96%; (e) LiOH·H₂O, THF/MeOH/H₂O (3:1:1), 25 °C, 16 h, 98%.

Scheme 6.

Reagents and conditions: (a) EDCI, HOBT, *i*-Pr₂NEt, CH₂Cl₂, 25 °C, 16 h (49–95%); (b) for **3e**, TFA, CH₂Cl₂, 25 °C, 2 h, 98%.

Table 1Enzyme inhibitory and antiviral activity of inhibitors **3a–h**.

Entry	Inhibitor structure	K_i	IC_{50}^a
		(nM)	
1	3a	0.21	31
2	3b	0.63	581
3	3c	0.64	23
4	3d	0.107	32
5	3e	5.08	600
6	3f	0.51	290

Entry	Inhibitor structure	K_i	IC_{50}^a
		(nM)	
7	3g	629	> 1000
8	3h	70	> 1000

^aDarunavir (**1**) exhibited K_i = 16 pM, antiviral IC_{50} = 3.2 nM

Table 2Enzyme inhibitory and antiviral activity of inhibitors **4a–l**.

Entry	Inhibitor structure	K_i	$IC_{50}^{a,b}$
		(nM)	
1	4a	30.8	> 1000
2	4b	0.025	69
3	4c	1.61	189
4	4d	0.79	164
5	4e	0.53	221

Entry	Inhibitor structure	K_i	$IC_{50}^{a,b}$
		(nM)	
6	4f	0.76	32
7	4g	0.80	327
8	4h	0.41	33
9	4i	23.6	nt
10	4j	2.50	nt
11	4k	0.49	280

Entry	Inhibitor structure	K_i	$IC_{50}^{a,b}$
		(nM)	
12	4I	0.40	109

^aDarunavir (1) exhibited $K_i = 16$ pM, antiviral $IC_{50} = 3.2$ nM

^bnt = not tested.