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Eur J Med Chem. Author manuscript; available in PMC 2017 July 07.

Published in final edited form as:

Eur J Med Chem. 2017 January 27; 126: 502–516. doi:10.1016/j.ejmech.2016.11.027.

Structure-Based Exploration and Exploitation of the S_4 Subsite of Norovirus 3CL Protease in the Design of Potent and Permeable Inhibitors

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Abstract

Human noroviruses are the primary cause of epidemic and sporadic acute gastroenteritis. The worldwide high morbidity and mortality associated with norovirus infections, particularly among the elderly, immunocompromised patients and children, constitute a serious public health concern. There are currently no approved human vaccines or norovirus-specific small-molecule therapeutics or prophylactics. Norovirus 3CL protease has recently emerged as a potential therapeutic target for the development of anti-norovirus agents. We hypothesized that the S_4 subsite of the enzyme may provide an effective means of designing potent and cell permeable inhibitors of the enzyme. We report herein the structure-guided exploration and exploitation of the S_4 subsite of norovirus 3CL protease in the design and synthesis of effective inhibitors of the protease.

1. Introduction

Human noroviruses belong to the family *Caliciviridae* and are the leading cause of acute gastroenteritis worldwide [1–2]. They are associated with high morbidity and a heavy economic burden [3–5]. In developing countries the mortality rate among children <5 years old due to diarrheal disease caused by noroviruses is estimated to account for 71,000 deaths annually [6–8]. Combating norovirus infections presents a challenge because of the facile foodborne and waterborne transmission of noroviruses, as well as their high genetic diversity

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and environmental stability [9–10]. The problem is further exacerbated by the current lack of diagnostics and norovirus-specific therapeutics and prophylactics, or vaccines. Thus, there is an urgent and unmet medical need for the discovery and development of anti-norovirus small-molecule therapeutics and prophylactics [11–14], as well as effective vaccines [15–16].

Both viral and host factors can in principle serve as a launching pad for the discovery of anti-norovirus therapeutics [11–14,17–20]. Prominent viral targets that are particularly well-suited as targets for anti-noroviral drug development include the viral-encoded 3CL protease (3CLpro) and RNA dependent RNA polymerase (RdRp) because of the essential roles they play in viral replication [18]. Following translation of the viral genome, the viral polyprotein is cleaved by 3CLpro, a cysteine protease with a prototypical catalytic triad (Cys 139, His30, Glu54) and a strong preference for a P_1 Gln (or Glu) residue [21], to generate structural and nonstructural proteins [22–24] Norovirus 3CLpro has been the focus of intense investigations and an array of inhibitors of 3CLpro that display anti-norovirus activity have been reported [11–14,19], including peptidyl [25–26] and macrocyclic [27–28] transition state inhibitors and transition state mimics [29]. Importantly, *in vivo* proof of concept in a mouse model using a dipeptidyl transition state inhibitor of norovirus 3CLpro has also been demonstrated [25]. We describe herein the structure-guided exploration of the S_4 subsite of 3CLpro and the subsequent deployment of structure-activity relationship and biochemical studies in the design and discovery of potent and permeable inhibitors of the enzyme.

2. Results and Discussion

2.1. Inhibitor design rationale

The design and optimization of the inhibitors was informed by the utilization of available crystal structures and the parallel acquisition of additional high resolution X-ray crystal structures. Thus, inspection of previously-determined high resolution X-ray crystal structures of dipeptidyl inhibitors bound to norovirus 3CL protease (PDB accession codes 4XBB, 4XBC and 4XBD) [25] revealed that the “cap” R group in inhibitor (I) projects toward the S_4 subsite of the enzyme and suggested that its close proximity to a string of hydrophobic amino acids (Ala158, Ala160, Val168 and Ile109) can be exploited through appropriate cap modifications, including the use of sulfonamide functionalities. Additional design considerations included the use of key recognition elements, such as a Gln surrogate [30] and a cyclohexyl methyl residue at the P_1 and P_2 positions, respectively, capable of engaging in H-bonding and hydrophobic interactions. Finally, based on the recently-demonstrated *in vivo* efficacy of aldehyde bisulfite adducts [31], this moiety was incorporated into inhibitor (I) and, for comparative purposes, the corresponding aldehyde inhibitors were also included.

2.2. Chemistry

The synthesis of inhibitors ***6a-7m*** was accomplished using the reaction sequence shown in Scheme 1. Briefly, N-Boc-L-cyclohexyl methyl alanine was coupled to glutamine surrogate ***8*** [30] to obtain ***2***, followed by removal of the Boc protective group to yield intermediate ***3*** which was subsequently reacted with a series of sulfonyl chlorides to yield sulfonamide

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esters ***4a–m.*** Reduction with lithium borohydride generated the corresponding alcohols ***5a–m***. Dess-Martin periodinane oxidation of ***5a–m*** proceeded smoothly to yield aldehydes ***6a–m*** which were transformed to the corresponding aldehyde bisulfite adducts ***7a–m***. The synthesized sulfonamide derivatives are listed in Table 1. Carbamates ***12a–c*** were synthesized according to Scheme 2 via reaction of intermediate ***3*** with an appropriate chloroformate and further elaboration to yield the corresponding aldehydes and aldehyde bisulfite adducts, which are listed in Table 2.

2.3. Biochemical Studies

The inhibitory activity of the synthesized compounds against NV 3CLpro and their anti-norovirus activity in a cell-based replicon system, were evaluated as described in the experimental section [32]. The determined IC_{50}, EC_{50} and CC_{50} values are listed in Tables 1 and 2 and they are the average of at least two determinations.

Inspection of the results in Table 1 permit the following inferences to be drawn: (a) the potency of the aldehyde bisulfite adducts is comparable to those of the precursor aldehydes, an observation noted previously [25,33]. Advantages accrued through the use of aldehyde bisulfite adducts include higher solubility and metabolic stability, as well as low cytotoxicity [31]. Whether the bisulfite adducts exert their action by functioning as transition state mimics or latent precursors of the aldehyde functionality, or both, has not been established as yet and is currently under investigation; (b) a significant increase in potency (15-fold) was observed when the phenyl ring is moved further into the S_4 pocket (Table 1, compare the EC_{50} values of compounds ***7a, 7b*** and ***7c***) and an additional 4-fold gain in potency was realized by extending the linker further (Table 1, compound ***7l***). These observations are congruent with the results of X-ray crystallographic studies with inhibitor ***7l***. Crystallization of NV 3CLpro with inhibitor ***7l*** yielded hexagonal and orthorhombic crystals and the electron density clearly shows the sulfur atom of the active site Cys139 residue bound covalently to the carbonyl carbon of the P_1 residue of inhibitor ***7l***, thereby establishing the mechanism of action of this class of inhibitors (Figure 2). The electrostatic surface representation shows the aryl ring to be optimally nestled in the hydrophobic S_4 pocket of the enzyme and engaged in hydrophobic interactions with Ile109, Val168, Ala160 and Ala159 (Figure 3). Inhibitor ***7l*** is bound to the active site of the enzyme via a network of hydrogen bonds in the manner of an antiparallel β-sheet and includes inhibitor backbone H-bonds with Gln110 and Ala158 which serve to position and orient the inhibitor correctly with respect to the catalytic residues (Figures 4 and 5). Additionally, a H-bond with His30 serves to stabilize the tetrahedral adduct and two critical H-bonds involving the P_1 Gln surrogate ring oxygen with His157 and Thr134 are clearly evident; (c) halogen substitutions in the aromatic ring were generally beneficial (Table 1, compounds ***6d/7d, 6e/7e*** and ***6g/7g***); (d) potency also improved when a 4-biphenyl or 2-pthalimidoethane moiety was used (Table 1, compounds ***6j/7j*** and ***6k/7k***); (e) the significance of hydrophobic interactions involving R and the S_4 subsite was further probed by using an n-octyl chain cap, resulting in inhibitors displaying submicromolar ED_{50} values (Table 1, compounds ***6m/7m).*** The structural determinants accounting for the higher potency of compounds ***6m/7m*** were identified by obtaining a high resolution X-ray crystal structure of the NV 3CLpro:***7m*** complex (Figure 6). Inhibitor ***7m*** adopts a binding mode in the active site similar to ***7l*** (Figure 7) which is

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clearly evident from the superposition of the two inhibitors in the active site of the enzyme (Figure 8). Furthermore, inhibitor ***7m*** engages in identical H-bond interactions as inhibitor ***7l*** (Figure 9), hence the comparable potencies. Importantly, the n-octyl chain encompasses the S_4 subsite and participates in hydrophobic interactions with the string of hydrophobic amino acids that make up the S_4 pocket (Figure 10). The importance of hydrophobic interactions is similarly evident in carbamate derivatives ***11c/12c*** (Table 2); and, (f) an eight to ten-fold increase in potency was realized when the sulfonamide linkage was replaced by a carbamate moiety (Table 2, compounds ***11a/12a*** versus compounds ***6h/7h*** in Table 1).

3. Conclusion

Structural, synthetic, biochemical, and cell-based studies have been employed to illuminate the S_4 subsite of NV 3CLpro and to gain insight and understanding into the significance and impact of hydrophobic interactions on pharmacological activity. In principle, further gains in potency can be realized through the use of constrained R moieties that mitigate the entropic penalty incurred in using long aliphatic chains. The results reported herein provide a sound framework and a solid foundation for conducting further studies aimed at identifying anti-norovirus drug candidates.

4. Experimental section

4.1. General

Reagents and dry solvents were purchased from various chemical suppliers (Chem-Impex, Acros Organics, TCI America, Aldrich, and Bachem) and were used as obtained. Silica gel (230–450 mesh) used for flash chromatography was purchased from Sorbent Technologies (Atlanta, GA). Thin layer chromatography was performed using Analtech silica gel plates (Newark, DE). Visualization was accomplished using UV light and/or iodine. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 using a Varian XL-400 spectrometer and are reported relative to TMS (δ_H= 0.00 ppm). Chemical shifts are reported in ppm and spin multiplicities are represented by the following signals: s (singlet), d (doublet), dd (doublets of doublet), t (triplet), q (quartet) and m (multiplet). Melting points were recorded on a Mel-Temp apparatus and are uncorrected. High resolution mass spectrometry (HRMS) was performed at the University of Kansas Mass Spectrometry lab using an LCT Premier mass spectrometer (Waters, Milford, MA) equipped with a time of flight mass analyzer and an electrospray ion source or in-house using a G6230B TOF MS (Agilent Technologies, Santa Clara, CA). The purity of the synthesized compounds was established using HPLC and was >95%.

4.1.1. Synthesis of compound 2—To a solution of compound **1** (10 mmol) in dry DMF (20 mL) was added EDCI (2.40 g, 12.5 mmol, 1.25 eq), HOBt (1.92 g, 12.5 mmol, 1.25 eq) and the mixture was stirred for 30 minutes at room temperature. In a separate flask, a solution of deprotected glutamine surrogate **8** (2.23 g, 10 mmol) in DMF (15 mL) cooled to 0–5 °C was treated with N,N-diisopropylethylamine (DIEA) (9.5 g, 40 mmol, 4 eq), stirred for 30 minutes, and then added to the reaction mixture containing (L) N-Boc cyclohexylalanine. The reaction mixture was stirred for 12 h while monitoring the reaction by TLC. The solvent was removed and the residue was dissolved in ethyl acetate (200 mL) and washed with 10% aqueous citric acid (2 × 40 mL). The ethyl acetate layer was further

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washed with saturated $NaHCO_3$ (40 mL), followed by saturated NaCl (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to yield a yellow-colored oily product. Purification by flash chromatography yielded ester **2** as a white solid.

4.1.1.1. Methyl (S)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-cyclohexylpropanamido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 2: White solid (yield 77%); M.p 72–74 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.84 – 1.04 (m, 2 H), 1.11 – 1.31 (m, 4 H), 1.44 (s, 9 H), 1.60 – 1.75 (m, 6 H), 1.78 – 1.94 (m, 3 H), 2.15 – 2.28 (m, 1 H), 2.35 – 2.48 (m, 2 H), 3.26 – 3.40 (m, 2 H), 3.73 (s, 3 H), 4.21 – 4.34 (m, 1 H), 4.53 (ddd, *J*=11.13, 7.37, 3.91 Hz, 1 H), 5.03 (d, *J*=7.23 Hz, 1 H), 6.34 (s, 1 H), 7.55 (d, *J*=5.66 Hz, 1 H). HRMS (ESI) calcd for $C_{22}H_{37}N_3O_6$: [M-]: 439.2682. Found: 439.2866.

4.1.2. Synthesis of compound 3—To a solution of **c**ompound **2** (10 mmol) in dry DCM (5 mL) was added 4M HCl in dioxane (10 mL) and the mixture was stirred for 2 h at room temperature. The solvent was removed and the residue was dried under high vacuum to yield the hydrochloride salt as a pale white solid.

4.1.2.1. Methyl (S)-2-((S)-2-amino-3-cyclohexylpropanamido)-3-((S)-2-oxopyrroli din-3-yl)propanoate hydrochloride 3: Pale white solid (yield 100%); M.p 60–62 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.87 (quin, *J*=10.76 Hz, 2 H), 1.09 – 1.29 (m, 3 H), 1.34 – 1.45 (m, 2 H), 1.52 – 1.60 (m, 2 H), 1.65 (d, *J*=8.25 Hz, 4 H), 1.80 (d, *J*=12.03 Hz, 1 H), 2.01 – 2.13 (m, 1 H), 2.12 – 2.24 (m, 2 H), 2.38 – 2.48 (m, 2 H), 3.07 – 3.23 (m, 2 H), 3.58 (s, 3 H), 3.79 (d, *J*=5.30 Hz, 1 H), 4.25 (br. s, 1 H), 4.36 – 4.46 (m, 1 H), 7.69 (s, 1 H), 8.33 (s, 1 H), 9.00 (d, *J*=7.74 Hz, 1 H). HRMS (ESI) calcd for $C_{17}H_{30}ClN_3O_4$: [M-]: 375.1925. Found: 375.1920.

4.1.3. Synthesis of compounds 4a–m—To a solution of compound **3** (5 mmol) in dry DCM (20 mL) kept at 0 °C was added triethylamine (TEA) (1.3 mL,11 mmol, 2.2 eq) slowly with stirring. The appropriate sulfonyl chloride derivative (5.5 mmol, 1.1 eq) was added and reaction mixture was stirred for 12 h at 35 °C. The solvent was removed and the residue was dissolved in ethyl acetate (50 mL) and washed with water (2 × 25 mL). The ethyl acetate layer was further washed with saturated NaCl (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to yield a crude product. Purification by flash chromatography yielded esters **4a–m.**

4.1.3.1. Methyl (S)-2-((S)-3-cyclohexyl-2-(phenylsulfonamido)propanamido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4a: White solid (yield 73%); M.p 45–47 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.67 – 0.79 (m, 1 H), 0.82 – 0.94 (m, 1 H), 0.98 – 1.07 (m, 2 H), 1.10 – 1.22 (m, 1 H), 1.31 – 1.41 (m, 1 H), 1.43 – 1.65 (m, 6 H), 1.73 – 1.88 (m, 2 H), 2.02 – 2.13 (m, 2 H), 2.19 – 2.29 (m, 1 H), 2.34 (ddd, *J*=12.40, 8.40, 4.00 Hz, 1 H), 3.38 (dd, *J*=8.93, 4.74 Hz, 2 H), 3.70 (s, 3 H), 3.84 (td, *J*=9.30, 4.93 Hz, 1 H), 4.27 (ddd, *J*=11.23, 6.98, 3.86 Hz, 1 H), 5.30 (s, 1 H), 5.97 (d, *J*=9.18 Hz, 1 H), 6.44 (s, 1 H), 7.44 – 7.50 (m, 2 H), 7.51 – 7.57 (m, 1 H), 7.87 (d, *J*=7.23 Hz, 2 H). HRMS (ESI) calcd for $C_{23}H_{33}N_3O_6S$: [M+H]: 480.2168 Found: 480.2147.

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4.1.3.2. Methyl (S)-2-((S)-3-cyclohexyl-2-((phenylmethyl)sulfonamido)propan amido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4b: White solid (yield 77%); M.p 41–43 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.82 – 0.98 (m, 2 H), 1.07 – 1.30 (m, 3 H), 1.42 – 1.51 (m, 2 H), 1.59 – 1.71 (m, 5 H), 1.77 (d, *J*=12.45 Hz, 1 H), 1.82 – 1.96 (m, 2 H), 2.17 (ddd, *J*=14.13, 11.74, 5.66 Hz, 1 H), 2.34 – 2.44 (m, 1 H), 2.45 – 2.54 (m, 1 H), 3.31 – 3.39 (m, 2 H), 3.73 (s, 3 H), 3.94 – 4.04 (m, 1 H), 4.22 – 4.33 (m, 2 H), 4.44 – 4.53 (m, 1 H), 5.51 (d, *J*=8.98 Hz, 1 H), 6.15 (s, 1 H), 7.33 – 7.39 (m, 3 H), 7.41 – 7.47 (m, 2 H), 7.99 (d, *J*=6.74 Hz, 1 H). HRMS (ESI) calcd for $C_{24}H_{35}N_3O_6S$: [M+H]: 494.2325 Found: 494.2302.

4.1.3.3. Methyl (S)-2-((S)-3-cyclohexyl-2-((2-phenylethyl)sulfonamido)propan amido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4c: White solid (yield 82%); M.p 45–47 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.84 – 1.06 (m, 2 H), 1.09 – 1.30 (m, 4 H), 1.47 – 1.59 (m, 2 H), 1.61 – 1.74 (m, 4 H), 1.75 – 1.94 (m, 3 H), 2.13 (ddd, *J*=14.08, 11.96, 5.69 Hz, 1 H), 2.19 – 2.29 (m, 1 H), 2.30 – 2.42 (m, 1 H), 3.06 – 3.16 (m, 2 H), 3.17 – 3.34 (m, 4 H), 3.64 (s, 3 H), 4.10 (td, *J*=9.02, 4.86 Hz, 1 H), 4.39 – 4.48 (m, 1 H), 5.68 (d, *J*=9.47 Hz, 1 H), 6.24 (s, 1 H), 7.17 – 7.25 (m, 2 H), 7.25 – 7.35 (m, 3 H), 8.15 (d, *J*=6.79 Hz, 1 H). HRMS (ESI) calcd for $C_{25}H_{37}N_3O_6S$: [M+H]: 508.2481 Found: 508.2460.

4.1.3.4. Methyl (S)-2-((S)-3-cyclohexyl-2-((4-fluorophenyl)sulfonamido)propan amido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4d: White solid (yield 78%); M.p 63–65 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.76 (d, *J*=10.40 Hz, 1 H), 0.85 – 0.96 (m, 1 H), 1.08 (d, *J*=9.52 Hz, 2 H), 1.13 – 1.22 (m, 1 H), 1.35 – 1.55 (m, 2 H), 1.55 – 1.66 (m, 5 H), 1.71 (br. s., 1 H), 1.79 – 1.92 (m, 2 H), 1.99 – 2.11 (m, 1 H), 2.19 – 2.28 (m, 1 H), 2.36 (ddd, *J*=12.55, 8.47, 4.37 Hz, 1 H), 3.39 (dd, *J*=8.93, 4.64 Hz, 2 H), 3.70 (s, 3 H), 3.81 (td, *J*=9.28, 4.93 Hz, 1 H), 4.21 – 4.29 (m, 1 H), 6.00 (d, *J*=9.23 Hz, 1 H), 6.31 (s, 1 H), 7.15 (t, *J*=8.54 Hz, 2 H), 7.86 – 7.92 (m, 2 H), 7.95 (d, *J*=6.59 Hz, 1 H). HRMS (ESI) calcd for $C_{23}H_{32}FN_3O_6S$: [M+H]: 498.2074 Found: 498.2050.

4.1.3.5. Methyl (S)-2-((S)-2-((3-chlorophenyl)sulfonamido)-3-cyclohexylpropan amido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4e: White solid (yield 81%); M.p 58–59 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.73 – 0.82 (m, 1 H), 0.84 – 0.94 (m, 1 H), 1.06 (t, *J*=8.98 Hz, 2 H), 1.13 – 1.20 (m, 1 H), 1.40 (d, *J*=16.50 Hz, 1 H), 1.44 – 1.50 (m, 1 H), 1.51 – 1.65 (m, 4 H), 1.72 (br. s., 2 H), 1.79 – 1.89 (m, 2 H), 1.97 – 2.08 (m, 1 H), 2.23 – 2.30 (m, 1 H), 2.35 (d, *J*=11.52 Hz, 1 H), 3.35 – 3.42 (m, 2 H), 3.68 (s, 3 H), 3.85 (d, *J*=4.20 Hz, 1 H), 4.15 – 4.23 (m, 1 H), 5.98 (d, *J*=9.08 Hz, 1 H), 6.27 (s, 1 H), 7.40 – 7.45 (m, 1 H), 7.47 – 7.53 (m, 1 H), 7.75 (d, *J*=7.62 Hz, 1 H), 7.83 (s, 1 H), 8.16 (d, *J*=5.96 Hz, 1 H). HRMS (ESI) calcd for $C_{23}H_{32}ClN_3O_6S$: [M+H]: 514.1779 Found: 514.1738.

4.1.3.6. Methyl (S)-2-((S)-2-((4-chlorophenyl)sulfonamido)-3-cyclohexylpropan amido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4f: White solid (yield 76%); M.p 52–54 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.70 – 0.83 (m, 1 H), 0.89 (q, *J*=11.00 Hz, 1 H), 1.03 – 1.12 (m, 1 H), 1.13 – 1.22 (m, 1 H), 1.37 – 1.54 (m, 2 H), 1.57 – 1.68 (m, 5 H), 1.71 (br. s., 1 H), 1.80 – 1.91 (m, 2 H), 1.97 – 2.09 (m, 2 H), 2.17 – 2.26 (m, 1 H), 2.32 – 2.45 (m, 1 H), 3.36 – 3.46 (m, 2 H), 3.70 (s, 3 H), 3.80 (td, *J*=9.25, 4.98 Hz, 1 H), 4.16 – 4.25 (m, 1 H), 6.00 (d, *J*=9.28 Hz, 1 H), 6.30 (s, 1 H), 7.44 (d, *J*=8.59 Hz, 2 H), 7.81 (d,

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J=8.59 Hz, 2 H), 8.04 (d, *J*=6.35 Hz, 1 H). HRMS (ESI) calcd for $C_{23}H_{32}ClN_3O_6S$: [M+H]: 514.1779 Found: 514.1770.

4.1.3.7. Methyl (S)-2-((S)-2-(((4-chlorophenyl)methyl)sulfonamido)-3-cyclohexyl propanamido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4g: White solid (yield 71%); M.p 38–39 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.83 – 0.98 (m, 2 H), 1.08 – 1.29 (m, 3 H), 1.40 – 1.52 (m, 2 H), 1.60 – 1.71 (m, 5 H), 1.77 (d, *J*=12.30 Hz, 1 H), 1.84 – 1.98 (m, 2 H), 2.09 – 2.20 (m, 1 H), 2.34 – 2.45 (m, 1 H), 2.46 – 2.53 (m, 1 H), 3.37 (dd, *J*=8.81, 4.66 Hz, 2 H), 3.73 (s, 3 H), 3.95 – 4.03 (m, 1 H), 4.18 – 4.31 (m, 2 H), 4.40 – 4.48 (m, 1 H), 5.67 (d, *J*=8.93 Hz, 1 H), 6.21 (s, 1 H), 7.32 – 7.35 (m, 2 H), 7.38 – 7.42 (m, 2 H), 8.10 (d, *J*=6.49 Hz, 1 H). HRMS (ESI) calcd for $C_{24}H_{34}ClN_3O_6S$: [M+H]: 528.1835 Found: 528.1879.

4.1.3.8. Methyl (S)-2-((S)-2-((2-(3-chlorophenyl)ethyl)sulfonamido)-3-cyclohexyl propanamido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4h: White solid (yield 71%); M.p 63–65 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.85 – 1.05 (m, 2 H), 1.12 – 1.31 (m, 3 H), 1.49 – 1.58 (m, 2 H), 1.62 – 1.75 (m, 6 H), 1.79 – 1.96 (m, 2 H), 2.13 (ddd, *J*=14.10, 12.00, 5.79 Hz, 1 H), 2.25 – 2.34 (m, 1 H), 2.36 – 2.45 (m, 1 H), 3.03 – 3.16 (m, 2 H), 3.21 – 3.29 (m, 2 H), 3.30 – 3.39 (m, 2 H), 3.68 (s, 3 H), 4.05 – 4.16 (m, 1 H), 4.39 – 4.47 (m, 1 H), 5.80 (d, *J*=9.52 Hz, 1 H), 6.28 (s, 1 H), 7.10 (d, *J*=6.83 Hz, 1 H), 7.19 – 7.26 (m, 3 H), 8.24 (d, *J*=6.69 Hz, 1 H). HRMS (ESI) calcd for $C_{25}H_{36}ClN_3O_6S$: [M+H]: 542.2092 Found: 542.2033.

4.1.3.9. Methyl (S)-2-((S)-3-cyclohexyl-2-(thiophene-2-sulfonamido)propanamido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4i: White solid (yield 62%); M.p 45–47 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.72 – 0.85 (m, 1 H), 0.86 – 0.95 (m, 1 H), 1.04 – 1.15 (m, 2 H), 1.16 – 1.27 (m, 1 H), 1.35 – 1.46 (m, 1 H), 1.50 (dd, *J*=9.45, 4.76 Hz, 1 H), 1.54 – 1.67 (m, 5 H), 1.79 – 1.92 (m, 3 H), 2.04 – 2.18 (m, 1 H), 2.28 – 2.42 (m, 2 H), 3.35 – 3.41 (m, 2 H), 3.71 (s, 3 H), 3.95 (td, *J*=9.19, 4.91 Hz, 1 H), 4.34 (ddd, *J*=11.28, 7.08, 3.81 Hz, 1 H), 6.17 (d, *J*=8.93 Hz, 1 H), 6.53 (br. s., 1 H), 7.04 (dd, *J*=4.83, 3.91 Hz, 1 H), 7.56 (dd, *J*=4.96, 1.00 Hz, 1 H), 7.61 (dd, *J*=3.64, 1.05 Hz, 1 H), 7.89 (d, *J*=6.88 Hz, 1 H). HRMS (ESI) calcd for $C_{21}H_{31}N_3O_6S_2$: [M+H]: 486.1733 Found: 486.1785.

4.1.3.10. Methyl (S)-2-((S)-2-([1,1′-biphenyl]-4-sulfonamido)-3-cyclohexylpropan amido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4j: White solid (yield 62%); M.p 89–92 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.73 (d, *J*=10.50 Hz, 1 H), 0.81 – 0.93 (m, 1 H), 0.98 – 1.07 (m, 2 H), 1.09 – 1.18 (m, 1 H), 1.31 – 1.43 (m, 1 H), 1.45 – 1.64 (m, 6 H), 1.67 – 1.84 (m, 3 H), 1.96 – 2.05 (m, 1 H), 2.08 – 2.19 (m, 2 H), 3.01 – 3.10 (m, 1 H), 3.17 – 3.25 (m, 1 H), 3.66 (s, 3 H), 3.81 (td, *J*=9.21, 4.86 Hz, 1 H), 4.16 – 4.24 (m, 1 H), 5.92 (d, *J*=9.13 Hz, 1 H), 6.18 (s, 1 H), 7.37 – 7.42 (m, 1 H), 7.43 – 7.48 (m, 2 H), 7.57 (d, *J*=7.32 Hz, 2 H), 7.66 (d, *J*=8.40 Hz, 2 H), 7.91 (d, *J*=8.40 Hz, 2 H), 8.00 (d, *J*=6.49 Hz, 1 H). HRMS (ESI) calcd for $C_{29}H_{37}N_3O_6S$: [M+H]: 556.2481 Found: 556.2430.

4.1.3.11. methyl (S)-2-((S)-3-cyclohexyl-2-((2-(1,3-dioxoisoindolin-2-yl)ethyl)sulfon amido)propanamido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4k: White solid (yield 72%); M.p 59–60 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.85 – 1.03 (m, 2 H), 1.09 – 1.24 (m, 3 H), 1.58 (td, *J*=9.35, 5.74 Hz, 2 H), 1.63 – 1.77 (m, 5 H), 1.79 – 1.87 (m, 2 H),

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1.94 (ddd, *J*=14.26, 7.49, 3.69 Hz, 1 H), 2.20 (ddd, *J*=14.04, 11.80, 5.77 Hz, 1 H), 2.35 – 2.45 (m, 1 H), 2.48 – 2.58 (m, 1 H), 3.30 – 3.47 (m, 4 H), 3.67 (s, 3 H), 4.03 – 4.19 (m, 2 H), 4.32 (dt, *J*=14.40, 7.03 Hz, 1 H), 4.43 – 4.54 (m, 1 H), 5.91 (d, *J*=9.11 Hz, 1 H), 6.37 (s, 1 H), 7.67 – 7.76 (m, 2 H), 7.81 – 7.89 (m, 2 H), 8.23 (d, *J*=6.71 Hz, 1 H). HRMS (ESI) calcd for $C_{27}H_{36}N_4O_8S$: [M+H]: 577.2332 Found: 577.2340.

4.1.3.12. Methyl (S)-2-((S)-3-cyclohexyl-2-((3-(4-methoxyphenoxy)propyl)sulfonamido)propanamido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4l: White solid (yield 67%); M.p 49–51 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.86 – 1.02 (m, 2 H), 1.10 – 1.30 (m, 3 H), 1.47 – 1.59 (m, 2 H), 1.62 – 1.74 (m, 5 H), 1.79 – 1.90 (m, 2 H), 1.93 (dt, *J*=7.03, 3.56 Hz, 1 H), 2.13 (ddd, *J*=14.13, 11.79, 5.96 Hz, 1 H), 2.26 (dd, *J*=6.13, 3.25 Hz, 2 H), 2.31 – 2.39 (m, 1 H), 2.42 – 2.52 (m, 1 H), 3.17 – 3.26 (m, 2 H), 3.26 – 3.36 (m, 2 H), 3.70 (s, 3 H), 3.76 (s, 3 H), 4.00 (t, *J*=5.86 Hz, 2 H), 4.05 – 4.15 (m, 1 H), 4.39 – 4.50 (m, 1 H), 5.63 (d, *J*=9.42 Hz, 1 H), 6.16 (s, 1 H), 6.81 (s, 4 H), 8.23 (d, *J*=6.59 Hz, 1 H). HRMS (ESI) calcd for $C_{27}H_{41}N_3O_8S$: [M+H]: 568.2693 Found: 568.2633.

4.1.3.13. Methyl (S)-2-((S)-3-cyclohexyl-2-(octylsulfonamido)propanamido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 4m: White solid (yield 75%); M.p 56–58 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.88 (t, *J*=6.79 Hz, 4 H) 0.91 – 1.04 (m, 2 H), 1.09 – 1.34 (m, 9 H), 1.36 – 1.44 (m, 1 H), 1.46 – 1.56 (m, 1 H), 1.58 – 1.65 (m, 6 H), 1.69 (d, *J*=14.06 Hz, 4 H), 1.77 – 1.89 (m, 2 H), 1.90 – 1.99 (m, 1 H), 2.09 – 2.21 (m, 1 H), 2.38 – 2.54 (m, 2 H), 3.00 (t, *J*=7.96 Hz, 2 H), 3.35 – 3.43 (m, 2 H), 3.73 (s, 3 H), 3.99 – 4.09 (m, 1 H), 4.42 – 4.50 (m, 1 H), 5.34 (d, *J*=9.32 Hz, 1 H), 6.04 (s, 1 H), 8.13 (d, *J*=6.54 Hz, 1 H). HRMS (ESI) calcd for $C_{25}H_{46}N_3O_6S$: [M+H]: 516.3107 Found: 516.3112.

4.1.4. Synthesis of alcohols 5a–m—To a solution of ester **4** (5 mmol) in anhydrous THF (30 mL) was added dropwise lithium borohydride (2M in THF, 7.5 mL, 15 mmol), followed by absolute ethyl alcohol (15 mL), and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then acidified using 5% HCl and the pH was adjusted to ~2. Removal of the solvent left a residue which was taken up in ethyl acetate (100 mL) and the organic layer was washed with brine (25 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to yield compounds **5a–m.**

4.1.4.1. (S)-3-cyclohexyl-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)-2-(phenylsulfonamido)propanamide 5a: White solid (yield 95%); M.p 48–51 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.59 – 0.73 (m, 2 H), 0.76 – 0.94 (m, 2 H), 0.96 – 1.18 (m, 3 H), 1.31 (br. s, 1 H), 1.39 – 1.64 (m, 6 H), 1.75 – 1.87 (m, 1 H), 1.96 – 2.04 (m, 2 H), 2.30 – 2.44 (m, 2 H), 3.32 – 3.40 (m, 2 H), 3.42 – 3.49 (m, 1 H), 3.57 (dd, *J*=11.25, 3.25 Hz, 1 H), 3.69 – 3.78 (m, 1 H), 3.84 – 3.93 (m, 1 H), 6.28 (d, *J*=8.10 Hz, 1 H), 6.37 (s, 1 H), 7.48 – 7.53 (m, 2 H), 7.55 – 7.60 (m, 1 H), 7.71 (d, *J*=7.32 Hz, 1 H), 7.89 (d, *J*=7.47 Hz, 2 H). HRMS (ESI) calcd for $C_{22}H_{33}N_3O_5S$: [M+H]: 452.2219 Found: 452.2210.

4.1.4.2. (S)-3-cyclohexyl-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)-2-((phenylmethyl)sulfonamido)propanamide 5b: White solid (yield 94%); M.p 45–47 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.81 – 0.96 (m, 2 H), 1.07 – 1.23 (m, 4 H), 1.37 (br. s., 1 H), 1.43 – 1.52 (m, 1 H), 1.56 (dd, *J*=13.72, 6.74 Hz, 2 H), 1.62 – 1.76 (m, 6

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H), 1.77 – 1.86 (m, 1 H), 2.38 (td, *J*=5.93, 3.47 Hz, 1 H), 2.46 (d, *J*=6.40 Hz, 1 H), 3.31 (d, *J*=7.62 Hz, 2 H), 3.54 – 3.60 (m, 1 H), 3.65 (d, *J*=8.49 Hz, 1 H), 3.83 – 3.93 (m, 1 H), 3.97 – 4.06 (m, 1 H), 4.26 (s, 2 H), 5.85 (d, *J*=7.08 Hz, 1 H), 6.26 (s, 1 H), 7.32 – 7.38 (m, 3 H), 7.38 – 7.43 (m, 2 H), 7.66 (d, *J*=7.18 Hz, 1 H). HRMS (ESI) calcd for $C_{23}H_{35}N_3O_5S$: [M +H]: 466.2376 Found: 466.2360.

4.1.4.3. (S)-3-cyclohexyl-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)-2-((2-phenylethyl)sulfonamido)propanamide 5c: White solid (yield 94%); M.p 49–50 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.84 – 1.01 (m, 2 H), 1.10 – 1.23 (m, 4 H), 1.55 (d, *J*=7.23 Hz, 2 H), 1.60 – 1.74 (m, 6 H), 1.77 – 1.87 (m, 2 H), 2.20 – 2.30 (m, 1 H), 2.33 – 2.42 (m, 1 H), 3.05 – 3.15 (m, 2 H), 3.18 – 3.33 (m, 2 H), 3.59 (d, *J*=18.50 Hz, 2 H), 3.83 (br. s., 1 H), 4.01 (d, *J*=4.39 Hz, 2 H), 4.14 – 4.22 (m, 2 H), 6.11 (d, *J*=5.42 Hz, 1 H), 6.45 (s, 1 H), 7.16 – 7.23 (m, 2 H), 7.23 – 7.33 (m, 3 H), 7.79 (d, *J*=6.59 Hz, 1 H). HRMS (ESI) calcd for $C_{24}H_{37}N_3O_5S$: [M+H]: 480.2532 Found: 480.2508.

4.1.4.4. (S)-3-cyclohexyl-2-((4-fluorophenyl)sulfonamido)-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 5d: White solid (yield 96%); M.p 66–68 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.63 – 0.77 (m, 1 H), 0.79 – 0.90 (m, 1 H), 0.91 – 1.18 (m, 3 H), 1.34 (d, *J*=11.81 Hz, 1 H), 1.42 – 1.52 (m, 2 H), 1.52 – 1.67 (m, 6 H), 1.79 – 1.89 (m, 1 H), 2.01 (d, *J*=6.40 Hz, 1 H), 2.27 – 2.42 (m, 2 H), 3.33 – 3.41 (m, 2 H), 3.46 – 3.55 (m, 2 H), 3.58 (br. s., 1 H), 3.68 – 3.78 (m, 1 H), 3.85 – 4.01 (m, 1 H), 6.33 (s, 1 H), 7.18 (t, *J*=8.28 Hz, 2 H), 7.80 (d, *J*=7.20 Hz, 1 H), 7.91 (dd, *J*=8.47, 5.00 Hz, 2 H), 8.25 (d, *J*=5.82 Hz, 1 H). HRMS (ESI) calcd for $C_{22}H_{32}FN_3O_5S$: [M+H]: 470.2125 Found: 470.2115.

4.1.4.5. (S)-2-((3-chlorophenyl)sulfonamido)-3-cyclohexyl-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 5e: White solid (yield 94%); M.p 61–63 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.67 – 0.80 (m, 2 H), 0.86 (q, *J*=11.23 Hz, 1 H), 0.94 – 1.19 (m, 3 H), 1.38 – 1.51 (m, 3 H), 1.52 – 1.67 (m, 4 H), 1.84 (t, *J*=8.01 Hz, 2 H), 1.93 – 2.01 (m, 1 H), 2.32 – 2.43 (m, 2 H), 3.33 – 3.42 (m, 2 H), 3.44 – 3.50 (m, 1 H), 3.54 – 3.60 (m, 1 H), 3.77 (dt, *J*=8.86, 4.60 Hz, 1 H), 3.81 – 3.88 (m, 1 H), 4.21 (br. s, 1 H), 6.28 (s, 1 H), 7.45 (t, *J*=7.79 Hz, 1 H), 7.49 – 7.56 (m, 1 H), 7.78 (d, *J*=7.57 Hz, 1 H), 7.84 – 7.89 (m, 1 H), 7.97 (d, *J*=5.57 Hz, 1 H), 8.17 (d, *J*=7.20 Hz, 1 H). HRMS (ESI) calcd for $C_{22}H_{32}ClN_3O_5S$: [M+H]: 486.1829 Found: 486.1809.

4.1.4.6. (S)-2-((4-chlorophenyl)sulfonamido)-3-cyclohexyl-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 5f: White solid (yield 93%); M.p 56–57 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.65 – 0.77 (m, 1 H), 0.78 – 0.93 (m, 1 H), 0.94 – 1.19 (m, 3 H), 1.34 – 1.42 (m, 1 H), 1.43 – 1.52 (m, 2 H), 1.53 – 1.64 (m, 5 H), 1.76 – 1.90 (m, 2 H), 1.91 – 2.02 (m, 1 H), 2.24 – 2.33 (m, 1 H), 2.38 (dd, *J*=8.30, 3.32 Hz, 1 H), 3.34 – 3.44 (m, 2 H), 3.45 – 3.52 (m, 1 H), 3.58 (d, *J*=8.15 Hz, 1 H), 3.82 (br. s, 1 H), 3.95 (d, *J*=3.91 Hz, 1 H), 4.17 – 4.25 (m, 1 H), 5.97 (d, *J*=5.20 Hz, 1 H), 6.33 (s, 1 H), 7.41 – 7.50 (m, 2 H), 7.79 – 7.89 (m, 2 H), 8.05 (d, *J*=7.20 Hz, 1 H). HRMS (ESI) calcd for $C_{22}H_{32}ClN_3O_5S$: [M+H]: 486.1829 Found: 486.1811.

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4.1.4.7. (S)-2-(((4-chlorophenyl)methyl)sulfonamido)-3-cyclohexyl-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 5g: White solid (yield 95%); M.p 47–48 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.82 – 0.95 (m, 2 H), 1.11 – 1.21 (m, 2 H), 1.37 (d, *J*=4.42 Hz, 1 H), 1.43 – 1.50 (m, 1 H), 1.53 – 1.61 (m, 3 H), 1.62 – 1.75 (m, 6 H), 1.83 (dd, *J*=11.42, 9.67 Hz, 1 H), 2.00 (br. s., 1 H), 2.35 – 2.52 (m, 2 H), 3.30 – 3.36 (m, 2 H), 3.55 – 3.61 (m, 1 H), 3.64 – 3.71 (m, 1 H), 3.84 – 3.93 (m, 1 H), 4.00 (d, *J*=3.56 Hz, 1 H), 4.23 (d, *J*=4.96 Hz, 2 H), 5.81 (d, *J*=8.40 Hz, 1 H), 6.11 (s, 1 H), 7.30 – 7.33 (m, 1 H), 7.34 – 7.36 (m, 3 H), 7.80 (d, *J*=7.03 Hz, 1 H). HRMS (ESI) calcd for $C_{23}H_{34}ClN_3O_5S$: [M+H]: 500.1986 Found: 500.1978.

4.1.4.8. (S)-2-((2-(3-chlorophenyl)ethyl)sulfonamido)-3-cyclohexyl-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 5h: White solid (yield 96%); M.p 67–69 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.86 – 1.02 (m, 2 H), 1.09 – 1.21 (m, 2 H), 1.45 – 1.65 (m, 4 H), 1.70 (d, *J*=10.25 Hz, 5 H), 1.76 – 1.86 (m, 2 H), 1.99 (d, *J*=5.81 Hz, 1 H), 2.25 – 2.35 (m, 1 H), 2.35 – 2.45 (m, 1 H), 3.04 – 3.16 (m, 2 H), 3.19 – 3.28 (m, 2 H), 3.29 – 3.37 (m, 2 H), 3.49 (br. s, 1 H), 3.54 – 3.60 (m, 1 H), 3.62 – 3.69 (m, 1 H), 3.95 – 4.05 (m, 2 H), 5.87 (d, *J*=9.03 Hz, 1 H), 6.17 (s, 1 H), 7.10 (d, *J*=6.74 Hz, 1 H), 7.20 – 7.23 (m, 2 H), 7.24 (br. s., 1 H), 7.94 (d, *J*=7.03 Hz, 1 H). HRMS (ESI) calcd for $C_{24}H_{36}ClN_3O_5S$: [M+H]: 514.2142 Found: 514.2148.

4.1.4.9. (S)-3-cyclohexyl-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)-2-(thiophene-2-sulfonamido)propanamide 5i: White solid (yield 96%); M.p 48–50 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.65 – 0.84 (m, 2 H), 0.95 – 1.14 (m, 3 H), 1.29 – 1.38 (m, 4 H), 1.47 – 1.64 (m, 6 H), 1.67 – 1.78 (m, 1 H), 2.01 – 2.12 (m, 2 H), 3.04 – 3.19 (m, 2 H), 3.21 – 3.29 (m, 2 H), 3.63 (dd, *J*=6.96, 4.17 Hz, 1 H), 3.77 (q, *J*=7.53 Hz, 1 H), 4.65 (t, *J*=5.49 Hz, 1 H), 7.09 – 7.14 (m, 1 H), 7.53 – 7.54 (m, 1 H), 7.55 (s, 1 H), 7.73 (d, *J*=8.64 Hz, 1 H), 7.87 (dd, *J*=4.88, 1.03 Hz, 1 H), 8.08 (d, *J*=8.35 Hz, 1 H). HRMS (ESI) calcd for $C_{20}H_{31}N_3O_5S_2$: [M+H]: 458.1783 Found: 458.1739.

4.1.4.10. (S)-2-([1,1′-biphenyl]-4-sulfonamido)-3-cyclohexyl-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 5j: White solid (yield 92%); M.p 93–95 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.61 – 0.71 (m, 1 H), 0.72 – 0.82 (m, 1 H), 0.84 – 0.95 (m, 1 H), 0.98 – 1.12 (m, 2 H), 1.25 – 1.38 (m, 3 H), 1.40 – 1.53 (m, 4 H), 1.56 (d, *J*=9.28 Hz, 2 H), 1.66 – 1.79 (m, 2 H), 2.02 – 2.12 (m, 2 H), 2.86 – 2.97 (m, 1 H), 3.00 – 3.08 (m, 1 H), 3.09 – 3.16 (m, 1 H), 3.27 (dd, *J*=10.04, 4.70 Hz, 1 H), 3.56 – 3.66 (m, 1 H), 3.70 – 3.80 (m, 1 H), 4.64 (t, *J*=5.00 Hz, 1 H), 6.53 (s, 1 H), 7.41 – 7.46 (m, 1 H), 7.49 (d, *J*=5.30 Hz, 1 H), 7.51 – 7.55 (m, 2 H), 7.68 – 7.75 (m, 2 H), 7.82 – 7.89 (m, 4 H), 7.95 (d, *J*=8.37 Hz, 1 H). HRMS (ESI) calcd for $C_{28}H_{37}N_3O_5S$: [M+H]: 528.2532 Found: 528.2568.

4.1.4.11. (S)-3-cyclohexyl-2-((2-(1,3-dioxoisoindolin-2-yl)ethyl)sulfonamido)-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 5k: White solid (yield 94%); M.p 65–66 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.83 – 1.01 (m, 2 H), 1.06 – 1.21 (m, 4 H), 1.41 – 1.74 (m, 6 H), 1.80 (d, *J*=11.74 Hz, 2 H), 1.95 – 2.02 (m, 2 H), 2.30 – 2.51 (m, 2 H), 3.24 – 3.38 (m, 2 H), 3.50 – 3.59 (m, 3 H), 3.64 (d, *J*=4.15 Hz, 1 H), 3.84 – 3.97 (m, 1 H), 4.03 (br. s., 1 H), 4.21 – 4.34 (m, 1 H), 4.64 (s, 2 H), 5.88 (s, 1 H), 7.33 – 7.41

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(m, 2 H), 7.48 – 7.56 (m, 1 H), 7.59 (d, *J*=6.18 Hz, 1 H), 7.71 – 7.76 (m, 1 H), 8.22 (d, *J*=7.30 Hz, 1 H). HRMS (ESI) calcd for $C_{26}H_{36}N_4O_7S$: [M+H]: 549.2383 Found: 549.2363.

4.1.4.12. (S)-3-cyclohexyl-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)-2-((3-(4-methoxyphenoxy)propyl)sulfonamido)propanamide 5l: White solid (yield 94%); M.p 58–61 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.87 – 1.02 (m, 2 H), 1.10 – 1.23 (m, 2 H), 1.42 – 1.58 (m, 3 H), 1.59 – 1.74 (m, 6 H), 1.78 – 1.87 (m, 2 H), 2.00 – 2.09 (m, 1 H), 2.20 – 2.30 (m, 2 H), 2.36 (ddd, *J*=9.18, 6.18, 3.34 Hz, 1 H), 2.41 – 2.51 (m, 1 H), 3.16 – 3.26 (m, 2 H), 3.27 – 3.33 (m, 2 H), 3.36 (d, *J*=6.05 Hz, 1 H), 3.54 – 3.61 (m, 1 H), 3.63 – 3.71 (m, 1 H), 3.76 (s, 3 H), 3.94 – 4.05 (m, 4 H), 5.75 (d, *J*=8.84 Hz, 1 H), 6.01 (s, 1 H), 6.78 – 6.86 (m, 4 H), 7.96 (d, *J*=6.98 Hz, 1 H). HRMS (ESI) calcd for $C_{26}H_{41}N_3O_7S$: [M+H]: 540.2743 Found: 540.2773.

4.1.4.13. (S)-3-cyclohexyl-N-((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)-2-(octylsulfonamido)propanamide 5m: White solid (yield 91%); M.p 65–66 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.88 (t, *J*=6.71 Hz, 3 H) 0.93 – 1.03 (m, 2 H), 1.10 – 1.21 (m, 2 H), 1.22 – 1.33 (m, 10 H), 1.36 – 1.44 (m, 2 H), 1.44 – 1.51 (m, 1 H), 1.52 – 1.58 (m, 1 H), 1.59 – 1.75 (m, 5 H), 1.76 – 1.90 (m, 4 H), 2.01 – 2.11 (m, 2 H), 2.37 – 2.53 (m, 2 H), 2.99 (t, *J*=7.96 Hz, 2 H), 3.33 – 3.40 (m, 2 H), 3.54 – 3.61 (m, 1 H), 3.64 – 3.70 (m, 1 H), 3.93 (td, *J*=9.08, 5.22 Hz, 1 H), 3.97 – 4.06 (m, 1 H), 5.66 (d, *J*=8.98 Hz, 1 H), 6.12 (s, 1 H), 7.82 (d, *J*=7.27 Hz, 1 H). HRMS (ESI) calcd for $C_{24}H_{45}N_3O_5S$: [M+H]: 488.3158 Found: 488.3143.

4.1.5. Synthesis of aldehydes 6a–m—Compound ***5*** (5 mmol) was dissolved in anhydrous dichloromethane (50 mL) under a nitrogen atmosphere and cooled to 0 °C. Dess-Martin periodinane reagent (3.18 g, 7.5 mmol, 1.5 eq) was added to the reaction mixture with stirring. The ice bath was removed and the reaction mixture was stirred at room temperature for 3 h (monitoring by TLC indicated complete disappearance of the starting material). A solution of 10% aqueous sodium thiosulfate (20 mL) was added and the solution was stirred for 15 minutes. The aqueous layer was removed and the organic layer was washed with 10% aqueous sodium thiosulfate (20 mL), followed by saturated aqueous sodium bicarbonate (2 × 20 mL), water (2 × 20 mL) and brine (20 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The yellow residue was purified by flash chromatography using silica gel (methylene chloride/ethyl acetate/methanol) to yield a white solid ***6a–m***.

4.1.5.1. (S)-3-cyclohexyl-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)-2-(phenylsulfonamido)propanamide 6a: White solid (yield 74%); M.p 69–71 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.73 (dd, *J*=11.52, 2.93 Hz, 1 H), 0.86 (td, *J*=11.91, 9.37 Hz, 1 H), 0.93 – 1.21 (m, 3 H), 1.24 – 1.36 (m, 1 H), 1.47 (ddd, *J*=14.16, 9.47, 4.88 Hz, 2 H), 1.51 – 1.66 (m, 4 H), 1.71 – 1.78 (m, 2 H), 1.81 – 1.92 (m, 2 H), 2.31 – 2.42 (m, 2 H), 3.34 – 3.43 (m, 2 H), 3.88 (td, *J*=9.28, 4.88 Hz, 1 H), 4.11 (dt, *J*=8.89, 6.10 Hz, 1 H), 6.14 (d, *J*=8.59 Hz, 1 H), 6.41 (s, 1 H), 7.45 – 7.58 (m, 3 H), 7.86 – 7.91 (m, 2 H), 8.36 (d, *J*=5.86 Hz, 1 H), 9.23 (d, *J*=0.78 Hz, 1 H). HRMS (ESI) calcd for $C_{22}H_{31}N_3O_5S$: [M-]: 449.1984. Found: 449.1981.

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4.1.5.2. (S)-3-cyclohexyl-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)-2-((phenylmethyl)sulfonamido)propanamide 6b: White solid (yield 76%); M.p 62–64 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.80 – 0.97 (m, 2 H), 1.09 – 1.30 (m, 3 H), 1.36 – 1.55 (m, 2 H), 1.57 – 1.79 (m, 6 H), 1.81 – 1.90 (m, 1 H), 1.92 – 2.02 (m, 2 H), 2.34 – 2.43 (m, 1 H), 2.47 – 2.57 (m, 1 H), 3.31 – 3.39 (m, 2 H), 3.98 (td, *J*=8.50, 6.05 Hz, 1 H), 4.22 – 4.32 (m, 2 H), 4.33 – 4.41 (m, 1 H), 5.61 (d, *J*=8.59 Hz, 1 H), 6.25 (s, 1 H), 7.33 – 7.38 (m, 3 H), 7.40 – 7.45 (m, 2 H), 8.26 (d, *J*=6.25 Hz, 1 H), 9.49 (s, 1 H). HRMS (ESI) calcd for $C_{23}H_{33}N_3O_5S$: [M-]: 463.2141 Found: 463.2143.

4.1.5.3. (S)-3-cyclohexyl-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)-2-((2-phenylethyl)sulfonamido)propanamide 6c: White solid (yield 74%); M.p 60–62 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.84 – 1.04 (m, 2 H), 1.09 – 1.30 (m, 2 H), 1.43 – 1.61 (m, 2 H), 1.62 – 1.76 (m, 6 H), 1.78 – 1.89 (m, 2 H), 1.89 – 2.07 (m, 2 H), 2.20 – 2.33 (m, 1 H), 2.34 – 2.46 (m, 1 H), 3.02 – 3.17 (m, 2 H), 3.19 – 3.43 (m, 4 H), 4.12 (td, *J*=8.96, 5.42 Hz, 1 H), 4.33 (dt, *J*=10.23, 5.28 Hz, 1 H), 5.72 (d, *J*=9.08 Hz, 1 H), 6.30 (br. s., 1 H), 7.16 – 7.25 (m, 3 H), 7.26 – 7.34 (m, 2 H), 8.45 (d, *J*=5.96 Hz, 1 H), 9.48 (s, 1 H). HRMS (ESI) calcd for $C_{24}H_{35}N_3O_5S$: [M-]: 477.2297. Found: 477.2296.

4.1.5.4. (S)-3-cyclohexyl-2-((4-fluorophenyl)sulfonamido)-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 6d: White solid (yield 68%); M.p 68–70 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.64 – 0.91 (m, 2 H), 0.95 – 1.20 (m, 3 H), 1.29 – 1.38 (m, 1 H), 1.42 – 1.66 (m, 6 H), 1.71 – 1.77 (m, 1 H), 1.81 – 1.92 (m, 2 H), 2.28 – 2.47 (m, 2 H), 3.34 – 3.44 (m, 2 H), 3.79 – 3.89 (m, 1 H), 4.12 (q, *J*=7.08 Hz, 1 H), 6.15 (d, *J*=8.69 Hz, 1 H), 6.35 (br. s., 1 H), 7.11 – 7.23 (m, 2 H), 7.87 – 7.95 (m, 3 H), 8.44 (d, *J*=5.52 Hz, 1 H), 9.33 (s, 1 H). HRMS (ESI) calcd for $C_{22}H_{30}N_3O_5FS$: [M-]: 467.1890. Found: 467.1889.

4.1.5.5. (S)-2-((3-chlorophenyl)sulfonamido)-3-cyclohexyl-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 6e: White solid (yield 76%); M.p 52–53 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.70 – 0.96 (m, 3 H), 1.00 – 1.23 (m, 4 H), 1.33 – 1.43 (m, 1 H), 1.45 – 1.71 (m, 6 H), 1.80 – 1.91 (m, 2 H), 2.33 – 2.48 (m, 2 H), 3.35 – 3.45 (m, 2 H), 3.85 – 3.95 (m, 1 H), 3.99 – 4.09 (m, 1 H), 6.11 (d, *J*=8.84 Hz, 1 H), 6.27 (br. s., 1 H), 7.41 – 7.49 (m, 1 H), 7.49 – 7.57 (m, 1 H), 7.75 – 7.81 (m, 1 H), 7.85 – 7.90 (m, 1 H), 8.67 (d, *J*=4.98 Hz, 1 H), 9.27 (s, 1 H). HRMS (ESI) calcd for $C_{22}H_{30}N_3O_5ClS$: [M-]: 483.1595. Found: 483.1593.

4.1.5.6. (S)-2-((4-chlorophenyl)sulfonamido)-3-cyclohexyl-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 6f: White solid (yield 76%); M.p 72–74 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.69 – 0.93 (m, 2 H), 0.97 – 1.22 (m, 4 H), 1.25 – 1.38 (m, 1 H), 1.43 – 1.72 (m, 6 H), 1.81 – 1.91 (m, 2 H), 2.02 – 2.15 (m, 1 H), 2.24 – 2.34 (m, 1 H), 2.35 – 2.44 (m, 1 H), 3.36 – 3.47 (m, 2 H), 3.83 (td, *J*=9.01, 5.18 Hz, 1 H), 4.03 – 4.15 (m, 1 H), 6.26 (d, *J*=8.69 Hz, 1 H), 6.38 (s, 1 H), 7.41 – 7.53 (m, 2 H), 7.82 (d, *J*=8.54 Hz, 2 H), 8.51 (d, *J*=5.37 Hz, 1 H), 9.33 (s, 1 H). HRMS (ESI) calcd for $C_{22}H_{30}N_3O_5ClS$: [M-]: 483.1595. Found: 483.1591.

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4.1.5.7. (S)-2-(((4-chlorophenyl)methyl)sulfonamido)-3-cyclohexyl-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 6g: White solid (yield 75%); M.p 52–53 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.80 – 0.97 (m, 2 H), 1.10 – 1.31 (m, 4 H), 1.36 – 1.54 (m, 1 H), 1.56 – 1.78 (m, 6 H), 1.80 – 1.90 (m, 1 H), 1.92 – 1.98 (m, 2 H), 2.36 – 2.46 (m, 1 H), 2.47 – 2.57 (m, 1 H), 3.32 – 3.42 (m, 2 H), 3.92 – 4.00 (m, 1 H), 4.12 (q, *J*=7.14 Hz, 2 H), 4.28 – 4.37 (m, 1 H), 5.69 (d, *J*=8.59 Hz, 1 H), 6.23 (s, 1 H), 7.30 – 7.43 (m, 4 H), 8.39 (d, *J*=5.66 Hz, 1 H), 9.49 (s, 1 H). HRMS (ESI) calcd for $C_{23}H_{33}N_3O_5ClS$: [M-]: 497.1751. Found: 497.1754.

4.1.5.8. (S)-2-((2-(3-chlorophenyl)ethyl)sulfonamido)-3-cyclohexyl-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 6h: White solid (yield 73%); M.p 53–55 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.84 – 1.03 (m, 2 H), 1.08 – 1.31 (m, 4 H), 1.43 – 1.57 (m, 2 H), 1.60 – 1.86 (m, 6 H), 1.89 – 1.97 (m, 2 H), 2.24 – 2.36 (m, 1 H), 2.36 – 2.48 (m, 1 H), 3.00 – 3.13 (m, 2 H), 3.16 – 3.41 (m, 4 H), 4.06 – 4.14 (m, 1 H), 4.27 – 4.36 (m, 1 H), 5.71 (d, *J*=9.28 Hz, 1 H), 6.22 (s, 1 H), 7.08 (d, *J*=6.83 Hz, 1 H), 7.16 – 7.24 (m, 3 H), 8.56 (d, *J*=5.76 Hz, 1 H), 9.47 (s, 1 H). HRMS (ESI) calcd for $C_{24}H_{34}N_3O_5ClS$: [M-]: 511.1908. Found: 511.1907.

4.1.5.9. (S)-3-cyclohexyl-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)-2-(thiophene-2-sulfonamido)propanamide 6i: White solid (yield 75%); M.p 68–69 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.70 – 0.95 (m, 2 H), 1.01 – 1.25 (m, 3 H), 1.30 – 1.41 (m, 1 H), 1.46 – 1.70 (m, 6 H), 1.74 – 2.00 (m, 4 H), 2.32 – 2.51 (m, 2 H), 3.40 (d, *J*=9.08 Hz, 2 H), 3.98 (td, *J*=9.01, 4.64 Hz, 1 H), 4.15 – 4.25 (m, 1 H), 6.32 (d, *J*=8.49 Hz, 1 H), 6.48 (br. s., 1 H), 7.03 – 7.10 (m, 1 H), 7.55 – 7.60 (m, 1 H), 7.63 (dd, *J*=3.52, 0.98 Hz, 1 H), 8.35 (d, *J*=5.86 Hz, 1 H), 9.31 (s, 1 H). HRMS (ESI) calcd for $C_{20}H_{29}N_3O_5S_2$: [M-]: 455.1549. Found: 455.1546.

4.1.5.10. (S)-2-([1,1′-biphenyl]-4-sulfonamido)-3-cyclohexyl-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 6j: White solid (yield 75%); M.p 56–58 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.68 – 0.80 (m, 2 H), 0.84 – 0.90 (m, 1 H), 0.91 – 0.97 (m, 1 H), 0.98 – 1.08 (m, 1 H), 1.09 – 1.20 (m, 2 H), 1.31 – 1.44 (m, 1 H), 1.45 – 1.68 (m, 6 H), 1.72 – 1.81 (m, 1 H), 1.81 – 1.89 (m, 1 H), 1.90 – 2.00 (m, 1 H), 2.17 – 2.37 (m, 2 H), 3.15 – 3.25 (m, 1 H), 3.27 – 3.38 (m, 1 H), 3.87 (td, *J*=9.00, 4.91 Hz, 1 H), 4.04 – 4.16 (m, 1 H), 6.13 (d, *J*=8.45 Hz, 1 H), 6.37 (br. s., 1 H), 7.39 – 7.44 (m, 1 H), 7.45 – 7.51 (m, 1 H), 7.58 (d, *J*=7.13 Hz, 2 H), 7.68 (d, *J*=8.35 Hz, 2 H), 7.94 (d, *J*=8.35 Hz, 2 H), 8.44 (d, *J*=5.61 Hz, 1 H), 9.30 (s, 1 H). HRMS (ESI) calcd for $C_{28}H_{35}N_3O_5S$: [M-]: 525.2297. Found: 525.2297.

4.1.5.11. (S)-3-cyclohexyl-2-((2-(1,3-dioxoisoindolin-2-yl)ethyl)sulfonamido)-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 6k: White solid (yield 73%); M.p 59–61 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.80 – 1.04 (m, 2 H), 1.09 – 1.28 (m, 3 H), 1.43 – 1.73 (m, 6 H), 1.80 (br. s., 2 H), 1.93 – 2.02 (m, 1 H), 2.16 – 2.23 (m, 1 H), 2.31 – 2.46 (m, 2 H), 2.48 – 2.62 (m, 1 H), 3.24 – 3.55 (m, 4 H), 3.63 – 3.76 (m, 1 H), 4.04 – 4.19 (m, 2 H), 4.25 – 4.40 (m, 1 H), 5.81 (d, *J*=8.69 Hz, 1 H), 6.23 (t, *J*=7.64 Hz, 1 H), 7.45 – 7.61 (m, 1 H), 7.67 – 7.79 (m, 1 H), 7.84 (dd, *J*=5.37, 3.03 Hz, 1 H), 7.94 (d, *J*=7.62 Hz, 1

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H), 8.57 (d, *J*=5.86 Hz, 1 H), 9.48 (s, 1 H). HRMS (ESI) calcd for $C_{26}H_{34}N_4O_7S$: [M-]: 546.2148. Found: 546.2144.

4.1.5.12. (S)-3-cyclohexyl-2-((3-(4-methoxyphenoxy)propyl)sulfonamido)-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 6l: White solid (yield 73%); M.p 47–49 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.85 – 1.03 (m, 2 H), 1.06 – 1.31 (m, 2 H), 1.44 – 1.60 (m, 2 H), 1.62 – 1.76 (m, 6 H), 1.79 – 1.88 (m, 2 H), 1.91 – 2.00 (m, 2 H), 2.22 – 2.31 (m, 2 H), 2.31 – 2.41 (m, 1 H), 2.44 – 2.55 (m, 1 H), 3.15 – 3.28 (m, 2 H), 3.28 – 3.39 (m, 2 H), 3.76 (s, 3 H), 3.96 – 4.03 (m, 2 H), 4.09 (td, *J*=9.01, 5.42 Hz, 1 H), 4.29 – 4.37 (m, 1 H), 5.58 (d, *J*=9.18 Hz, 1 H), 6.11 (s, 1 H), 6.77 – 6.87 (m, 4 H), 8.53 (d, *J*=5.66 Hz, 1 H), 9.49 (s, 1 H). HRMS (ESI) calcd for $C_{26}H_{39}N_3O_7S$: [M-]: 537.2509. Found: 537.2508.

4.1.5.13. (S)-3-cyclohexyl-2-(octylsulfonamido)-N-((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)propanamide 6m: sticky solid (yield 74%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.84 – 1.03 (m, 4 H), 1.11 – 1.35 (m, 8 H), 1.38 (d, *J*=6.44 Hz, 2 H), 1.48 – 1.61 (m, 3 H), 1.62 – 1.77 (m, 8 H), 1.77 – 1.89 (m, 4 H), 1.94 – 2.02 (m, 2 H), 2.38 – 2.48 (m, 1 H), 2.48 – 2.59 (m, 1 H), 3.00 (t, *J*=7.96 Hz, 2 H), 3.34 – 3.43 (m, 2 H), 4.05 (td, *J*=9.01, 5.22 Hz, 1 H), 4.33 – 4.41 (m, 1 H), 5.52 (d, *J*=9.18 Hz, 1 H), 6.21 (s, 1 H), 8.38 (d, *J*=5.96 Hz, 1 H), 9.51 (s, 1 H). HRMS (ESI) calcd for $C_{24}H_{43}N_3O_5S$: [M-]: 485.2923. Found: 485.2922.

4.1.6. Synthesis of bisulfite adducts 7a–m—To a solution of aldehyde ***6*** (5 mmol) in dry ethyl acetate (20 mL) was added absolute ethanol (12 mL) with stirring, followed by a solution of sodium bisulfite (540 mg; 5 mmol) in water (5 mL). The reaction mixture was stirred for 3 h at 50 °C and then allowed to cool to room temperature. The precipitate was vacuum filtered and the solid was thoroughly washed with absolute ethanol. The filtrate was dried over anhydrous sodium sulfate, filtered, and concentrated to yield a yellowish oil which was treated with ethyl ether (2 × 50 mL) to form a white solid. The white solid was stirred with ethyl ether (30 mL) and ethyl acetate (15 mL) for 5 minutes. Careful removal of the solvent using a pipette yielded compounds ***7a–m.***

4.1.6.1. Sodium (2S)-2-((S)-3-cyclohexyl-2-(phenylsulfonamido)propanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7a: White solid (yield 68%); M.p 154–156 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.57 – 0.82 (m, 2 H), 0.91 (d, *J*=9.18 Hz, 1 H), 1.00 – 1.14 (m, 2 H), 1.16 – 1.25 (m, 1 H), 1.29 (d, *J*=8.40 Hz, 1 H), 1.51 (d, *J*=11.33 Hz, 6 H), 1.68 – 1.87 (m, 1 H), 1.89 – 2.10 (m, 2 H), 2.95 – 3.15 (m, 2 H), 3.71 – 3.81 (m, 2 H), 3.87 (d, *J*=3.91 Hz, 1 H), 4.07 (t, *J*=9.23 Hz, 1 H), 5.35 (d, *J*=5.57 Hz, 1 H), 5.41 (d, *J*=5.86 Hz, 1 H), 7.45 (br. s., 1 H), 7.49 – 7.65 (m, 4 H), 7.71 – 7.93 (m, 3 H). HRMS (ESI) calcd for $C_{22}H_{32}N_3O_8S_2Na$: [M-]: 553.1529. Found: 553.1531.

4.1.6.2. Sodium (2S)-2-((S)-3-cyclohexyl-2-((phenylmethyl)sulfonamido)propanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7b: White solid (yield 76%); M.p 137–139 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.85 (br. s., 2 H), 1.01 – 1.28 (m, 3 H), 1.31 – 1.48 (m, 2 H), 1.51 – 1.83 (m, 6 H), 1.86 – 2.26 (m, 4 H), 2.90 – 3.03 (m, 1 H), 3.11 (d, *J*=5.86 Hz, 1 H), 3.84 – 4.03 (m, 2 H), 4.19 – 4.30 (m, 1 H), 4.33 (s, 1 H), 5.46 (d, *J*=5.47 Hz, 1 H), 5.57 (d, *J*=6.25 Hz, 1 H), 7.35 (br. s., 5 H), 7.46 (d, *J*=8.20

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Hz, 1 H), 7.51 (br. s., 1 H), 7.73 (d, *J*=8.98 Hz, 1 H), 7.97 (d, *J*=8.98 Hz, 1 H). HRMS (ESI) calcd for $C_{23}H_{34}N_3O_8S_2Na$: [M-]: 567.1685. Found: 567.1682.

4.1.6.3. Sodium (2S)-2-((S)-3-cyclohexyl-2-((2-phenylethyl)sulfonamido)propanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7c: White solid (yield 65%); M.p 74–76 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.78 – 0.98 (m, 2 H), 1.04 – 1.29 (m, 4 H), 1.44 (br. s., 3 H), 1.65 (br. s., 4 H), 1.76 – 1.93 (m, 2 H), 2.00 (br. s., 1 H), 2.16 (br. s., 1 H), 2.69 (d, *J*=8.40 Hz, 1 H), 2.90 (d, *J*=9.96 Hz, 2 H), 3.05 (br. s., 4 H), 3.84 (br. s., 1 H), 3.96 (br. s., 1 H), 4.17 – 4.30 (m, 1 H), 7.26 (d, *J*=4.59 Hz, 5 H), 7.47 (br. s., 1 H), 7.59 – 7.64 (m, 1 H), 7.94 (br. s., 1 H), 8.64 (d, *J*=7.03 Hz, 1 H). HRMS (ESI) calcd for $C_{24}H_{36}N_3O_8S_2Na$: [M-]: 581.1842. Found: 581.1841.

4.1.6.4. Sodium (2S)-2-((S)-3-cyclohexyl-2-((4-fluorophenyl)sulfonamido)propanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7d: White solid (yield 78%); M.p 75–77 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.64 – 0.84 (m, 2 H), 0.88 – 0.99 (m, 1 H), 1.04 – 1.13 (m, 3 H), 1.16 – 1.26 (m, 2 H), 1.28 – 1.39 (m, 2 H), 1.55 (d, *J*=9.57 Hz, 4 H), 1.69 – 1.85 (m, 1 H), 1.89 – 1.96 (m, 1 H), 2.05 – 2.14 (m, 1 H), 3.00 – 3.16 (m, 2 H), 3.54 – 3.63 (m, 1 H), 3.70 – 3.81 (m, 1 H), 3.98 – 4.08 (m, 1 H), 5.31 (dd, *J*=16.23, 5.93 Hz, 1 H), 7.32 – 7.46 (m, 2 H), 7.53 (d, *J*=4.74 Hz, 1 H), 7.63 – 7.70 (m, 1 H), 7.74 (s, 1 H), 7.79 – 7.90 (m, 2 H), 7.95 (br. s., 1 H). HRMS (ESI) calcd for $C_{22}H_{31}N_3O_8FS_2Na$: [M-]: 571.1434. Found: 571.1435.

4.1.6.5. Sodium (2S)-2-((S)-2-((3-chlorophenyl)sulfonamido)-3-cyclohexylpropanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate (7e): *White solid (y*ield 78%); M.p 74–75 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.64 – 0.84 (m, 3 H), 0.91 (d, *J*=6.40 Hz, 1 H), 1.01 – 1.21 (m, 4 H), 1.28 – 1.37 (m, 1 H), 1.42 – 1.63 (m, 4 H), 1.67 – 1.87 (m, 2 H), 1.88 – 2.14 (m, 2 H), 3.01 – 3.21 (m, 2 H), 3.75 – 3.90 (m, 1 H), 4.01 – 4.15 (m, 1 H), 4.26 – 4.42 (m, 1 H), 5.27 (d, *J*=5.27 Hz, 1 H), 6.02 – 6.10 (m, 1 H), 7.44 (br. s., 1 H), 7.50 – 7.60 (m, 1 H), 7.61 – 7.69 (m, 1 H), 7.70 – 7.75 (m, 1 H), 7.76 – 7.83 (m, 1 H), 8.05 – 8.17 (m, 1 H), 8.48 – 8.60 (m, 1 H). HRMS (ESI) calcd for $C_{22}H_{31}N_3O_8ClS_2Na$: [M-]: 587.1139. Found: 587.1134.

4.1.6.6. Sodium (2S)-2-((S)-2-((4-chlorophenyl)sulfonamido)-3-cyclohexylpropanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7f: White soild (yield 61%); M.p 152–153 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.64 – 0.86 (m, 2 H), 1.01 – 1.12 (m, 4 H), 1.15 – 1.27 (m, 1 H), 1.29 – 1.37 (m, 2 H), 1.39 – 1.62 (m, 6 H), 1.71 – 1.93 (m, 2 H), 1.97 – 2.09 (m, 1 H), 3.01 – 3.13 (m, 2 H), 3.73 – 3.81 (m, 1 H), 3.87 (dd, *J*=5.53, 2.18 Hz, 1 H), 4.00 – 4.12 (m, 1 H), 5.28 – 5.39 (m, 1 H), 7.45 (d, *J*=1.88 Hz, 1 H), 7.56 – 7.65 (m, 2 H), 7.73 – 7.87 (m, 3 H), 8.03 (d, *J*=13.23 Hz, 1 H). HRMS (ESI) calcd for $C_{22}H_{31}N_3O_8ClS_2Na$: [M-]: 587.1139. Found: 587.1137.

4.1.6.7. Sodium (2S)-2-((S)-2-(((4-chlorophenyl)methyl)sulfonamido)-3-cyclohexylpropanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7g: White solid (yield 61%); M.p 76–78 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.84 (br. s., 2 H), 1.04 – 1.24 (m, 6 H), 1.37 (d, *J*=8.59 Hz, 2 H), 1.65 (d, *J*=8.98 Hz, 6 H), 1.84 – 1.97 (m, 1 H), 2.08 – 2.35 (m, 3 H), 2.95 (d, *J*=7.42 Hz, 1 H), 3.04 – 3.18 (m, 1 H), 3.57 (dd, *J*=13.18,

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6.83 Hz, 1 H), 3.74 (q, *J*=6.77 Hz, 1 H), 3.84 – 3.99 (m, 1 H), 4.17 – 4.38 (m, 1 H), 7.41 (br. s., 3 H), 7.50 – 7.55 (m, 1 H), 7.65 (br. s., 1 H), 7.92 (d, *J*=8.69 Hz, 1 H), 8.64 (d, *J*=6.83 Hz, 1 H). HRMS (ESI) calcd for $C_{23}H_{33}N_3O_8ClS_2Na$: [M-]: 601.1295. Found: 601.1295.

4.1.6.8. Sodium (2S)-2-((S)-2-((2-(3-chlorophenyl)ethyl)sulfonamido)-3-cyclohexyl propanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7h: White solid (yield 71%); M.p 78–80 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.87 (br. s., 2 H), 1.02 – 1.23 (m, 5 H), 1.30 – 1.53 (m, 4 H), 1.54 – 1.71 (m, 4 H), 1.74 – 1.87 (m, 2 H), 1.92 – 2.10 (m, 1 H), 2.65 – 2.79 (m, 1 H), 2.86 – 3.00 (m, 2 H), 3.02 – 3.17 (m, 2 H), 3.73 (q, *J*=7.08 Hz, 1 H), 3.85 – 4.02 (m, 2 H), 4.20 (d, *J*=7.57 Hz, 1 H), 4.68 – 4.78 (m, 1 H), 7.21 – 7.37 (m, 3 H), 7.42 – 7.51 (m, 1 H), 7.55 – 7.62 (m, 1 H), 7.69 (d, *J*=8.79 Hz, 1 H), 7.90 – 7.98 (m, 1 H). HRMS (ESI) calcd for $C_{24}H_{35}N_3O_8ClS_2Na$: [M-]: 615.1452. Found: 615.1459.

4.1.6.9. Sodium (2S)-2-((S)-3-cyclohexyl-2-(thiophene-2-sulfonamido)propan amido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7i: White solid (yield 67%); M.p 156–158 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.64 – 0.83 (m, 2 H), 0.92 – 1.13 (m, 4 H), 1.16 – 1.25 (m, 1 H), 1.28 – 1.38 (m, 1 H), 1.55 (d, *J*=11.38 Hz, 6 H), 1.70 – 1.95 (m, 2 H), 1.97 – 2.15 (m, 2 H), 2.97 – 3.19 (m, 2 H), 3.74 – 3.84 (m, 1 H), 3.84 – 3.94 (m, 1 H), 4.05 – 4.17 (m, 1 H), 5.31 (t, *J*=6.93 Hz, 1 H), 7.09 (t, *J*=4.00 Hz, 1 H), 7.45 (s, 1 H), 7.54 – 7.60 (m, 1 H), 7.73 (d, *J*=8.93 Hz, 1 H), 7.82 – 7.87 (m, 1 H), 8.05 (br. s., 1 H). HRMS (ESI) calcd for $C_{20}H_{30}N_3O_8S_3Na$: [M-]: 599.1093. Found: 599.1094.

4.1.6.10. Sodium (2S)-2-((S)-2-([1,1′-biphenyl]-4-sulfonamido)-3-cyclohexylpropan amido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7j: White solid (yield 59%); M.p 154–156 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.66 (d, *J*=4.98 Hz, 1 H), 0.76 (d, *J*=10.64 Hz, 1 H), 0.87 (br. s., 1 H), 1.00 – 1.13 (m, 4 H), 1.16 – 1.24 (m, 1 H), 1.29 – 1.39 (m, 3 H), 1.41 – 1.62 (m, 6 H), 1.73 – 1.84 (m, 1 H), 1.89 – 2.05 (m, 1 H), 2.85 – 2.93 (m, 1 H), 2.97 – 3.09 (m, 1 H), 3.61 (dd, *J*=10.20, 3.37 Hz, 1 H), 3.71 – 3.83 (m, 1 H), 3.87 – 3.94 (m, 1 H), 5.31 (t, *J*=5.08 Hz, 1 H), 7.38 (d, *J*=3.81 Hz, 1 H), 7.42 – 7.47 (m, 2 H), 7.51 (d, *J*=2.05 Hz, 2 H), 7.58 – 7.66 (m, 1 H), 7.69 – 7.78 (m, 2 H), 7.80 – 7.90 (m, 2 H), 7.94 (d, *J*=7.42 Hz, 1 H). HRMS (ESI) calcd for $C_{28}H_{36}N_3O_8S_2Na$: [M-]: 629.1842. Found: 629.1844.

4.1.6.11 Sdium (2S)-2-((S)-3-cyclohexyl-2-((2-(1,3-dioxoisoindolin-2-yl)ethyl)sulfon amido)propanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7k: White solid (yield 76%); M.p 74–76 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.85 (br. s., 2 H), 0.99 – 1.27 (m, 5 H), 1.41 (br. s., 2 H), 1.53 – 1.74 (m, 6 H), 1.80 – 1.92 (m, 1 H), 2.05 – 2.31 (m, 2 H), 2.96 – 3.14 (m, 2 H), 3.54 – 3.65 (m, 1 H), 3.74 (q, *J*=7.13 Hz, 2 H), 3.94 (d, *J*=6.05 Hz, 3 H), 4.31 – 4.41 (m, 1 H), 6.03 – 6.15 (m, 1 H), 6.63 (dd, *J*=9.03, 2.10 Hz, 1 H), 7.43 – 7.55 (m, 2 H), 7.59 – 7.74 (m, 1 H), 7.83 – 7.94 (m, 2 H), 8.62 – 8.71 (m, 1 H). HRMS (ESI) calcd for $C_{26}H_{35}N_4O_{10}S_2Na$: [M-]: 650.1692. Found: 650.1693.

4.1.6.12. Sodium (2S)-2-((S)-3-cyclohexyl-2-((3-(4-methoxyphenoxy)propyl)sulfon amido)propanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7l: White solid (yield 63%); M.p 65–67 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.85 (d,

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J=10.74 Hz, 2 H), 1.02 – 1.30 (m, 3 H), 1.33 – 1.50 (m, 2 H), 1.52 – 1.69 (m, 6 H), 1.71 – 1.84 (m, 2 H), 1.88 – 2.22 (m, 5 H), 2.93 – 3.04 (m, 2 H), 3.05 – 3.17 (m, 2 H), 3.69 (s, 3 H), 3.80 (br. s., 1 H), 3.85 – 3.92 (m, 1 H), 3.95 – 4.07 (m, 2 H), 4.22 (br. s., 1 H), 5.47 (d, *J*=5.86 Hz, 1 H), 6.85 (s, 4 H), 7.37 – 7.51 (m, 1 H), 7.65 (d, *J*=8.98 Hz, 1 H), 7.94 (d, *J*=8.89 Hz, 1 H). HRMS (ESI) calcd for $C_{26}H_{40}N_3O_{10}S_2Na$: [M-]: 641.2053. Found: 641.2051.

4.1.6.13. Sodium (2S)-2-((S)-3-cyclohexyl-2-(octylsulfonamido)propanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 7m: White solid (yield 65%); M.p 58–60 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.82 – 0.92 (m, 4 H), 1.04 – 1.19 (m, 2 H), 1.24 (br. s., 6 H), 1.36 – 1.50 (m, 2 H), 1.63 (d, *J*=11.42 Hz, 6 H), 1.77 (d, *J*=10.84 Hz, 2 H), 1.86 – 2.04 (m, 2 H), 2.05 – 2.14 (m, 3 H), 2.15 – 2.25 (m, 1 H), 2.83 – 2.96 (m, 2 H), 3.00 – 3.08 (m, 2 H), 3.14 (t, *J*=8.35 Hz, 2 H), 3.76 (d, *J*=8.30 Hz, 1 H), 3.85 (t, *J*=4.88 Hz, 2 H), 3.92 – 4.06 (m, 1 H), 4.22 (t, *J*=9.62 Hz, 1 H), 5.41 (d, *J*=5.86 Hz, 1 H), 7.21 – 7.29 (m, 1 H), 7.47 (d, *J*=5.47 Hz, 1 H), 7.62 (d, *J*=9.08 Hz, 1 H), 7.88 (d, *J*=8.98 Hz, 1 H). HRMS (ESI) calcd for $C_{24}H_{44}N_3O_8S_2Na$: [M-]: 589.2468. Found: 589.2466.

4.1.7. Synthesis of esters 9a–c—To a solution of the hydrochloride salt of ester **3** (10 mmol) in anhydrous THF (40 mL) kept at 0 °C was added slowly N,N-diisopropylethylamine (DIEA) (2.6 mL,20 mmol, 2 eq) with stirring. The appropriate chloroformate (11 mmol, 1.1 eq) was added to the solution and the reaction mixture was stirred for 16 h at room temperature. The solvent was removed and the residue was dissolved in ethyl acetate (100 mL) and washed with water (2 × 50 mL). The ethyl acetate layer was further washed with 5% HCl (2 × 50 mL) and saturated NaCl (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to yield a crude product which was purification by flash chromatography to yield esters **9a–c.**

4.1.7.1. Methyl (S)-2-((S)-2-(((3-chlorophenethoxy)carbonyl)amino)-3-cyclohexylpropan amido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 9a: White solid (yield 73%); M.p 43–45 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.84 – 1.02 (m, 2 H), 1.09 – 1.23 (m, 3 H), 1.38 (d, *J*=2.64 Hz, 1 H), 1.45 – 1.53 (m, 1 H), 1.69 (d, *J*=10.20 Hz, 6 H), 1.79 – 1.95 (m, 2 H), 2.12 – 2.22 (m, 1 H), 2.36 – 2.46 (m, 2 H), 2.87 – 2.94 (m, 2 H), 3.34 (d, *J*=6.88 Hz, 2 H), 3.73 (s, 3 H), 4.20 – 4.35 (m, 3 H), 4.48 (ddd, *J*=11.12, 7.07, 3.88 Hz, 1 H), 5.29 (d, *J*=8.25 Hz, 1 H), 6.18 (s, 1 H), 7.08 – 7.13 (m, 1 H), 7.19 – 7.26 (m, 3 H), 7.84 (d, *J*=5.52 Hz, 1 H). HRMS (ESI) calcd for $C_{26}H_{37}ClN_3O_6$: [M+H]: 522.2371 Found: 522.2372.

4.1.7.2. Methyl (8S,11S)-8-(cyclohexylmethyl)-6,9-dioxo-11-(((S)-2-oxopyrrolidin-3-yl)methyl)-1-phenyl-2,5-dioxa-7,10-diazadodecan-12-oate9b: White solid (yield 67%); M.p 59–61 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.86 – 1.03 (m, 2 H), 1.09 – 1.29 (m, 4 H), 1.34 – 1.44 (m, 2 H), 1.45 – 1.55 (m, 1 H), 1.60 – 1.73 (m, 5 H), 1.75 – 1.95 (m, 2 H), 2.10 – 2.22 (m, 1 H), 2.35 – 2.46 (m, 2 H), 3.30 (d, *J*=8.40 Hz, 2 H), 3.67 (q, *J*=4.65 Hz, 2 H), 3.72 (s, 3 H), 4.18 – 4.28 (m, 2 H), 4.32 (d, *J*=5.66 Hz, 1 H), 4.49 (ddd, *J*=11.05, 7.02, 3.95 Hz, 1 H), 4.56 (s, 2 H), 5.40 (d, *J*=8.30 Hz, 1 H), 6.11 (s, 1 H), 7.28 – 7.31 (m, 1 H),

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7.34 (s, 4 H), 7.76 (d, *J*=6.83 Hz, 1 H). HRMS (ESI) calcd for $C_{27}H_{39}N_3O_7$: [M+H]: 518.2866 Found: 518.2874.

4.1.7.3. Methyl (S)-2-((S)-3-cyclohexyl-2-undecanamidopropanamido)-3-((S)-2-oxopyrrolidin-3-yl)propanoate 9c: White solid (yield 60%); M.p 67–68 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.88 (t, *J*=6.44 Hz, 4 H), 0.91 – 1.03 (m, 2 H), 1.10 – 1.21 (m, 2 H), 1.23 – 1.36 (m, 14 H), 1.37 – 1.44 (m, 1 H), 1.45 – 1.54 (m, 1 H), 1.57 – 1.66 (m, 2 H), 1.70 (d, *J*=8.59 Hz, 2 H), 1.78 – 1.98 (m, 5 H), 2.11 – 2.26 (m, 2 H), 2.37 – 2.48 (m, 2 H), 3.35 (d, *J*=7.81 Hz, 2 H), 3.73 (s, 3 H), 3.98 – 4.12 (m, 2 H), 4.33 (d, *J*=4.78 Hz, 1 H), 4.46 – 4.56 (m, 1 H), 5.21 (d, *J*=7.91 Hz, 1 H), 6.23 (s, 2 H), 7.77 (d, *J*=5.66 Hz, 1 H). HRMS (ESI) calcd for $C_{28}H_{49}N_3O_6$: [M+H]: 524.3700 Found: 524.3712.

4.1.8. Synthesis of alcohols 10a–c—To a solution of ester **9** (5 mmol) in anhydrous THF (30 mL) was added dropwise lithium borohydride (2M in THF, 7.5 mL, 15 mmol), followed by absolute ethyl alcohol (15 mL), and the reaction mixture was stirred at room temperature overnight. The reaction mixture was acidified with 5% HCl and the pH adjusted to ~2. Removal of the solvent left a residue which was taken up in ethyl acetate (100 mL). The organic layer was washed with brine (25 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to yield compounds **10a–c.**

4.1.8.1. 3-chlorophenethyl ((S)-3-cyclohexyl-1-(((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)amino)-1-oxopropan-2-yl)carbamate 10a: White solid (yield 93%); M.p 55–56 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.86 – 0.96 (m, 2 H), 1.18 (d, *J*=17.43 Hz, 3 H), 1.29 – 1.37 (m, 2 H), 1.39 – 1.52 (m, 1 H), 1.66 (br. s., 6 H), 1.76 – 1.88 (m, 2 H), 2.00 (br. s., 1 H), 2.34 – 2.49 (m, 2 H), 2.84 – 2.94 (m, 2 H), 3.33 (d, *J*=8.18 Hz, 2 H), 3.61 (d, *J*=15.55 Hz, 2 H), 3.94 – 4.02 (m, 1 H), 4.19 – 4.31 (m, 3 H), 5.36 (d, *J*=7.23 Hz, 1 H), 6.20 (s, 1 H), 7.10 (d, *J*=6.47 Hz, 1 H), 7.17 – 7.25 (m, 3 H), 7.77 (d, *J*=7.80 Hz, 1 H). HRMS (ESI) calcd for $C_{25}H_{36}ClN_3O_5$: [M+H]: 494.2422 Found: 494.2412.

4.1.8.2. 2-(benzyloxy)ethyl ((S)-3-cyclohexyl-1-(((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)amino)-1-oxopropan-2-yl)carbamate 10b: White solid (yield 97%); M.p 68–69 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.83 – 1.04 (m, 2 H), 1.11 – 1.24 (m, 3 H), 1.31 – 1.41 (m, 1 H), 1.51 (dd, *J*=8.59, 5.27 Hz, 2 H), 1.68 (d, *J*=9.28 Hz, 6 H), 1.80 (d, *J*=8.79 Hz, 2 H), 2.02 (br. s., 1 H), 2.33 – 2.46 (m, 2 H), 3.23 – 3.34 (m, 2 H), 3.55 – 3.61 (m, 3 H), 3.66 (s, 2 H), 3.94 – 4.05 (m, 1 H), 4.24 (s, 2 H), 4.55 (s, 2 H), 5.59 (d, *J*=5.66 Hz, 1 H), 6.33 (s, 1 H), 7.28 – 7.41 (m, 5 H), 7.64 (d, *J*=6.44 Hz, 1 H). HRMS (ESI) calcd for $C_{26}H_{39}N_3O_6$: [M+H]: 490.2917 Found: 490.2907.

4.1.8.3. N-((S)-3-cyclohexyl-1-(((S)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)amino)-1-oxopropan-2-yl)undecanamide 10c: White solid (yield 97%); M.p 78–80 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.88 (t, *J*=6.20 Hz, 3 H), 0.96 (d, *J*=10.94 Hz, 2 H), 1.09 – 1.19 (m, 3 H), 1.20 – 1.34 (m, 14 H), 1.51 (d, *J*=7.42 Hz, 1 H), 1.58 – 1.73 (m, 10 H), 1.80 (d, *J*=10.15 Hz, 2 H), 2.03 (br. s., 1 H), 2.36 – 2.55 (m, 2 H), 3.30 – 3.41 (m, 2 H), 3.63 (s, 3 H), 4.04 (t, *J*=7.00 Hz, 2 H), 4.19 – 4.28 (m, 1 H), 5.34 (d, *J*=7.80 Hz, 1 H), 6.45 (s, 1 H), 7.67 (d, *J*=5.60 Hz, 1 H). HRMS (ESI) calcd for $C_{27}H_{49}N_3O_5$: [M+H]: 496.3750 Found: 496.3745.

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4.1.9. Synthesis of aldehydes 11a–c—Compound **10** (5 mmol) was dissolved in anhydrous dichloromethane (50 mL) under a nitrogen atmosphere and cooled to 0°C. Dess-Martin periodinane reagent (3.18 g, 7.5 mmol, 1.5 eq) was added to the reaction mixture with stirring. The ice bath was removed and the reaction mixture was stirred at room temperature for 3 h (monitoring by TLC indicated complete disappearance of the starting material). A solution of 10% aqueous sodium thiosulfate (20 mL) was added and the solution was stirred for another 15 minutes. The aqueous layer was removed and the organic layer was washed with 10% aqueous sodium thiosulfate (20 mL), followed by saturated aqueous sodium bicarbonate (2 × 20 mL), water (2 × 20 mL) and brine (20 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The yellow residue was purified by flash chromatography (silica gel/methylene chloride/ethyl acetate/methanol) to yield aldehydes ***11a–c***.

4.1.9.1. 3-chlorophenethyl ((S)-3-cyclohexyl-1-oxo-1-(((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)amino)propan-2-yl)carbamate 11a: White solid (yield 76%); M.p 45–47 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.86 – 1.01 (m, 2 H), 1.10 – 1.27 (m, 2 H), 1.30 – 1.45 (m, 2 H), 1.47 – 1.58 (m, 1 H), 1.60 – 1.89 (m, 6 H), 1.92 – 2.00 (m, 2 H), 2.01 – 2.10 (m, 1 H), 2.36 – 2.51 (m, 2 H), 2.91 (t, *J*=6.69 Hz, 2 H), 3.31 – 3.43 (m, 2 H), 4.16 – 4.37 (m, 4 H), 5.25 (d, *J*=8.20 Hz, 1 H), 5.96 (br. s., 1 H), 7.10 (d, *J*=6.35 Hz, 1 H), 7.17 – 7.25 (m, 3 H), 8.36 (d, *J*=5.57 Hz, 1 H), 9.49 (s, 1 H). HRMS (ESI) calcd for $C_{25}H_{34}ClN_3O_5$: [M-]: 491.2187. Found: 491.2198.

4.1.9.2. 2-(benzyloxy)ethyl ((S)-3-cyclohexyl-1-oxo-1-(((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)amino)propan-2-yl)carbamate 11b: White solid (yield 78%); M.p 38–40 °C; ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.84 – 1.04 (m, 2 H), 1.11 – 1.30 (m, 2 H), 1.39 (d, *J*=3.22 Hz, 1 H), 1.48 – 1.57 (m, 1 H), 1.71 (dd, *J*=13.57, 8.69 Hz, 6 H), 1.78 – 1.88 (m, 2 H), 1.93 – 1.98 (m, 1 H), 2.01 – 2.09 (m, 1 H), 2.32 – 2.52 (m, 2 H), 3.25 – 3.36 (m, 2 H), 3.67 (t, *J*=4.54 Hz, 4 H), 4.25 (d, *J*=2.93 Hz, 1 H), 4.30 – 4.39 (m, 1 H), 4.51 – 4.62 (m, 2 H), 5.37 (d, *J*=8.01 Hz, 1 H), 5.99 (br s, 1 H), 7.34 (s, 5 H), 8.24 (d, *J*=4.88 Hz, 1 H), 9.49 (s, 1 H). HRMS (ESI) calcd for $C_{26}H_{37}N_3O_6$: [M-]: 487.2682. Found: 487.2683.

4.1.9.3. N-((S)-3-cyclohexyl-1-oxo-1-(((S)-1-oxo-3-((S)-2-oxopyrrolidin-3-yl)propan-2-yl)amino) propan-2-yl)undecanamide 11c: Sticky solid (yield 71%); ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ ppm 0.88 (t, *J*=6.71 Hz, 2 H), 0.91 – 1.01 (m, 2 H), 1.07 – 1.36 (m, 16 H), 1.49 – 1.64 (m, 4 H), 1.69 (d, *J*=5.71 Hz, 6 H), 1.77 – 1.90 (m, 2 H), 1.91 – 1.99 (m, 1 H), 2.02 – 2.14 (m, 1 H), 2.27 – 2.58 (m, 3 H), 3.31 – 3.42 (m, 2 H), 3.94 – 4.12 (m, 2 H), 4.28 – 4.39 (m, 1 H), 4.41 – 4.53 (m, 1 H), 5.20 – 5.27 (m, 1 H), 6.16 – 6.24 (m, 1 H), 8.21 – 8.28 (m, 1 H), 9.57 (s, 1 H). HRMS (ESI) calcd for $C_{27}H_{47}N_3O_5$: [M-]: 493.3516. Found: 493.3518.

4.1.10. Synthesis of bisulfite adducts 12a–c—To a solution of aldehyde **11** (5 mmol) in dry ethyl acetate (20 mL) was added absolute ethanol (12 mL) with stirring, followed by a solution of sodium bisulfite (540 mg; 5 mmol) in water (5 mL). The reaction mixture was stirred for 3 h at 50 °C. The reaction mixture was allowed to cool to room temperature and then vacuum filtered. The solid was thoroughly washed with absolute ethanol and the filtrate

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was dried over anhydrous sodium sulfate, filtered, and concentrated to yield a yellowish oil. The oily product was treated with ethyl ether (2 × 50 mL) to form white solid. The white solid was stirred with ethyl ether (30 mL) and ethyl acetate (15 mL) for 5 minutes. Careful removal of the solvent using a pipette yielded compounds ***12a–c***.

4.1.10.1. Sodium (2S)-2-((S)-2-(((3-chlorophenethoxy)carbonyl)amino)-3-cyclohexylprop anamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 12a: White solid (yield 68%); M.p 76–78 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.76 – 0.92 (m, 2 H), 1.02 – 1.17 (m, 3 H), 1.25 (d, *J*=13.38 Hz, 1 H), 1.40 (d, *J*=4.69 Hz, 2 H), 1.51 – 1.75 (m, 6 H), 1.80 – 2.02 (m, 1 H), 2.03 – 2.23 (m, 2 H), 2.87 (d, *J*=3.81 Hz, 2 H), 2.99 – 3.08 (m, 1 H), 3.08 – 3.20 (m, 1 H), 3.69 – 3.87 (m, 2 H), 3.88 – 4.04 (m, 1 H), 4.14 (d, *J*=6.54 Hz, 3 H), 5.43 – 5.48 (m, 1 H), 7.19 – 7.38 (m, 4 H), 7.47 (br s, 1 H), 7.55 (dd, *J*=14.94, 9.08 Hz, 2 H). HRMS (ESI) calcd for $C_{25}H_{35}ClN_3O_8SNa$: [M-]: 595.1731. Found: 595.1733.

4.1.10.2. Sodium (8S,11S)-8-(cyclohexylmethyl)-12-hydroxy-6,9-dioxo-11-(((S)-2-oxo pyrrolidin-3-yl)methyl)-1-phenyl-2,5-dioxa-7,10-diazadodecane-12-sulfonate 12b: White solid (yield 73%); M.p 47–50 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.78 – 0.94 (m, 2 H), 1.01 – 1.22 (m, 4 H), 1.32 (br s, 1 H), 1.39 – 1.51 (m, 2 H), 1.53 – 1.72 (m, 6 H), 1.86 – 1.94 (m, 1 H), 2.00 – 2.34 (m, 2 H), 2.99 – 3.18 (m, 2 H), 3.60 (d, *J*=3.71 Hz, 4 H), 3.75 (q, *J*=7.13 Hz, 1 H), 4.00 – 4.22 (m, 2 H), 4.50 (s, 2 H), 7.25 – 7.38 (m, 5 H), 7.43 (d, *J*=7.13 Hz, 1 H), 7.54 (br s, 1 H), 7.64 (br s, 1 H), 8.47 (d, *J*=7.03 Hz, 1 H). HRMS (ESI) calcd for $C_{26}H_{38}N_3O_9SNa$: [M-]: 591.2226. Found: 591.2227.

4.1.10.3. Sodium (2S)-2-((S)-3-cyclohexyl-2-undecanamidopropanamido)-1-hydroxy-3-((S)-2-oxopyrrolidin-3-yl)propane-1-sulfonate 12c: White solid (yield 67%); M.p 53–55 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 0.86 (t, *J*=6.13 Hz, 2 H), 1.07 – 1.14 (m, 2 H), 1.25 (br s, 16 H), 1.39 (d, *J*=19.72 Hz, 2 H), 1.52 (br s, 5 H), 1.62 (br. s., 6 H), 1.87 – 2.02 (m, 2 H), 2.12 (d, *J*=7.23 Hz, 2 H), 3.01 – 3.20 (m, 2 H), 3.82 – 4.00 (m, 2 H), 4.05 (br. s., 1 H), 4.17 – 4.29 (m, 1 H), 5.29 – 5.36 (m, 1 H), 5.73 – 5.78 (m, 1 H), 7.14 – 7.19 (m, 1 H), 7.47 (d, *J*=9.15 Hz, 1 H), 7.58 (s, 1 H). HRMS (ESI) calcd for $C_{27}H_{48}N_3O_7SNa$: [M-]: 597.3060. Found: 597.3063.

4.2. X-ray crystallographic studies. Crystallization and data collection

Purified norovirus 3CL Protease (NV 3CLpro) in 100 mM NaCl, 20 mM Tris pH 8.0 (11 mg/mL) was used for preparation of the inhibitor complexes. Stock solutions (100 mM) of each inhibitor were prepared in DMSO and added to NV 3CLpro by mixing 7 μL of the inhibitor (3 mM) with 243 μL (0.5 mM) of NV 3CLpro and incubating on ice for 1 hour. All crystallization experiments were conducted Compact Jr. (Rigaku Reagents) sitting drop vapor diffusion plates at 20 °C using equal volumes of protein and crystallization solution equilibrated against 75 μL of the latter. Hexagonal NV 3CLpro:***7l*** (h) and orthorhombic crystals NV 3CLpro:***7l*** (o) were obtained for the NV 3CLpro:***7l*** complex in 2–3 days from the Index HT screen (Hampton Research) condition G5 (25% (w/v) PEG 3350, 100 mM Tris pH 8.5, 200 mM lithium sulfate) and Wizard 3–4 screen (Rigaku Reagents) condition A10 (20% (w/v) PEG 3350, 200 mM sodium thiocyanate) respectively. Crystals of the complex

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with inhibitor ***7m*** (NV 3CLpro**:7m**) were also obtained from the Index HT G5 condition. Samples were transferred to a fresh drop composed of 80% crystallization solution and 20% (v/v) PEG 200 and stored in liquid nitrogen. X-ray diffraction data were collected at the Advanced Photon Source beamline 17-ID using a Dectris Pilatus 6M pixel array detector.

4.3. Structure Solution and Refinement

Intensities were integrated using XDS [34,35] via Autoproc [36] and the Laue class analysis and data scaling were performed with Aimless [37]. Structure solution was conducted by molecular replacement with Phaser [38] using a previously determined structure of inhibitor bound to NV 3CLpro (PDB: 3UR9) [32] as the search model. Each crystal form contained two molecules in the asymmetric unit. Structure refinement and manual model building were conducted with Phenix [39] and Coot [40], respectively. Disordered side chains were truncated to the point for which electron density could be observed. Structure validation was conducted with Molprobity [41] and figures were prepared using the CCP4MG package [42]. Structure superposition was conducted with GESAMT [43].

4.4. FRET protease assays

The FRET protease assay was performed by preparing stock solutions of the substrate (Edans-DFHLQ/GP-Dabcyl) and inhibitor in DMSO and diluting into assay buffer which was comprised of 20mM HEPES buffer, pH 8, containing NaCl (200 mM), 0.4 mM EDTA, glycerol (60%), and 6 mM dithiothreitol (DTT). The protease was mixed with serial dilutions of each compound or with DMSO in 25 μL of assay buffer and incubated at 37°C for 30 min, followed by the addition of 25 μL of assay buffer containing substrate. Fluorescence readings were obtained using an excitation wavelength of 360 nm and an emission wavelength of 460 nm on a fluorescence microplate reader (FLx800; Biotec, Winoosk, VT) 1 h following the addition of substrate. Relative fluorescence units (RFU) were determined by subtracting background values (substrate-containing well without protease) from the raw fluorescence values, as described previously [25,31–32]. The dose-dependent FRET inhibition curves were fitted with a variable slope by using GraphPad Prism software (GraphPad, La Jolla, CA) in order to determine the IC_{50} values of the inhibitors.

4.5. Cell-based inhibition assays

The effects of each inhibitor on virus replication were examined in the NV replicon harboring cells (HG23 cells) [32]. Briefly, confluent and semi-confluent cells were incubated with medium containing DMSO (<0.1%) or each compound (up to 100 μM) for 48 h. After the incubation, total RNA was extracted and viral genome was quantitated with real-time quantitative RT-PCR (qRT-PCR). The EC_{50} values were determined by GraphPadPrism software [32].

4.6. Nonspecific cytotoxic effects

The cytotoxic dose for 50% cell death (CC_{50}) for each compound was determined for HG23 cells used in this study. Confluent cells grown in 96-well plates were treated with various concentrations (1 to 100 μM) of each compound for 72 h. Cell cytotoxicity was measured by

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a CytoTox 96 nonradioactive cytotoxicity assay kit (Promega, Madison, WI) and crystal violet staining. The in vitro therapeutic index was calculated by dividing the CC_{50} by the EC_{50}.

4.7. Accession Codes

Coordinates and structure factors were deposited to the Worldwide Protein Data Bank (wwPDB) with the accession codes: NV 3CLpro-7l (h) (5T6D), NV 3CLpro-7l (o) (5T6F), and NV 3CLpro-7m (5T6G).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The generous financial support of this work by the National Institutes of Health (AI109039) is gratefully acknowledged. Use of the University of Kansas Protein Structure Laboratory was supported by a grant from the National Institute of General Medical Sciences (P30GM110761) from the National Institutes of Health. Use of the IMCA-CAT beamline 17-ID at the Advanced Photon Source was supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Hauptman-Woodward Medical Research Institute. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under contract no. DE-AC02-06CH11357.

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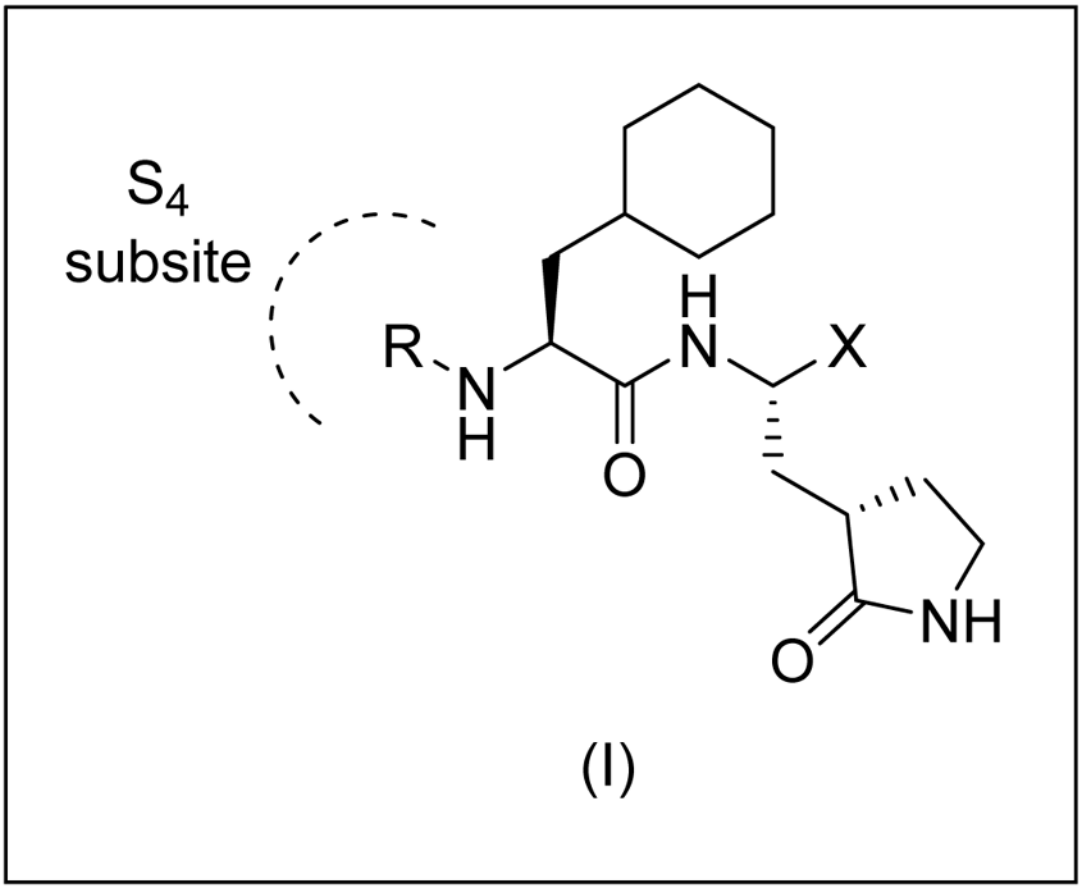

Fig. 1.
General structure of inhibitor (I)

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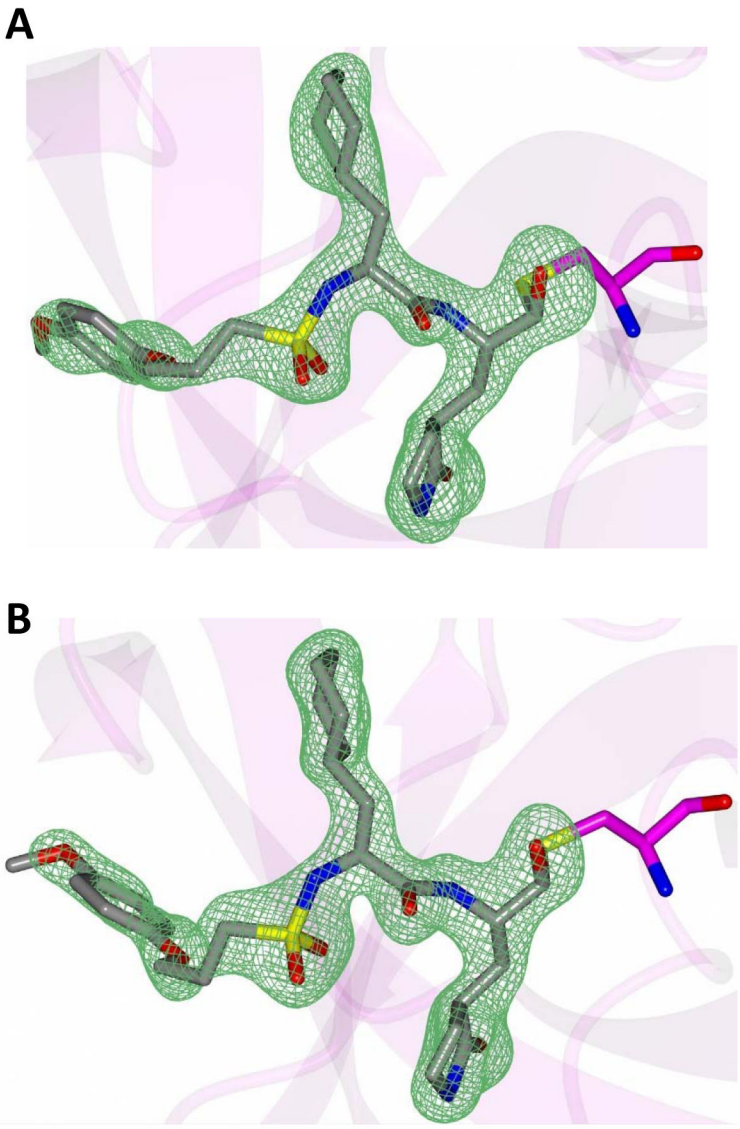

Fig. 2.

Fo-Fc omit map of inhibitor ***7l*** (green mesh) contoured at 3σ for (**A**) NV 3CLpro:***7l*** (hexagonal) and **B**) NV 3CLpro:***7l*** (orthorhombic).

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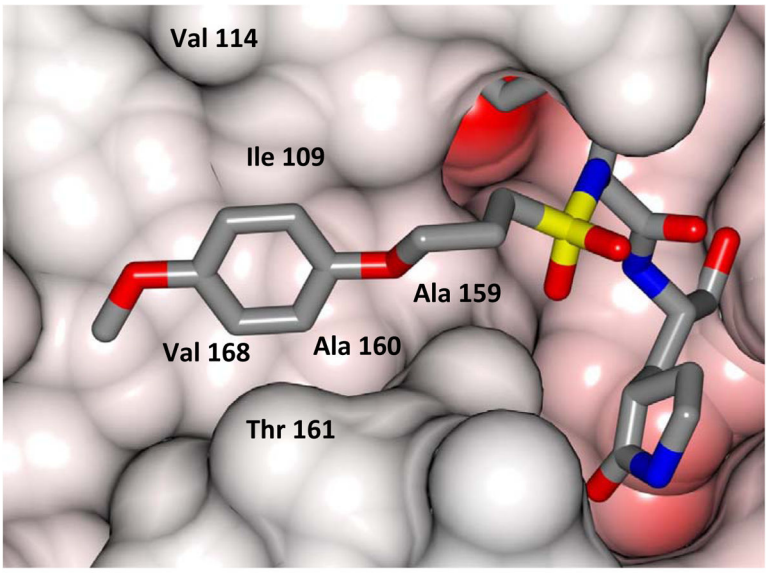

Fig. 3.

Electrostatic surface representation of NV 3CLpro showing the aryl ring region of inhibitor ***7l*** positioned in the hydrophobic S_4 pocket.

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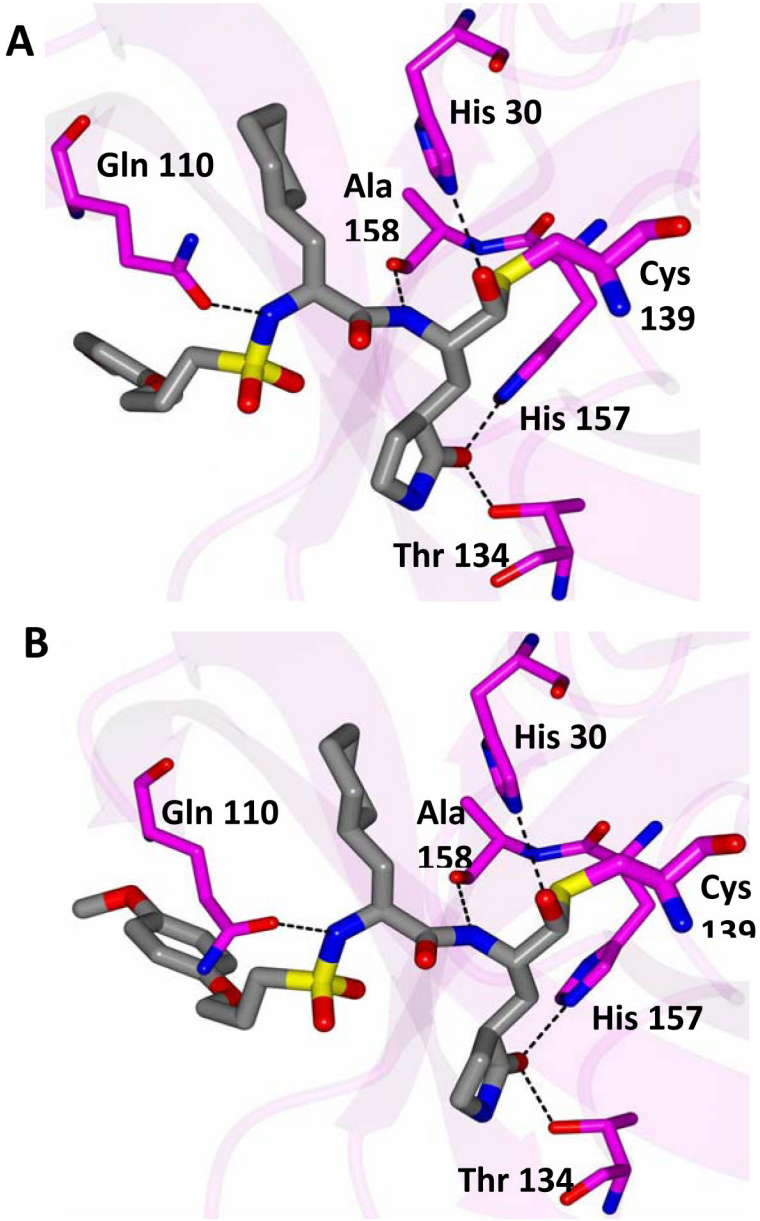

Fig. 4.

Hydrogen bond interactions (dashed lines) for (**A**) NV 3CLpro:***7l*** (hexagonal) and **B**) NV 3CLpro:***7l*** (orthorhombic).

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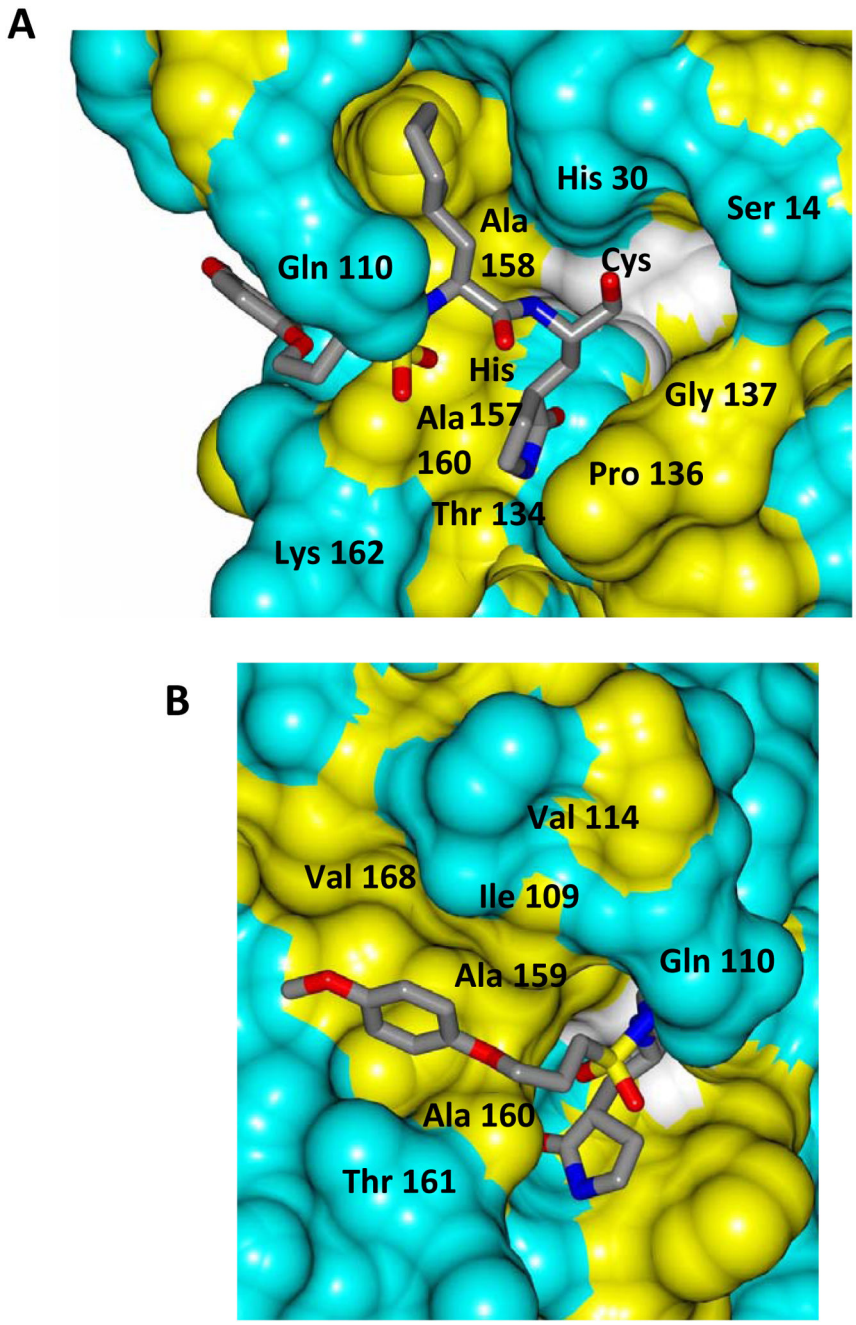

Fig. 5.

Surface representation of NV 3CLpro:***7l*** (hexagonal) with neighboring residues colored yellow (nonpolar), cyan (polar), and white (weakly polar). **A**) View of the inhibitor in the S_1/S_2 pocket and **B**) the S_4 pocket.

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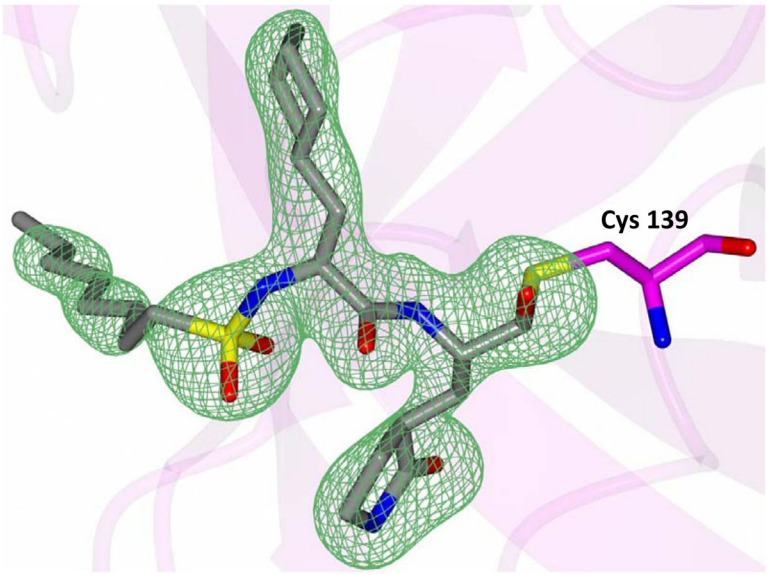

Fig. 6.
Fo-Fc omit map of inhibitor ***7m*** (green mesh) contoured at 3σ.

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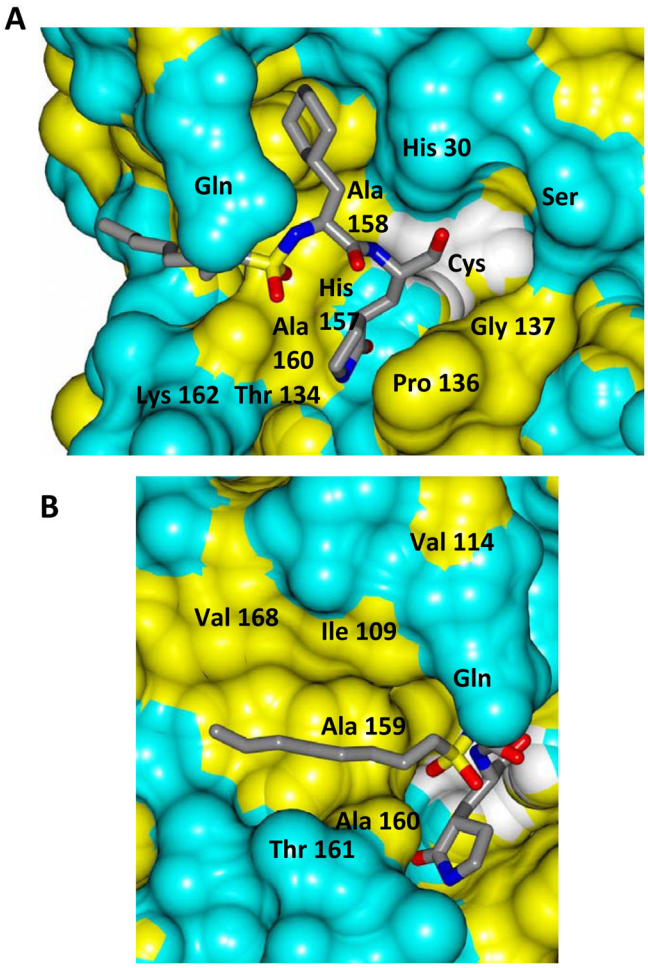

Fig. 7.

Surface representation of NV 3CLpro:***7m*** with neighboring residues colored yellow (nonpolar), cyan (polar), and white (weakly polar). **A**) View of the inhibitor in the S_1/S_2 pocket and **B**) the S_4 pocket.

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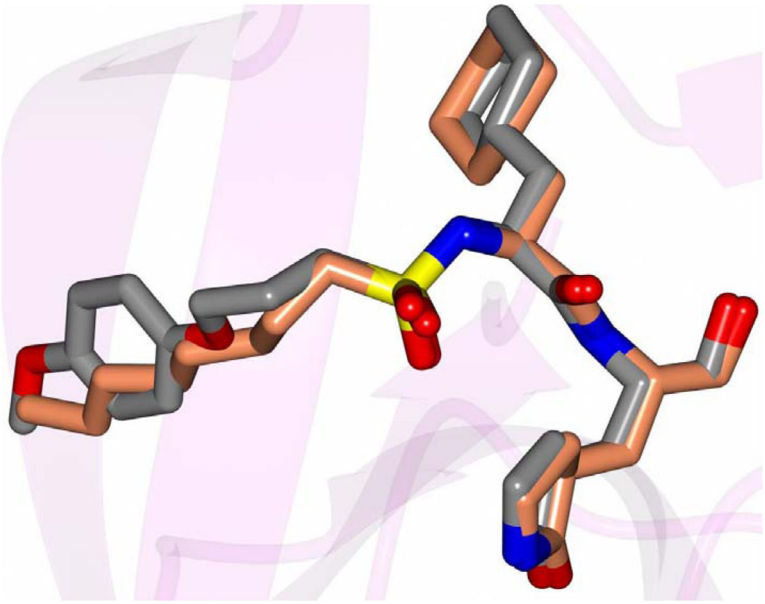

Fig. 8.

Superposition of inhibitor ***7l*** (gray) and ***7m*** (coral) which adopt similar binding modes in the NV 3CLpro active site

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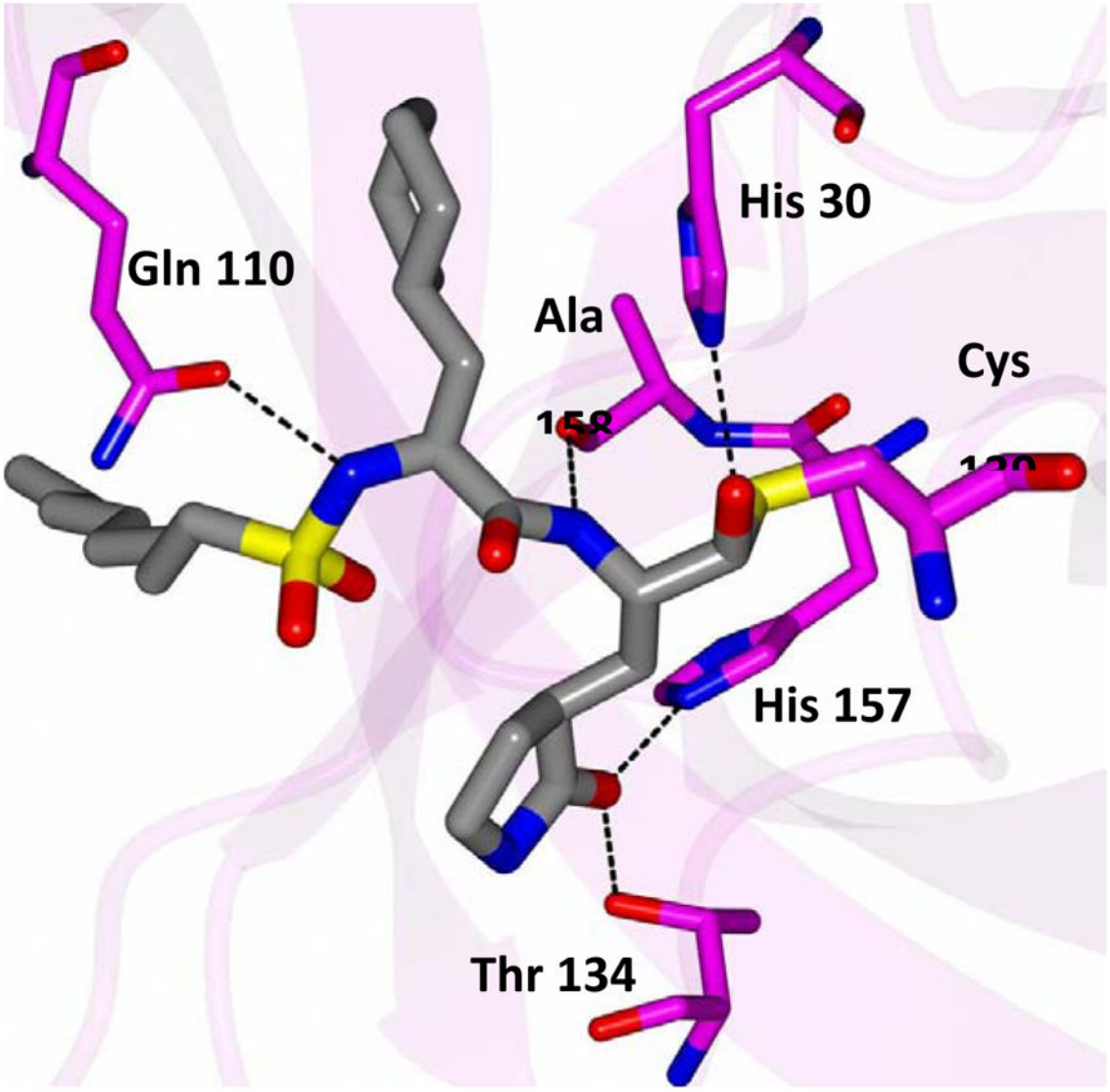

Fig. 9.
Hydrogen bond interactions (dashed lines) for NV 3CLpro:***7m***.

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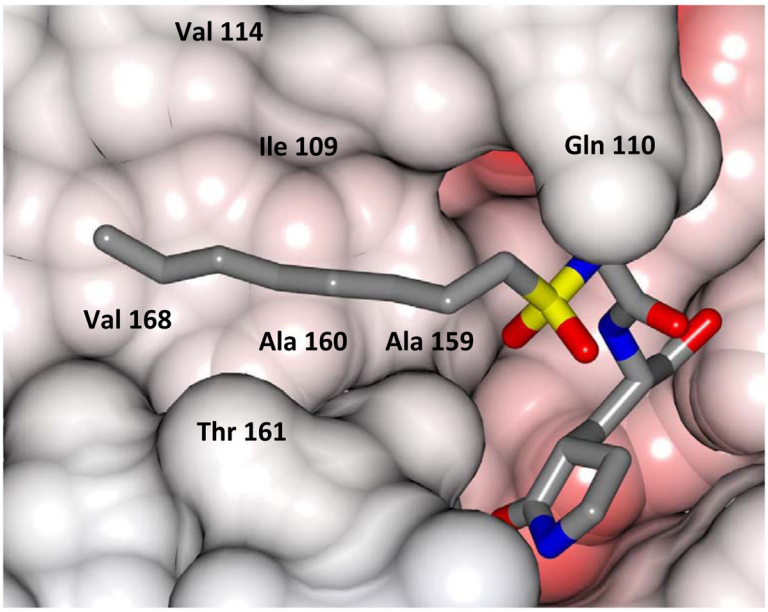

Fig. 10.

Electrostatic surface representation of NV 3CLpro showing the aliphatic chain of inhibitor ***7m*** positioned in a hydrophobic S_4 pocket.

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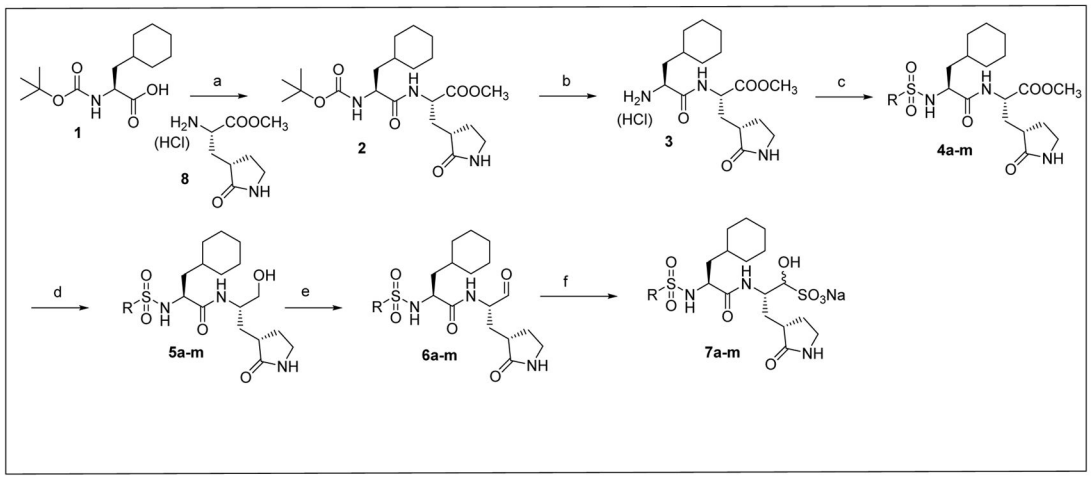

Scheme 1.

Synthesis of Inhibitors ***6a–m*** and ***7a–m***

[a]EDCI/HOBt/DIEA/DMF then **8**; [b]4M HCl in dioxane/2h; [c]RSO_2Cl/TEA/DCM/35°C; [d]2M $LiBH_4$/THF/CH_3OH;[e]Dess-Martin periodinane/DCM; [f]C_2H_5OH/EtOAc/$NaHSO_3$

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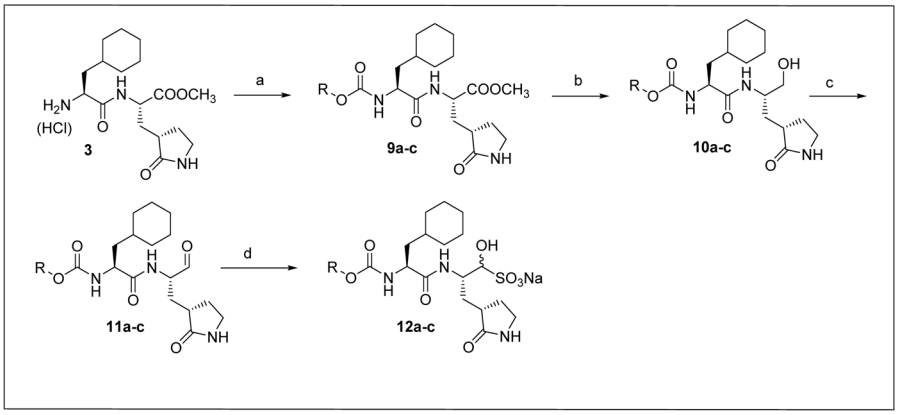

Scheme 2.

Synthesis of Inhibitors ***11a–c*** and ***12a–c***

[a]ROCOCl/TEA/DCM/ reflux 3 hrs; [b]2M $LiBH_4$/THF/CH_3OH;[c]Dess-Martin periodinane/DCM; [d]C_2H_5OH/EtOAc/$NaHSO_3$

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Table 1

Activity of compounds ***6a–m*** and ***7a–m*** against NV 3CL protease and replicon harboring cells.

Compound	R	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
6a	C_6H_5	8.5	9.5	> 100
7a		13.5	12.3	> 100
6b	$(C_6H_5)\ CH_2$	4.5	4.1	> 100
7b		4.3	4.3	> 100
6c	$(C_6H_5)\ CH_2CH_2$	1.8	0.7	> 100
7c		2.1	0.8	> 100
6d	*p*-F (C_6H_4)	4.8	2	> 100
7d		6.2	2.1	> 100
6e	*m*-Cl (C_6H_4)	5.5	2.3	> 100
7e		5.7	1.9	> 100
6f	*p*-Cl (C_6H_4)	5.8	3.5	> 100
7f		5.1	7.2	> 100
6g	*p*-Cl (C_6H_4) CH_2	1.8	2.4	> 100
7g		1.2	2.2	> 100
6h	*m*-Cl (C_6H_4) CH_2CH_2	2.3	1.1	> 100
7h		1.9	0.8	> 100
6i	2-Thiophene	10.4	8.5	> 100
7i		8.5	8.1	> 100
6j	Biphenyl-4-	1.3	1.3	> 100
7j		1.1	1.2	> 100
6k	2-Phthalimidoethane	2.5	2.3	> 100
7k		2.6	2.1	> 100
6l	p-$CH_3O(C_6H_4)O(CH_2)_3$	1.8	0.2	> 100
7l		1.6	0.2	> 100
6m	$CH_3(CH_2)_7$	1.2	0.3	33.8
7m		1.1	0.2	35.4

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Table 2

Activity of compounds ***11a–c*** and ***12a–c*** against NV 3CL protease and replicon harboring cells

Compound	R	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
11a	*m-Cl* $(C_6H_4)CH_2CH_2$	0.6	0.1	43.2
12a		0.5	0.1	46.7
11b	$(C_6H_5)CH_2O(CH_2)_2$	2.1	0.2	> 100
12b		1.6	0.3	> 100
11c	CH_3 $(CH_2)_9$	0.3	0.1	32.1
12c		0.4	0.2	34.5

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Table 3

Crystallographic Data for NV 3CL Protease-Inhibitor Structures

	NV 3CLpro:7l (h)	NV 3CLpro:7l (o)	NV 3CLPro:7m
Data Collection			
Unit-cell parameters (Å, °)	$a=b$=59.53, c=356.80	a=37.51, b=64.80, c=125.37	$a=b$=59.58, c=357.70
Space group	$P6_122$	$P2_12_12_1$	$P6_122$
Resolution (Å)[1]	49.53–2.10 (2.16–2.10)	45.05–1.90 (1.94–1.90)	49.57–2.45 (2.55–2.45)
Wavelength (Å)	1.0000	1.0000	1.0000
Temperature (K)	100	100	100
Observed reflections	434,656	158,261	276,664
Unique reflections	23,382	24,911	15,076
$<I/\sigma(I)>$[1]	13.4 (1.9)	13.7 (2.1)	13.2 (1.9)
Completeness (%)[1]	100 (99.9)	99.9 (100)	100 (100)
Multiplicity[1]	18.6 (18.9)	6.4 (5.8)	18.4 (19.5)
R_{merge} (%)[1, 2]	20.7 (186.3)	9.9 (92.6)	25.1 (197.3)
R_{meas} (%)[1, 4]	21.3 (191.5)	10.7 (101.8)	25.8 (202.6)
R_{pim} (%)[1, 4]	4.9 (43.8)	4.2 (41.6)	6.0 (45.5)
$CC_{1/2}$ [1, 5]	0.998 (0.897)	0.999 (0.834)	0.997 (0.714)
Refinement			
Resolution (Å) [1]	47.30–2.10	35.94–1.90	49.57–2.45
Reflections (working/test)[1]	22,094/1,115	23,626/1,210	14,228/727
R_{factor} / R_{free} (%)[1,3]	19.3/24.3	19.4/24.9	18.8/26.1
No. of atoms (Protein/Ligand/Water)	2,440/74/108	2,400/63/104	2,473/63/74
Model Quality			
R.m.s deviations			
Bond lengths (Å)	0.010	0.010	0.008
Bond angles (°)	1.040	1.031	0.924
Average B-factor (Å^2)			
All Atoms	32.6	35.4	38.1
Protein	32.3	35.3	38.1
Ligand	35.5	38.3	36.7
Water	38.2	37.2	39.7
Coordinate error(maximum likelihood) (Å)	0.23	0.22	0.28
Ramachandran Plot			
Most favored (%)	96.9	96.0	94.9
Additionally allowed (%)	3.1	3.7	4.8

[1] Values in parenthesis are for the highest resolution shell.

[2] $R_{merge} = \Sigma_{hkl}\Sigma_i |I_i(hkl) - <I(hkl)>| / \Sigma_{hkl}\Sigma_i I_i(hkl)$, where $I_i(hkl)$ is the intensity measured for the ith reflection and $<I(hkl)>$ is the average intensity of all reflections with indices hkl.

[3] $R_{factor} = \Sigma_{hkl} ||F_{obs}(hkl)| - |F_{calc}(hkl)|| / \Sigma_{hkl} |F_{obs}(hkl)|$; Rfree is calculated in an identical manner using 5% of randomly selected reflections that were not included in the refinement.

[4] R_{meas} = redundancy-independent (multiplicity-weighted) R_{merge}[37, 44]. R_{pim} = precision-indicating (multiplicity-weighted) R_{merge}[45, 46].

[5] $CC_{1/2}$ is the correlation coefficient of the mean intensities between two random half-sets of data [47, 48].

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