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Design, synthesis and preliminary biological studies of pyrrolidine derivatives as Mcl-1 inhibitors

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ABSTRACT

Anti-apoptotic proteins, such as B-cell lymphoma (Bcl-2) protein, myeloid cell leukemia sequence 1 (Mcl-1) protein, are potential targets for cancer treatment. In the studies, a series of pyrrolidine derivatives were developed as potent Mcl-1 inhibitors. The preliminary biological studies suggested that most of target compounds exhibit good abilities for targeting Mcl-1 protein. Among them, compound **21** (K_i = 0.53 μM) exhibited equal inhibitory activities towards Mcl-1 protein compared to positive control gossypol (K_i = 0.39 μM). This compound also possessed good antiproliferative activities against MDA-MB-231 and PC-3 cancer cells.

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1. Introduction

As a program of cell suicide, apoptosis is essential for eliminating useless or dangerous cells and maintaining proper tissue development.[1] In addition, aberrant apoptosis can result in many pathological process, such as neurodegenerative diseases, myelodysplastic syndromes, acquired immunodeficiency syndrome, autoimmune diseases and cancers.[2] Therefore, modulating apoptosis by targeting the key regulators in apoptotic pathways may be an attractive approach to cancer therapy.[3]

In mitochondria-mediated apoptotic pathway, B-cell lymphoma (Bcl-2) family proteins are key regulators of apoptosis through acting on the outer mitochondrial membrane (OMM) to adjust its permeability and then releasing cell death factors, such as cytochrome c.[4] This family is comprised of anti-apoptotic proteins and pro-apoptotic proteins. The anti-apoptotic members, such as Bcl-2, Bcl-X_L, Bcl-w, Mcl-1, share three or four Bcl-2 homology (BH) domains. In the pro-apoptotic subfamily, it can be further divided into the multidomains proteins and BH3-only proteins. The multidomains proteins usually include the members of Bax, Bak, Bok/Mtd, Bcl-X_S and Bcl-G_L which have two or three BH domains. The BH3-only proteins can convey death signals to trigger apoptosis and have only one BH3 domain structure, which include Bad, Bid, Bik/Nbk, Bim/Bod, Blk, Hrk/DP5, Noxa, Puma/Bbc3, Bnip3, Bmf and Bcl-G_S.[5] Abnormal over-expression of anti-apoptotic (pro-survival) proteins may make tumor cells to acquire the resistance to anti-tumor agents by evading apoptotic pathways.[6]

As an important member of anti-apoptotic (pro-survival) proteins subfamily, Mcl-1 protein could lead to the survival and immortalization of cells when introduced exogenously.[7] In 2001, Zhou et al. found that mice with a human Mcl-1 transgene exhibited high incidence of B-cell lymphoma after an extended period.[8] In addition, overexpression of Mcl-1 protein was observed in many human cancers, such as lung, breast, prostate, pancreatic, ovarian and cervical cancers,[9–13] which is a mechanism for tumor cells to resist conventional chemotherapy agents.[14] Therefore, inhibiting Mcl-1 protein could be a promising strategy to suppress tumor cells. So far, many small-molecule Mcl-1 inhibitors have been designed and synthesized such as S1,[15,16] polyphenol derivatives (Gossypol),[17] indole derivatives (GX15-070,[18] A-1210477[19]) (Fig. 1).

In 2008, professor Xing designed and synthesized a novel Bcl-2 proteins inhibitor **WL-276** based on the scaffold of rhodanine.[20] And this compound could suppress PC-3 cell growth in a dose-dependent manner effectively. To investigate SARs of **WL-276**, we previous synthesized a series of **WL-276** derivatives. The results showed that modifications of **WL-276** effectively enhanced binding affinities for Mcl-1 over other Bcl-2 proteins and their water solubility needed to be improved.[21] In our recent screening, pyrrolidine derivatives **1** exhibited a moderate Mcl-1 inhibition

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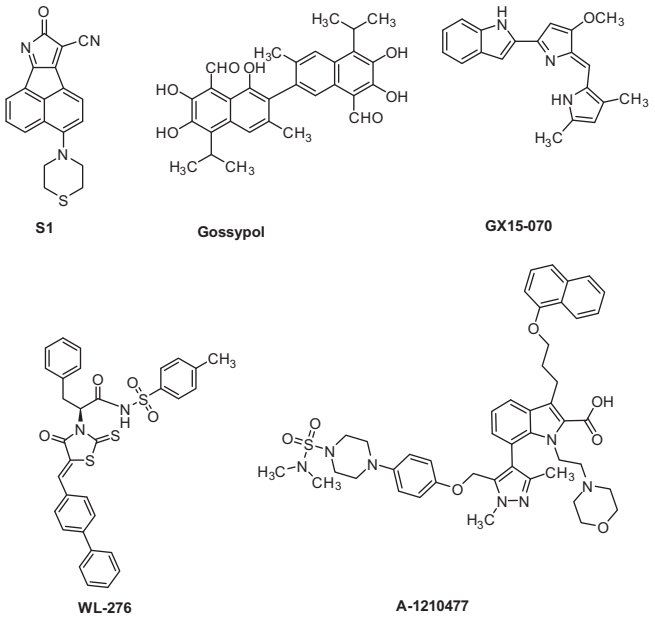


Figure 1. The structures of reported Mcl-1 inhibitors.

(K_i = 8.4 μM) and displayed good water solubility, which was suitable for further research. Therefore, a series of pyrrolidine derivatives was designed based on the structures **1** (Fig. 2). In this paper, we will demonstrate the synthesis, binding affinities to Mcl-1 protein and anti-proliferative activity against some tumor cell lines of these compounds.

2. Chemistry

The synthesis route of pyrrolidine derivatives was showed in Scheme 1. (2*S*,4*R*)-4-hydroxyproline **2** was as the starting material to get intermediate **3** by amino and carboxyl protection. Then intermediate **3** was reacted with various substituted phenols by Mitsunobu reaction to get key intermediates **4a**–**4d**. Different N-Boc-amino acids **5a**–**5d** coupled with different substituted bensulfonamides to yield intermediates **6a**–**6k** by mixed anhydrides method.[22] Finally, the key intermediates **4a**–**4d** was treated with different amino acid methyl ester hydrochlorides to get target compounds **1**, **7**–**15**. The intermediates **6a**–**6k** was first removed the protection of Boc group by ethyl acetate saturated with HCl gas. Sequential reacting with the key intermediates **4a**–**4d**, the target compounds **16**–**31** was obtained by mixed anhydrides method.

3. Results and discussion

We synthesized a series of pyrrolidine derivatives according to the structures of compound **1**. Their competitive binding affinities for Mcl-1 protein were obtained by fluorescence polarization assays (FPAs). The results of K_i value were listed in Table 1. According to the preliminary data, some active compounds (**16**–**31**) usually had biphenyl groups on R_1, aromatic amino acid side chains on R_2, and different substituted benzenesulfonamides on R_3. Especially, compounds containing 3-NO_2-4-Cl substituent on R_3 effectively increased potency against Mcl-1 protein. For example, compound **18** (K_i = 0.94 μM) exhibited almost 9-fold more potent than compound **1** (K_i = 8.4 μM). And these compounds with phenyl or 4-bromophenyl on R_2 showed better activities compared to 3-indolyl and *p*-hydroxy phenyl substitutions, such as compounds **18**, **21**, **25**, **31**. The compound **21** was the most potent compound

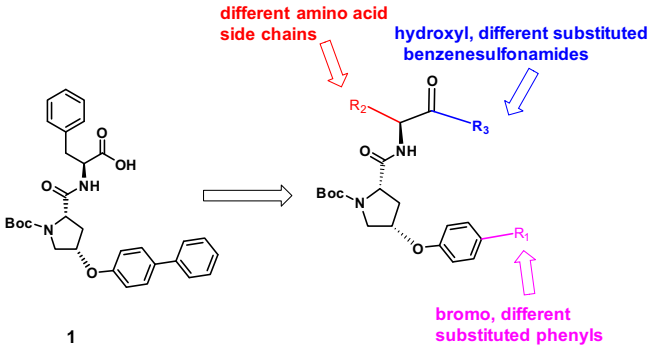
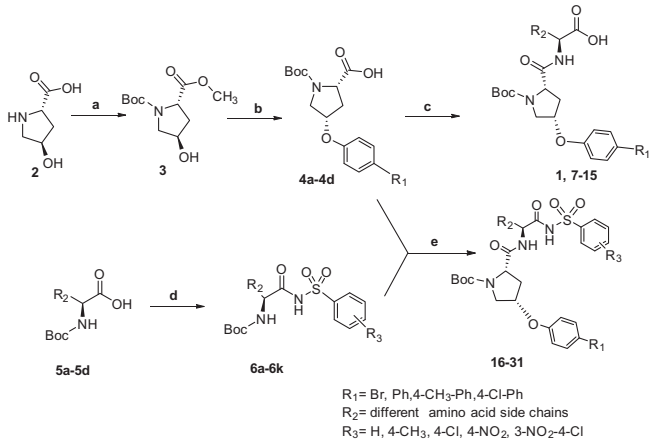


Figure 2. The structures of **1** and the target compounds.



Scheme 1. Reagents and conditions: (a) (i) MeOH, CH_3COCl; (ii) $(Boc)_2O$, Et_3N, CH_2Cl_2; (b) (i) PPh_3, DEAD, substituted phenols R_1-Ph-OH, THF, rt, 12 h; (ii) 1 M NaOH, THF, rt, overnight; (c) (i) NMM, isobutyl chloroformate, THF; (ii) 1 M NaOH, THF, rt, overnight; (d) NMM, isobutylchloroformate, NaH, benzenesulfonamide, −20 °C to rt, 4 h; (e) (i) ethyl acetate saturated with HCl, rt, overnight; (ii) NMM, isobutylchloroformate, THF.

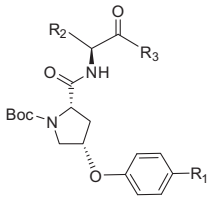
in the series, and the binding affinity of the compound **21** (K_i = 0.53 μM) showed 15 times more potent than compound **1**.

Docking studies were performed so as to study the interaction between Mcl-1 protein and active compound **21** using Surflex-Dock (Fig. 3). The docking results showed that biphenyl group of compound **21** could mimetic the α-helix of BH3-only protein, Bim (Fig. 3a). Acyl–sulfonyl group could form two hydrogen bonds with Arg263 and one hydrogen bond with Asn260. In addition, the carbonyl of pyrrolidine ring interacted with Thr266 by one hydrogen bond. (Fig. 3b).

To explore if pyrrolidine derivatives had inhibitory activities for other anti-apoptotic Bcl-2 family members, we selected four representative compounds to test the inhibitory activities against Bcl-2 and Bcl-X_L proteins (Table 2). The results showed that all tested compounds had good inhibitory activities to the anti-apoptotic proteins of Bcl-2 family. And these compounds displayed more potent binding affinity on Mcl-1 protein than other two Bcl-2 proteins except compound **29**. In addition, compound **28** exhibited a profile for selectively inhibiting Mcl-1 protein with 8.2-fold versus Bcl-2 protein and 15.5-fold versus Bcl-X_L protein.

In further studies, three human tumor cell lines, MDA-MB-231 (breast cancer cell), PC-3 (prostatic cancer cell) and K562 (chronic myelogenous leukemia cell), were chosen to evaluate the antiproliferative activities of active compounds **21**, **28**, **29** and **30** by MTT assay. Their IC_{50} values were listed in Table 3. The reported Mcl-1 inhibitor, Gossypol, was evaluated as the positive control in the

Table 1
The binding affinities of pyrrolidine derivatives to Mcl-1 protein



Compd	R_1	R_2	R_3	Mcl-1 K_i[a] (μM)
1	Ph	Ph-CH_2–	OH	8.4 ± 0.13
7	Br	Ph-CH_2–	OH	>10
8	Br	H	OH	>10
9	Ph	H	OH	>10
10	Br	Ph	OH	>10
11	Ph	Ph	OH	>10
12	Br	Isopropyl	OH	>10
13	Ph	Isopropyl	OH	>10
14	Br	Isobutyl	OH	>10
15	Ph	Isobutyl	OH	>10
16	Ph	Ph-CH_2–	Ph-SO_2NH–	6.4 ± 1.9
17	Ph	Ph-CH_2–	4-CH_3– Ph-SO_2NH–	3.3 ± 0.53
18	Ph	Ph-CH_2–	3-NO_2-4-Cl– Ph-SO_2NH–	0.94 ± 0.15
19	Ph	4-Br-Ph-CH_2–	Ph-SO_2NH–	1.2 ± 0.12
20	Ph	4-Br-Ph-CH_2–	4-CH_3– Ph-SO_2NH–	0.99 ± 0.12
21	Ph	4-Br-Ph-CH_2–	3-NO_2-4-Cl– Ph-SO_2NH–	0.53 ± 0.09
22	Ph	4-Br-Ph-CH_2–	4-NO_2– Ph-SO_2NH–	2.2 ± 0.72
23	Ph	4-Br-Ph-CH_2–	4-Cl– Ph-SO_2NH–	0.74 ± 0.11
24	Ph	1*H*-Indole-3-CH_2–	Ph-SO_2NH–	3.2 ± 0.60
25	Ph	1*H*-Indole-3-CH_2–	3-NO_2-4-Cl– Ph-SO_2NH–	1.2 ± 0.32
26	4-CH_3-Ph	Ph-CH_2–	Ph-SO_2NH–	1.6 ± 0.32
27	4-CH_3-Ph	Ph-CH_2–	4-CH_3– Ph-SO_2NH–	1.9 ± 0.26
28	4-CH_3-Ph	Ph-CH_2–	3-NO_2-4-Cl– Ph-SO_2NH–	0.56 ± 0.18
29	4-CH_3-Ph	4-Br-Ph-CH_2–	3-NO_2-4-Cl– Ph-SO_2NH–	0.63 ± 0.20
30	4-Cl-Ph	4-Br-Ph-CH_2–	3-NO_2-4-Cl– Ph-SO_2NH–	0.58 ± 0.07
31	Ph	4-OH-Ph-CH_2–	3-NO_2-4-Cl– Ph-SO_2NH–	2.2 ± 0.62
Gossypol				0.39 ± 0.04

[a] Each value is the result of three separate experiments.

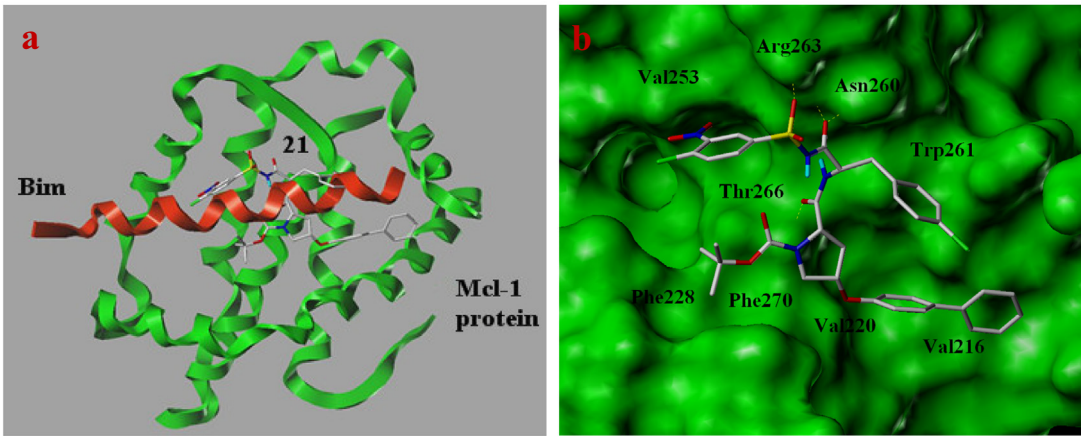


Figure 3. (a) Docked compound **21** with Mcl-1 protein complexed with Bim protein. (b) The docking mode of compound **21** in the hydrophobic groove of Mcl-1 protein.

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

Compd	Bcl-X_L K_i[a] (μM)	Bcl-2 K_i[a] (μM)	Mcl-1 K_i[a] (μM)
21	2.2 ± 0.06	0.93 ± 0.06	0.53 ± 0.09
28	8.7 ± 2.5	4.6 ± 0.15	0.56 ± 0.18
29	1.2 ± 0.36	0.55 ± 0.05	0.63 ± 0.20
30	2.1 ± 0.17	0.83 ± 0.04	0.58 ± 0.07
Gossypol	0.77 ± 0.40	0.56 ± 0.06	0.39 ± 0.04

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

Table 3
Antiproliferative activities of representative compounds

Compd	IC_{50}[a] (μM)		
	MDA-MB-231	PC-3	K562
21	13.6 ± 0.61	10.7 ± 1.1	23.0 ± 1.9
28	15.4 ± 1.7	13.5 ± 0.42	22.4 ± 1.8
29	14.9 ± 2.4	13.8 ± 0.55	22.1 ± 1.5
30	15.8 ± 1.9	11.2 ± 0.65	21.9 ± 0.60
Gossypol	9.42 ± 0.43	5.78 ± 0.36	5.91 ± 0.77

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

same condition. The result showed that all the four compounds had IC_{50} values of over 20 μM for K562 cell line and similar inhibition on MDA-MB-231 and PC-3 cell lines. In addition, compound **21** possesses similar growth inhibition towards MDA-MB-231 compared to Gossypol.

4. Conclusions

Based on the recent screening, we developed a novel Mcl-1 inhibitor, compound **1**, with a new scaffold of pyrrolidine. Different pyrrolidine derivatives were designed and synthesized by introducing different amino acid side chains, biphenyl groups and benzenesulfonamides. According to the data of protein binding assay and anti-proliferative assay, compound **21** displayed the promising inhibitory activity against Mcl-1 protein and tumor cell lines. These results suggest that further optimization of pyrrolidine derivatives could lead to new anti-tumor agents.

5. Experiment section

5.1. General chemistry information

All the starting materials used were commercially available and used directly. The products were purified by recrystallization or silica gel chromatography (200–300 mesh). The completion of reactions were monitored by conventional thin-layer chromatography analysis on 0.25 mm silica gel plates (60GF-254) and observed under UV light (254 nM or 365 nM) or iodine vapor. Melting points were tested on an electrothermal melting point apparatus without correction. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured with a Bruker DRX spectrometer (600 MHz) and a Bruker Avance spectrometer (300 MHz or 400 MHz). Chemical shifts are expressed in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard, and coupling constants (*J* values) are given in hertz (Hz). The splitting patterns were described as s (singlet), d (doublet), dd (doublet doublet), t (triplet), q (quartet), m (multiplet), and br s (broad singlet). High-resolution mass spectral (HRMS) data were displayed as *m*/*z* on an Agilent 6510 Quadrupole Time-of-Flight LC/MS deliver. The following yields were for optimized products.

5.1.1. (2*S*,4*R*)-1-*tert*-Butyl 2-methyl 4-hydroxypyrrolidine-1,2-dicarboxylate (3)

Acetyl chloride (14 mL, 198 mmol) was slowly added into the anhydrous methanol (100 mL) in an ice bath. After stirred for 30 min, compound **2** (7.87 g, 60.0 mmol) was added to the solution. Then the reaction was refluxed for 4 h in an oil bath. After the methanol was removed under reduced pressure, about 10 mL acetone was added into the residue and filtered to give 8.67 g of (2*S*,4*R*)-methyl 4-hydroxypyrrolidine-2-carboxylate hydrochloride as a white powder. Yield: 88%, mp: 160–164 °C. Di-*tert*-butyl dicarbonate (11.42 g, 52.3 mmol) in 50 mL dichloromethane was slowly added to the solution of (2*S*,4*R*)-methyl 4-hydroxypyrrolidine-2-carboxylate hydrochloride (8.64 g, 47.6 mmol), triethylamine (13.5 mL, 96.0 mmol) in 150 mL dichloromethane. After stirred overnight at room temperature, the reaction mixture was concentrated under reduced pressure and dissolved with EtOAc. The EtOAc layer was washed two times by 1 M citric acid, saturated sodium bicarbonate and brine, respectively. Finally, the organic layer was dried over anhydrous $MgSO_4$ and concentrated to obtain compound **3** as a white powder. Yield: 88%, mp: 93–94 °C. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.41 (s, 9H), 2.06–2.09 (m, 1H), 2.27–2.34 (m, 1H), 3.43–3.64 (m, 2H), 3.73 (s, 3H), 4.37–4.48 (m, 2H), ESI-MS *m*/*z*: 246.3 $(M+H)^+$.

5.1.2. (2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(4-bromophenoxy) pyrrolidine-2-carboxylic acid (4a)

The reaction mixture of compound **3** (9.8 g, 40 mmol), 4-bromophenol (8.30 g, 48 mmol) and triphenylphosphine (15.7 g, 60 mmol) in 100 mL anhydrous THF was placed at 0 °C. Then diethyl azodicarboxylate (DEAD, 9.40 g, 60 mmol) in 20 mL anhydrous was added to the above solution dropwise. After the reaction was warm to room temperature and stirred for 12 h, THF was removed and extracted by EtOAc. The EtOAc phase was washed by 0.5 M NaOH, 1 M citric acid and brine successively. After dried over anhydrous $MgSO_4$, the solution was concentrated to yield colorless oil liquid. Then the oil was dissolved in 100 mL mixed-solvent (THF/1 M NaOH = 1:1) and stirred overnight at room temperature. When the organic solvent was removed, the residue was acidified with 1 M HCl until pH 2–3. The formed precipitation was collected and recrystallized from MeOH/H_2O to get compound **4a** as a white lamellar crystal. Yield: 62%, mp: 105–107 °C. ^{1}H NMR (DMSO-d_6, 600 MHz), δ 1.35 (s, 9H), 2.14–2.20 (m, 1H), 2.52–2.56 (m, 1H), 3.39–3.42 (m, 1H), 3.64–3.70 (m, 1H), 4.22–4.26 (m, 1H), 5.02 (s, 1H), 6.84 (d, *J* = 7.8 Hz, 2H), 7.44 (d, *J* = 7.8 Hz, 2H), 12.51 (s, 1H), ESI-MS *m*/*z*: 385.1 $(M-H)^-$.

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

5.1.2.1. (2*S*,4*S*)-4-(4-Phenylphenoxy)-1-(*tert*-butoxycarbonyl) pyrrolidine-2-carboxylic acid (4b). White lamellar crystal, yield: 41%, mp: 158–160 °C. ^{1}H NMR (DMSO-d_6, 400 MHz), δ 1.42 (s, 9H), 2.22 (d, *J* = 13.2 Hz, 1H), 2.55–2.63 (m, 1H), 3.43–3.46 (m, 1H), 3.71–3.78 (m, 1H), 4.26–4.32 (m, 1H), 5.05 (s, 1H), 6.95–6.97 (m, 2H), 7.31 (t, *J* = 7.4 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.61 (d, *J* = 7.1 Hz, 2H), 12.47 (s, 1H), ESI-MS *m*/*z*: 382.5 $(M-H)^-$.

5.1.2.2. (2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(4-(4′-methyl)phenylphenoxy)pyrrolidine-2-carboxylic acid (4c). White lamellar crystal, yield: 40%, mp: 152–154 °C. ^{1}H NMR (DMSO-d_6, 400 MHz), δ 1.41 (s, 9H), 2.24 (d, *J* = 13.6 Hz, 1H), 2.32 (s, 3H), 2.53–2.64 (m, 1H), 3.41–3.46 (m, 1H), 3.70–3.75 (m, 1H), 4.26–4.31 (m, 1H), 5.04–5.06 (m, 1H), 6.92–6.95 (m, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.57 (d, *J* = 8.6 Hz, 2H), 12.49 (s, 1H), ESI-MS *m*/*z*: 396.4 $[M-H]^-$.

5.1.2.3. (2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(4-(4′-chloro)phenylphenoxy)pyrrolidine-2-carboxylic acid (4d). White lamellar crystal, yield: 43%, mp: 162–164 °C. ^{1}H NMR (DMSO-d_6, 600 MHz), δ 1.41 (s, 9H), 2.20–2.22 (m, 1H), 2.59–2.62 (m, 1H), 3.41–3.45 (m, 1H), 3.71–3.77 (m, 1H), 4.29 (dd, *J* = 21 Hz, 9.6 Hz, 1H), 5.06–5.09 (m, 1H), 6.94–6.97 (m, 2H), 7.48 (d, *J* = 7.8 Hz, 2H), 7.61 (d, *J* = 7.8 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 2H), 12.54 (s, 1H), ESI-MS *m*/*z*: 416.5 $[M-H]^-$.

5.1.3. (*S*)-*tert*-Butyl(1-oxo-3-phenyl-1-(phenylsulfonamido) propan-2-yl)carbamate (6a)

N-Methylmorpholine (0.45 g, 4.4 mmol) and isobutyl chloroformate (0.60 g, 4.4 mmol) was added into a solution of *N*-Boc-*L*-phenylalanine (1.06 g, 4 mmol) in 20 mL anhydrous THF at −20 °C. After stirred for 1 h, a solution of benzenesulfonamide (0.94 g, 6 mmol) and NaH (0.48 g, 12 mmol) in 15 mL anhydrous THF was added and stirred overnight at room temperature. Then the solvent was removed under reduced pressure and the right amount of EtOAc was added. The solution was washed by 1 M citric acid, brine and dried over anhydrous $MgSO_4$ successively. After removal of EtOAc, the crude product was further purified by column chromatography (petroleum ether/ethyl acetate = 5:1) to yield pure compound **6a** as a white powder. Yield: 68%, mp: 133–135 °C. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.38 (s, 9H), 2.93 (dd,

J = 14.1 Hz, 6.9 Hz, 1H), 3.05 (dd, J = 14.1 Hz, 6.3 Hz, 1H), 4.31 (s, 1H), 4.90 (s, 1H), 7.02–7.04 (m, 2H), 7.04–7.23 (m, 3H), 7.52–7.57 (m, 2H), 7.57–7.66 (m, 1H), 8.05 (d, J = 7.5 Hz, 2H), 9.34 (s, 1H), ESI-MS m/z: 403.1 $(M-H)^-$.

Compounds **6b–6j** were synthesized following the procedure described above.

5.1.3.1. (*S*)-*tert*-Butyl-(1-(4-methylphenylsulfonamido)-1-oxo-3-phenylpropan-2-yl)carbamate (6b). White powder, the pure compound **6b** was not obtained by column chromatography and it was used into the next step directly.

5.1.3.2. (*S*)-*tert*-Butyl-(1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-phenylpropan-2-yl)carbamate (6c). Yellow powder, yield: 80%, mp: 147–149 °C. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.40 (s, 9H), 2.96 (dd, J = 14.1 Hz, 7.8 Hz, 1H), 3.07 (dd, J = 14.1 Hz, 6.3 Hz, 1H), 4.20–4.27 (m, 1H), 4.81 (d, J = 6.6 Hz, 1H), 7.07–7.10 (m, 2H), 7.26–7.28 (m, 3H), 7.73 (d, J = 8.4 Hz, 1H), 8.19 (dd, J = 8.4 Hz, 2.1 Hz, 1H), 8.48 (d, J = 2.1 Hz, 1H), 9.50 (s, 1H), ESI-MS m/z: 482.1 $(M-H)^-$.

5.1.3.3. (*S*)-*tert*-Butyl(3-(4-bromophenyl)-1-oxo-1-(phenylsulfonamido)propan-2-yl)carbamate (6d). White powder, yield: 87%, mp: 119–121 °C. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 1.26 (s, 9H), 2.57–2.61 (m, 1H), 2.79–2.85 (m, 1H), 4.06–4.13 (m, 1H), 7.07 (br s, 1H), 7.16 (d, J = 8.1 Hz, 2H), 7.42 (d, J = 8.1 Hz, 2H), 7.58–7.66 (m, 2H), 7.70 (t, J = 7.2 Hz, 1H), 7.88–7.95 (m, 2H), 12.43 (s, 1H), ESI-MS m/z: 483.3 $(M-H)^-$.

5.1.3.4. (*S*)-*tert*-Butyl(3-(4-bromophenyl)-1-(4-methylphenylsulfonamido)-1-oxopropan-2-yl)carbamate (6e). White powder, yield: 85%, mp: 114–115 °C. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 1.31 (s, 9H), 2.35 (s, 3H), 2.68–2.75 (m, 1H), 2.89–2.95 (m, 1H), 3.84–3.86 (m, 1H), 6.04 (br s, 1H), 6.92 (d, J = 8.1 Hz, 2H), 7.21–7.35 (m, 4H), 7.65 (d, J = 8.1 Hz, 2H), 12.34 (s, 1H), ESI-MS m/z: 497.4 $(M-H)^-$.

5.1.3.5. (*S*)-*tert*-Butyl(3-(4-bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamate (6f). Yellow powder, yield: 72%, mp: 116–118 °C. ^{1}H-NMR (DMSO-d_6, 300 MHz), δ 1.31 (s, 9H), 2.68–2.75 (m, 1H), 2.91–2.98 (m, 1H), 3.82–3.89 (m, 1H), 6.01 (d, J = 7.8 Hz, 1H), 6.94 (d, J = 8.1 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 7.82 (d, J = 8.1 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 8.32 (s, 1H), ESI-MS m/z: 562.2 $(M-H)^-$.

5.1.3.6. (*S*)-*tert*-Butyl(3-(4-bromophenyl)-1-(4-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamate (6g). Yellow powder, the pure compound **7g** was also not obtained by column chromatography and it was used into the next step directly.

5.1.3.7. (*S*)-*tert*-Butyl(3-(4-bromophenyl)-1-(4-chlorophenylsulfonamido)-1-oxopropan-2-yl)carbamate (6h). White powder, yield: 75%, mp: 121–123 °C. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 1.31 (s, 9H), 2.70–2.77 (m, 1H), 2.91–2.97 (m, 1H), 3.80–3.86 (m, 1H), 5.88 (d, J = 7.5 Hz, 1H), 6.89 (d, J = 8.1 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 6.9 Hz, 2H), 7.74 (d, J = 8.7 Hz, 2H), ESI-MS m/z: 517.3 $(M-H)^-$.

5.1.3.8. (*S*)-*tert*-Butyl(3-(1*H*-indol-3-yl)-1-oxo-1-(phenylsulfonamido)propan-2-yl)carbamate (6i). Yellow powder, yield: 80%, mp: 95–97 °C. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.39 (s, 9H), 3.19–3.21 (m, 2H), 4.38 (br s, 1H), 4.89 (br s, 1H), 6.96 (s, 1H), 7.13 (t, J = 7.2 Hz, 1H), 7.22–7.24 (m, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.52–7.57 (m, 3H), 7.66 (t, J = 7.5 Hz, 1H), 8.03 (d, J = 7.5 Hz, 2H), 8.08 (br s, 1H), 8.94 (br s, 1H), ESI-MS m/z: 442.5 $(M-H)^-$.

5.1.3.9. (*S*)-*tert*-Butyl(1-(4-chloro-3-nitrophenylsulfonamido)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamate (6j). Yellow powder, yield: 85%, mp: 90–92 °C. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.40 (s, 9H), 3.18–3.20 (d, J = 6.6 Hz, 2H), 4.38–4.40 (m, 1H), 5.00 (d, J = 6.9 Hz, 1H), 6.97 (s, 1H), 7.09 (t, J = 7.5 Hz, 1H), 7.22 (t, J = 7.2 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 2.1 Hz, 1H), 8.15 (d, J = 2.1 Hz, 1H), 8.42 (d, J = 2.1 Hz, 1H), 9.43 (br s, 1H), ESI-MS m/z: 521.3 $(M-H)^-$.

5.1.3.10. (*S*)-*tert*-Butyl(3-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamate (6k). *tert*-Butyl dimethylsilyl chloride (1.13 g, 7.5 mmol) and imidazole (2.04 g, 30 mmol) was added into a solution of *N*-Boc-*L*-tyrosine (1.40 g, 5 mmol) in 30 mL *N*,*N*-dimethylformamide (DMF) at 0 °C. After stirred for 1 h at 0 °C, the mixture was allowed to stir overnight at room temperature. Then the reaction mixture was transferred to a separating funnel by adding to much water and extracted with EtOAc two times. The combined organic layer was washed by 1 M citric acid, brine, dried over anhydrous $MgSO_4$ and concentrated to yield colorless oil. Finally, the resulting oil was treated with the procedure of the synthesis of compound **6a** to obtain yellow oil compound **6k**. Yield: 78%, ^{1}H NMR ($CDCl_3$, 600 MHz), δ 0.18 (s, 6H), 0.97 (s, 9H), 1.40 (s, 9H), 2.86–2.90 (m, 1H), 2.97–2.99 (m, 1H), 4.23 (s, 1H), 4.90 (s, 1H), 6.71 (d, J = 7.8 Hz, 2H), 6.92 (d, J = 7.8 Hz, 2H), 7.23 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 7.8 Hz, 1H), 8.51 (s, 1H), 9.76 (s, 1H), ESI-MS m/z: 612.5 $(M-H)^-$.

5.1.4. (*S*)-2-((2*S*,4*S*)-4-(4-Phenylphenoxy)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)-3-phenylpropanoic acid (1)

The intermediate **4b** (0.38 g, 1 mmol) was dissolved in 15 mL anhydrous THF at −20 °C. 10 min later, NMM (0.22 g, 2.2 mmol) and isobutylchloroformate (0.16 g, 1.1 mmol) were added successively. After stirred for 1 h, phenylalanine methyl ester hydrochloride (0.20 g, 1.1 mmol) was added and the reaction was warmed slowly to rt and stirred overnight. The solvent was removed under low pressure. The resulting residue was extracted with EtOAc and washed with 1 M citric acid, saturated $NaHCO_3$ and brine, respectively. After dried over $MgSO_4$ and concentrated, the obtained oil was dissolved in 10 mL mixed-solvent (THF/1 M NaOH = 1:1) and stirred for 4 h at room temperature. When the reaction was completed, the solvent was removed and the residue was acidified with 1 M HCl until pH 2–3. The generated white precipitation was collected and recrystallized from MeOH/H_2O to yield compound **1** as a white crystal. Yield: 88%, mp: 202–204 °C. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 1.29 (s, 9H), 1.99–2.20 (m, 1H), 2.61 (br s, 1H), 2.91–2.98 (m, 1H), 3.04–3.10 (m, 1H), 3.37–3.42 (m, 1H), 3.82–3.87 (m, 1H), 4.18–4.28 (m, 1H), 4.46 (br s, 1H), 5.00 (br s, 1H), 6.96 (d, J = 8.7 Hz, 2H), 7.16–7.24 (m, 5H), 7.28–7.33 (m, 1H), 7.43 (t, J = 7.8 Hz, 2H), 7.57–7.62 (m, 4H), 7.78–7.84 (m, 1H), 12.76 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 176.57, 174.63, 172.11, 156.34, 140.56, 135.69, 134.73, 130.92, 129.43, 128.74, 128.60, 128.37, 127.17, 126.84, 126.76, 115.90, 81.66, 75.67, 60.04, 52.97, 37.86, 28.09, 20.71, 19.18. HRMS (AP-ESI) m/z calcd for $C_{31}H_{34}N_2O_6$ $[M-H]^-$: 529.2339, found: 529.2340.

Compounds **7–15** were synthesized following the procedure described above.

5.1.4.1. (*S*)-2-((2*S*,4*S*)-4-(4-Bromophenoxy)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)-3-phenylpropanoic acid (7). White crystal, yield: 87%, mp: 185–187 °C. ^{1}H NMR (DMSO-d_6, 300 MHz), δ 1.24 (s, 9H), 1.93–1.98 (m, 1H), 2.57 (s, 1H), 2.89–2.96 (m, 1H), 3.02–3.09 (m, 1H), 3.37–3.38 (m, 1H), 3.77–3.83 (m, 1H), 4.16 (br s, 1H), 4.44 (br s, 1H), 4.93 (s, 1H), 6.82 (d, J = 8.7 Hz, 2H), 7.14–7.26 (m, 5H), 7.40–7.46 (m, 2H),

7.80–7.83 (m, 1H), 12.71 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 174.08, 171.89, 155.88, 135.62, 132.54, 129.37, 128.58, 127.17, 117.42, 113.92, 81.75, 75.84, 59.95, 53.10, 52.84, 37.73, 28.09. HRMS (AP-ESI) m/z calcd for $C_{25}H_{29}BrN_2O_6$ $[M-H]^-$: 531.1131, found: 531.1133.

5.1.4.2. 2-((2*S*,4*S*)-4-(4-Bromophenoxy)-1-(*tert*-butoxycarbonyl) pyrrolidine-2-carboxamido)acetic acid (8). White crystal, yield: 32%, mp: 72–74 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.45 (s, 9H), 2.45–2.69 (m, 2H), 3.75 (s, 2H), 4.00–4.13 (m, 2H), 4.46 (br s, 1H), 4.84 (s, 1H), 6.67 (d, J = 8.8 Hz, 2H), 7.11 (br s, 1H), 7.73 (d, J = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 172.72, 171.98, 155.64, 154.99, 132.52, 117.42, 113.89, 81.91, 75.33, 59.96, 52.77, 41.46, 35.82, 28.25.HRMS (AP-ESI) m/z calcd for $C_{18}H_{23}BrN_2O_6$ $[M-H]^-$: 441.0661, found: 441.0677.

5.1.4.3. 2-((2*S*,4*S*)-4-(4-Phenylphenoxy)-1-(*tert*-butoxycarbonyl) pyrrolidine-2-carboxamido)acetic acid (9). White crystal, yield: 95%, mp: 195–197 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.46 (s, 9H), 2.48–2.71 (m, 2H), 3.76–3.82 (m, 2H), 4.00–4.20 (m, 2H), 4.46 (br s, 1H), 4.91 (s, 1H), 6.84 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 4.0 Hz, 1H), 7.27–7.31 (m, 1H), 7.37 (t, J = 7.2 Hz, 2H), 7.46–7.50 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 172.94, 172.20, 156.05, 155.34, 140.56, 134.73, 128.73, 128.37, 126.83, 126.76, 115.86, 81.84, 75.14, 60.11, 52.86, 41.50, 28.26.HRMS (AP-ESI) m/z calcd for $C_{24}H_{28}N_2O_6$ $[M-H]^-$: 439.1869, found: 439.1876.

5.1.4.4. (*S*)-2-((2*S*,4*S*)-4-(4-Bromophenoxy)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)-2-phenylacetic acid (10). White crystal, yield: 50%, mp: 96–98 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.47 (s, 9H), 2.18–2.42 (m, 1H), 2.48–2.66 (m, 1H), 3.66 (s, 2H), 4.45 (br s, 1H), 4.74 (s, 1H), 5.57 (d, J = 6.8 Hz, 1H), 6.20–6.35 (m, 2H), 7.18 (d, J = 7.0 Hz, 2H), 7.29–7.33 (m, 3H), 7.38 (d, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 172.90, 171.79, 155.53, 155.37, 136.43, 132.30, 128.97, 128.50, 127.52, 117.46, 113.78, 82.00, 75.66, 60.16, 56.40, 52.91, 29.68, 28.19. HRMS (AP-ESI) m/z calcd for $C_{24}H_{27}BrN_2O_6$ $[M-H]^-$: 517.0974, found: 517.0982.

5.1.4.5. (*S*)-2-((2*S*,4*S*)-4-(4-Phenylphenoxy)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)-2-phenylacetic acid (11). White crystal, yield: 39%, mp: 211–213 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.48 (s, 9H), 2.23–2.42 (m, 1H), 2.55–2.70 (m, 1H), 3.71–3.78 (m, 2H), 4.48 (br s, 1H), 4.85 (s, 1H), 5.60 (d, J = 6.8 Hz, 1H), 6.45–6.53 (m, 2H), 7.25 (s, 1H), 7.29–7.32 (m, 5H), 7.39–7.42 (m, 4H), 7.48 (d, J = 7.6 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 173.56, 172.03, 156.00, 155.34, 140.65, 134.60, 129.02, 128.73, 128.55, 128.20, 127.55, 126.81, 126.78, 115.87, 81.82, 75.56, 60.37, 56.49, 53.05, 29.70, 28.21. HRMS (AP-ESI) m/z calcd for $C_{30}H_{32}N_2O_6$ $[M-H]^-$: 515.2182, found: 515.2206.

5.1.4.6. (*S*)-2-((2*S*,4*S*)-4-(4-Bromophenoxy)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)-3-methylbutanoic acid (12). White crystal, yield: 33%, mp: 80–82 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 0.93 (dd, J = 11.2 Hz, 6.8 Hz, 6H), 1.46 (s, 9H), 2.21–2.23 (m, 1H), 2.33–2.51 (m, 1H), 2.56–2.89 (m, 1H), 3.72–3.86 (m, 2H), 4.49 (d, J = 5.6 Hz, 2H), 4.80 (s, 1H), 6.68 (d, J = 8.8 Hz, 2H), 7.35 (d, J = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 174.82, 172.15, 156.13, 155.22, 132.48, 117.49, 113.87, 81.75, 76.29, 60.21, 57.25, 53.15, 31.29, 28.18, 19.03, 17.80. HRMS (AP-ESI) m/z calcd for $C_{21}H_{29}BrN_2O_6$ $[M-H]^-$: 483.1131, found: 483.1138.

5.1.4.7. (*S*)-2-((2*S*,4*S*)-4-(4-Phenylphenoxy)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)-3-methylbutanoic acid (13). White crystal, yield: 90%, mp: 185–186 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 0.96 (dd, J = 12.8 Hz, 6.8 Hz, 6H), 1.46 (s, 9H), 2.24–2.25 (m, 1H), 2.35–2.59 (m, 1H), 2.60–2.90 (m, 1H), 3.80 (s, 2H), 4.41–4.55 (m, 2H), 4.89 (s, 1H), 6.87 (d, J = 8.4 Hz, 2H), 7.29–7.32 (m, 1H), 7.39–7.43 (m, 2H), 7.49–7.54 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 175.01, 172.22, 156.55, 140.57, 134.68, 128.74, 128.30, 126.84, 126.76, 115.95, 81.67, 76.05, 60.36, 57.31, 53.27, 31.35, 29.69, 28.20, 19.06, 17.91. HRMS (AP-ESI) m/z calcd for $C_{27}H_{34}N_2O_6$ $[M-H]^-$: 481.2339, found: 481.2361.

5.1.4.8. (*S*)-2-((2*S*,4*S*)-4-(4-Bromophenoxy)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)-4-methylpentanoic acid (14). White crystal, yield: 85%, mp: 152–154 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 0.90–0.93 (m, 6H), 1.46 (s, 9H), 1.55–1.60 (m, 1H), 1.65–1.73 (m, 2H), 2.24–2.46 (m, 1H), 2.54–2.79 (m, 1H), 3.74 (s, 2H), 4.45 (br s, 1H), 4.58 (br s, 1H), 4.80 (s, 1H), 6.68 (d, J = 8.8 Hz, 2H), 7.36 (d, J = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 178.79, 175.02, 158.87, 135.39, 132.49, 120.33, 117.43, 116.83, 84.73, 79.01, 63.01, 55.95, 53.80, 44.52, 31.07, 27.56, 25.59, 24.89. HRMS (AP-ESI) m/z calcd for $C_{22}H_{31}BrN_2O_6$ $[M-H]^-$: 497.1287, found: 497.1314.

5.1.4.9. (*S*)-2-((2*S*,4*S*)-4-(4-Phenylphenoxy)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)-4-methylpentanoic acid (15). White crystal, yield: 95%, mp: 182–184 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 0.91–0.96 (m, 6H), 1.47 (s, 9H), 1.59–1.63 (m, 1H), 1.69–1.75 (m, 2H), 2.33–2.56 (m, 1H), 2.60–2.86 (m, 1H), 3.78 (s, 2H), 4.61 (br s, 1H), 4.63 (br s, 1H), 4.89 (s, 1H), 6.86 (d, J = 8.4 Hz, 2H), 7.29–7.33 (m, 1H), 7.39 (t, J = 7.6 Hz, 2H), 7.49–7.54 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 176.39, 176.23, 156.35, 155.25, 140.55, 134.74, 128.74, 128.32, 126.85, 126.76, 115.86, 81.68, 75.81, 60.17, 53.16, 50.86, 41.70, 30.57, 28.17, 24.67, 22.68, 22.03, 20.69, 19.17. HRMS (AP-ESI) m/z calcd for $C_{28}H_{36}N_2O_6$ $[M-H]^-$: 495.2495, found: 495.2505.

5.1.5. (2*S*,4*S*)-*tert*-Butyl 4-(4-phenylphenoxy)-2-(((*S*)-1-oxo-3-phenyl-1-(phenylsulfonamido)propan-2-yl)carbamoyl) pyrrolidine-1-carboxylate (16)

The intermediate **6a** (0.49 g, 1.2 mmol) was dissolved in EtOAc saturated with HCl and stirred at room temperature overnight. Then the generated hydrochloride precipitate was collected and dried for the next step. The other intermediate **4b** (0.38 g, 1 mmol) was dissolved in 10 mL anhydrous THF at −20 °C. 10 min later, *N*-methylmorpholine (0.22 g, 2.2 mmol) and isobutyl chloroformate (0.16 g, 1.1 mmol) was added successively. After 1 h, the hydrochloride precipitate previously dried was added and the reaction mixture was warm slowly to rt and stirred until no starting materials was existed. Then the solvent was evaporated under reduced pressure and was extracted with EtOAc, washed with 1 M citric acid, saturated $NaHCO_3$, brine and dried over $MgSO_4$. The concentrated residue was purified by column chromatography (petroleum ether/ethyl acetate = 2:1) to obtain the compound **16** as white powder. Yield: 40%, mp: 94–96 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.38 (s, 9H), 2.48–2.51 (m, 1H), 2.63 (d, J = 7.1 Hz, 1H), 2.91 (d, J = 10.1 Hz, 1H), 3.40 (d, J = 12.6 Hz, 1H), 3.71 (d, J = 11.5 Hz, 1H), 3.76 (d, J = 11.2 Hz, 1H), 4.46 (d, J = 10.3 Hz, 1H), 4.83 (s, 1H), 4.94 (s, 1H), 6.71 (d, J = 7.3 Hz, 1H), 6.91 (d, J = 7.7 Hz, 2H), 7.01 (d, J = 7.5 Hz, 2H), 7.14 (m, 2H), 7.22 (m, 1H), 7.34 (t, J = 7.0 Hz, 1H), 7.45 (t, J = 6.8 Hz, 2H), 7.56–7.57 (m, 6H), 7.65–7.66 (m, 1H), 8.06 (d, J = 6.8 Hz, 2H), 10.00 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.58, 169.88, 156.16, 140.37, 138.87, 135.10, 134.75, 133.65, 129.45, 128.93, 128.80, 128.70, 128.63, 128.57, 127.50, 126.99, 126.77, 115.91, 82.30, 60.13, 53.24, 36.80, 34.66, 28.17. HRMS (AP-ESI) m/z calcd for $C_{37}H_{39}N_3O_7S$ $[M-H]^-$ 668.2430, found: 668.2436.

Compounds **17**–**31** were synthesized following the procedure described above.

5.1.5.1. (2*S*,4*S*)-*tert*-Butyl-4-(4-phenylphenoxy)-2-(((*S*)-1-(4-methylphenylsulfonamido)-1-oxo-3-phenylpropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (17). White powder, yield: 31%, mp: 89–91 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.37 (s, 9H), 2.46 (s, 3H), 2.49–2.50 (m, 1H), 2.61 (d, J = 14.1 Hz, 1H), 2.90 (d, J = 13.0 Hz, 1H), 3.41 (d, J = 13.2 Hz, 1H), 3.70–3.71 (m, 1H), 3.75 (d, J = 10.7 Hz, 1H), 4.44 (d, J = 10.1 Hz, 1H), 4.79 (br s, 1H), 4.92 (s, 1H), 6.66 (d, J = 5.9 Hz, 1H), 6.90 (d, J = 5.7 Hz, 2H), 7.04 (d, J = 6.4 Hz, 2H), 7.14–7.15 (m, 2H), 7.21–7.23 (m, 1H), 7.33–7.34 (m, 3H), 7.42–7.44 (m, 2H), 7.55–7.56 (m, 4H), 7.93 (d, J = 5.2 Hz, 2H), 9.87 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.44, 169.95, 156.18, 144.63, 140.38, 135.92, 135.08, 134.80, 129.48, 129.32, 128.89, 128.80, 128.71, 128.56, 127.47, 126.99, 126.77, 115.91, 82.29, 60.12, 53.11, 36.81, 34.66, 28.17, 21.66. HRMS (AP-ESI) m/z calcd for $C_{38}H_{41}N_3O_7S$ $[M-H]^-$ 682.2587, found: 682.2600.

5.1.5.2. (2*S*,4*S*)-*tert*-Butyl-4-(4-phenylphenoxy)-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-phenylpropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (18). Yellow powder, yield: 35%, mp: 106–109 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.35 (s, 9H), 2.50–2.51 (m, 1H), 2.56 (d, J = 14.2 Hz, 1H), 2.92–2.94 (m, 1H), 3.37–3.38 (m, 1H), 3.68–3.40 (m, 1H), 3.77 (d, J = 11.8 Hz, 1H), 4.40 (d, J = 8.7 Hz, 1H), 4.73 (s, 1H), 4.91 (s, 1H), 6.65 (d, J = 7.4 Hz, 1H), 6.86 (d, J = 8.2 Hz, 2H), 7.04 (d, J = 7.4 Hz, 2H), 7.17 (t, J = 7.5 Hz, 2H), 7.23 (d, J = 7.3 Hz, 1H), 7.33 (t, J = 7.4 Hz, 1H), 7.43 (t, J = 7.5 Hz, 2H), 7.53–7.54 (m, 4H), 7.74 (d, J = 8.6 Hz, 1H), 8.23 (d, J = 8.4 Hz, 1H), 8.48 (s, 1H), 10.21 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.67, 170.42, 156.04, 147.52, 140.27, 138.81, 134.48, 132.93, 132.68, 132.49, 129.32, 128.99, 128.83, 128.61, 127.77, 127.06, 126.76, 126.03, 115.88, 82.60, 60.14, 53.28, 36.81, 34.70, 28.14, 18.95. HRMS (AP-ESI) m/z calcd for $C_{37}H_{37}ClN_4O_9S$ $[M-H]^-$ 747.1892, found: 747.1901.

5.1.5.3. (2*S*,4*S*)-*tert*-Butyl-4-(4-phenylphenoxy)-2-(((*S*)-3-(4-bromophenyl)-1-oxo-1-(phenylsulfonamido)propan-2-yl)carbamoyl) pyrrolidine-1-carboxylate (19). White powder, yield: 51%, mp: 96–98 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.40 (s, 9H), 2.45–2.46 (m, 1H), 2.62 (d, J = 13.4 Hz, 1H), 2.84 (d, J = 10.7 Hz, 1H), 3.32 (d, J = 11.4 Hz, 1H), 3.71–3.74 (m, 2H), 4.44 (d, J = 9.5 Hz, 1H), 4.80 (s, 1H), 4.91 (s, 1H), 6.62 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.0 Hz, 2H), 6.88 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 6.9 Hz, 2H), 7.32 (t, J = 7.3 Hz, 1H), 7.42 (d, J = 7.4 Hz, 2H), 7.53–7.54 (m, 6H), 7.65 (t, J = 7.0 Hz, 1H), 8.04 (d, J = 6.1 Hz, 2H), 9.96 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.99, 169.54, 156.13, 140.27, 138.79, 135.18, 133.73, 131.90, 131.17, 128.81, 128.75, 128.63, 127.03, 126.77, 121.59, 115.94, 81.93, 60.05, 53.36, 52.84, 36.34, 34.55, 28.12. HRMS (AP-ESI) m/z calcd for $C_{37}H_{38}BrN_3O_7S$ $[M-H]^-$ 746.1536, found: 746.1546.

5.1.5.4. (2*S*,4*S*)-*tert*-Butyl-4-(4-phenylphenoxy)-2-(((*S*)-3-(4-bromophenyl)-1-(4-methylphenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (20). White powder, yield: 37%, mp: 95–97 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.38 (s, 9H), 2.44 (s, 3H), 2.46–2.47 (m, 1H), 2.59 (d, J = 13.7 Hz, 1H), 2.82 (d, J = 7.6 Hz, 1H), 3.32 (d, J = 15.4 Hz, 1H), 3.69–3.74 (m, 2H), 4.41 (d, J = 10.1 Hz, 1H), 4.75 (s, 1H), 4.88 (s, 1H), 6.56 (d, J = 7.9 Hz, 1H), 6.86–6.88 (m, 4H), 7.20 (d, J = 7.4 Hz, 2H), 7.30–7.32 (m, 3H), 7.41 (t, J = 7.6 Hz, 2H), 7.51–7.52 (m, 4H), 7.89 (d, J = 7.0 Hz, 2H), 9.85 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.69, 169.69, 156.14, 144.77, 140.28, 135.82, 135.15, 133.74, 131.84, 131.19, 129.36, 128.81, 128.66, 128.61, 127.02, 126.77, 121.54, 115.93, 82.40, 60.04, 53.36, 52.83, 36.36, 34.37, 28.12, 21.66. HRMS (AP-ESI) m/z calcd for $C_{38}H_{40}BrN_3O_7S$ $[M-H]^-$ 760.1692, found: 760.1695.

5.1.5.5. (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 (21). Yellow powder, yield: 41%, mp: 97–99 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.39 (s, 9H), 2.46–2.50 (m, 1H), 2.58 (d, J = 13.9 Hz, 1H), 2.90 (d, J = 8.6 Hz, 1H), 3.27 (d, J = 11.0 Hz, 1H), 3.68 (d, J = 11.3 Hz, 1H), 3.77 (d, J = 11.8 Hz, 1H), 4.41 (d, J = 10.4 Hz, 1H), 4.73 (s, 1H), 4.90 (s, 1H), 6.68 (d, J = 5.9 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H), 6.93 (d, J = 7.02 Hz, 2H), 7.28 (d, J = 7.0 Hz, 2H), 7.33 (t, J = 7.2 Hz, 1H), 7.43 (t, J = 7.3 Hz, 2H), 7.53–7.55 (m, 4H), 7.71 (d, J = 8.1 Hz, 1H), 8.18 (d, J = 7.7 Hz, 1H), 8.52 (s, 1H), 10.34 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.83, 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, 53.23, 36.30, 34.60, 28.09. HRMS (AP-ESI) m/z calcd for $C_{37}H_{36}BrClN_4O_9S$ $[M-H]^-$ 825.0997, found: 825.1006.

5.1.5.6. (2*S*,4*S*)-*tert*-Butyl-4-(4-phenylphenoxy)-2-(((*S*)-3-(4-bromophenyl)-1-(4-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (22). Yellow powder, yield: 30%, mp: 128–130 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.35 (s, 9H), 2.48–2.51 (m, 1H), 2.57 (d, J = 13.8 Hz, 1H), 2.90 (dd, J = 13.8 Hz, 4.2 Hz, 1H), 3.31 (d, J = 11.4 Hz, 1H), 3.69 (d, J = 9 Hz, 1H), 3.79 (d, J = 12.6 Hz, 1H), 4.41 (d, J = 9.6 Hz, 1H), 4.71 (s, 1H), 4.91 (s, 1H), 6.64 (d, J = 7.8 Hz, 1H), 6.87 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 7.8 Hz, 2H), 7.32 (d, J = 8.4 Hz, 2H), 7.34 (t, J = 7.2 Hz, 1H), 7.43 (t, J = 7.2 Hz, 2H), 7.54–7.56 (m, 4H), 8.24 (d, J = 8.4 Hz, 2H), 8.37 (d, J = 8.4 Hz, 2H), 10.20 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 172.18, 169.8, 156.00, 150.69, 144.22, 140.16, 135.41, 133.45, 132.05, 131.12, 130.01, 128.85, 128.68, 127.13, 126.76, 123.94, 121.90, 115.96, 82.77, 60.09, 53.36, 53.21, 36.11, 34.36, 28.12. HRMS (AP-ESI) m/z calcd for $C_{37}H_{37}BrN_4O_9S$ $[M-H]^-$ 791.1386, found: 791.1387.

5.1.5.7. (2*S*,4*S*)-*tert*-Butyl-4-(4-phenylphenoxy)-2-(((*S*)-3-(4-bromophenyl)-1-(4-chlorophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (23). White powder, yield: 26%, mp: 125–127 °C. ^{1}H NMR ($(CD_3)_2CO$, 600 MHz), δ 1.35 (s, 9H), 2.19–2.27 (m, 1H), 2.53–2.54 (m, 1H), 2.91–2.92 (m, 1H), 3.03 (dd, J = 13.2 Hz, 4.8 Hz, 1H), 3.51–3.56 (m, 1H), 3.75–3.76 (m, 1H), 4.21–4.26 (m, 1H), 4.60–4.70 (m, 1H), 5.00 (br s, 1H), 6.74–6.88 (m, 2H), 6.98 (d, J = 7.8 Hz, 2H), 7.20–7.23 (m, 3H), 7.33 (t, J = 7.8 Hz, 2H), 7.49–7.52 (m, 4H), 7.58 (d, J = 7.8 Hz, 2H), 7.89 (d, J = 7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.84, 169.69, 156.06, 140.44, 140.23, 137.17, 133.64, 131.97, 131.14, 130.15, 129.05, 128.83, 128.66, 127.08, 126.77, 115.95, 82.58, 60.07, 53.36, 52.92, 28.11.HRMS (AP-ESI) m/z calcd for $C_{37}H_{37}BrClN_3O_7S$ $[M-H]^-$ 780.1146, found: 780.1149.

5.1.5.8. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-3-(1*H*-indol-3-yl)-1-oxo-1-(phenylsulfonamido)propan-2-yl)carbamoyl)-4-(4-phenylphenoxy)pyrrolidine-1-carboxylate (24). Yellow powder, yield: 28%, mp: 115–117 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.11 (s, 9H), 2.53–2.55 (m, 1H), 2.61 (d, J = 14.3 Hz, 1H), 3.15 (dd, J = 14.8 Hz, 4.1 Hz, 1H), 3.56 (dd, J = 14.8 Hz, 3.4 Hz, 1H), 3.62 (dd, J = 11.9 Hz, 3.7 Hz, 1H), 3.76 (d, J = 12.1 Hz, 1H), 4.41 (d, J = 9.5 Hz, 1H), 4.87–4.88 (m, 1H), 4.97 (s, 1H), 6.81 (s, 1H), 6.85–6.86 (m, 2H), 7.00 (d, J = 8.4 Hz, 2H), 7.17 (t, J = 7.4 Hz, 1H), 7.33–7.36 (m, 2H), 7.44 (t, J = 7.6 Hz, 2H), 7.53–7.59 (m, 6H), 7.65 (d, J = 7.1 Hz, 2H), 8.05 (d, J = 7.7 Hz, 1H), 8.07 (s, 1H), 9.92 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.49, 170.91, 156.29, 155.77, 140.41, 139.08,

136.07, 135.05, 133.55, 128.80, 128.62, 127.54, 126.97, 126.78, 124.31, 122.39, 120.03, 118.22, 115.72, 111.69, 108.08, 82.13, 60.25, 54.78, 52.88, 34.83, 27.71, 26.67. HRMS (AP-ESI) m/z calcd for $C_{39}H_{40}N_4O_7S$ $[M-H]^-$ 707.2539, found: 707.2546.

5.1.5.9. (2*S*,4*S*)-*tert*-Butyl-4-(4-phenylphenoxy)-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (25). Yellow powder, yield: 33%, mp: 123–125 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.09 (s, 9H), 2.53–2.60 (m, 2H), 3.14 (dd, J = 15.0 Hz, 4.2 Hz, 1H), 3.52 (d, J = 15.0 Hz, 1H), 3.62 (d, J = 8.4 Hz, 1H), 3.78 (d, J = 6.0 Hz, 1H), 4.37 (d, J = 8.4 Hz, 1H), 4.84 (s, 1H), 4.96 (s, 1H), 6.74 (s, 1H), 6.83–6.88 (m, 2H), 6.95 (d, J = 7.8 Hz, 1H), 7.17 (t, J = 7.2 Hz, 1H), 7.26–7.36 (m, 2H), 7.42 (t, J = 7.2 Hz, 2H), 7.54–7.56 (m, 4H), 7.62 (d, J = 7.8 Hz, 1H), 7.73 (d, J = 7.8 Hz, 1H), 8.12 (br s, 1H), 8.24 (d, J = 8.4 Hz, 1H), 8.33 (s, 1H), 10.19 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.48, 156.16, 155.94, 147.61, 140.33, 139.03, 136.13, 135.20, 133.20, 132.35, 128.82, 128.67, 127.36, 127.03, 126.77, 125.69, 123.84, 122.65, 120.19, 118.13, 115.70, 111.81, 108.23, 82.39, 60.28, 54.87, 52.97, 34.82, 27.65, 26.61. HRMS (AP-ESI) m/z calcd for $C_{39}H_{38}ClN_5O_9S$ $[M-H]^-$ 786.2001, found: 786.1996.

5.1.5.10. (2*S*,4*S*)-*tert*-Butyl-4-(4-(4′-methyl)phenylphenoxy)-2-(((*S*)-1-oxo-3-phenyl-1-(phenylsulfonamido)propan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (26). White powder, yield: 35%, mp: 86–88 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.37 (s, 9H), 2.41 (s, 3H), 2.47–2.51 (m, 1H), 2.61 (d, J = 14.4 Hz, 1H), 2.90 (dd, J = 13.7 Hz, 5.0 Hz, 1H), 3.43 (dd, J = 13.7 Hz, 3.2 Hz, 1H), 3.70–3.72 (m, 1H), 3.76 (d, J = 11.8 Hz, 1H), 4.44 (d, J = 9.9 Hz, 1H), 4.79 (s, 1H), 4.92 (s, 1H), 6.63 (d, J = 8.2 Hz, 1H), 6.89 (d, J = 8.1 Hz, 2H), 7.03 (d, J = 7.14 Hz, 2H), 7.16 (t, J = 7.4 Hz, 2H), 7.22–7.23 (m, 1H), 7.26 (d, J = 7.9 Hz, 2H), 7.46 (d, J = 7.7 Hz, 2H), 7.53–7.57 (m, 4H), 7.67 (t, J = 7.3 Hz, 1H), 8.07 (d, J = 7.6 Hz, 2H), 9.91 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.99, 170.03, 155.95, 138.89, 137.50, 136.74, 135.11, 134.76, 133.64, 129.52, 129.45, 128.93, 128.70, 128.63, 128.36, 127.49, 126.61, 115.91, 82.30, 65.68, 60.14, 53.25, 36.81, 34.68, 29.70, 28.17, 21.06. HRMS (AP-ESI) m/z calcd for $C_{38}H_{41}N_3O_7S$ $[M-H]^-$ 682.2587, found: 682.2587.

5.1.5.11. (2*S*,4*S*)-*tert*-Butyl-4-(4-(4′-methyl)phenylphenoxy)-2-(((*S*)-1-(4-methylphenylsulfonamido)-1-oxo-3-phenylpropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (27). White powder, yield: 34%, mp: 96–98 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.35 (s, 9H), 2.38 (s, 3H), 2.44 (s, 3H), 2.47–2.48 (m, 1H), 2.58 (d, J = 14.8 Hz, 1H), 2.89 (dd, J = 13.8 Hz, 4.3 Hz, 1H), 3.39 (d, J = 11.2 Hz, 1H), 3.67 (d, J = 14.4 Hz, 1H), 3.72 (d, J = 10.9 Hz, 1H), 4.41 (d, J = 9.1 Hz, 1H), 4.75 (s, 1H), 4.89 (s, 1H), 6.81 (d, J = 7.7 Hz, 1H), 6.86 (d, J = 8.0 Hz, 2H), 7.02 (d, J = 7.3 Hz, 2H), 7.14 (t, J = 7.7 Hz, 2H), 7.19–7.24 (m, 3H), 7.31 (d, J = 7.6 Hz, 2H), 7.43 (d, J = 7.9 Hz, 2H), 7.51 (d, J = 7.9 Hz, 2H), 7.92 (d, J = 7.7 Hz, 2H), 9.84 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.38, 170.03, 155.97, 144.61, 137.52, 136.73, 135.94, 135.06, 134.82, 129.51, 129.49, 129.32, 128.88, 128.69, 128.34, 127.45, 126.61, 115.91, 82.25, 60.13, 53.23, 36.84, 34.68, 28.17, 21.65, 21.06, 19.20. HRMS (AP-ESI) m/z calcd for $C_{39}H_{43}N_3O_7S$ $[M-H]^-$ 696.2743, found: 696.2749.

5.1.5.12. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-phenylpropan-2-yl)carbamoyl)-4-(4-(4′-methyl)phenylphenoxy)pyrrolidine-1-carboxylate (28). Yellow powder, yield: 38%. Mp: 90–92 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.35 (s, 9H), 2.39 (s, 3H), 2.48–2.51 (m, 1H), 2.55 (d, J = 13.8 Hz, 1H), 2.93 (dd, J = 14.4 Hz, 5.4 Hz, 1H), 3.37 (dd, J = 13.8 Hz, 4.8 Hz, 1H), 3.67 (dd, J = 12.6 Hz, 3.0 Hz, 1H), 3.76 (d, J = 12.6 Hz, 1H), 4.39 (d, J = 8.4 Hz, 1H), 4.70–4.74 (m, 1H), 4.90 (s, 1H), 6.64 (s, 1H), 6.84 (d, J = 8.3 Hz, 2H), 7.04 (d, J = 7.2 Hz, 2H), 7.17 (t, J = 7.2 Hz, 2H), 7.23 (m, 3H), 7.43 (d, J = 7.8 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.73 (d, J = 8.4 Hz, 1H), 8.23 (d, J = 7.8 Hz, 1H), 8.46 (s, 1H), 10.21 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.59, 170.43, 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 $C_{38}H_{39}ClN_4O_9S$ $[M-H]^-$ 761.2048, found: 761.2052.

5.1.5.13. (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 (29). Yellow powder, yield: 31%, mp: 111–113 °C. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 1.39 (s, 9H), 2.39 (s, 3H), 2.47–2.60 (m, 2H), 2.90 (dd, J = 13.8 Hz, 5.1 Hz, 1H), 3.31 (d, J = 13.2 Hz, 1H), 3.68 (dd, J = 12.0 Hz, 3.0 Hz, 1H), 3.77 (d, J = 12.3 Hz, 1H), 4.40 (d, J = 9.0 Hz, 1H), 4.70–4.73 (m, 1H), 4.90 (s, 1H), 6.61 (m, 1H), 6.84 (d, J = 8.4 Hz, 2H), 6.94 (d, J = 7.5 Hz, 2H), 7.23 (m, 2H), 7.31 (m, 2H), 7.44 (d, J = 7.8 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 8.1 Hz, 1H), 8.20 (d, J = 7.8 Hz, 1H), 8.53 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 172.14, 169.69, 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 $C_{38}H_{38}BrClN_4O_9S$ $[M-H]^-$ 839.1153, found: 839.1159.

5.1.5.14. (2*S*,4*S*)-*tert*-Butyl-2-(((*S*)-3-(4-bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)carbamoyl)-4-(4-(4′-chloro)phenylphenoxy)pyrrolidine-1-carboxylate (30). Yellow powder, yield: 26%. Mp: 125–127 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.40 (s, 9H), 2.47–2.50 (m, 1H), 2.57 (d, J = 14.4 Hz, 1H), 2.90 (d, J = 13.8 Hz, 1H), 3.30 (d, J = 10.8 Hz, 1H), 3.69 (d, J = 11.4 Hz, 1H), 3.77 (d, J = 11.4 Hz, 1H), 4.40 (d, J = 10.2 Hz, 1H), 4.73 (s, 1H), 4.90 (s, 1H), 6.64 (d, J = 7.2 Hz, 1H), 6.85 (d, J = 8.4 Hz, 2H), 6.94 (d, J = 7.8 Hz, 2H), 7.30 (d, J = 7.2 Hz, 2H), 7.40 (d, J = 7.8 Hz, 2H), 7.47 (d, J = 7.8 Hz, 2H), 7.51 (d, J = 7.8 Hz, 2H), 7.73 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 7.2 Hz, 1H), 8.53 (s, 1H), 10.26 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 171.80, 170.02, 156.21, 147.54, 138.64, 134.11, 133.42, 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 $C_{37}H_{35}BrCl_2N_4O_9S$ $[M-H]^-$ 859.0607, found: 859.0610.

5.1.5.15. (2*S*,4*S*)-*tert*-Butyl-4-(4-phenylphenoxy)-2-(((*S*)-1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (31). Yellow powder, yield 88%. Mp: 116–118 °C. ^{1}H NMR ($CDCl_3$, 600 MHz), δ 1.38 (s, 9H), 2.48–2.52 (m, 2H), 2.84 (dd, J = 13.8 Hz, 4.2 Hz, 1H), 3.31 (d, J = 11.4 Hz, 1H), 3.69 (d, J = 10.2 Hz, 1H), 3.81 (d, J = 12.6 Hz, 1H), 4.39 (d, J = 9.6 Hz, 1H), 4.69 (s, 1H), 4.91 (s, 1H), 5.38 (br s, 1H), 6.61 (d, J = 7.8 Hz, 2H), 6.69 (d, J = 6.6 Hz, 1H), 6.88–6.89 (m, 4H), 7.32 (t, J = 7.2 Hz, 1H), 7.42 (t, J = 7.8 Hz, 2H), 7.53–7.55 (m, 4H), 7.72 (d, J = 7.8 Hz, 1H), 8.82 (d, J = 8.4 Hz, 1H), 8.45 (s, 1H), 10.19 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 172.34, 170.56, 156.01, 155.61, 147.46, 140.21, 138.71, 135.24, 132.95, 132.69, 132.56, 130.52, 128.83, 128.61, 127.06, 126.74, 125.98, 125.84, 115.93, 115.87, 82.76, 60.16, 53.73, 53.27, 35.71, 34.77, 28.06. HRMS (AP-ESI) m/z Calcd for $C_{37}H_{37}ClN_4O_{10}S$ $[M-H]^-$ 763.1841. Found: 763.1830.

5.2. In vitro binding assay for Bcl-2 proteins

Fluorescence polarization assay was used to test the activities to Bcl-2 family proteins. A Bid-BH_3 peptide was marked with

5-carboxyfluorescein succinimidyl ester (FAM) as the fluorescence labeled peptide (5-FAM-QEDIIRNIARHLAQVGDSMDRSIPPG).

The assay was started from mixing the Mcl-1 protein with test compounds in PBS (pH 7.3, 140 mM NaCl, 2.7 mM KCl, 10 mM $NHPO_4$ and 1.8 mM KH_2PO_4) and preincubated for 30 min at room temperature in the dark. Then 20 μL 5-FAM-Bid-BH3 peptide solution in PBS was added and generated a final total volume of 200 μL and incubated another 20 min. The final concentration of Mcl-1 protein and 5-FAM-Bid-BH3 peptide were 195 nM and 10 nM, respectively. Finally 60 μL above solution and 60 μL calibration solution (1 nmol/L fluorescein and 10 mmol/L NaOH) were respectively, added into a black 384-well plate (Three parallel groups). Then the polarization values (milipolarization units, mP) were obtained by using the Tecan GENios-Pro Injector Reader (Tecan Group Ltd). The excitation wavelength was at 485 nm and the emission wavelength was at 535 nm. The measured data were processed with the GraphPad Prism software to get IC_{50} value and calculate K_i according to the literature method.[23]

The methods of binding assay for Bcl-2 protein and Bcl-X_L protein were almost the same as the above procedure except that the final concentrations of Bcl-2 and Bcl-X_L were 376 nM and 170 nM, respectively. In addition, the tested pyrrolidine derivatives were prepared at seven concentrations (1 nM, 10 nM, 100 nM, 1 μM, 10 μM, 50 μM, 100 μM) in dimethylsulfoxide (DMSO) solvent.

5.3. Cell culture and antiproliferative assay

Three human tumor cell lines (MDA-MB-231, PC-3 and K562) were incubated at the condition of 5% CO_2 at 37 °C with the RPMI1640 medium supplemented with 10% fetal bovine serum. When cells were at logarithmic growth phase, cancer cells were seeded in 96-well plates at 5000 cells per well and incubated for 12 h. Then a range of concentrations of tested compounds were added into 96-well plates and incubated for 48 h before adding 10 μL MTT (5 mg/mL) per well. After 4 h, the medium was removed and 150 μL DMSO/well was added. The absorbance was measured using a microtiter-plate reader (Thermo Varioskan Flash) at 570 nm after mixing for 5 min. The IC_{50} values were calculated according to the inhibitory ratios.

5.4. Docking study

The docking study was performed based on the Surflex-Dock program in Sybyl 7.3. The parameters were set at default values except that the structure of compound **21** was added Gasteiger–Hückel charges. The Mcl-1 protein (PDB code: 2PQK) was downloaded from the Protein Data Bank (PDB).

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