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Improved binding affinities of pyrrolidine derivatives as Mcl-1 inhibitors by modifying amino acid side chains

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ABSTRACT

As an important member of anti-apoptotic Bcl-2 protein, myeloid cell leukemia sequence 1 (Mcl-1) protein is an attractive target for cancer therapy. In this study, a new series of pyrrolidine derivatives as Mcl-1 inhibitors were developed by mainly modifying the amino acid side chain of compound **1**. Among them, compound **18** ($K_i = 0.077 \mu$ M) exhibited better potent inhibitory activities towards Mcl-1 protein compared to positive control **Gossypol** ($K_i = 0.18 \mu$ M). In addition, compound **40** possessed good antiproliferative activities against PC-3 cells ($K_i = 8.45 \mu$ M), which was the same as positive control **Gossypol** ($K_i = 7.54 \mu$ M).

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1. Introduction

As we all know, apoptosis which can be characterized by some morphological changes, such as cells shrink, DNA cleavage, is critical for embryogenesis, tissue homeostasis and defense against pathogens.¹ However, deregulation of apoptosis contributes to tumorgenesis, autoimmune and degenerative diseases.² Therefore, a better understanding of its regulation in normal and tumor cells will lay a foundation for developing more potent and selective anti-tumor agents.

The B-cell lymphoma-2 (Bcl-2) family proteins are essential regulators in the regulation of the intrinsic apoptotic pathway, including anti-apoptotic and pro-apoptotic proteins. Anti-apoptotic (pro-survival) proteins such as Bcl-2, Bcl-X_L and Mcl-1 could inhibit the activity of pro-apoptotic members by binding the BH3 domain of pro-apoptotic members (Bax, Bak and BH3-only proteins).³ The BH3-only proteins such as Bim, Bid and Noxa, are an important subgroup of pro-apoptotic members, which could neutralize the inhibitory activity of anti-apoptotic proteins and also directly contributes to the activation of Bax and Bak.⁴ The relative ratio of anti-apoptotic and pro-apoptotic members is in a dynamic equilibrium in normal cells. However, cancer cells can evade apoptosis by overexpressing one or more anti-apoptotic proteins.⁵

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http://dx.doi.org/10.1016/j.bmc.2016.10.020 0968-0896/© 2016 Elsevier Ltd. All rights reserved. Among these anti-apoptotic proteins, Mcl-1 protein is one of the overexpressed proteins that are present in cancer cells.⁶ And its distinct role in tumorgenesis and development has been proved in many Mcl-1 transgenic mice models.⁷ And the specific downregulation of Mcl-1 by certain gene silencing approaches can induce some cancer cell types to apoptosis.^{8–11} Consequently, suppressing the function of Mcl-1 protein may be an attractive approach for cancer therapy.

In previous study, based on the lead compound WL-276 and scaffold hopping strategy, we designed compound 1 which had a new scaffold, pyrrolidine. It displayed the best inhibitory activity to Mcl-1 protein, with a K_i value of 0.53 μ M.¹² However, the inhibitory activity for Mcl-1 protein should be improved compared to positive Gossypol and other reported lead compounds. Given that the interfaces between the anti-apoptotic and pro-apoptotic proteins are large and flexible, it is more difficult to target than enzyme/substrate interactions that are involved in smaller and more defined active sites.¹³ Therefore, in order to enhance the binding affinity for Mcl-1 protein, we used many non-classical amino acids obtaining by etherification and arylation on the aryl ring of L-4-Br phenylalanine to undertake a new round of structural modifications. In addition, to explore if the Boc protecting group on the pyrrolidine ring was essential for activities, we removed the Boc protecting group to synthesize some derivatives (Fig. 1). Herein, we will introduce synthesis, preliminary bioactivity and structure-activity relationships of the new pyrrolidine derivatives as Mcl-1 inhibitors in detail.

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Figure 1. The design of target compounds.

2. Results and discussion

2.1. Chemistry

Schemes 1 and 2 showed the synthetic route of the pyrrolidine derivatives as Mcl-1 inhibitors. L-Tyrosine was as the starting material to synthesis intermediate 2 by amino and carboxyl protections. Then intermediate 2 was reacted with different halohydrocarbons by Williamson reaction and hydrolyzed by the NaOH solution to obtain compounds **4a–4h**. In addition, L-tyrosine was successively protected by Cu²⁺ cation, reacted with different substituted benzyl bromides and protected by Boc group to yield compounds **4i–4k**. Compound **4k** and *N*-Boc-4-Br-phenylanine were treated by protection of carboxyl group and Suzuki reaction to get intermediate **4l–4n**. The target compounds **1, 10–47** were got

by the methods which were previously reported.¹² Then compounds **1**, **10–12** were removed the protecting group of Boc to yield the target compounds **48–51**.

2.2. Binding assay to Mcl-1 protein

The binding affinities for Mcl-1 protein of these pyrrolidine derivatives were examined by using fluorescence polarization assays (FPAs). We modified compound **1** by four parts, that is, amino acid side chain, biphenyl group, sulfonamide moiety and Boc protecting group on the scaffold of pyrrolidine. The results were summarized in Table 1. According to the results of binding assays, some important SAR information could be described as the following: (1) when there were no substituents on R_1 and R_3 position, the activities for Mcl-1 protein were poor except compound 33, such as 13, 19-20. (2) when there were bulky substituents on R₂ position, compounds with no substituents on R₁ position displayed better inhibitory activities, such as 18, 28. (3) When there were ether bonds on R₂ position and 3-NO₂-4-Cl group on R₃ position, the binding affinities for Mcl-1 protein of compounds with Cl group on R₁ position were better except compounds 30, 40. However, when the ether bonds were removed and directly replaced by aryl groups, the activities were reduced, such as 43, 45. The ether may afford a suitable change in the direction of the aryl ring of L-4-Br phenylalanine, which was a possible explanation for the activities. (4) When we removed the Boc protecting group, the binding affinities for Mcl-1 protein were reduced significantly, such as 48-50. The results indicated that the Boc group was necessary for binding affinities. It was that the Boc



R'=*n*-Pr,*i*-Pr,*i*-Bu,Hex,Ph-(CH₂)₂,Naph-2-CH₂,4-NO₂-Ph,TBDMS; R''= Ph, 4-OCH₃-Ph;

Scheme 1. Reagents and conditions: (a) (i) MeOH, CH₃COCl; (ii) (Boc)₂O, Et₃N, CH₂Cl₂; (b) (i) K₂CO₃, DMSO, 30 °C, overnight or imidazole, TBDMSCl, DMF, 0 °C–rt; (ii) 1 M NaOH, THF, 60–80 °C, 1.5 h; (c) (i) CuSO₄-5H₂O, NaOH, EDTA-2Na, CH₃OH, H₂O; (ii) (Boc)₂O, Et₃N, CH₂Cl₂; (d–e) (i) MeOH, CH₃COCl; (ii) (Boc)₂O, Et₃N, CH₂Cl₂; (iii) Pd(OAc)₂, Na₂CO₃, DMF, H₂O, TBAB.

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Scheme 2. Reagents and conditions: (e) (i) MeOH, CH₃COCl; (ii) (Boc)₂O, Et₃N,CH₂Cl₂; (f) (i) PPh₃, DEAD, various biphenols, THF, rt, 12 h; (ii) 1 M NaOH, THF, rt, overnight; (g) HATU, DIEA, DCM, 0 °C-rt; (h) (i) ethyl acetate saturated with HCl, rt, overnight; ii. NMM, isobutyl chloroformate, NaH, different benzenesulfonamides, -20 °C to rt, 4 h; (i) ethyl acetate saturated with HCl, rt, overnight; saturated NaHCO₃.

group may display certain interactions with some amino acid residues or make the whole scaffold enrich the active pocket of Mcl-1 protein better. Among these pyrrolidine derivatives, compound **18** had the most potent inhibitory activities for Mcl-1 protein ($K_i = 77$ nM) which displayed approximately 10 times more potent than compound **1** ($K_i = 0.53 \mu$ M) and over two times potent than the positive control **Gossypol** ($K_i = 0.18 \mu$ M). As we all know, the interactions between proteins and proteins involved large and flexible interfaces. The structure of compound **18** was bigger than compound **1** so that it may occupied more space of Mcl-1 protein active pocket, which maybe a reason for the improved inhibitory activities for Mcl-1 protein.

2.3. Docking study

To further understand the interactions between these compounds and Mcl-1 protein, we chose the most potent compound **18** to dock with Mcl-1 protein in the active pocket by using Surflex-Dock in silico. As shown in Figure 2, biphenyl group of compound **18** could mimetic the α -helix of Bim protein (Fig. 2a). Acyl-sulfonyl group could form three hydrogen bonds with Arg263 and Trp261. In addition, the Boc protecting group on pyrrolidine ring could form another one hydrogen bond with Thr266. The result may be a reason for that the Boc protection group was essential for binding affinities for Mcl-1 protein (Fig. 2b).

2.4. Binding assays for other anti-apoptotic Bcl-2 proteins

In order to explore whether the new series of pyrrolidine derivatives also could bind to other anti-apoptotic Bcl-2 proteins, five active compounds (**18**, **27**, **34**, **36**, **40**) were also chosen to evaluate their inhibitory activities against Bcl-X_L and Bcl-2 proteins by FPAs. As demonstrated in Table 2, these compounds exhibited good binding affinities for Bcl-2 and Mcl-1 proteins almost as the same as the positive control **Gossypol**. However these compounds displayed better potency in inhibiting Mcl-1 protein than the other two proteins, especially compound **18**. To our surprise, these compounds had no inhibitory activities against Bcl-X_L protein except **18**, whereas **Gossypol** displayed certain degree inhibitory potency

against $Bcl-X_L$ protein. Therefore, other four compounds could be potent Bcl-2/ Mcl-1 inhibitors.

2.5. Cell antiproliferative assay

As aberrant expression of anti-apoptotic Bcl-2 proteins could be observed in many human malignancies, such as breast cancer, prostate and lemkemia,^{14,15} three human cancer cells including MDA-MB-231 (breast cancer cell), PC-3 (prostatic cancer cell) and K562 (chronic myelogenous leukemia cell) were selected to test their antiproliferative activities of compounds **18**, **27**, **34**, **36**, **40** by using MTT assay and **Gossypol** was as the reference compound. The antiproliferative activities were tabulated as IC₅₀ values in Table 3. The data in Table 3 showed that all of them had poorer inhibitory activities against MDA-MB-231 and K562 cells compared to **Gossypol**. To our delight, they could inhibit the growth of PC-3 cells effectively. Especially, compound **40** displayed similar antiproliferative activities against PC-3 cells with **Gossypol**.

3. Conclusions

In brief, through mainly extending the aryl ring of amino acid side chain on compound **1**, we had designed and synthesized a new series of pyrrolidine derivatives as Mcl-1 inhibitors. Some of them displayed better binding affinities for Mcl-1 protein than compound **1**. Moreover, the most active compound **18** had a K_i value of 77 nM, which was over two times potent than the positive control **Gossypol**. In addition, compound **40** could effectively inhibit the growth of PC-3 cells. Notably, we also found that the Boc protection group of pyrrolidine scaffold was essential for binding for Mcl-1 protein. These data may be significance of guidance to find more potent Mcl-1 inhibitor.

4. Experiment section

4.1. General chemistry information

All chemicals and solvent were purchased from commercial suppliers and used directly unless other stated. Thin-layer

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Table 1

The binding affinities of pyrrolidine derivatives for Mcl-1 protein



Compd	R ₁	R ₂	R ₃	R ₄	Mcl-1 $K_i (\mu M)^a$
1	-H	-Br	3-NO ₂ -4-Cl	-Boc	0.53 ± 0.09
10	-CH ₃	-Br	3-NO ₂ -4-Cl	-Boc	0.63 ± 0.20
11	-Cl	-Br	3-NO ₂ -4-Cl	-Boc	0.58 ± 0.07
12	-CH ₃	-H	3-NO ₂ -4-Cl	-Boc	0.56 ± 0.18
13	-H	n-Pr-O	-H	-Boc	4.0 ± 0.67
14	-CH ₃	n-Pr-O	4-NO ₂	-Boc	0.90 ± 0.11
15	-H	n-Pr-O	4-NO ₂	-Boc	4.7 ± 1.1
16	-CH ₃	n-Pr-O	3-NO ₂ -4-Cl	-Boc	0.74 ± 0.09
17	-Cl	n-Pr-O	3-NO ₂ -4-Cl	-Boc	0.72 ± 0.14
18	-H	TBDMS-0	3-NO ₂ -4-Cl	-Boc	0.077 ± 0.0042
19	-H	i-Pr-O	-H	-Boc	8.1 ± 2.1
20	-H	i-Bu-O	-H	-Boc	2.0 ± 0.021
21	-Cl	i-Bu-O	3-NO2-4-Cl	-Boc	0.50 ± 0.04
22	-CH ₃	i-Bu-O	3-NO2-4-Cl	-Boc	0.71 ± 0.08
23	-CH ₃	i-Bu-O	4-NO ₂	-Boc	0.79 ± 0.15
24	-Cl	Hex-O	3-NO ₂ -4-Cl	-Boc	0.43 ± 0.10
25	-CH ₃	Hex-O	3-NO2-4-Cl	-Boc	0.78 ± 0.14
26	-CH ₃	$Ph-(CH_2)_2-O$	3-NO2-4-Cl	-Boc	0.41 ± 0.03
27	-Cl	Ph-(CH ₂) ₂ -O	3-NO ₂ -4-Cl	-Boc	0.26 ± 0.02
28	-H	Naph-2-CH ₂ -O	3-NO ₂ -4-Cl	-Boc	0.34 ± 0.05
29	-CH ₃	Naph-2-CH ₂ -O	3-NO ₂ -4-Cl	-Boc	0.50 ± 0.07
30	-Cl	Naph-2-CH ₂ -O	3-NO ₂ -4-Cl	-Boc	>10
31	-Cl	4-NO ₂ -Ph-O	3-NO ₂ -4-Cl	-Boc	0.54 ± 0.05
32	-H	Bn-O	3-NO ₂ -4-Cl	-Boc	0.78 ± 0.18
33	-H	Bn-O	-H	-Boc	0.59 ± 0.15
34	-Cl	Bn-O	3-NO ₂ -4-Cl	-Boc	0.22 ± 0.017
35	-CH ₃	Bn-O	3-NO ₂ -4-Cl	-Boc	0.80 ± 0.27
36	-H	4-Br-Bn-O	3-NO ₂ -4-Cl	-Boc	0.32 ± 0.03
37	-CH ₃	4-Br-Bn-O	3-NO ₂ -4-Cl	-Boc	0.46 ± 0.05
38	-Cl	4-Br-Bn-O	3-NO ₂ -4-Cl	-Boc	0.33 ± 0.03
39	-H	4-NO ₂ -Bn-O	3-NO ₂ -4-Cl	-Boc	0.58 ± 0.06
40	-CH ₃	4-NO ₂ -Bn-O	3-NO ₂ -4-Cl	-Boc	0.30 ± 0.09
41	-Cl	4-NO ₂ -Bn-O	3-NO ₂ -4-Cl	-Boc	0.68 ± 0.06
42	-CH ₃	Ph	3-NO ₂ -4-Cl	-Boc	0.77 ± 0.10
43	-Cl	Ph	3-NO ₂ -4-Cl	-Boc	>10
44	-CH ₃	4-OCH ₃ -Ph	3-NO ₂ -4-Cl	-Boc	0.50 ± 0.03
45	-Cl	4-OCH ₃ -Ph	3-NO ₂ -4-Cl	-Boc	>10
46	-CH ₃	4-(4-OCH ₃ -Ph)-Bn-O	3-NO ₂ -4-Cl	-Boc	0.56 ± 0.08
47	-Cl	4-(4-OCH ₃ -Ph)-Bn-O	3-NO ₂ -4-Cl	-Boc	0.48 ± 0.04
48	-H	-Br	3-NO ₂ -4-Cl	-H	N.A. ^b
49	-CH ₃	-Br	3-NO ₂ -4-Cl	-H	N.A. ^b
50	-Cl	-Br	3-NO ₂ -4-Cl	-H	N.A. ^b
51	-CH ₃	-H	3-NO ₂ -4-Cl	-H	1.3 ± 0.26
Gossypol					0.18 ± 0.01

^a Each value is the result of three separate experiments.

^b No activity.

chromatography (TLC) was undertook on 0.25 mm silica gel plates (60 GF-254) to monitor the completion of all the reactions and visualize the spots under UV light (254 nM or 365 nM) or iodine vapor. All the melting points were determined on an electrothermal melting point apparatus without correction. ESI-MS were measured on an Aglient-1100 series LC/MSD trap spectrometer. HRMS spectra were performed on an Aglient 6510 Quadrupole Time-of-Flight LC/MS deliver. ¹H NMR and ¹³C NMR spectra were measured with a Bruker DRX spectrometer (600 MHz) and a

Bruker Avance spectrometer (300 MHz or 400 MHz). Chemical shifts are given in parts per million (ppm) with tetramethylsilane (TMS) as an internal standard, and coupling constants (*J* values) are given in hertz (Hz). The splitting patterns were assigned as s (singlet), d (doublet), dd (doublet doublet), t (triplet), q (quartet), m (multiplet), and br s (broad singlet). All products were purified by recrystallization or column chromatography (silica gel 200–300 mesh). The following yields were generated after purification.

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Figure 2. (a) Compound 18 docked with Mcl-1 protein complexed with Bim protein in ribbon. (b) Surface representations of compound 18 in the hydrophobic groove of Mcl-1 protein.

 Table 2

 The binding affinities of representative pyrrolidine derivatives for three Bcl-2 proteins

Compd	Bcl-X _L	Bcl-2	Mcl-1
	K _i (μM) ^a	K _i (µM) ^a	K _i (µM) ^a
18 27 34 36	1.3 ± 0.65 N.A. ^b N.A. ^b N.A. ^b	$\begin{array}{c} 0.93 \pm 0.07 \\ 0.67 \pm 0.08 \\ 0.51 \pm 0.031 \\ 0.68 \pm 0.04 \\ 0.75 \pm 0.00 \end{array}$	$\begin{array}{c} 0.077 \pm 0.0042 \\ 0.26 \pm 0.02 \\ 0.22 \pm 0.017 \\ 0.32 \pm 0.03 \\ 0.30 \pm 0.00 \end{array}$
40	1.4 ± 0.54	0.75 ± 0.09	0.30 ± 0.09
Gossypol		0.45 ± 0.03	0.18 ± 0.01

^a Each value was reproduced in three independent assays and expressed with standard deviations.

^b No activity.

4.1.1. (S)-Methyl-2-((*tert*-butoxycarbonyl)amino)-3-(4-hydroxy-phenyl)propanoate (2)

In an ice bath, acetyl chloride (21.32 mL, 300 mmol) was added into 200 mL anhydrous methanol slowly. After stirred for 30 min, L-tyrosine (18.1 g, 100 mmol) was added into the solution. Then the solution was refluxed for 4 h in an oil bath and the solvent was removed under reduced pressure. The residue was added some acetone and filtered to give 22.78 g of L-tyrosine methyl ester hydrochloride as a white powder, yield: 99%. Di-tert-butyl dicarbonate (11.34 g, 52 mmol) in 50 mL 1,4-dioxane was slowly added into the solution of L-tyrosine methyl ester hydrochloride (9.26 g, 40 mmol), K₂CO₃ (8.28 g, 60 mmol) in 160 mL mixture solvent $(1,4-dioxane-H_2O = 1:1)$. After stirred overnight at room temperature, the reaction mixture was concentrated under reduced pressure and EtOAc was added. The EtOAc layer was washed by 1 N citric acid, saturated NaHCO₃ and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated to give compound **2** as a white powder. It could be directly used in the further steps without purification.

Гa	ıble	3	

Antiproliferative activities of representative compounds

Compd	$IC_{50} (\mu M)^a$		
	MDA-MB-231	PC-3	K562
18	23.4 ± 0.80	10.2 ± 2.4	17.3 ± 1.2
27	25.2 ± 1.3	13.2 ± 1.3	17.8 ± 2.1
34	20.6 ± 2.8	9.06 ± 0.46	17.0 ± 2.1
36	21.3 ± 2.5	10.6 ± 1.4	16.4 ± 2.5
40	16.9 ± 0.54	8.45 ± 1.8	16.6 ± 2.4
Gossypol	9.23 ± 1.2	7.54 ± 0.28	5.60 ± 0.29

^a Each value was reproduced in three independent assays and expressed with standard deviations.

4.1.2. (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4-propoxyphenyl) propanoic acid (4a)

To a solution of compound **2** (4.43 g, 15 mmol) and K_2CO_3 (4.14 g, 30 mmol) in 80 mL DMSO, 1-iodopropane (4.37 mL, 45 mmol) was added into the mixture slowly. After stirred overnight at 30 °C, 200 mL H₂O was added into the solution and extracted with EtOAc. The combined EtOAc layer was washed by 1 N NaOH solution and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated to get yellow oil. Then the yellow oil was dissolved in 60 mL THF and 60 mL 1 N NaOH solution. After stirred for 1.5 h in 60-80 °C, the solvent was removed under reduced pressure. The residue was acidified with 1 N HCl until pH 2-3 and then extracted with EtOAc. The organic layer was dried over anhydrous MgSO₄ and concentrated to give crude residue. The residue was purified by silica gel chromatography (petroleum ether-ethyl acetate = 20:1, 0.2% HOAc) to generate 2.72 g compound 8a as a white powder. Yield: 56%, mp: 76-78 °C.¹H NMR (DMSO- d_6 , 300 MHz), δ 12.54 (s, 1H), 7.15 (d, J = 8.7 Hz, 2H), 7.05 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 8.7 Hz, 2H), 4.06–3.95 (m, 1H), 3.90 (t, J = 6.3 Hz, 2H), 2.96–2.90 (m, 1H), 2.78–2.70 (m, 1H), 1.76– 1.64 (m, 2H), 1.32 (s, 9H), 0.99 (t, J = 7.5 Hz, 3H).

Compounds **4b–4h** were synthesized following the procedure described above.

4.1.2.1. (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4-isopropoxyphenyl)propanoic acid (4b). White powder, yield: 51%, mp: 88–90 °C. ¹H NMR (DMSO- d_6 , 300 MHz), δ 12.55 (br s, 1H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.05 (d, *J* = 8.1 Hz, 1H), 6.82 (d, *J* = 8.4 Hz, 2H), 4.60–4.48 (m, 1H), 4.07–3.96 (m, 1H), 2.96–2.90 (m, 1H), 2.77–2.69 (m, 1H), 1.32 (s, 9H), 1.24 (d, *J* = 6.0 Hz, 6H).

4.1.2.2. (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4-isobutoxyphenyl)propanoic acid (4c). White powder, yield: 29%, mp: 93– 95 °C. ¹H NMR (DMSO- d_6 , 300 MHz), δ 12.54 (br s, 1H), 7.15 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 2H), 4.07– 4.00 (m, 1H), 3.70 (d, *J* = 6.6 Hz, 2H), 2.97–2.91 (m, 1H), 2.79–2.71 (m, 1H), 2.05–1.91 (m, 1H), 1.33 (s, 9H), 0.97 (d, *J* = 6.6 Hz, 6H).

4.1.2.3. (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4-(hexyloxy)phenyl)propanoic acid (4d). White powder, yield: 46%, mp: 74–76 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.54 (br s, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 6.84 (d, *J* = 8.0 Hz, 2H), 4.93 (d, *J* = 8.0 Hz, 1H), 4.61–4.55 (m, 1H), 3.94 (t, *J* = 6.4 Hz, 2H), 3.14–3.00 (m, 2H), 1.80–1.73 (m, 2H), 1.42 (s, 9H), 1.35–1.33 (m, 6H), 0.92 (t, *J* = 6.8 Hz, 3H).

4.1.2.4. (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4-phenethoxyphenyl)propanoic acid (4e). White powder, yield: 29%, mp: 101–103 °C. ¹H NMR (CDCl₃, 400 MHz), δ 7.33–7.28 (m, 4H), 7.23–7.22 (m, 1H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.84 (d, *J* = 8.4 Hz, 2H), 4.91 (d, *J* = 8.0 Hz, 1H), 4.55–4.53 (m, 1H), 4.16 (t, *J* = 7.2 Hz, 1H), 3.14–3.04 (m, 4H), 1.42 (s, 9H).

4.1.2.5. (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4-(naphthalen-2-ylmethoxy)phenyl)propanoic acid (4f). White powder, yield: 61%, mp: 145–147 °C. ¹H NMR (CDCl₃, 400 MHz), δ 7.85– 7.81 (m, 4H), 7.52–7.46 (m, 3H), 7.12 (d, *J* = 8.0 Hz, 2H), 6.97 (d, *J* = 8.0 Hz, 2H), 5.19 (s, 2H), 4.93 (d, *J* = 8.0 Hz, 1H), 4.57 (d, *J* = 6.0 Hz, 1H), 3.15–3.02 (m, 2H), 1.42 (s, 9H).

4.1.2.6. (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4-(4-nitrophenoxy)phenyl)propanoic acid (4g). White powder, yield: 36%, mp: $125-127 \,^{\circ}$ C. ¹H NMR (CDCl₃, 400 MHz), δ 8.23 (d, *J* = 9.2 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.06 (d, *J* = 8.0 Hz, 2H), 7.04 (d, *J* = 9.2 Hz, 2H), 5.02 (d, *J* = 8.0 Hz, 1H), 4.66-4.64 (m, 1H), 3.29-3.24 (m, 1H), 3.12-3.07 (m, 1H), 1.45 (s, 9H).

4.1.2.7. (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)propanoic acid (4h). Colorless oily liquid, yield: 65%. ¹H NMR (CDCl₃, 300 MHz), δ 7.04 (d, *J* = 8.4 Hz, 2H), 6.77 (d, *J* = 8.4 Hz, 2H), 4.91 (d, *J* = 7.8 Hz, 1H), 4.56–4.37 (m, 1H), 3.16–2.91 (m, 2H), 1.42 (s, 9H), 0.97 (s, 9H), 0.18 (s, 6H).

4.1.3. (*S*)-3-(4-(Benzyloxy)phenyl)-2-((*tert*-butoxycarbonyl) amino)propanoic acid (4i)

To a solution of L-tyrosine (12.5 g, 69 mmol) in 2 N NaOH solution (69 mL), 1 N copper sulfate solution (34.5 mL) was added and heated at 60 °C for 1.5 h. Then 200 mL methanol and benzyl bromide (9.1 mL, 75.9 mmol) were added into the reaction mixture. After stirred vigorously for 3 h at 60 °C, the reaction mixture was cooled to room temperature. The precipitate was filtered and washed by proper amount of water, methanol and ether to get blue solid. Then the solid was added into the solution of 55.77 g/L EDTA-2Na solution (368 mL). After stirred overnight at 60 °C, the reaction mass was cooled to room temperature. The precipitate was filtered and washed by water to give 24.3 g pale white solid. Di-tert-butyl dicarbonate (7.9 g, 36 mmol) was slowly added into a solution of 13.6 g pale white and Et₃N (12.5 mL, 90 mmol) in 200 mL mixed solvent (1,4-dioxane- $H_2O = 2:1$) at 0 °C. After stirred overnight at room temperature, the reaction mixture was concentrated under reduced pressure and acidified with HCl solution until pH 2–3. The mixture was extracted with EtOAc. The combined EtOAc was washed by brine and dried over anhydrous MgSO₄. After the solvent was concentrated to give white oil, the obtained residue was purified by silica gel chromatography (petroleum etherethyl acetate = 8:1, 0.2% HOAc) to generate 6.5 g compound 4i as a white powder. Yield: 46%, mp: 112–114 °C. ¹H NMR (DMSO-*d*₆, 300 MHz), δ 12.52 (s, 1H), 7.45–7.30 (m, 5H), 7.17 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 8.1 Hz, 1H), 6.93 (d, J = 8.7 Hz, 2H), 5.06 (s, 2H), 4.06-3.98 (m, 1H), 2.96-2.90 (m, 1H), 2.78-2.70 (m, 1H), 1.32 (s, 9H).

Compounds **4j-4k** were synthesized following the procedure described above.

4.1.3.1. (*S*)-3-(4-((4-Bromobenzyl)oxy)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (4j). White powder, yield: 44%, mp: 116–117 °C. ¹H NMR (CDCl₃, 400 MHz), δ 7.51 (d, *J* = 8.4 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.00 (d, *J* = 8.4 Hz, 2H), 5.00 (s, 2H), 4.92–4.90 (m, 1H), 4.57–4.55 (m, 1H), 3.15–3.01 (m, 2H), 1.42 (s, 9H). **4.1.3.2.** (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4-((4-nitrobenzyl)oxy)phenyl)propanoic acid (4k). Pale yellow powder, yield: 33%, mp: 82–84 °C. ¹H NMR (CDCl₃, 400 MHz), δ 8.24 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 8.8 Hz, 2H), 5.14 (s, 2H), 4.96 (d, *J* = 7.2 Hz, 1H), 4.56 (d, *J* = 4.8 Hz, 1H), 3.25–3.02 (m, 2H), 1.41 (s, 9H).

4133 (*S*)-3-([1,1'-Biphenyl]-4-yl)-2-((*tert*-butoxycarbonyl) amino)propanoic acid (41). *N*-Boc-4-Br-phenylanine was the starting material to obtain *N*-Boc-4-Br-phenylanine methyl ester by the procedure of compound 2. N-Boc-4-Br-phenylanine methyl ester (3.58 g, 10 mmol), phenyl boronic acid (1.46 g, 12 mmol), Na₂CO₃ (4.24 g, 40 mmol), Pd(OAc)₂ (0.03 g, 0.1 mmol), TBAB (3.22 g, 10 mmol) were added into 60 mL mixture solvent (DMF-H₂O = 1:1). After stirred overnight at 80 °C, lots of water was added into the reaction solution and extracted with EtOAc. The EtOAc laver was washed by 1 N citric acid and brine. The organic layer was dried over anhydrous MgSO₄ and concentrated to give the crude product. Finally, it was purified by silica gel chromatography (petroleum ether-ethyl acetate = 6:1, 0.2% HOAc) to generate 1.6 g compound **4l** as a white solid. Yield: 47%, mp: 126–128 °C. ¹H NMR (CDCl₃, 400 MHz), δ 7.60–7.55 (m, 4H), 7.45-7.41 (m, 2H), 7.36-7.34 (m, 1H), 7.32-7.27 (m, 2H), 4.98 (d, *I* = 7.6 Hz, 1H), 4.66 (d, *I* = 6.0 Hz, 1H), 3.27–3.11 (m, 2H), 1.42 (s, 9H).

Compounds **4m**–**4n** were synthesized following the procedure described above.

4.1.3.4. (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4'-methoxy-[1,1'-biphenyl]-4-yl)propanoic acid (4m). White powder, yield: 64%, mp: 158–160 °C. ¹H NMR (CDCl₃, 400 MHz), δ 7.44– 7.41 (m, 4H), 7.19 (d, *J* = 8.0 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 4.90 (d, *J* = 7.6 Hz, 1H), 4.66 (d, *J* = 6.4 Hz, 1H), 3.78 (s, 3H), 3.18–2.90 (m, 2H), 1.36 (s, 9H).

4.1.3.5. (*S*)-2-((*tert*-Butoxycarbonyl)amino)-3-(4-((4'-methoxy-[1,1'-biphenyl]-4-yl)methoxy)phenyl)propanoic acid (4n). White powder, yield: 84%, mp: 174–176 °C. ¹H NMR (CDCl₃, 400 MHz), δ 7.56–7.51 (m, 4H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H), 6.98–6.93 (m, 4H), 5.06 (s, 2H), 4.93 (d, *J* = 7.6 Hz, 1H), 4.58 (d, *J* = 6.0 Hz, 1H), 3.15 (s, 3H), 3.16–3.04 (m, 2H), 1.42 (s, 9H).

4.1.4. (2*S*,4*R*)-1-*tert*-Butyl-2-methyl4-hydroxypyrrolidine-1,2-dicarboxylate (7)

Compound **7** was synthesized following the procedure of compound **2**. White powder, yield: 58%, mp: 92–93 °C. ¹H NMR (CDCl₃, 400 MHz), δ 4.49–4.38 (m, 2H), 3.73 (s, 3H), 3.66–3.44 (m, 2H), 2.33–2.19 (m, 2H), 2.10–2.04 (m, 2H), 1.42 (s, 9H).

4.1.5. (2*S*,4*S*)-4-([1,1'-Biphenyl]-4-yloxy)-1-(*tert*-butoxycarbonyl) pyrrolidine-2-carboxylic acid (8a)

In an ice bath, DEAD (0.65 mL, 4 mmol) in 10 mL anhydrous THF was added slowly into the solution of compound **7** (1.18 g, 4.8 mmol), [1,1'-biphenyl]-4-ol (0.68 g, 4 mmol), PPh₃ (1.05 g, 4 mmol) and Et₃N (0.55 mL, 4 mmol) in 20 mL anhydrous THF for 1 h. After stirred overnight at room temperature, the reaction mixture was concentrated under reduced pressure and extracted with EtOAc. The EtOAc layer was washed by 1 N NaOH, 1 N citric acid and brine. Then the organic layer was dried over anhydrous MgSO₄ and concentrated to give white crude residue. The residue was purified by silica gel chromatography (petroleum ether–ethyl acetate = 10:1) to generate 1.1 g white powder. The solution of 1.1 g white powder in 11 mL THF was treated with 11 mL 1 N NaOH.

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After stirred for 1.5 h in 60–80 °C, the solvent was removed under reduced pressure. The residue was acidified with 1 N HCl until pH 2–3 and extracted with EtOAc. The organic layer was dried over anhydrous MgSO₄ and concentrated to give crude residue. Finally, the residue was purified by recrystallized from methanol/water to give 0.6 g compound **8a** as a white needle solid. Yield: 40%, mp: 158–160 °C. ¹H NMR (DMSO-*d*₆, 400 MHz), δ 12.52 (s, 1H), 7.61 (d, *J* = 7.1 Hz, 2H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.45 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 1H), 6.98–6.94 (m, 2H), 5.07–5.06 (m, 1H), 4.32–4.26 (m, 1H), 3.79–3.71 (m, 1H), 3.47–3.42 (m, 1H), 2.62–2.58 (m, 1H), 2.24 (d, *J* = 13.6 Hz, 1H), 1.42 (s, 9H), ESI-MS *m/z*: 382.5 (M–H)[–].

Compounds **8b–8c** were synthesized following the procedure described above.

4.1.5.1. (25,4S)-1-(*tert***-Butoxycarbonyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)pyrolidine-2-carboxylic acid (8b).** White flake solid, yield: 33%, mp: $152-154 \,^{\circ}$ C. ¹H NMR (DMSO- d_6 , 400 MHz), δ 12.49 (s, 1H), 7.57 (d, $J = 8.6 \,$ Hz, 2H), 7.51 (d, $J = 8.0 \,$ Hz, 2H), 7.24 (d, $J = 8.0 \,$ Hz, 2H), 6.95–6.92 (m, 2H), 5.05–5.04 (m, 1H), 4.31–4.26 (m, 1H), 3.75–3.70 (m, 1H), 3.46–3.41 (m, 1H), 2.64–2.53 (m, 1H), 2.32 (s, 3H), 2.24 (d, $J = 13.6 \,$ Hz, 1H), 1.41 (s, 9H), ESI-MS m/z: 396.4 [M–H]⁻.

4.1.5.2. (25,4S)-1-(*tert*-Butoxycarbonyl)-4-((4'-chloro-[1,1'**biphenyl**]-4-yl)oxy)pyrolidine-2-carboxylic acid (8c). White flake solid, yield: 37%, mp: 162–164 °C. ¹H NMR (DMSO- d_6 , 400 MHz), δ 12.55 (s, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.7 Hz, 2H), 7.49 (d, J = 8.6 Hz, 2H), 6.97–6.94 (m, 2H), 5.07– 5.06 (m, 1H), 4.32–4.26 (m, 1H), 3.78–3.71 (m, 1H), 3.46–3.41 (m, 1H), 2.65–2.55 (m, 1H), 2.23 (d, J = 13.2 Hz, 1H), 1.41 (s, 9H), ESI-MS m/z: 416.5 [M–H]⁻.

4.1.6. (*S*)-*tert*-Butyl(3-(4-bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamate (9a)

The solution of compound **3** (3.4 g, 10 mmol) in 60 mL anhydrous DCM was reacted with 4-chloro-3-nitrobenzenesulfonamide (2.6 g, 11 mmol), HATU (4.56 g, 12 mmol) and ethyldiisopropylamine (2.58 g, 20 mmol) and stirred at room temperature for 6 h. Solvent was removed under reduced pressure. The reaction mixture was extracted with EtOAc. The combined organic phases were washed with 1 N citric acid and brine, dried over MgSO4, filtered and concentrated to generate the yellow oil. Finally, it was purified by silica gel chromatography (petroleum ether–ethyl acetate = 6:1, 0.2% HOAc) to generate 4.1 g compound **9a** as a white powder. Yield: 73%, mp: 144–146 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.82 (s, 1H), 8.51 (d, *J* = 2.0 Hz, 1H), 8.17–8.15 (m, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 8.4 Hz, 2H), 4.92 (br s, 1H), 4.26–4.25 (m, 1H), 3.06–3.01 (m, 1H), 2.91–2.85 (m, 1H), 1.40 (s, 9H).

Compounds **9b–9t** were synthesized following the procedure described above.

4.1.6.1. (*S*)-*tert*-Butyl(1-(4-chloro-3-nitrophenylsulfonamido)-**1-oxo-3-phenylpropan-2-yl)carbamate** (9b). Pale yellow powder, yield: 98%, mp: 132–134 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.56 (s, 1H), 8.49 (d, *J* = 2.4 Hz, 1H), 8.21–8.18 (m, 1H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.30–7.27 (m, 2H), 7.24–7.22 (m, 1H), 7.08–7.07 (m, 2H), 4.87–4.83 (m, 1H), 4.29–4.20 (m, 1H), 3.10–3.05 (m, 1H), 2.99–2.93 (m, 1H), 1.40 (s, 9H).

4.1.6.2. (*S*)-*tert*-Butyl(1-oxo-1-(phenylsulfonamido)-3-(4-propoxyphenyl)propan-2-yl)carbamate (9c). White powder, yield: 51%, mp: 108–110 °C. ¹H NMR (CDCl₃, 300 MHz), δ 9.16 (s, 1H), 8.06 (d, *J* = 7.5 Hz, 2H), 7.69 (t, *J* = 7.5 Hz, 1H), 7.58 (t,

J = 7.5 Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 6.77 (d, J = 8.4 Hz, 2H), 4.81 (d, J = 6.6 Hz, 1H), 4.22 (br s, 1H), 3.90 (t, J = 6.6 Hz, 2H), 3.00–2.87 (m, 2H), 1.85–1.76 (m, 2H), 1.39 (s, 9H), 0.99 (t, J = 7.2 Hz, 3H).

4.1.6.3. (*S*)-*tert*-Butyl(1-(4-nitrophenylsulfonamido)-1-oxo-3-(4-propoxyphenyl)propan-2-yl) carbamate (9d). Pale yellow powder, yield: 61%, mp: 246–248 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.49 (s, 1H), 8.38 (d, *J* = 8.8 Hz, 2H), 8.23 (d, *J* = 8.8 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.78 (d, *J* = 8.4 Hz, 2H), 4.83 (d, *J* = 6.8 Hz, 1H), 4.20 (d, *J* = 6.8 Hz, 1H), 3.89 (t, *J* = 6.5 Hz, 2H), 3.00–2.88 (m, 2H), 1.82–1.77 (m, 2H), 1.40 (s, 9H), 1.05 (t, *J* = 8.1 Hz, 3H).

4.1.6.4. (*S*)-*tert*-Butyl(1-(4-chloro-3-nitrophenylsulfonamido)-**1-oxo-3-(4-propoxyphenyl)propan-2-yl)carbamate (9e).** Pale yellow powder, yield: 32%, mp: 136–137 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.58 (s, 1H), 8.50 (d, J = 2.1 Hz, 1H), 8.20 (dd, J_1 = 8.4 Hz, J_2 = 2.1 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.4 Hz, 2H), 4.87 (d, J = 6.9 Hz, 1H), 4.24–4.17 (m, 1H), 3.90 (t, J = 6.6 Hz, 2H), 3.02–2.87 (m, 2H), 1.86–1.74 (m, 2H), 1.41 (s, 9H), 1.06 (t, J = 7.2 Hz, 3H).

4.1.6.5. (*S*)-*tert*-Butyl(3-(4-isopropoxyphenyl)-1-oxo-1-(phenyl-sulfonamido)propan-2-yl)carbamate (9f). White powder, yield: 43%, mp: 133–134 °C. ¹H NMR (DMSO- d_6 , 400 MHz), δ 12.35 (s, 1H), 7.92 (d, *J* = 7.8 Hz, 2H), 7.74–7.59 (m, 3H), 7.10 (d, *J* = 8.4 Hz, 2H), 7.03 (d, *J* = 8.1 Hz, 1H), 6.77 (d, *J* = 8.4 Hz, 2H), 4.60–4.48 (m, 1H), 4.13–4.06 (m, 1H), 2.77–2.72 (m, 1H), 2.55–2.51 (m, 1H), 1.32 (s, 9H), 1.24 (d, *J* = 6.0 Hz, 6H).

4.1.6.6. (*S*)-*tert*-Butyl(3-(4-isobutoxyphenyl)-1-oxo-1-(phenyl-sulfonamido)propan-2-yl)carbamate (9g). White powder, yield: 36%, mp: 122–124 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.27 (s, 1H), 8.06 (d, *J* = 7.6 Hz, 2H), 7.68–7.64 (m, 1H), 7.57–7.53 (m, 2H), 6.93 (d, *J* = 8.4 Hz, 2H), 6.76 (d, *J* = 8.4 Hz, 2H), 4.88 (d, *J* = 7.2 Hz, 1H), 4.26 (s, 1H), 3.68 (d, *J* = 6.4 Hz, 2H), 2.98–2.89 (m, 2H), 2.09–2.01 (m, 1H), 1.39 (s, 9H), 1.02 (d, *J* = 6.8 Hz, 6H).

4.1.6.7. (*S*)-*tert*-Butyl(1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-isobutoxyphenyl)-1-oxopropan-2-yl)carbamate (9h). White powder, yield: 51%, mp: 100–102 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.65 (s, 1H), 8.50 (d, *J* = 2.0 Hz, 1H), 8.17 (dd, *J*₁ = 8.4 Hz, *J*₂ = 2.0 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.79 (d, *J* = 8.4 Hz, 2H), 4.88 (d, *J* = 6.8 Hz, 1H), 4.21 (d, *J* = 6.8 Hz, 1H), 3.69 (d, *J* = 6.8 Hz, 2H), 2.97–2.88 (m, 2H), 2.08–2.02 (m, 1H), 1.40 (s, 9H), 1.03 (d, *J* = 6.8 Hz, 6H).

4.1.6.8. (*S*)-*tert*-Butyl(3-(4-isobutoxyphenyl)-1-(4-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamate (9i). White powder, yield: 41%, mp: 172–174 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.56 (s, 1H), 8.38 (d, *J* = 8.8 Hz, 2H), 8.23 (d, *J* = 8.8 Hz, 2H), 6.97 (d, *J* = 8.0 Hz, 2H), 6.78 (d, *J* = 8.0 Hz, 2H), 4.87 (d, *J* = 6.8 Hz, 1H), 4.22 (d, *J* = 6.4 Hz, 1H), 3.68 (d, *J* = 6.8 Hz, 2H), 2.99–2.87 (m, 2H), 2.12–2.02 (m, 1H), 1.41 (s, 9H), 1.03 (d, *J* = 6.8 Hz, 6H).

4.1.6.9. (*S*)-*tert*-Butyl(1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-(hexyloxy)phenyl)-1-oxopropan-2-yl)carbamate

(9j). Pale yellow powder, yield: 89%, mp: 98–100 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.59 (s, 1H), 8.50 (d, J = 2.4 Hz, 1H), 8.20 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H), 7.75 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 8.0 Hz, 2H), 6.79 (d, J = 8.0 Hz, 2H), 4.86 (d, J = 6.8 Hz, 1H), 4.27–4.14 (m, 1H), 3.93 (d, J = 6.4 Hz, 2H), 3.01–2.89 (m, 2H), 1.80–1.73 (m, 2H), 1.45–1.43 (m, 2H), 1.41 (s, 9H), 1.35–1.25 (m, 4H), 0.92 (t, J = 6.8 Hz, 3H).

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!4.1.6.10. (*S*)-*tert*-Butyl(1-(4-chloro-3-nitrophenylsulfonamido)-**1-oxo-3-(4-phenethoxyphenyl)propan-2-yl)carbamate** (9k). White powder, yield: 73%, mp: 141–143 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.68 (s, 1H), 8.49 (d, *J* = 2.0 Hz, 1H), 8.17 (dd, *J*₁ = 8.4 Hz, *J*₂ = 1.8 Hz, 1H), 7.71 (d, *J* = 8.8 Hz, 1H), 7.34–7.27 (m, 4H), 7.24–7.22 (m, 1H), 6.96 (d, *J* = 8.4 Hz, 2H), 6.77 (d, *J* = 8.4 Hz, 2H), 4.89 (br s, 1H), 4.22 (d, *J* = 6.0 Hz, 1H), 4.15 (t, *J* = 7.2 Hz, 2H), 3.12 (t, *J* = 7.2 Hz, 2H), 2.99–2.86 (m, 2H), 1.40 (s, 9H).

4.1.6.11. (*S*)-*tert*-Butyl(1-(4-chloro-3-nitrophenylsulfonamido)-**3-(4-(naphthalen-2-ylmethoxy)phenyl)-1-oxopropan-2-yl)carbamate (9l).** Pale yellow powder, yield: 38%, mp: 104–105 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.78 (s, 1H), 8.49 (s, 1H), 8.17 (d, *J* = 8.4 Hz, 1H), 7.86–7.82 (m, 4H), 7.70 (d, *J* = 8.4 Hz, 1H), 7.53– 7.47 (m, 3H), 6.98 (d, *J* = 8.0 Hz, 2H), 6.90 (d, *J* = 8.0 Hz, 2H), 5.18 (s, 2H), 4.93 (br s, 1H), 4.23 (br s, 1H), 3.00–2.85 (m, 2H), 1.39 (s, 9H).

4.1.6.12. (*S*)-*tert*-Butyl(1-(4-chloro-3-nitrophenylsulfonamido)-**3-(4-(4-nitrophenoxy)phenyl)-1-oxopropan-2-yl)carbamate** (**9m**). Pale yellow powder, yield: 49%, mp: 116–118 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.66 (s, 1H), 8.52 (d, *J* = 2.4 Hz, 1H), 8.22–8.19 (m, 3H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.05–6.99 (m, 4H), 4.91 (d, *J* = 6.4 Hz, 1H), 4.32–4.28 (m, 1H), 3.15–3.10 (m, 1H), 3.01–2.95 (m, 1H), 1.42 (s, 9H).

4.1.6.13. (*S*)-*tert*-Butyl(3-(4-(benzyloxy)phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamate

(9n). Pale yellow powder, yield: 87%, mp: 212–214 °C. ¹H NMR (DMSO- d_6 , 300 MHz), δ 8.32 (d, J = 1.8 Hz, 1H), 8.00 (dd, $J_1 = 8.4$ Hz, $J_2 = 1.8$ Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.45–7.29 (m, 5H), 6.87 (d, J = 8.4 Hz, 2H), 6.74 (d, J = 8.4 Hz, 2H), 5.85 (d, J = 7.5 Hz, 1H), 5.02 (s, 2H), 3.85–3.79 (m, 1H), 2.92–2.86 (m, 1H), 2.73–2.66 (m, 1H), 1.31 (s, 9H).

4.1.6.14. (*S*)-*tert*-Butyl(3-(4-(benzyloxy)phenyl)-1-oxo-1-(phenylsulfonamido)propan-2-yl)carbamate (90). White powder, yield: 37%, mp: 140–142 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.25 (s, 1H), 8.05 (d, *J* = 7.6 Hz, 2H), 7.67–7.63 (m, 1H), 7.55– 7.51 (m, 2H), 7.43–7.31 (m, 5H), 6.95 (d, *J* = 8.4 Hz, 2H), 6.84 (d, *J* = 8.4 Hz, 2H), 5.02 (s, 2H), 4.87 (d, *J* = 7.6 Hz, 1H), 4.26 (br s, 1H), 2.99–2.87 (m, 2H), 1.39 (s, 9H).

4.1.6.15. (*S*)-*tert*-Butyl(3-(4-((4-bromobenzyl)oxy)phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamate (9p). White powder, yield: 45%, mp: 146–148 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.65 (s, 1H), 8.50 (d, *J* = 2.4 Hz, 1H), 8.20 (dd, *J*₁ = 8.8 Hz, *J*₂ = 2.4 Hz, 1H), 7.75 (d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.8 Hz, 2H), 7.01 (d, *J* = 8.0 Hz, 2H), 6.86 (d, *J* = 8.8 Hz, 2H), 4.99 (s, 2H), 4.83 (d, *J* = 7.2 Hz, 1H), 4.21–4.19 (m, 1H), 3.02–2.89 (m, 2H), 1.40 (s, 9H).

4.1.6.16. (*S*)-*tert*-Butyl(1-(4-chloro-3-nitrophenylsulfonamido)-**3-(4-((4-nitrobenzyl)oxy)phenyl)-1-oxopropan-2-yl)carbamate** (**9q**). Yellow powder, yield: 87%, mp: 142–144 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.53 (s, 1H), 8.49 (d, *J* = 2.0 Hz, 1H), 8.26– 8.20 (m, 3H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.01 (d, *J* = 8.0 Hz, 2H), 5.15 (s, 2H), 4.83 (d, *J* = 6.8 Hz, 1H), 4.23–4.18 (m, 1H), 3.04–2.91 (m, 2H), 1.40 (s, 9H).

4.1.6.17. (*S***)**-*tert*-Butyl(3-([1,1'-biphenyl]-4-yl)-1-(4-chloro-3nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamate (9r). Pale yellow powder, yield: 91%, mp: 104–106 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.61 (s, 1H), 8.46 (d, *J* = 2.0 Hz, 1H), 8.13 (dd, *J*₁ = 8.4 Hz, *J*₂ = 2.4 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.49 (d, *J* = 7.2 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.39 (t, *J* = 7.2 Hz, 2H), 7.30 (t, *J* = 7.2 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 2H), 4.84 (d, *J* = 6.8 Hz, 1H), 4.27–4.19 (m, 1H), 3.08–3.03 (m, 1H), 2.96–2.90 (m, 1H), 1.34 (s, 9H).

4.1.6.18. (*S*)-*tert*-Butyl(1-(4-chloro-3-nitrophenylsulfonamido)-**3**-(4'-methoxy-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamate (9s). Pale yellow powder, yield: 48%, mp: 86–88 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.71 (s, 1H), 8.52 (d, *J* = 2.0 Hz, 1H), 8.19 (dd, *J*₁ = 8.4 Hz, *J*₂ = 1.6 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.50–7.44 (m, 4H), 7.13 (d, *J* = 8.0 Hz, 2H), 6.98 (d, *J* = 8.8 Hz, 2H), 4.90 (d, *J* = 6.8 Hz, 1H), 4.29 (d, *J* = 6.8 Hz, 1H), 3.85 (s, 3H), 3.12–3.07 (m, 1H), 3.01–2.96 (m, 1H), 1.40 (s, 9H).

4.1.6.19. (*S*)-*tert*-Butyl(1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-((4'-methoxy-[1,1'-biphenyl]-4-yl)methoxy)phenyl)-1-oxopropan-2-yl)carbamate (9t). Pale yellow powder, yield: 43%, mp: 181–183 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.60 (s, 1H), 8.50 (s, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.58–7.52 (m, 4H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.01–6.97 (m, 4H), 6.90 (d, *J* = 8.0 Hz, 2H), 5.06 (s, 2H), 4.87 (d, *J* = 6.8 Hz, 1H), 4.24–4.21 (m, 1H), 3.85 (s, 3H), 3.02–2.89 (m, 2H), 1.40 (s, 9H).

4.1.6.20. (*S*)-*tert*-Butyl-(3-(4-((tert-butyldimethylsilyl)oxy)phenyl)-1-(4-chloro-3-nitrobenzamido)-1-oxopropan-2-yl)carba-

mate (9u). Pale yellow oily liquid, yield: 78%. ¹H NMR (CDCl₃, 600 MHz), δ 9.76 (s, 1H), 8.51 (s, 1H), 8.16 (d, *J* = 7.8 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 6.92 (d, *J* = 7.8 Hz, 2H), 6.71 (d, *J* = 7.8 Hz, 2H), 4.90 (s, 1H), 4.23 (s, 1H), 2.99–2.97 (m, 1H), 2.90–2.86 (m, 1H), 1.40 (s, 9H), 0.97 (s, 9H), 0.18 (s, 6H).

4.1.7. (2*S*,4*S*)-*tert*-Butyl-4-(4-phenylphenoxy)-2-(((*S*)-3-(4-bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (1)

Compound 9a (0.68 g, 1.2 mmol) was dissolved in 20 mL EtOAc saturated with HCl gas and stirred overnight at room temperature. Then the generated hydrochloride precipitate was collected and used for the further step. *N*-methylmorpholine (0.22 g, 2.2 mmol) and isobutyl chloroformate (0.16 g, 1.1 mmol) were added into a solution of compound 8a (0.38 g, 1 mmol) in 10 mL anhydrous THF at -20 °C. After stirred for 1 h, the hydrochloride precipitate above was added and stirred overnight at room temperature. Then THF was evaporated under reduced pressure and extracted by EtOAc. The EtOAc layer was washed with 1 N citric acid, brine and dried over anhydrous MgSO₄. The concentrated residue was purified by column chromatography (petroleum ether-ethyl acetate = 3:1-2:1, 0.2% HOAc) to obtain the compound **1** as a white powder. Yield: 41%, mp: 97–99 °C. ¹H NMR (CDCl₃, 600 MHz), δ 10.34 (s, 1H), 8.52 (s, 1H), 8.18 (d, J = 7.7 Hz, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.55–7.53 (m, 4H), 7.43 (t, J = 7.3 Hz, 2H), 7.33 (t, J = 7.2 Hz, 1H), 7.28 (d, J = 7.0 Hz, 2H), 6.93 (d, J = 7.02 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 6.68 (d, J = 5.9 Hz, 1H), 4.90 (s, 1H), 4.73 (s, 1H), 4.41 (d, J = 10.4 Hz, 1H), 3.77 (d, J = 11.8 Hz, 1H), 3.68 (d, J = 11.3 Hz, 1H), 3.27 (d, J = 11.0 Hz, 1H), 2.90 (d, J = 8.6 Hz, 1H), 2.58 (d, J = 13.9 Hz, 1H), 2.50–2.46 (m, 1H), 1.39 (s, 9H). ¹³C NMR $(CDCl_3, 100 \text{ MHz}), \delta$ 171.96, 170.04, 155.99, 147.54, 140.18, 138.69, 135.40, 133.42, 132.80, 132.55, 132.05, 131.08, 128.85, 128.69, 127.11, 126.77, 126.13, 121.95, 115.96, 82.80, 60.06, 53.36, 53.22, 36.30, 34.60, 28.09. HRMS (AP-ESI) m/z Calcd for C₃₇-H₃₆BrClN₄O₉S [M–H]⁻ 825.0997, found: 825.1006.

Compounds **10–46** were synthesized following the procedure described above.

4.1.7.1. (2*S*,**4***S*)-*tert*-Butyl-2-(((*S*)-3-(4-bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)-4-(4-(4'-methyl)phenylphenoxy)pyrrolidine-1-carboxylate (10). Yellow powder, yield: 31%, mp: 111–113 °C. ¹H NMR (CDCl₃, 300 MHz), δ 8.53 (s, 1H), 8.20 (d, *J* = 7.8 Hz, 1H), 7.72 (d, *J* = 8.1 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 7.8 Hz, 2H), 7.31 (m, 2H), 7.23 (m, 2H), 6.94 (d, *J* = 7.5 Hz, 2H), 6.84 (d, *J* = 8.4 Hz, 2H), 6.61 (m, 1H), 4.90 (s, 1H), 4.73–4.70 (m, 1H), 4.40 (d, *J* = 9.0 Hz, 1H), 3.77 (d, *J* = 12.3 Hz, 1H), 3.68 (dd, *J* = 12.0 Hz, 3.0 Hz, 1H), 3.31 (d, *J* = 13.2 Hz, 1H), 2.90 (dd, *J* = 13.8 Hz, 5.1 Hz, 1H), 2.60–2.47 (m, 2H), 2.39 (s, 3H), 1.39 (s, 9H).¹³C NMR (CDCl₃, 100 MHz), δ 171.75, 169.87, 155.77, 147.54, 138.70, 137.30, 136.89, 133.41, 132.79, 132.54, 132.04, 131.09, 130.91, 129.56, 128.85, 128.46, 126.60, 126.13, 121.94, 115.97, 82.82, 65.57, 60.06, 53.37, 53.22, 36.29, 30.59, 28.08, 21.06. HRMS (AP-ESI) *m*/*z* Calcd for C₃₈H₃₈BrClN₄O₉S [M–H]⁻ 839.1153, found: 839.1159.

4.1.7.2. ((25,4S)-tert-Butyl-2-(((S)-3-(4-bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)car-bamoyl)-4-(4-(4'-chloro)phenylphenoxy)pyrrolidine-1-car-

boxvlate (11). Yellow powder, yield: 26%, mp: 125-127 °C. ¹H NMR (CDCl₃, 600 MHz), δ 10.26 (s, 1H), 8.53 (s, 1H), 8.20 (d, / = 7.2 Hz, 1H), 7.73 (d, / = 8.4 Hz, 1H), 7.51 (d, *J* = 7.8 Hz, 2H), 7.47 (d, *J* = 7.8 Hz, 2H), 7.40 (d, *J* = 7.8 Hz, 2H), 7.30 (d, J = 7.2 Hz, 2H), 6.94 (d, J = 7.8 Hz, 2H), 6.85 (d, I = 8.4 Hz, 2H), 6.64 (d, I = 7.2 Hz, 1H), 4.90 (s, 1H), 4.73 (s, 1H), 4.40 (d, *J* = 10.2 Hz, 1H), 3.77 (d, *J* = 11.4 Hz, 1H), 3.69 (d, J = 11.4 Hz, 1H), 3.30 (d, J = 10.8 Hz, 1H), 2.90 (d, J = 13.8 Hz, 1H), 2.57 (d, *J* = 14.4 Hz, 1H), 2.50–2.47 (m, 1H), 1.40 (s, 9H).¹³C NMR (CDCl₃, 100 MHz), δ 172.25, 169.51, 156.21, 147.54, 138.64, 134.11, 133.41, 133.20, 133.08, 132.83, 132.74, 132.55, 132.04, 131.05, 128.98, 128.56, 127.99, 126.10, 121.95, 116.03, 82.84, 60.04, 53.35, 53.22, 36.32, 34.54, 28.31, 28.08. HRMS (AP-ESI) *m*/*z* Calcd for C₃₇H₃₅BrCl₂-N₄O₉S [M–H]⁻ 859.0607, found: 859.0610.

4.1.7.3. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenyl-sulfonamido)-1-oxo-3-phenylpropan-2-yl)carbamoyl)-4-(4-(4'-methyl)phenylphenoxy)pyrrolidine-1-carboxylate

(12). Yellow powder, yield: 38%, mp: 90-92 °C. ¹H NMR $(CDCl_3, 600 \text{ MHz}), \delta 10.21 \text{ (s, 1H)}, 8.46 \text{ (s, 1H)}, 8.23 \text{ (d,}$ *I* = 7.8 Hz, 1H), 7.73 (d, *I* = 8.4 Hz, 1H), 7.51 (d, *I* = 8.4 Hz, 2H), 7.43 (d, J = 7.8 Hz, 2H), 7.23 (m, 3H), 7.17 (t, J = 7.2 Hz, 2H), 7.04 (d, J = 7.2 Hz, 2H), 6.84 (d, J = 8.3 Hz, 2H), 6.64 (s, 1H), 4.90 (s, 1H), 4.70-4.74 (m, 1H), 4.39 (d, J=8.4 Hz, 1H), 3.76 (d, *I* = 12.6 Hz, 1H), 3.67 (dd, *I* = 12.6 Hz, 3.0 Hz, 1H), 3.37 (dd, *J* = 13.8 Hz, 4.8 Hz, 1H), 2.93 (dd, *J* = 14.4 Hz, 5.4 Hz, 1H), 2.55 (d, J = 13.8 Hz, 1H), 2.48-2.51 (m, 1H), 2.39 (s, 3H), 1.35 (s, 9H).¹³C NMR (CDCl₃, 100 MHz), δ 171.59, 170.12, 155.81, 147.52, 138.80, 137.39, 136.83, 135.25, 134.46, 132.93, 132.69, 132.48, 130.90, 129.54, 129.34, 129.00, 128.40, 127.78, 126.59, 126.04, 115.89, 82.60, 65.56, 60.15, 53.29, 36.74, 34.74, 30.59, 28.14, 21.05. HRMS (AP-ESI) *m*/*z* Calcd for C₃₈H₃₉ClN₄O₉S [M–H][–] 761.2048, found: 761.2052.

4.1.7.4. (2*S*,4*S*)-*tert*-Butyl4-([1,1'-biphenyl]-4-yloxy)-2-(((*S*)-1-oxo-1-(phenylsulfonamido)-3-(4-propoxyphenyl)propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (13). Pale yellow powder, yield: 35%, mp: 94–96 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.88 (s, 1H), 8.06 (d, *J* = 7.6 Hz, 2H), 7.64–7.62 (m, 1H), 7.54–7.52 (m, 6H), 7.44–7.40 (m, 2H), 7.33–7.30 (m, 1H), 6.93–6.89 (m, 4H), 6.67 (d, *J* = 8.2 Hz, 2H), 6.61 (br s, 1H), 4.91 (s, 1H), 4.70 (br s, 1H), 4.43 (d, *J* = 9.0 Hz, 1H), 3.84–3.68 (m, 4H), 3.38 (d, *J* = 13.0 Hz, 1H), 2.82–2.78 (m, 1H), 2.61 (d, *J* = 14.4 Hz, 1H), 2.49– 2.44 (m, 1H), 1.79–1.74 (m, 2H), 1.35 (s, 9H), 1.03 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.48, 170.28, 158.56, 156.15, 140.35, 138.94, 135.16, 133.61, 130.48, 128.79, 128.67, 128.58,126.99, 126.76, 126.16, 115.94, 114.85, 82.30, 69.33, 60.16, 53.25, 36.01, 34.72, 29.70, 28.11, 22.58, 10.56. HRMS (AP- ESI) m/z Calcd for $C_{40}H_{45}N_3O_8S$ $[M-H]^-$ 726.2849, found: 726.2846.

4.1.7.5. (2*S*,4*S*)-*tert*-Butyl4-((4'-methyl-[1,1'-biphenyl]-4-yl) oxy)-2-(((*S*)-1-(4-nitrophenylsulfonamido)-1-oxo-3-(4-propox-yphenyl)propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate

(14). Pale yellow powder, yield: 38%, mp: 88–90 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.11 (s, 1H), 8.37 (d, J = 8.6 Hz, 2H), 8.24 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.24 (d, J = 8.0 Hz, 2H), 7.00 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.0 Hz, 2H), 6.65 (d, J = 7.3 Hz, 1H), 4.91 (s, 1H), 4.63 (br s, 1H), 4.40 (d, J = 9.6 Hz, 1H), 3.85–3.78 (m, 2H), 3.73–3.67 (m, 2H), 3.35 (d, J = 11.6 Hz, 1H), 2.87–2.82 (m,1H), 2.53–2.49 (m, 2H), 2.38 (s, 3H), 1.80–1.75 (m, 2H), 1.36 (s, 9H), 1.04 (t, J = 7.4 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz), δ 175.45, 171.68, 170.57, 158.73, 155.82, 150.63, 144.39, 137.37, 136.83, 135.27, 130.43, 130.00, 129.54, 128.39, 126.58, 125.88, 123.88, 115.93, 114.92, 82.55, 69.39, 60.17, 53.55, 53.27, 35.99, 34.73, 28.10, 22.57, 21.04, 20.53, 10.53. HRMS (AP-ESI) m/z Calcd for C₄₁H₄₆N₄-O₁₀S [M–H]⁻ 785.2856, found: 785.2860.

4.1.7.6. (2*S*,4*S*)-*tert*-Butyl4-([1,1'-biphenyl]-4-yloxy)-2-(((*S*)-1-(4-nitrophenylsulfonamido)-1-oxo-3-(4-propoxyphenyl)pro-

pan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (15). Pale yellow powder, yield: 23%, mp: 90-92 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.12 (s, 1H), 8.36 (d, J = 8.6 Hz, 2H), 8.24 (d, J = 8.6 Hz, 2H), 7.55–7.52 (m, 4H), 7.44 (t, J = 7.4 Hz, 2H), 7.34– 7.30 (m, 1H), 6.99 (d, J = 8.4 Hz, 2H), 6.89 (d, J = 8.4 Hz, 2H), 6.72-6.65 (m, 3H), 4.92 (s, 1H), 4.67-4.62 (m, 1H), 4.41 (dd, $J_1 = 2.5 \text{ Hz}, J_2 = 9.9 \text{ Hz}, 1\text{H}$, 3.84–3.79 (m, 3H), 3.71–3.67 (m, 1H), 3.35 (d, J = 11.5 Hz, 1H), 2.87-2.83 (m, 1H), 2.57-2.49 (m, 2H), 1.78–1.76 (m, 2H), 1.36 (s, 9H), 1.04 (t, J = 7.4 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz), *δ* 171.64, 170.58, 158.74, 156.04, 150.64, 144.39, 140.25, 135.29, 130.43, 130.00, 128.82, 128.61, 127.07, 126.74, 125.87, 123.89, 115.93, 114.93, 82.59, 69.39, 60.18, 53.54, 53.27, 35.96, 34.75, 29.69, 28.10, 22.56, 10.53. HRMS (AP-ESI) m/ *z* Calcd for C₄₀H₄₄N₄O₁₀S [M–H]⁻ 771.2700, found: 771.2708.

4.1.7.7. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsul-fonamido)-1-oxo-3-(4-propoxyphenyl)propan-2-yl)carbamoyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-carboxy-

Pale yellow powder, yield: 44%, mp: 96–98 °C. ¹H late (16). NMR (CDCl₃, 400 MHz), δ 10.16 (s, 1H), 8.50 (d, I = 2.0 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.24 (d, J = 8.0 Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 6.69–6.62 (m, 3H), 4.91 (s, 1H), 4.71-4.66 (m, 1H), 4.40-4.37 (m, 1H), 3.86-3.79 (m, 3H), 3.70–3.66 (m, 1H), 3.39–3.30 (m, 1H), 2.86 (dd, $J_1 = 5.2$ Hz, $J_2 = 14.0$ Hz, 1H), 2.57–2.45 (m, 2H), 2.39 (s, 3H), 1.82–1.73 (m, 2H), 1.34 (s, 9H), 1.04 (d, J = 7.6 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.41, 170.71, 158.79, 155.80, 147.47, 138.86, 137.38, 136.83, 135.26, 132.99, 132.68, 132.51, 130.39, 129.55, 128.42, 126.60, 126.10, 125.74, 115.90, 114.86, 82.61, 69.34, 60.16, 53.30, 35.95, 34.71, 29.71, 28.06, 22.58, 21.06, 10.56. HRMS (AP-ESI) m/z Calcd for C₄₁H₄₅ClN₄O₁₀S [M–H]⁻ 819.2467, found: 819.2474.

4.1.7.8. (2S,4S)-*tert*-Butyl-2-(((S)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-(4-propoxyphenyl)propan-2-yl)carbamoyl)-**4-((4'-chloro-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-carboxylate (17).** Pale yellow powder, yield: 58%, mp: 104–106 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.14 (s, 1H), 8.50 (d, *J* = 2.0 Hz, 1H), 8.25 (d, *J* = 8.8 Hz, 1H), 7.74 (d, *J* = 8.8 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 8.4 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.69–6.63 (m, 3H), 4.92 (s, 1H), 4.71–

726.2844.

4.66 (m, 1H), 4.40–4.38 (m, 1H), 3.86–3.78 (m, 3H), 3.72–3.69 (m, 1H), 3.36–3.30 (m, 1H), 2.85 (dd, J_1 = 5.2 Hz, J_2 = 14.0 Hz, 1H), 2.58–2.45 (m, 2H), 1.82–1.73 (m, 2H), 1.35 (s, 9H), 1.04 (d, J = 7.6 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.40, 170.59, 158.78, 156.23, 155.92, 147.47, 138.83, 138.72, 133.96, 133.12, 132.98, 132.70, 132.50, 130.38, 128.96, 128.51, 127.98, 126.07, 125.71, 115.95, 114.84, 82.63, 69.35, 60.10, 53.29, 35.99, 34.59, 29.70, 28.05, 22.58, 10.55. HRMS (AP-ESI) m/z Calcd for C₄₀H₄₂Cl₂N₄O₁₀S [M–H]⁻ 839.1920, found: 839.1918.

4.1.7.9. (2*S*,4*S*)-*tert*-Butyl-4-([1,1′-biphenyl]-4-yloxy)-2-(((*S*)-3-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-1-(4-chloro-3-nitro-phenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-

1-carboxylate (18). Pale yellow powder, yield: 34%, mp: 102– 104 °C. ¹H NMR (CDCl₃, 600 MHz), δ 10.2 (s, 1H), 8.52 (s, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.55–7.53 (m, 4H), 7.42 (t, *J* = 7.2 Hz, 2H), 7.32 (t, *J* = 7.2 Hz, 1H), 6.88–6.92 (m, 4H), 6.69 (d, *J* = 6.6 Hz, 1H), 6.63 (d, *J* = 7.8 Hz, 2H), 4.93 (s, 1H), 4.70 (s, 1H), 4.39 (d, *J* = 9.6 Hz, 1H), 3.79 (d, *J* = 12.0 Hz, 1H), 3.70 (d, *J* = 11.4 Hz, 1H), 3.32 (d, *J* = 12.0 Hz, 1H), 2.84 (d, *J* = 9.0 Hz, 1H), 2.57–2.55 (d, *J* = 13.8 Hz, 1H), 2.51–2.49 (m, 1H), 1.37 (s, 9H), 0.96 (s, 9H), 0.15–0.14 (m, 6H). ¹³C NMR (DMSO-*d*₆, 100 MHz), δ 171.48, 170.68, 156.42, 153.63, 153.22, 147.00, 139.63, 139.03, 132.80, 132.29, 130.50, 130.16, 128.92, 128.72, 127.67, 126.65, 126.08, 125.00, 119.22, 115.70, 78.95, 73.76, 58.70, 54.21, 51.24, 35.84, 27.86, 27.53, 25.40, 17.74, -4.73. HRMS (AP-ESI) *m/z* Calcd for C₄₃H₅₁ClN₄O₁₀SSi [M–H]⁻ 877.2705, Found: 877.2708.

4.1.7.10. (2S,4S)-*tert*-Butyl4-([1,1'-biphenyl]-4-yloxy)-2-(((S)-3-(4-isopropoxyphenyl)-1-oxo-1-(phenylsulfonamido)propan-2yl)carbamoyl)pyrrolidine-1-carboxylate (19). Pale yellow powder, yield: 30%, mp: 93–95 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.86 (s, 1H), 8.07 (d, *J* = 7.5 Hz, 2H), 7.66 (t, *J* = 7.3 Hz, 1H), 7.55– 7.53 (m, 6H), 7.44–7.41 (m, 2H), 7.34–7.30 (m, 1H), 6.91–6.89 (m, 4H), 6.64–6.62 (m, 3H), 4.91 (s, 1H), 4.72 (br s, 1H), 4.46 (m, 2H), 3.78–3.68 (m, 2H), 3.38 (d, *J* = 12.4 Hz, 1H), 2.81–2.77 (m, 1H), 2.61 (d, *J* = 14.2 Hz, 1H), 2.50–2.47 (m, 1H), 1.35 (s, 9H), 1.29 (d, *J* = 4.1 Hz, 6H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.21, 170.28, 157.36, 156.16, 155.76, 140.37, 138.94, 135.15, 133.61, 130.53, 128.79, 128.67, 128.58, 126.98, 126.76, 126.09, 115.94, 82.30, 69.66, 60.16, 53.23, 36.02, 34.75, 29.65, 28.11, 22.06. HRMS (AP-

4.1.7.11. (2*S*,4*S*)-*tert*-Butyl4-([1,1′-biphenyl]-4-yloxy)-2-(((*S*)-3-(4-isobutoxyphenyl)-1-oxo-1-(phenylsulfonamido)propan-2-

ESI) m/z Calcd for C₄₀H₄₅N₃O₈S [M-H]⁻ 726.2849, found:

yl)carbamoyl)pyrrolidine-1-carboxylate (20). White powder, yield: 20%, mp: 86–88 °C. ¹H NMR (CDCl₃, 400 MHz), *δ* 9.82 (s, 1H), 8.07 (d, *J* = 7.7 Hz, 2H), 7.67–7.63 (m, 1H), 7.60–7.54 (m, 6H), 7.44 (t, *J* = 7.4 Hz, 2H), 7.34–7.32 (m, 1H), 6.93–6.90 (m, 4H), 6.67 (d, *J* = 8.2 Hz, 2H), 6.60 (d, *J* = 8.2 Hz, 1H), 4.92 (s, 1H), 4.70 (br s, 1H), 4.43 (d, *J* = 9.4 Hz, 1H), 3.80–3.66 (m, 2H), 3.65–3.58 (m, 2H), 3.41 (d, *J* = 12.2 Hz, 1H), 3.02 (br s,1H), 2.80–2.76 (m, 1H), 2.61 (d, *J* = 13.2 Hz, 1H), 2.50–2.48 (m, 1H), 1.34 (s, 9H), 1.02 (d, *J* = 6.7 Hz, 6H). ¹³C NMR (CDCl₃, 100 MHz), *δ* 171.18, 170.22, 158.67, 156.16, 155.75, 140.35, 138.96, 135.15, 133.61, 130.48, 128.79, 128.67, 128.59, 126.99, 126.77, 126.10, 115.93, 114.86, 82.28, 74.25, 60.16, 53.27, 36.02, 34.73, 28.28, 28.11, 19.27, 19.25. HRMS (AP-ESI) *m*/*z* Calcd for $C_{41}H_{47}N_3O_8S$ [M–H]⁻ 740.3006, found: 740.3025.

4.1.7.12. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenyl-sulfonamido)-3-(4-isobutoxyphenyl)-1-oxopropan-2-yl)car-bamoyl)-4-((4'-chloro-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-carboxylate (21). Pale yellow powder, yield: 51%, mp: 102–104 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.14 (s, 1H), 8.50 (d,

J = 2.0 Hz, 1H), 8.25 (d, *J* = 8.4 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.51–7.45 (m, 4H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.00–6.87 (m, 4H), 6.69–6.63 (m, 3H), 4.93 (s, 1H), 4.70–4.66 (m, 1H), 4.41–4.38 (m, 1H), 3.85–3.69 (m, 2H), 3.66–3.57 (m, 2H), 3.36–3.34 (m, 1H), 2.84 (dd, *J*₁ = 5.2 Hz, *J*₂ = 14.0 Hz, 1H), 2.59–2.46 (m, 2H), 2.09–1.99 (m, 1H), 1.34 (s, 9H), 1.02 (d, *J* = 6.8 Hz, 6H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.34, 170.60, 158.88, 156.23, 155.92, 147.47, 138.87, 138.72, 133.95, 133.12, 133.00, 132.70, 132.50, 130.38, 128.96, 128.53, 127.99, 126.07, 125.65, 115.94, 114.83, 82.64, 74.22, 60.09, 53.31, 35.99, 34.58, 29.71, 28.29, 28.05, 19.27, 19.23. HRMS (AP-ESI) *m*/*z* Calcd for C₄₁H₄₄Cl₂N₄O₁₀S [M–H]⁻ 853.2077, found: 853.2076.

4.1.7.13. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsul-fonamido)-3-(4-isobutoxyphenyl)-1-oxopropan-2-yl)car-

bamoyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-carboxvlate (22). Pale yellow powder, yield: 42%, mp: 100–102 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.14 (s, 1H), 8.51 (d, J = 2.0 Hz, 1H), 8.26 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 2H), 6.69 (d, J = 8.8 Hz, 2H), 6.63 (d, J = 7.6 Hz, 1H), 4.91 (s, 1H), 4.71-4.64 (m, 1H), 4.40-4.37 (m, 1H), 3.82 (d, J = 12.0 Hz, 1H), 3.71-3.66 (m, 1H), 3.64-3.57 (m, 2H), 3.39-3.34 (m, 1H), 2.84 $(dd, J_1 = 5.2 Hz, J_1 = 14.0 Hz, 1H), 2.57-2.45 (m, 2H), 2.39 (s, 10.10 Hz)$ 3H), 2.09–2.01 (m, 1H), 1.33 (s, 9H), 1.02 (d, J = 6.8 Hz, 6H). ^{13}C NMR (CDCl₃, 100 MHz), δ 171.33, 170.75, 158.89, 155.93, 155.79, 147.46, 138.85, 137.37, 136.84, 135.25, 133.02, 132.69, 132.51, 130.39, 129.55, 128.44, 126.61, 126.11, 125.66, 115.88, 114.85, 82.62, 74.20, 60.15, 53.32, 35.93, 34.70, 29.72, 28.29, 28.06, 21.08, 19.29, 19.25. HRMS (AP-ESI) m/z Calcd for C₄₂H₄₇ClN₄O₁₀S [M–H]⁻ 833.2623, found: 833.2626.

(23). Pale yellow powder, yield: 53%, mp: 104–105 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.12 (s, 1H), 8.36 (d, *J* = 8.4 Hz, 2H), 8.24 (d, *J* = 8.4 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 6.99 (d, *J* = 8.0 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 2H), 6.71–6.62 (m, 3H), 4.92 (s, 1H), 4.65 (br s, 1H), 4.40 (d, *J* = 10.0 Hz, 1H), 3.82 (d, *J* = 12.0 Hz, 1H), 3.71–3.58 (m, 3H), 3.39–3.29 (m, 1H), 2.86 (dd, *J*₁ = 5.2 Hz, *J*₂ = 14.0 Hz, 1H), 2.58–2.45 (m, 2H), ¹³C NMR (CDCl₃, 100 MHz), δ 171.58, 170.62, 158.84, 155.81, 150.61, 144.38, 137.35, 136.85, 130.93, 130.45, 130.02, 129.55, 128.86, 128.42, 126.59, 125.76, 123.89, 115.89, 114.90, 82.58, 74.26, 65.58, 60.17, 53.30, 35.96, 34.70, 29.71, 28.29, 28.10, 21.06, 19.24. HRMS (AP-ESI) *m/z* Calcd for C₄₂-H_{48N4}Q₀₁₀S [M–H]⁻ 799.3013, found: 799.3015.

4.1.7.15. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-(hexyloxy)phenyl)-1-oxopropan-2-yl)car-bamoyl)-4-((4'-chloro-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-

carboxylate (24). Pale yellow powder, yield: 47%, mp: 84– 86 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.14 (s, 1H), 8.50 (d, *J* = 2.0 Hz, 1H), 8.25 (d, *J* = 8.4 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.51–7.44 (m, 4H), 7.40 (d, *J* = 8.4 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 6.69 (d, *J* = 8.4 Hz, 2H), 6.64 (d, *J* = 7.6 Hz, 1H), 4.92 (s, 1H), 4.68–4.66 (m, 1H), 4.41–4.38 (m, 1H), 3.89– 3.78 (m, 3H), 3.71–3.68 (m, 1H), 3.35–3.31 (m, 1H), 2.85 (dd, *J*₁ = 5.2 Hz, *J*₂ = 14.0 Hz, 1H), 2.58–2.46 (m, 2H), 1.77–1.70 (m, 2H), 1.49–1.41 (m, 2H), 1.35 (s, 9H), 1.35–1.24 (m, 4H), 0.92 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.39, 170.60, 158.79, 156.22, 155.92, 147.47, 138.83, 138.72, 133.96, 133.13,

132.98, 132.70, 132.50, 130.37, 128.96, 128.51, 127.98, 126.07, 125.69, 115.95, 114.83, 82.62, 67.89, 60.10, 53.41, 53.28, 35.98, 34.60, 31.59, 29.25, 28.06, 25.78, 22.60, 14.03. HRMS (AP-ESI) m/z Calcd for $C_{43}H_{48}Cl_2N_4O_{10}S$ [M–H]⁻ 881.2390, found: 881.2388.

4.1.7.16. (25,45)-*tert*-Butyl-2-(((5)-1-(4-chloro-3-nitrophenylsul-fonamido)-3-(4-(hexyloxy)phenyl)-1-oxopropan-2-yl)car-

bamoyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-Pale yellow powder, yield: 40%, mp: 90carboxylate (25). 92 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.15 (s, 1H), 8.51 (d, J = 2.0 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.24 (d, J = 8.0 Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.4 Hz, 2H), 6.69 (d, J = 8.4 Hz, 2H), 6.63 (d, J = 7.6 Hz, 1H), 4.91 (s, 1H), 4.67 (br s, 1H), 4.40-4.37 (m, 1H), 3.86-3.78 (m, 3H), 3.70-3.66 (m, 1H), 3.36–3.32 (m, 1H), 2.86 (dd, J_1 = 5.2 Hz, J_2 = 14.0 Hz, 1H), 2.57– 2.45 (m, 2H), 2.39 (s, 3H), 1.77-1.70 (m, 2H), 1.49-1.40 (m, 2H), 1.35 (s, 9H), 1.33–1.26 (m, 4H), 0.92 (t, I = 6.8 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz), *δ* 171.41, 170.73, 158.81, 155.94, 155.79, 147.46, 138.84, 137.37, 136.83, 135.25, 133.00, 132.69, 132.51, 130.39, 129.55, 128.42, 126.60, 126.12, 125.70, 115.89, 114.84, 82.60, 67.88, 60.16, 53.30, 35.95, 34.70, 31.61, 29.26, 28.07, 25.79, 22.62, 21.08, 14.05. HRMS (AP-ESI) m/z Calcd for C₄₄H₅₁-ClN₄O₁₀S [M–H][–] 861.2936, found: 861.2936.

4.1.7.17. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-(4-phenethoxyphenyl)propan-2-yl)car-bamoyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-

carboxylate (26). Pale yellow powder, yield: 68%, mp: 98-100 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.19 (s, 1H), 8.50 (s, 1H), 8.22 (d, J = 8.4 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.52 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.0 Hz, 2H), 7.33-7.26 (m, 3H), 7.25-7.19 (m, 4H), 6.94 (d, J = 8.0 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 6.71-6.63 (m, 3H), 4.89 (s, 1H), 4.68 (br s, 1H), 4.39 (d, J = 10.0 Hz, 1H), 4.07-4.02 (m, 2H), 3.79-3.65 (m, 2H), 3.34-3.30 (m, 1H), 3.07-3.00 (m, 2H), 2.86-2.81 (m, 1H), 2.56-2.43 (m, 2H), 2.38 (s, 3H), 1.28 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.45, 170.66, 158.44, 155.92, 155.78, 147.46, 138.82, 138.20, 137.37, 136.85, 135.24, 132.95, 132.70, 132.52, 130.42, 129.57, 129.03, 128.50, 128.43, 126.61, 126.54, 126.12, 126.05, 115.91, 114.89, 82.59, 68.53, 60.14, 53.30, 35.98, 35.80, 34.68, 28.35, 28.02, 21.09. HRMS (AP-ESI) m/z Calcd for C₄₆H₄₇ClN₄O₁₀S [M–H]⁻ 881.2623, found: 881.2623.

4.1.7.18. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-(4-phenethoxyphenyl)propan-2-yl)car-bamoyl)-4-((4'-chloro-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-

carboxylate (27). White powder, yield: 77%, mp: 103–104 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.15 (s, 1H), 8.50 (s, 1H), 8.23 (d, J = 8.4 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 7.49–7.43 (m, 4H), 7.39 (d, J = 8.4 Hz, 2H), 7.33–7.29 (m, 3H), 7.23–7.19 (m, 2H), 6.94 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 8.4 Hz, 2H), 6.72–6.62 (m, 3H), 4.90 (br s, 1H), 4.69–4.64 (m, 1H), 4.40–4.37 (m, 1H), 4.08–4.02 (m, 2H), 3.78–3.58 (m, 2H), 3.34–3.30 (m, 1H), 3.07–2.99 (m, 2H), 2.85–2.81 (m, 1H), 2.57–2.45 (m, 2H), 1.29 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.43, 170.54, 158.43, 156.21, 155.91, 147.45, 138.78, 138.71, 138.17, 133.95, 133.13, 132.97, 132.73, 132.52, 130.41, 129.02, 128.98, 128.51, 127.99, 126.56, 126.08, 115.95, 114.86, 82.61, 68.53, 60.08, 53.29, 36.02, 35.79, 34.56, 28.34, 28.01. HRMS (AP-ESI) *m*/*z* Calcd for C₄₅H₄₄Cl₂N₄O₁₀S [M–H]⁻ 901.2077, found: 901.2076.

4.1.7.19. (2*S*,**4***S*)-*tert*-Butyl4-([1,1'-biphenyl]-4-yloxy)-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-(naphthalen-2ylmethoxy)phenyl)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (28). Pale yellow powder, yield: 28%, mp: 108– 110 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.20 (s, 1H), 8.52 (d, J = 1.6 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 7.85–7.79 (m, 4H), 7.73 (d, J = 8.0 Hz, 1H), 7.53–7.48 (m, 7H), 7.45–7.39 (m, 2H), 7.34–7.30 (m, 1H), 6.99 (d, J = 8.4 Hz, 2H), 6.88–6.82 (m, 4H), 6.65 (d, J = 7.2 Hz, 1H), 5.12 (s, 2H), 4.88–4.81 (m, 1H), 4.69–4.65 (m, 1H), 4.41–4.37 (m, 1H), 3.78–3.73 (m, 1H), 3.68–3.64 (m, 1H), 3.33–3.30 (m, 1H), 2.91–2.86 (m, 1H), 2.56–2.48 (m, 2H), 1.38 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.55, 170.60, 158.37, 155.97, 147.45, 140.19, 138.77, 133.25, 133.06, 132.69, 132.50, 130.46, 128.82, 128.60, 128.40, 127.93, 127.73, 127.05, 126.73, 126.47, 126.27, 126.15, 126.09, 125.15, 115.90, 115.27, 82.61, 69.95, 60.12, 53.49, 53.26, 35.97, 34.63, 28.33, 28.10, 20.46. HRMS (AP-ESI) *m*/*z* Calcd for C₄₈H₄₅ClN₄O₁₀S [M–H]⁻ 903.2472, found: 903.2470.

4.1.7.20. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-(naphthalen-2-ylmethoxy)phenyl)-1-oxo-

propan-2-yl)carbamoyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy) pyrrolidine-1-carboxylate (29). White powder, yield: 44%, mp: 174–176 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.20 (s, 1H), 8.52 (s, 1H), 8.24 (d, J = 8.4 Hz, 1H), 7.85-7.80 (m, 4H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.52–7.41 (m, 7H), 7.22 (d, *J* = 8.0 Hz, 2H), 6.99 (d, I = 8.4 Hz, 2H, 6.84 (d, I = 8.0 Hz, 4H), 6.65 (d, I = 6.4 Hz, 1H), 5.12 (s, 2H), 4.87 (br s, 1H), 4.66 (br s, 1H), 4.40 (d, J = 8.0 Hz, 1H), 3.79-3.65 (m, 2H), 3.33-3.30 (m, 1H), 2.91-2.87 (m, 1H), 2.56-2.47 (m, 2H), 2.37 (s, 3H), 1.37 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.47, 170.57, 158.34, 155.72, 147.41, 138.74, 137.28, 136.78, 135.19, 134.07, 133.22, 133.04, 132.94, 132.66, 132.47, 130.43, 129.51, 128.36, 127.90, 127.70, 126.54, 126.25, 126.07, 125.13, 115.89, 115.23, 82.55, 69.91, 60.10, 53.41, 53.24, 35.89, 34.61, 28.07, 21.02. HRMS (AP-ESI) m/z Calcd for C₄₉H₄₇-ClN₄O₁₀S [M–H]⁻ 917.2623, found: 917.2623.

4.1.7.21. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-(naphthalen-2-ylmethoxy)phenyl)-1-oxo-

propan-2-yl)carbamoyl)-4-((4'-chloro-[1,1'-biphenyl]-4-yl)oxy) pyrrolidine-1-carboxylate (30). Pale vellow powder, vield: 50%, mp: 120–122 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.23 (s, 1H), 8.51 (s, 1H), 8.24 (d, J = 8.0 Hz, 1H), 7.85-7.80 (m, 4H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.50–7.34 (m, 9H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.86– 6.83 (m, 4H), 6.66 (br s, 1H), 5.11 (s, 2H), 4.87-4.81 (m, 1H), 4.69-4.64 (m, 1H), 4.41-4.38 (m, 1H), 3.79-3.65 (m, 2H), 3.31-3.25 (m, 1H), 2.91-2.86 (m, 1H), 2.57-2.44 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (CDCl₃, 100 MHz), δ 171.55, 158.35, 156.18, 155.90, 147.44, 138.75, 138.65, 134.08, 133.24, 133.06, 132.71, 132.50, 130.45, 128.94, 128.46, 128.40, 128.24, 127.94, 127.87, 127.73, 126.46, 126.32, 126.26, 126.17, 126.05, 125.13, 115.96, 115.23, 82.60, 81.85, 69.95, 60.08, 53.51, 53.24, 36.06, 34.54, 28.33, 28.10. HRMS (AP-ESI) m/z Calcd for $C_{48}H_{44}Cl_2N_4O_{10}S$ [M-H]⁻ 937.2077, found: 937.2077.

4.1.7.22. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-(4-nitrophenoxy)phenyl)-1-oxopropan-2-yl)-carbamoyl)-4-((4'-chloro-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-

1-carboxylate (31). Pale yellow powder, yield: 24%, mp: 118– 120 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.30 (s, 1H), 8.51 (s, 1H), 8.21–8.06 (m, 3H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.52–7.36 (m, 6H), 7.16 (d, *J* = 8.4 Hz, 2H), 6.96–6.82 (m, 7H), 4.92 (s, 1H), 4.76 (br s, 1H), 4.44 (d, *J* = 10.0 Hz, 1H), 3.78–3.61 (m, 2H), 3.28–3.13 (m, 1H), 3.05–3.00 (m, 1H), 2.60–2.45 (m, 2H), 1.44 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 172.39, 169.99, 162.74, 156.11, 154.33, 147.51, 142.87, 138.60, 138.55, 133.24, 132.91, 132.77, 132.60, 131.32, 131.21, 128.99, 128.51, 128.39, 127.91, 126.07, 125.95, 120.72, 117.29, 117.14, 115.98, 82.71, 60.08, 53.83, 53.33, 36.39, 34.54, 28.32, 28.19. HRMS (AP-ESI) *m/z* Calcd for C₄₃H₃₉Cl₂N₅O₁₂S [M–H][–] 918.1615, found: 918.1615.

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4.1.7.23. (25,45)-*tert*-Butyl4-([1,1'-biphenyl]-4-yloxy)-2-(((5)-3-(4-(benzyloxy)phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate

(32). Pale yellow powder, yield: 33%, mp: 102–104 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.25 (s, 1H), 8.51 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.54–7.52 (m, 4H), 7.43–7.30 (m, 8H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 6.78 (d, *J* = 8.2 Hz, 2H), 6.68 (d, *J* = 7.7 Hz, 1H), 4.95 (d, *J* = 15.6 Hz, 3H), 4.70 (br s, 1H), 4.41–4.38 (m, 1H), 3.80–3.66 (m, 2H), 3.34–3.29 (m,1H), 2.90–2.85 (m, 1H), 2.58–2.49 (m, 2H), 1.37 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 175.22, 171.85, 158.72, 155.99, 147.49, 132.97, 132.72, 132.52, 132.17, 132.14, 132.11, 132.08, 130.44, 128.84, 128.68, 128.62, 128.56, 128.07, 127.43, 127.09, 126.76, 115.98, 115.32, 82.91, 69.91, 60.22, 53.55, 35.88, 28.14. HRMS (AP-ESI) *m/z* Calcd for C₄₄H₄₃ClN₄O₁₀S [M–H][–] 853.2310, found: 853.2308.

4.1.7.24. (2S,4S)-tert-Butyl-4-([1,1'-biphenyl]-4-yloxy)-2-(((S)-3-(4-(benzyloxy)phenyl)-1-oxo-1-(phenylsulfonamido)propan-2yl)carbamoyl)pyrrolidine-1-carboxylate (33). White nowder, yield: 49%, mp: 100–102 °C. ¹H NMR (CDCl₃, 400 MHz), δ 9.90 (s, 1H), 8.05 (d, J = 7.0 Hz, 2H), 7.64–7.60 (m, 1H), 7.57–7.49 (m, 6H), 7.41-7.29 (m, 8H), 6.94 (d, J=8.4 Hz, 2H), 6.89 (d, *I* = 7.7 Hz, 2H), 6.74 (d, *I* = 8.4 Hz, 2H), 6.65 (d, *I* = 6.9 Hz, 1H), 4.94-4.90 (m, 3H), 4.74-4.72 (m, 1H), 4.44 (d, J = 9.4 Hz, 1H), 3.73 (d, J = 12.5 Hz, 2H), 3.35-3.32 (m, 1H), 2.84-2.82 (m,1H), 2.61–2.43 (m, 2H), 1.37 (s, 9H). 13 C NMR (CDCl₃, 100 MHz), δ 171.26, 170.07, 158.19, 156.14, 155.73, 140.31, 138.88, 136.76, 135.12, 133.62, 130.55, 128.79, 128.67, 128.59, 128.04, 127.40, 126.98, 126.76, 115.93, 115.15, 82.22, 69.89, 60.13, 53.24, 36.03, 34.64, 28.14. HRMS (AP-ESI) m/z Calcd for C44H45N3O8S [M-H]⁻ 774.2849, found: 774.2848.

4.1.7.25. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-3-(4-(benzyloxy)phenyl)-1-(3-chloro-4-nitrophenylsulfonamido)-1-oxopropan-2-yl)car-bamoyl)-4-((4'-chloro-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-

carboxylate (34). Pale yellow powder, yield: 38%, mp: 88– 90 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.21 (s, 1H), 8.51 (s, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.49–7.45 (m, 4H), 7.38–7.36 (m, 7H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.2 Hz, 2H), 6.79 (d, *J* = 8.2 Hz, 2H), 6.67 (d, *J* = 6.9 Hz, 1H), 4.95 (s, 2H), 4.90 (s, 1H), 4.70–4.65 (m, 1H), 4.41 (d, *J* = 9.7 Hz, 1H), 3.78–3.67 (m, 2H), 3.31 (d, *J* = 13.6 Hz, 1H), 2.90–2.85 (m, 1H), 2.58–2.48 (m, 2H), 1.37 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.83, 170.29, 158.40, 156.24, 147.52, 138.83, 138.71, 136.65, 133.14, 132.94, 132.70, 132.52, 130.45, 128.96, 128.61, 128.49, 128.07, 127.98, 127.42, 126.44, 126.07, 116.03, 115.23, 82.64, 69.89, 60.12, 53.62, 53.27, 36.04, 34.63, 28.11. HRMS (AP-ESI) *m/z* Calcd for C₄₄H₄₂Cl₂-N₄O₁₀S [M–H]⁻ 887.1920, found: 887.1918.

4.1.7.26. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-3-(4-(benzyloxy)phenyl)-1-(4chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-

carboxylate (35). Pale yellow powder, yield: 28%, mp: 100– 102 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.18 (s, 1H), 8.52 (d, *J* = 2.0 Hz, 1H), 8.24 (d, *J* = 7.6 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 2H), 7.38–7.32 (m, 5H), 7.23 (d, *J* = 8.0 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.79 (d, *J* = 8.4 Hz, 2H), 6.64 (d, *J* = 7.6 Hz, 1H), 4.98 (s, 2H), 4.90 (s, 1H), 4.71–4.63 (m, 1H), 4.40–4.37 (m, 1H), 3.80–3.73 (m, 1H), 3.71–3.65 (m, 1H), 3.35–3.31 (m, 1H), 2.89 (dd, *J*₁ = 5.2 Hz, *J*₁ = 14.0 Hz, 1H), 2.56–2.44 (m, 2H), 2.38 (s, 3H), 1.36 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.53, 170.58, 158.39, 155.79, 147.48, 138.84, 137.35, 136.81, 136.67, 135.25, 132.91, 132.66, 132.50, 130.46, 129.54, 128.59, 128.39, 128.04, 127.42, 126.59, 126.40, 126.09, 115.94, 115.21, 82.57, 69.86, 60.15, 53.47, 53.28, 35.98, 34.70, 28.35, 28.10, 21.05. HRMS (AP-ESI) m/z Calcd for $C_{45}H_{45}\text{-}\ClN_4O_{10}S~[M-H]^-$ 867.2467, found: 867.2469.

4.1.7.27. (2*S*,4*S*)-*tert*-Butyl4-([1,1'-biphenyl]-4-yloxy)-2-(((*S*)-3-(4-((4-bromobenzyl)oxy)phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-car-

boxylate (36). Pale yellow powder, yield: 54%, mp: 150–152 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.20 (s, 1H), 8.50 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.53–7.47 (m, 6H), 7.43–7.40 (m, 2H), 7.34–7.31 (m, 1H), 7.25 (d, *J* = 8.4 Hz, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.75 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 6.8 Hz, 1H), 4.91–4.87 (m, 3H), 4.70–4.68 (m, 1H), 4.41–4.38 (m, 1H), 3.78–3.67 (m, 2H), 3.31–3.27 (m, 1H), 2.91–2.86 (m, 1H), 2.57–2.46 (m, 2H), 1.36 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.55, 170.49, 158.05, 155.97, 147.44, 140.16, 138.75, 135.65, 135.21, 132.99, 132.71, 132.52, 131.70, 130.48, 128.97, 128.84, 128.59, 127.08, 126.72, 126.06, 121.92, 115.90, 115.13, 82.54, 69.02, 60.11, 53.48, 53.27, 36.00, 34.62, 28.08. HRMS (AP-ESI) *m/z* Calcd for C₄₄H₄₂BrClN₄O₁₀S [M–H][–] 931.1415, found: 931.1414.

4.1.7.28. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-3-(4-((4-bromobenzyl)oxy) phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)pyrro-

Idime-1-carboxylate (37). White powder, yield: 36%, mp: 174–176 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.23 (s, 1H), 8.50 (d, J = 6.4 Hz, 1H), 8.23 (d, J = 7.6 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H), 7.50–7.41 (m, 6H), 7.25–7.21 (m, 4H), 6.97–6.93 (m, 2H), 6.84–6.82 (m, 2H), 6.75–6.73 (m, 2H), 6.64–6.60 (m, 1H), 4.89–4.87 (m, 3H), 4.68 (br s, 1H), 4.40–4.35 (m, 1H), 3.78–3.66 (m, 2H), 3.30–3.26 (m, 1H), 2.89–2.86 (m, 1H), 2.56–2.45 (m, 2H), 2.39 (s, 3H), 1.36 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.64, 170.54, 158.08, 155.78, 147.48, 138.81, 137.31, 136.87, 135.70, 135.23, 132.97, 132.71, 132.53, 131.71, 130.50, 129.57, 128.99, 128.38, 126.72, 126.57, 126.08, 121.93, 115.96, 115.17, 82.55, 69.05, 60.15, 53.59, 53.30, 36.02, 34.69, 28.11, 21.08. HRMS (AP-ESI) *m*/*z* Calcd for C₄₅H₄₄BrClN₄O₁₀S [M–H]⁻ 945.1572, found: 945.1576.

4.1.7.29. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-3-(4-((4-bromobenzyl)oxy) phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)-4-((4'-chloro-[1,1'-biphenyl]-4-yl)oxy)pyrro-

Idine-1-carboxylate (38). Pale yellow powder, yield: 46%, mp: 106–108 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.18 (s, 1H), 8.50 (d, *J* = 1.6 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.50–7.43 (m, 6H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.4 Hz, 2H), 6.65 (d, *J* = 7.2 Hz, 1H), 4.90 (s, 3H), 4.69–4.65 (m, 1H), 4.41–4.38 (m, 1H), 3.78–3.67 (m, 2H), 3.32–3.28 (m, 1H), 2.90–2.86 (m, 1H), 2.57–2.48 (m, 2H), 1.36 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.52, 170.39, 158.06, 156.18, 155.90, 147.45, 138.75, 138.64, 135.64, 133.93, 133.13, 133.00, 132.73, 132.52, 131.72, 130.49, 128.97, 128.48, 127.95, 126.64, 126.04, 121.96, 115.96, 115.12, 82.57, 69.03, 60.06, 53.50, 53.28, 36.01, 34.50, 28.09. HRMS (AP-ESI) *m*/*z* Calcd for C₄₄H₄₁BrCl₂N₄O₁₀S [M–H]⁻ 965.1026, found: 965.1001.

4.1.7.30. (2*S*,4*S*)-*tert*-Butyl4-([1,1′-biphenyl]-4-yloxy)-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-((4-nitrobenzyl)oxy) phenyl)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxy-

late (39). Pale yellow powder, yield: 42%, mp: 108–110 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.23 (s, 1H), 8.51 (d, *J* = 2.0 Hz, 1H), 8.26–8.14 (m, 3H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.55–7.51 (m, 6H),

7.43–7.40 (m, 2H), 7.35–7.31 (m, 1H), 7.00 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 6.77 (d, *J* = 8.4 Hz, 2H), 6.67 (d, *J* = 7.2 Hz, 1H), 5.03 (s, 2H), 4.92–4.90 (m, 1H), 4.71–4.61 (m, 1H), 4.42 (d, *J* = 9.6 Hz, 1H), 3.80–3.67 (m, 2H), 3.31–3.26 (m, 1H), 2.94 (dd, *J*₁ = 4.8 Hz, *J*₂ = 14.0 Hz, 1H), 2.58–2.49 (m, 2H), 1.37 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.73, 170.48, 157.66, 156.01, 147.60, 147.48, 144.08, 140.13, 138.78, 135.23, 133.01, 132.73, 132.56, 130.60, 128.87, 128.57, 127.51, 127.13, 126.69, 126.03, 123.79, 115.97, 115.12, 82.49, 68.48, 60.14, 53.74, 53.31, 36.09, 34.63, 28.12. HRMS (AP-ESI) *m*/*z* Calcd for C₄₄H₄₂ClN₅O₁₂S [M–H][–] 898.2161, found: 898.2161.

4.1.7.31. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsul-fonamido)-3-(4-((4-nitrobenzyl)oxy)phenyl)-1-oxopropan-2-yl) carbamoyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-carboxylate (40). Pale yellow powder, yield: 29%, mp: 108–

110 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.20 (s, 1H), 8.50 (d, J = 2.4 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.21 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.8 Hz, 1H), 7.53–7.49 (m, 4H), 7.42 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 6.67 (d, J = 8.4 Hz, 2H), 6.65 (d, J = 6.4 Hz, 1H), 5.03 (s, 2H), 4.89 (s, 1H), 4.69–4.66 (m, 1H), 4.41–4.38 (m, 1H), 3.80–3.66 (m, 2H), 3.30–3.26 (m, 1H), 2.94–2.90 (m, 1H), 2.56–2.45 (m, 2H), 2.39 (s, 3H), 1.37 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.83, 170.57, 157.70, 155.78, 147.63, 147.53, 144.07, 138.91, 136.96, 135.29, 132.97, 132.69, 132.54, 130.59, 129.59, 128.35, 127.51, 126.54, 126.01, 123.79, 116.04, 115.20, 82.54, 68.52, 60.20, 53.82, 53.31, 36.01, 34.76, 28.14, 21.05. HRMS (AP-ESI) *m*/*z* Calcd for C₄₅-H₄₄ClN₅O₁₂S [M–H][–] 912.2317, found: 912.2317.

4.1.7.32. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-((4-nitrobenzyl)oxy)phenyl)-1-oxopropan-2-yl)carbamoyl)-4-((4'-chloro-[1,1'-biphenyl]-4-yl)oxy)pyrro-

Idime-1-carboxylate (41). Pale yellow powder, yield: 27%, mp: 114–115 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.17 (s, 1H), 8.49 (d, *J* = 1.6 Hz, 1H), 8.26–8.21 (m, 3H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.0 Hz, 2H), 6.78 (d, *J* = 8.4 Hz, 2H), 6.67 (d, *J* = 7.6 Hz, 1H), 5.05 (s, 2H), 4.91 (s, 1H), 4.69–4.65 (m, 1H), 4.41–4.39 (m, 1H), 3.79–3.68 (m, 2H), 3.31–3.27 (m, 1H), 2.92–2.89 (m, 1H), 2.58–2.48 (m, 2H), 1.37 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.70, 170.58, 157.66, 156.20, 147.61, 147.48, 144.05, 138.77, 138.64, 133.01, 132.75, 132.55, 130.64, 128.99, 128.46, 127.94, 127.50, 126.01, 123.81, 115.98, 115.08, 82.50, 68.48, 60.04, 53.69, 53.35, 36.10, 34.46, 28.11. HRMS (AP-ESI) *m/z* Calcd for C₄₄H₄₁Cl₂N₅O₁₂S [M–H]⁻ 932.1771, found: 932.1773.

4.1.7.33. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-3-([1,1'-biphenyl]-4-yl)-1-(4chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-

carboxylate (42). Pale yellow powder, yield: 51%, mp: 114– 116 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.25 (s, 1H), 8.55 (d, *J* = 2.0 Hz, 1H), 8.25 (d, *J* = 8.4 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.52–7.49 (m, 4H), 7.44–7.39 (m, 6H), 7.36–7.32 (m, 1H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.69 (d, *J* = 7.6 Hz, 1H), 4.89 (s, 1H), 4.76–4.73 (br s, 1H), 4.41–4.38 (m, 1H), 3.80 (d, *J* = 12.0 Hz, 1H), 3.68–3.65 (m, 1H), 3.42–3.38 (m, 1H), 3.02 (dd, *J*₁ = 4.8 Hz, *J*₂ = 14.0 Hz, 1H), 2.58–2.45 (m, 2H), 2.39 (s, 3H), 1.26 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.82, 170.42, 155.83, 147.51, 140.48, 139.95, 138.81, 137.34, 136.82, 135.23, 133.41, 132.85, 132.73, 132.54, 129.85, 129.54, 128.83, 128.40, 127.56, 127.47, 126.86, 126.59, 126.16, 115.97, 82.60, 60.12, 53.56, 53.31, 36.47, 34.65, 28.01, 21.07. HRMS (AP-ESI) *m/z* Calcd for C₄₄H₄₃ClN₄O₉S [M–H][–] 837.2361, found: 837.2363. 4.1.7.34. (2S,4S)-tert-Butyl-2-(((S)-3-([1,1'-biphenyl]-4-yl)-1-(4chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)-4-((4'-chloro-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1carboxylate (43). Pale yellow powder, yield: 62%, mp: 148-149 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.30 (s, 1H), 8.54 (s, 1H), 8.24 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.51-7.47 (m, 3H), 7.45-7.38 (m, 8H), 7.36-7.32 (m, 2H), 7.15 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 6.73 (d, J = 7.2 Hz, 1H), 4.91–4.90 (m, 1H), 4.77-4.73 (m, 1H), 4.43-4.40 (m, 1H), 3.78-3.67 (m, 2H), 3.39-3.34 (m, 1H), 3.03-2.98 (m, 1H), 2.59-2.46 (m, 2H), 1.20 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.85, 170.29, 156.25, 147.50, 140.44, 139.91, 138.78, 138.68, 133.89, 133.43, 133.09, 132.73, 132.53, 129.80, 128.93, 128.84, 128.46, 127.96, 127.57, 127.42, 126.83, 126.10, 116.01, 82.60, 60.06, 58.43, 53.66, 53.28, 36.51, 34.54, 28.00, 18.40. HRMS (AP-ESI) m/z Calcd for C₄₃H₄₀Cl₂N₄O₉S [M-H]⁻ 857.1815, found: 857.1814.

4.1.7.35. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(4'-methoxy-[1,1'-biphenyl]-4-yl)-1-oxopropan-2yl)carbamoyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)pyrro-

lidine-1-carboxylate (44). Pale yellow powder, yield: 62%, mp: 114–116 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.26 (s, 1H), 8.55 (d, *J* = 2.0 Hz, 1H), 8.25 (d, *J* = 8.4 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.46–7.37 (m, 6H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 6.68 (d, *J* = 7.6 Hz, 1H), 4.90 (br s, 1H), 4.76–4.71 (m, 1H), 4.41 (d, *J* = 10.0 Hz, 1H), 3.85 (s, 3H), 3.80–3.77 (m, 1H), 3.69–3.65 (m, 1H), 3.42–3.37 (m, 1H), 2.99–2.95 (m, 1H), 2.57–2.43 (m, 2H), 2.39 (s, 3H), 1.26 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.67, 170.50, 159.34, 155.79, 147.47, 140.11, 138.78, 137.32, 136.81, 135.21, 132.90, 132.73, 132.53, 129.77, 129.52, 128.39, 127.87, 126.98, 126.58, 126.15, 115.94, 114.24, 82.62, 60.11, 55.32, 53.28, 36.36, 34.64, 27.98, 21.06. HRMS (AP-ESI) *m/z* Calcd for C₄₅H₄₅ClN₄O₁₀S [M–H]⁻ 867.2467, found: 867.2468.

4.1.7.36. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(4'-methoxy-[1,1'-biphenyl]-4-yl)-1-oxopropan-2-yl)carbamoyl)-4-((4'-chloro-[1,1'-biphenyl]-4-yl)oxy)pyrro-

lidine-1-carboxylate (45). Pale yellow powder, yield: 56%, mp: 118–120 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.27 (s, 1H), 8.54 (s, 1H), 8.25 (d, *J* = 8.4 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.52–7.37 (m, 10H), 7.11 (d, *J* = 8.0 Hz, 2H), 6.95 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 6.71 (d, *J* = 7.2 Hz, 1H), 4.90 (s, 1H), 4.76–4.69 (m, 1H), 4.42 (d, *J* = 10.0 Hz, 1H), 3.84 (s, 3H), 3.79–3.76 (m, 1H), 3.72–3.66 (m, 1H), 3.39–3.34 (m, 1H), 3.00 (dd, *J*₁ = 4.8 Hz, *J*₂ = 14.0 Hz, 1H), 2.58–2.48 (m, 2H), 1.27 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.71, 170.37, 159.34, 156.21, 155.96, 147.46, 140.07, 138.74, 138.65, 133.90, 133.08, 132.90, 132.75, 132.53, 132.33, 129.75, 128.93, 128.47, 127.95, 127.84, 126.92, 126.11, 116.00, 114.25, 82.64, 60.05, 55.33, 53.48, 53.28, 36.42, 34.50, 27.98. HRMS (AP-ESI) *m*/*z* Calcd for C₄₄H₄₂Cl₂N₄O₁₀S [M–H]⁻ 887.1920, found: 887.1920.

4.1.7.37. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-((4'-methoxy-[1,1'-biphenyl]-4-yl)methoxy) phenyl)-1-oxopropan-2-yl)carbamoyl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)pyrrolidine-1-carboxylate (46). Pale yellow powder, yield: 44%, mp: 106–108 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.22 (s, 1H), 8.52 (s, 1H), 8.24 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.56–7.49 (m, 6H), 7.43 (d, *J* = 7.2 Hz, 4H), 7.23 (d, *J* = 8.0 Hz, 2H), 6.99–6.97 (m, 4H), 6.86–6.79 (m, 4H), 6.67 (d, *J* = 7.6 Hz, 1H), 4.98 (s, 2H), 4.90 (br s, 1H), 4.70–4.66 (m, 1H), 4.41–4.38 (m, 1H), 3.86 (s, 3H), 3.82–3.75 (m, 1H), 3.69–3.64 (m, 1H), 3.35–3.30 (m, 1H), 2.90 (dd, *J*₁ = 4.8 Hz, *J*₂ = 14.0 Hz, 1H), 2.57–2.46 (m, 2H), 2.38 (s, 3H), 1.38 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz), δ 171.50, 170.62, 159.27, 158.38, 155.76, 147.45,

140.64, 138.78, 137.32, 136.81, 135.22, 134.92, 133.19, 132.94, 132.69, 132.51, 130.46, 129.54, 128.39, 128.09, 127.96, 126.87, 126.58, 126.39, 126.10, 115.91, 115.19, 114.25, 82.59, 69.64, 60.13, 55.35, 53.45, 53.27, 35.98, 34.66, 28.11, 21.06. HRMS (AP-ESI) *m/z* Calcd for $C_{52}H_{51}ClN_4O_{11}S$ [M–H]⁻ 973.2885, found: 973.2884.

4.1.7.38. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsul-fonamido)-3-(4-((4'-methoxy-[1,1'-biphenyl]-4-yl)methoxy) phenyl)-1-oxopropan-2-yl)carbamoyl)-4-((4'-chloro-[1,1'-

biphenyl]-4-yl)oxy)pyrrolidine-1-carboxylate (47). Pale yellow powder, yield: 27%, mp: 110-112 °C. ¹H NMR (CDCl₃, 400 MHz), δ 10.22 (s, 1H), 8.52 (s, 1H), 8.25 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 8.4 Hz, 1H), 7.56-7.48 (m, 6H), 7.45-7.36 (m, 6H), 7.00–6.96 (m, 4H), 6.86 (d, J = 8.0 Hz, 2H), 6.81 (d, J = 8.0 Hz, 2H), 6.67 (d, J = 7.6 Hz, 1H), 4.98 (s, 2H), 4.91 (s, 1H), 4.71-4.67 (m, 1H), 4.42 (d, J = 9.6 Hz, 1H), 3.86 (s, 3H), 3.80-3.77 (m, 1H), 3.70-3.67 (m, 1H), 3.33–3.29 (m, 1H), 2.90 (dd, J_1 = 5.2 Hz, J_2 = 14.0 Hz, 1H), 2.58-2.49 (m, 2H), 1.38 (s, 9H). ¹³C NMR (DMSO-d₆, 100 MHz), δ 172.39, 171.20, 159.42, 157.59, 157.26, 147.59, 139.77, 139.51, 139.00, 135.93, 133.43, 132.85, 132.59, 132.05, 132.00, 131.18, 130.68, 129.24, 128.62, 128.37, 128.26, 128.18, 126.60, 125.61, 116.41, 114.84, 114.79, 79.53, 74.32, 69.28, 59.25, 55.64, 54.86, 36.32, 28.45, 28.08. HRMS (AP-ESI) m/z Calcd for C₅₁-H₄₈Cl₂N₄O₁₁S [M–H]⁻ 993.2339, found: 993.2340.

4.1.8. (2*S*,4*S*)-4-([1,1'-biphenyl]-4-yloxy)-*N*-((*S*)-3-(4bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1oxopropan-2-yl)pyrrolidine-2-carboxamide (48)

Compound 1 (0.4 g, 0.48 mmol) was dissolved in 10 mL EtOAc saturated with HCl gas and stirred overnight at room temperature. Then some amount of saturated NaHCO₃ solution was added and sequentially extracted with EtOAc. The EtOAc layer was washed by brine and dried over anhydrous MgSO₄. Finally, the solvent was evaporated under reduced pressure. The concentrated residue was purified by column chromatography (dichloromethanemethanol = 20:1) to obtain 0.16 g compound 48 as a yellow powder. Yield: 46%, mp: 134–136 °C. ¹H NMR (DMSO-*d*₆, 400 MHz), δ 10.20-10.09 (m, 1H), 8.92-8.83 (m, 2H), 8.52 (s, 1H), 8.13 (d. *I* = 8.8 Hz, 1H), 7.99 (d, *I* = 8.4 Hz, 1H), 7.63–7.60 (m, 4H), 7.46– 7.42 (m, 2H), 7.36–7.31 (m, 3H), 7.11 (d, J = 8.0 Hz, 2H), 6.96 (d, *I* = 8.8 Hz, 2H), 5.14 (s, 1H), 4.54–4.53 (m, 1H), 4.30–4.28 (m, 1H), 3.55-3.43 (m, 3H), 3.04-2.99 (m, 1H), 2.79-2.71 (m, 1H), 2.14-2.09 (m, 1H). 13 C NMR (DMSO- d_6 , 100 MHz), δ 170.82, 168.17, 156.24, 147.51, 140.13, 136.42, 133.96, 133.39, 132.79, 131.97, 131.38, 130.91, 129.34, 128.35, 127.35, 126.74, 125.47, 120.36, 116.72, 75.48, 58.32, 55.57, 50.55, 36.28, 35.99. HRMS (AP-ESI) m/ *z* Calcd for C₃₂H₂₈BrClN₄O₇S [M–H][–] 725.0472, found: 725.0471.

Compounds **49–51** were synthesized following the procedure described above.

4.1.8.1. (2*S*,4*S*)-*N*-((*S*)-3-(4-bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)-4-((4'-methyl-[1,1'-

biphenyl]-4-yl)oxy)pyrrolidine-2-carboxamide (49). Pale yellow powder, yield: 53%, mp: 158–160 °C. ¹H NMR (DMSO-*d*₆, 400 MHz), δ 8.32 (d, *J* = 2.0 Hz, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 8.04 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.4 Hz, 1H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.57 (d, *J* = 8.8 Hz, 2H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 6.96–6.90 (m, 4H), 4.98 (s, 1H), 4.26–4.21 (m, 1H), 3.94–3.93 (m, 1H), 3.43–3.39 (m, 1H), 3.16 (d, *J* = 12.0 Hz, 1H), 2.98–2.93 (m, 1H), 2.87–2.82 (m, 1H), 2.61–2.54 (m, 1H), 2.32 (s, 3H), 2.06–2.02 (m, 1H). ¹³C NMR (DMSO-*d*₆, 100 MHz), δ 174.94, 156.66, 146.90, 146.50, 137.65, 137.36, 136.44, 133.49, 132.65, 132.11, 131.72, 130.86, 129.92, 128.06, 127.03, 126.54, 124.77, 119.66, 116.49, 76.81, 59.31, 56.23, 51.62, 37.86, 36.53, 21.10. HRMS (AP-ESI) *m/z* Calcd for C₃₃H₃₀BrClN₄O₇S [M–H]⁻ 739.0629, found: 739.0632. 4.1.8.2. (2*S*,4*S*)-*N*-((*S*)-3-(4-Bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)-4-((4'-chloro-[1,1'-

biphenyl]-4-yl)oxy)pyrrolidine-2-carboxamide (50). Yellow powder, yield: 54%, mp: 138–140 °C. ¹H NMR (DMSO-*d*₆, 400 MHz), δ 8.32 (d, *J* = 2.0 Hz, 1H), 8.01 (dd, *J*₁ = 2.0 Hz, *J*₂ = 8.4 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.60 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.05 (d, *J* = 8.0 Hz, 2H), 6.96 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.0 Hz, 2H), 4.87 (s, 1H), 4.24–4.17 (m, 1H), 3.61–3.58 (m, 1H), 3.31–3.28 (m, 1H), 2.90 (d, *J* = 4.8 Hz, 2H), 2.82 (d, *J* = 10.0 Hz, 1H), 2.39–2.32 (m, 1H), 2.02 (s, 1H). ¹³C NMR (DMSO-*d*₆, 100 MHz), δ 175.16, 172.60, 157.84, 146.95, 146.43, 137.38, 132.74, 132.26, 131.96, 131.77, 131.59, 130.65, 129.24, 128.36, 128.29, 127.09, 124.71, 119.61, 116.25, 78.35, 60.17, 55.28, 52.67, 38.05, 36.78. HRMS (AP-ESI) *m*/*z* Calcd for C₃₂H₂₇BrCl₂N₄O₇S [M–H]⁻ 759.0088, found: 759.0083.

4.1.8.3. (2S,4S)-N-((S)-1-(4-Chloro-3-nitrophenylsulfonamido)-1-oxo-3-phenylpropan-2-yl)-4-((4'-methyl-[1,1'-biphenyl]-4-yl) oxy)pyrrolidine-2-carboxamide (51). Yellow powder, yield: 17%, mp: 156–158 °C. ¹H NMR (DMSO- d_6 , 400 MHz), δ 8.31 (d, *J* = 1.6 Hz, 1H), 8.28 (d, *J* = 8.0 Hz, 1H), 7.98–7.96 (m, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.52 (d, *J* = 8.0 Hz, 2H), 7.24 (d, J = 8.0 Hz, 2H), 7.09–7.04 (m, 3H), 6.99–6.89 (m, 4H), 5.03 (s, 1H), 4.29-4.24 (m, 1H), 4.05 (br s, 1H), 3.47-3.42 (m, 1H), 3.28 (d, J = 12.0 Hz, 1H), 3.03-2.99 (m, 1H), 2.86-2.81 (m, 1H), 2.71-2.65 (m, 1H), 2.32 (s, 3H), 2.06-2.03 (m, 1H). ¹³C NMR (DMSO-d₆, 100 MHz), δ 175.27, 156.73, 146.87, 146.60, 138.28, 137.35, 133.42, 132.65, 131.71, 129.92, 128.04, 126.97, 126.53, 126.35, 124.76, 116.47, 76.82, 59.32, 56.48, 51.57, 38.48, 36.52, 21.10. HRMS (AP-ESI) m/z Calcd for $C_{33}H_{31}CIN_4O_7S$ [M-H]⁻ 661.1524, found: 661.1521.

4.2. Binding assay for Bcl-2 family proteins

Fluorescence polarization technique was employed in the binding assay for Bcl-2 family proteins. In this assay, a fluorescencelabeled BH3 peptide was used as the tracer. The binding affinities of tested compounds were characterized quantitatively based on the changes of FP signals when the addition of the tested compounds at different concentrations.

In the competitive binding experiments for Mcl-1 protein, a 26-reside BH3 peptide derived from Bid protein (QEDIIRNIARH-LAQVGDSMDRSIPPG) was labeled at the N-terminus by 5-carboxyfluorescein succinimidyl ester (FAM) as the fluorescence labeled tracer (5-FAM-QEDIIRNIARHLAQVGDSMDRSIPPG).¹⁶ Mcl-1 protein and these tested compounds were preincubated in the PBS assay solution for 30 min in dark at room temperature. Then 20 µL 5-FAM-Bid-BH3 peptide solution in PBS was added into the solution to obtain a total volume of 200 µL and incubated for another 20 min. Finally, 60 µL solutions were added into every well of Corning 384-well, black, flat-bottom plates (Corning Inc.) and three wells per a concentration of every sample. The polarization values (milipolarization units, mP) were measured under the condition of an excitation wavelength at 485 nm and an emission wavelength at 535 nm using the Tecan GENios-Pro Injector Reader (Tecan Group Ltd). In these experiments, all tested pyrrolidine derivatives were prepared in dimethylsulfoxide (DMSO) at seven concentrations (1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 50 µM, 100 µM). The final concentrations of Mcl-1 protein and 5-FAM-Bid-BH3 peptide were 160 nM and 10 nM, respectively.

The binding assay for Bcl-2 protein was almost as well as that for Mcl-1 protein except that the total concentration of Bcl-2 protein was 230 nM. However, in the experiment for Bcl- X_L protein, a different 5-FAM-Bim-BH3 peptide (5-FAM-DMRPEIWIAQELRRIG-

DEFNAYYARR) was used as the fluorescence tracer. The total concentration of Bcl-X_L protein was 205 nM. The competitive inhibition constant (K_i) of each tested compound was calculated using the methods developed by Wang et al.¹⁷

4.3. MTT assay

MDA-MB-231 (breast cancer cell), PC-3 (prostatic cancer cell) and K562 (chronic myelogenous leukemia cells were cultured in RPMI1640/DEME medium containing 10% FBS and incubated in humidified incubator (37 °C, 5% CO₂). When these cancer cells were growing at logarithmic growth phase, they were seeded in the 96-well plates (5000 cells per well) and incubated for 12 h. Then a series of different concentrated tested compounds were added into the growing cells for another 48 h. 10 μ L MTT/well (5 mg/mL in PBS) solution was added and incubated for further 4 h. Finally, 150 μ L DMSO/well was added to dissolve the formed formazan and mixed for 5 min. A microtiter-plate reader (Thermo Varioskan Flash) was used to measure optical density of every well at 570 nm to obtain their anti-proliferative activities.

4.4. Docking study

Surflex-Dock program in Sybyl 7.3 was used to perform the molecular docking between compound **18** and Mcl-1 protein. Except compound **18** was added Gasteiger-Hückel charge, other parameters were set at default values. The Mcl-1 protein was downloaded from the Protein Data Bank (PDB code: 2PQK).

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A. Supplementary data

Supplementary data (the NMR spectra of all the target compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.10.020.

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