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Discovery and development of substituted tyrosine derivatives as Bcl-2/Mcl-1 inhibitors

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ABSTRACT

Anti-apoptotic Bcl-2 family proteins are vital for cancer cells to escape apoptosis, which make them attractive targets for cancer therapy. Recently, a lead compound **1** was found to modestly inhibit the binding of BH3 peptide to Bcl-2 protein with a K_i value of 5.2 μM. Based on this, a series of substituted tyrosine derivatives were developed and tested for their binding affinities to Bcl-2 protein. Results indicated that these compounds exhibited potent binding affinities to Bcl-2 and Mcl-1 protein but not to Bcl-X_L protein. Promisingly, compound **6i** inhibited the binding of BH3 peptide to Bcl-2 and Mcl-1 protein with a K_i value of 450 and 190 nM respectively, and showed obvious anti-proliferative activities against tested cancer cells.

1. Introduction

Apoptosis is vital for normal tissue homeostasis by removal of damaged or unwanted cells. It is generally considered that evasion of apoptosis is a critical attribute of cancer cells. Among all kinds of proteins involved in apoptosis, the B-cell lymphoma 2 (Bcl-2) family proteins are one of the most well-characterized ones. These proteins are pivotal regulators of the intrinsic apoptosis pathway by the way of governing mitochondrial outer membrane (MOM) integrity. There are three groups of structurally related Bcl-2 family proteins: the multi-domain anti-apoptotic proteins (Bcl-2, Bcl-X_L, Mcl-1, Bcl-W, A1/BFL-1), the pro-apoptotic BH3-only proteins (BIM, BID, PUMA, NOXA, BAD, BMF, BIK, HRK), and the multi-domain pro-apoptotic proteins (Bak and Bax). In response to apoptotic stimuli (*e.g.*, DNA damage and growth factor withdrawal), the BH3-only proteins are upregulated by transcriptional and/or posttranscriptional pathways. This will unbalance anti- and pro-apoptotic Bcl-2 family proteins, and then lead to activation of BAK and BAX. Activated BAX/BAK oligomerize and permeabilize the mitochondrial outer membrane. Subsequently, mitochondrial proteins (*e.g.*, cytochrome *c*) are released to cytosol, which triggers caspase cascades leading to cell death.[1–4]

However, cancer cells can upregulate anti-apoptotic Bcl-2 proteins in various different ways to escape apoptosis.[5] In this case, small molecule inhibitors targeting these anti-apoptotic proteins have been developed to restore apoptosis. Molecules which mimic the BH3 domain of the BH3-only proteins to bind anti-apoptotic Bcl-2 proteins are called 'BH3 mimetics'. It is well known that developing small molecules capable of effectively interfering the protein–protein interactions between pro- and anti-apoptotic Bcl-2 proteins is challenging. However, progress has been so far made.[6,7] For example, **Venetoclax/ABT-199** (Fig. 1) has been approved to treat patients with 17p deletion chronic lymphocytic leukemia (CLL) on April 11, 2016.[8] This shows great promise to develop novel small molecule Bcl-2 inhibitors to cancer treatment.

In our previous work, thiazolidinones, thiadiazoles and pyrrolidines have been developed as Bcl-2 inhibitors.[9–15] Interestingly, an intermediate (**1**, Fig. 1) to synthesize these compounds was found to modestly inhibit the binding of BH3 peptide to Bcl-2 protein with a K_i value of 5.2 μM. In our on-going studies, we developed a series of substituted tyrosine derivatives based on this compound. This paper shows the synthesis and biological evaluation of these tyrosine derivatives.

2. Chemistry

Tyrosine derivatives were synthesized as outlined in Scheme 1.[13,16] As starting material, L-tyrosine **2** was protected by Cu^{2+}, reacted with benzyl bromides, and then protected by Boc group to yield key intermediates **3a–3e**. Then intermediates **3a–3e** were treated with different benzenesulfonamides to give target compounds **4a–4m**. The Boc groups of **4a–4m** were removed to generate target compounds **5a–5f**. Compounds **5a–5f** did coupling reactions with carboxylic acids to obtain

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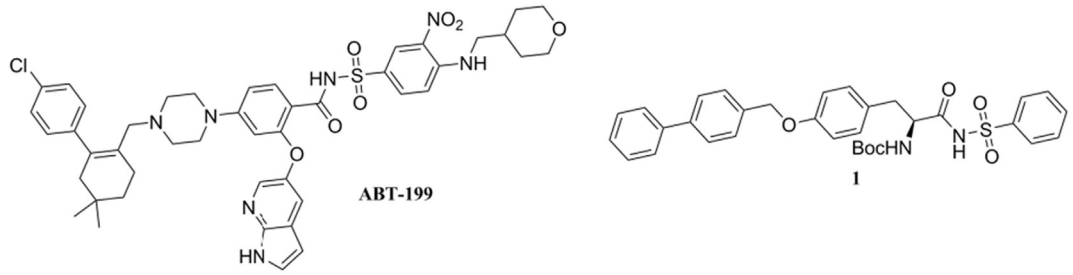
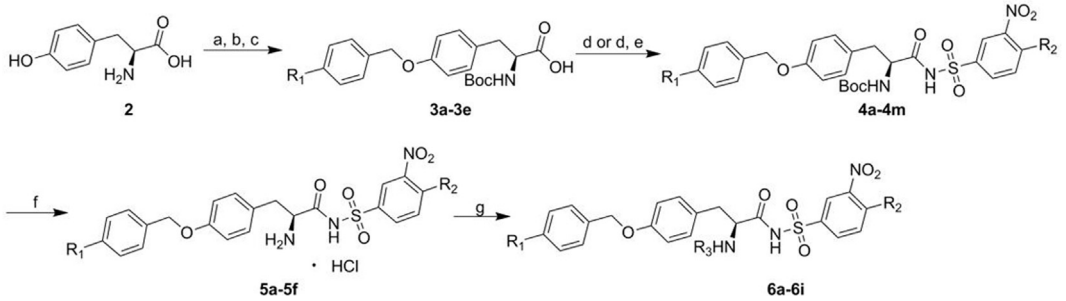


Fig. 1. Structures of **ABT-199** and **1**.



Scheme 1. Reagents and reaction conditions: (a) (i) 1 M $CuSO_4$, 2 M NaOH;(ii) benzyl bromides, MeOH; (b) EDTA •2Na; (c)$(Boc)_2O$, Et_3N, 1,4-Dioxane/H_2O; (d) (i) isobutyl chloroformate, NMM, THF; (ii) benzenesulfonamides, NaH, THF; (e) RNH_2, DMF; (f) ethyl acetate saturated with HCl; (g) carboxylic acids, TBTU, NMM, DMF.

target compounds **6a–6i**.

3. Results and discussion

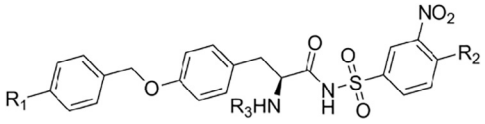
A total of twenty-nine target compounds were synthesized. Then these compounds were investigated for their binding affinities to Bcl-2 protein using the fluorescence polarization (FP) assays. Results were tabulated in Table 1 as K_i values. As we can see from Table 1, fourteen target compounds exhibited improved binding affinities to Bcl-2 protein compared to their predecessor **1**, and some of them were even better than **Gossypol**.

In this work, modifications on compound **1** were made mainly in three parts – biphenyl part (**R_1**), sulfonamide moiety (**R_2**) and the Boc group (**R_3**). Among these three parts, the Boc group (**R_3**) was vital for compound's binding affinity to Bcl-2 protein. The removal of this Boc group led to the total loss of compound's activity (*e.g.* **5a–5f**). However, the binding affinities could be restored when acyl groups were introduced to this position (*e.g.* **6a–6i**). Substitutions on sulfonamide moiety were beneficial to compound's potency. Compound **1** with no substitutions on sulfonamide moiety had a K_i value of 5.2 μM, while compounds with substitutions behaved much more better (*e.g.* **4a** 0.62 μM, **4l** 0.85 μM, **6i** 0.45 μM). But this was a different situation when it came to compounds with cycloalkyl amino groups on sulfonamide moiety (*e.g.* **4h**, **4i**, **6h**). In addition, changes on **R_1** had a little influence on compound's binding affinities compared with those on **R_2** and **R_3** (*e.g.* **4a** *vs* **4c** *vs* **4e**, **4f** *vs* **4g**, **6e** *vs* **6i**).

To understand how these compounds and Bcl-2 protein interacted with each other, representative compound **6i** were docked to the binding groove of Bcl-2 protein (PDB code: 4LVT) using Surflex-Dock software (Fig. 2a). Furthermore, the proposed interactions between **6i** and Bcl-2 protein in the binding groove were demonstrated in Fig. 2b.

Moreover, compounds **4a**, **6e** and **6i** were tested their binding affinities to Mcl-1 and Bcl-X_L protein (Table 2). As depicted in Table 2, these compounds exhibited no binding affinities to Bcl-X_L protein, but had potent binding affinities to Mcl-1 protein. It is promising because Bcl-X_L protein plays a key role in regulating platelets' life-span, and the inhibition of Bcl-X_L protein results in acute and reversible thrombocytopenia.[17] Furthermore, these three compounds were evaluated their

Table 1
The structures and Bcl-2 binding affinities of target compounds.



Compd.	R_1	R_2	R_3	K_i [a] (μM)
4a	Ph	Cl	Boc	0.62 ± 0.06
4b	H	Cl	Boc	N.A. [b]
4c	4-Me-Ph-	Cl	Boc	0.39 ± 0.03
4d	4-Cl-Ph-	Cl	Boc	0.58
4e	Ph-O-	Cl	Boc	0.70 ± 0.04
4f	H	Ph-$(CH_2)_3$-NH-	Boc	1.5 ± 0.06
4g	CN	Ph-$(CH_2)_3$-NH-	Boc	0.80 ± 0.08
4h	CN	Cyclopentyl-NH-	Boc	N.A.
4i	Ph	Cy-NH-	Boc	N.A.
4j	Ph	Ph-$(CH_2)_3$-NH-	Boc	N.A.
4k	4-Me-Ph-	Cy-CH_2-NH-	Boc	N.A.
4l	4-Me-Ph-	Ph-O-$(CH_2)_2$-NH-	Boc	0.85 ± 0.07
4m	Ph-O-	Cy-NH-	Boc	N.A.
5a	Ph	Cl	H	N.A.
5b	H	Ph-$(CH_2)_3$-NH-	H	N.A.
5c	CN	Ph-$(CH_2)_3$-NH-	H	N.A.
5d	CN	Cyclopentyl-NH-	H	N.A.
5e	Ph	Cy-NH-	H	N.A.
5f	Ph	Ph-$(CH_2)_3$-NH-	H	N.A.
6a	Ph	Cl	*n*-Amyl-CO-	0.56 ± 0.06
6b	Ph	Cl	Py-3-CO-	0.58 ± 0.01
6c	Ph	Cl	4-CH_3-Bn-CO-	1.1 ± 0.16
6d	Ph	Cl	Naph-2-CO-	0.57 ± 0.08
6e	H	Ph-$(CH_2)_3$-NH-	4-CH_3-Bn-CO-	0.45 ± 0.07
6f	CN	Ph-$(CH_2)_3$-NH-	Naph-2-CO-	N.A.
6g	CN	Cyclopentyl-NH-	4-Ph-Bn-CO-	0.61 ± 0.03
6h	Ph	Cy-NH-	Naph-2-CO-	N.A.
6i	Ph	Ph-$(CH_2)_3$-NH-	4-CH_3-Bn-CO-	0.45 ± 0.01
1				5.2 ± 0.45
Gossypol				0.88 ± 0.15

[a] K_i values were expressed as the mean ± standard deviation of three independent determinations.
[b] No activity.

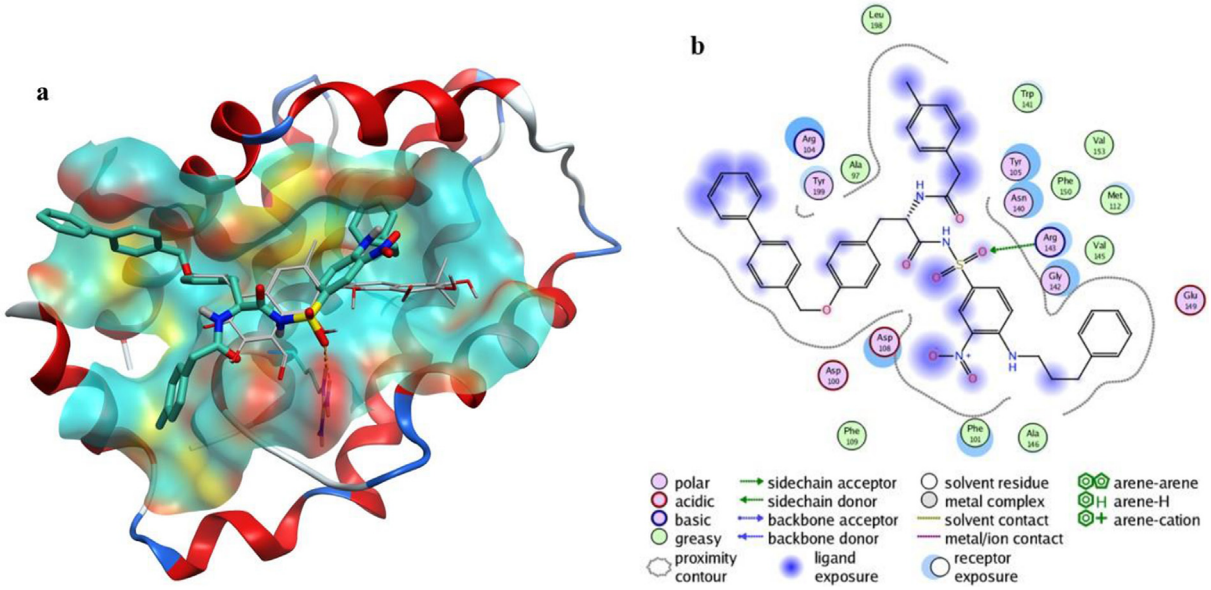


Fig. 2. (a) The docking mode of **6i** and **Gossypol** (grey) in the binding groove of Bcl-2 protein. (b) The proposed interactions between **6i** and Bcl-2 protein in the binding groove.

Table 2
Isoform binding affinities and anti-proliferative activities of representative compounds.

Compd.	K_i (μM)[a]		IC_{50} (μM)[a]				
	Bcl-X_L	Mcl-1	Jurkat	RS4;11	THP-1	Molt-4	HUEVC
4a	N.A.[b]	0.45 ± 0.03	23.49 ± 0.28	14.05 ± 2.86	27.25 ± 3.61	44.38 ± 0.92	> 50
6e	N.A.	0.50 ± 0.04	23.82 ± 0.45	16.80 ± 2.35	23.91 ± 2.00	> 50	> 100
6i	N.A.	0.19 ± 0.01	3.37 ± 0.51	3.39 ± 0.87	6.46 ± 0.17	8.36 ± 0.30	> 50
Gossypol	N.A.	0.53 ± 0.08	1.43 ± 0.25	1.23 ± 0.37	5.80 ± 0.65	1.94 ± 0.81	13.41 ± 1.48

[a] K_i and IC_{50} values were expressed as the mean ± standard deviation of three independent determinations.
[b] No activity.

anti-proliferative activities against four cancer cell lines and one normal human cell line using MTT assay (Table 2). The results showed that these compounds possessed certain anti-proliferative activities against tested cancer cell lines, but had less influence on the growth of normal human cell. Among these three compounds, compound **6i** displayed similar anti-proliferative activities against cancer cells compared to **Gossypol**.

In addition, compound **6i** was evaluated for its ability to induce apoptosis in the Jurkat cell line using annexin-V and propidium iodide (PI) double staining by flow cytometry (Fig. 3). It was found that compound **6i** induced apoptosis in a dose-dependent manner. Treatment of the Jurkat cells by 10 and 20 μM of **6i** for 48 h results in 6.2% and 22.2% of apoptotic cells (early + late) respectively, as compared to 2.6% of apoptotic cells in the DMSO control.

Caspase-3 activation is a crucial process involved in the intrinsic apoptosis pathway, so we determined the ability of compound **6i** to induce caspase-3 activity in the Jurkat cell line (Fig. 4). The obtained results showed that compound **6i** induced activation of caspase-3 activity in a dose-dependent manner. Importantly, these results correlate with the ability of **6i** to induce apoposis and inhibit Jurkat cell growth.

4. Conclusions

In conclusion, a series of substituted tyrosine derivatives were developed based on compound **1**. Fourteen of them exhibited improved binding affinities to Bcl-2 protein compared to **1**, and some of them were even better than **Gossypol**. It turns out that these compounds exhibited potent binding affinities to Bcl-2 and Mcl-1protein, but not to Bcl-X_L protein. Besides, they these compounds inhibit the growth of cancer cells. Promisingly, compound **6i** inhibited the binding of BH3 peptide to Bcl-2 and Mcl-1 protein with a K_i value of 450 and 190 nM respectively, and showed obvious anti-proliferative activities against tested cancer cells. In future, these results could pave the way for more potent Bcl-2/Mcl-1 inhibitors.

5. Experimental section

5.1. Chemistry: General procedures

Starting materials used in this work were commercially available. Unless otherwise specified, solvents and reagents used belong to analytical reagents. Reactions were all monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates (60GF – 254), and UV light (254 nM) was used to observed the spots. Products were purified by silica gel chromatography (200–300 mesh) or/and recrystallization. The RY-1 electrothermal melting point apparatus was used to measure melting points. Purities of target compounds were assessed by reverse-phase HPLC performed on Shimadzu LC-20AT with a Phenomenex Synergi C18 column (250 × 4.6 mm, 4 μm) and 1 mL/min flow. ^{1}H and ^{13}C NMR spectra were performed on a 400 MHz Brucker DRX spectrometer using TMS as an internal standard. Chemical shifts δ were showed in parts per million (ppm) and coupling constants *J* were given

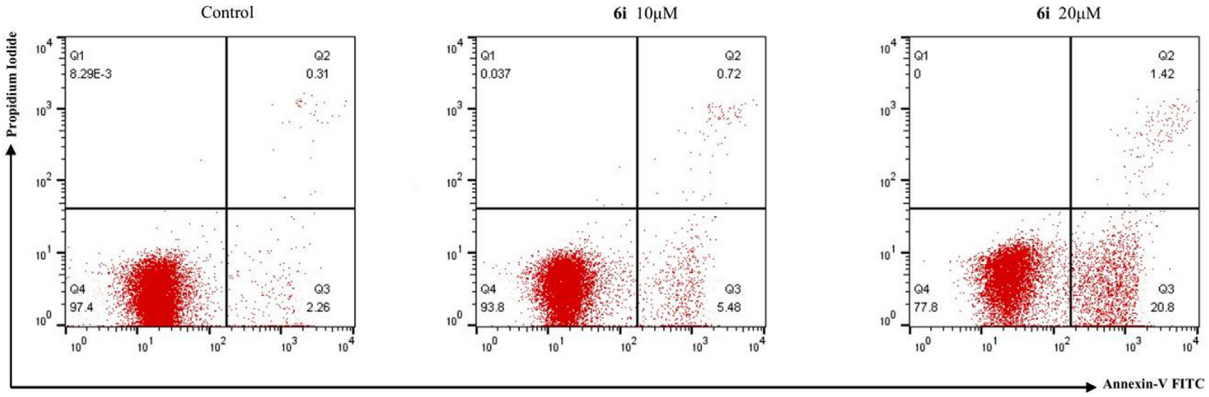


Fig. 3. Analysis of apoptosis induced by **6i** in the Jurkat cell line.

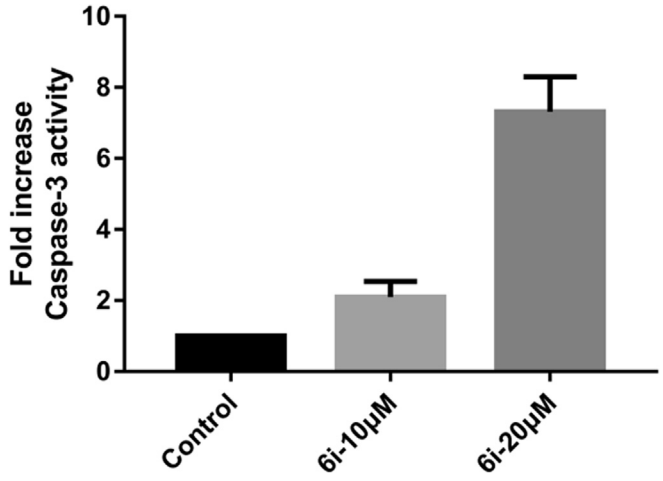


Fig. 4. Induction of caspase-3 by **6i** in the Jurkat cell line. Results shown are the mean and SEM from two separate experiments.

in hertz (Hz). Multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet). HRMS were conducted on an Agilent 6510 Quadrupole Time-of-Flight LC/MS deliver.

*5.1.1. (S)-3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid (**3a**)*

To a solution of L-tyrosine (5.83 g, 32.2 mmol) in 2 M NaOH solution (32.2 mL), 1 M copper sulfate solution (16.1 mL) was added, and the reaction mixture was stirred at 60 °C for 1 h. Then methanol (90 mL) and 4-phenylbenzyl bromide (8.75 g, 35.4 mmol) were added. The reaction mixture was stirred vigorously for 3 h at 60 °C. The precipitate was filtered, washed by water, and then added into 55.77 g/L EDTA·2Na solution (168 mL). The resulting mixture was stirred at 60 °C for 12 h.

After cooling down, the precipitate was filtered, washed by water, and then diluted with 1,4-dioxane/H_2O (2:1, 150 mL). Di-*tert*-butyl dicarbonate (9.13 g, 41.9 mmol) and Et_3N (14.5 mL, 104.6 mmol) was added at 0 °C. After stirred overnight at room temperature, the reaction mixture was acidified to pH 2–3 by 6 M HCl solution and extracted with EtOAc. The combined EtOAc was washed by brine and dried over $MgSO_4$. Then the solvent was evaporated in vacuum. Purification with silica gel column chromatography gave 7.56 g (52%) of **3a** as yellowish-brown powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (s, 1H), 7.73–7.60 (m, 4H), 7.56–7.42 (m, 4H), 7.37 (t, J = 7.3 Hz, 1H), 7.17 (d, J = 8.5 Hz, 2H), 7.07 (d, J = 8.3 Hz, 1H), 6.94 (d, J = 8.6 Hz, 2H), 5.12 (s, 2H), 4.11–3.92 (m, 1H), 2.94 (dd, J = 13.8, 4.4 Hz, 1H), 2.76 (dd, J = 13.7, 10.3 Hz, 1H), 1.41–1.21 (m, 9H).

Compounds **3b–3e** were synthesized following the procedure described above.

*5.1.2. (S)-3-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino) propanoic acid (**3b**)*

White powder, yield: 46%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (s, 1H), 7.52–7.27 (m, 5H), 7.16 (d, J = 8.5 Hz, 2H), 7.07 (d, J = 8.3 Hz, 1H), 6.92 (d, J = 8.6 Hz, 2H), 5.06 (s, 2H), 4.09–3.89 (m, 1H), 2.94 (dd, J = 13.8, 4.5 Hz, 1H), 2.75 (dd, J = 13.7, 10.4 Hz, 1H), 1.41–1.17 (m, 9H).

*5.1.3. (S)-2-((tert-butoxycarbonyl)amino)-3-(4-((4′-methyl-[1,1′-biphenyl]-4-yl)methoxy)phenyl) propanoic acid (**3c**)*

Yellowish-brown powder, yield: 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.54 (s, 1H), 7.65 (d, J = 8.3 Hz, 2H), 7.56 (d, J = 8.1 Hz, 2H), 7.50 (d, J = 8.3 Hz, 2H), 7.27 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 8.5 Hz, 2H), 7.04 (d, J = 8.3 Hz, 1H), 6.93 (d, J = 8.6 Hz, 2H), 5.10 (s, 2H), 4.10–3.96 (m, 1H), 2.94 (dd, J = 13.8, 4.5 Hz, 1H), 2.75 (dd, J = 13.7, 10.3 Hz, 1H), 2.34 (s, 3H), 1.36–1.20 (m, 9H).

*5.1.4. (S)-2-((tert-butoxycarbonyl)amino)-3-(4-((4-phenoxybenzyl)oxy) phenyl)propanoic acid (**3d**)*

White powder, yield: 19%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (s, 1H), 7.54–7.33 (m, 4H), 7.20–7.11 (m, 3H), 7.08–6.98 (m, 4H), 6.92 (d, J = 8.6 Hz, 2H), 5.03 (s, 2H), 4.10–3.97 (m, 1H), 2.94 (dd, J = 13.8, 4.4 Hz, 1H), 2.76 (dd, J = 13.6, 10.4 Hz, 1H), 1.36–1.24 (m, 9H).

*5.1.5. (S)-2-((tert-butoxycarbonyl)amino)-3-(4-((4-cyanobenzyl)oxy) phenyl)propanoic acid (**3e**)*

Colorless oil, yield: 55%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (s, 1H), 7.86 (d, J = 8.3 Hz, 2H), 7.63 (d, J = 8.3 Hz, 2H), 7.18 (d, J = 8.5 Hz, 2H), 7.08 (d, J = 8.3 Hz, 1H), 6.93 (d, J = 8.6 Hz, 2H), 5.19 (s, 2H), 4.09–4.03 (m, 1H), 2.96 (dd, J = 13.8, 4.4 Hz, 1H), 2.76 (dd, J = 13.7, 10.4 Hz, 1H), 1.42–1.22 (m, 9H).

*5.1.6. (S)-2-((tert-butoxycarbonyl)amino)-3-(4-((4′-chloro-[1,1′-biphenyl]-4-yl)methoxy)phenyl) propanoic acid (**3f**)*

White powder, yield: 21%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.56 (s, 1H), 7.77–7.63 (m, 4H), 7.59–7.46 (m, 4H), 7.17 (d, J = 8.5 Hz, 2H), 7.05 (d, J = 8.3 Hz, 1H), 6.94 (d, J = 8.6 Hz, 2H), 5.12 (s, 2H), 4.10–3.96 (m, 1H), 2.95 (dd, J = 13.8, 4.5 Hz, 1H), 2.76 (dd, J = 13.7, 10.3 Hz, 1H), 1.32 (s, 9H).

*5.1.7. Tert-butyl (S)-(3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-1-oxo-1-(phenylsulfonamido)propan- 2-yl) carbamate (**1**)*

To a solution of NaH (0.17 g, 4.2 mmol) in anhydrous THF (10 mL) at 0 °C was added benzenesulfonamide (0.29 g, 1.84 mmol). The mixture was stirred at 0 °C for 30 min and then at room temperature for another 4 h to give corresponding salt.

N-methyl morpholine (0.2 mL, 1.84 mmol) was added at −20 °C to a solution of **3a** (0.75 g, 1.68 mmol) in anhydrous THF (10 mL). After 15 min, isobutyl chloroformate (0.24 mL, 1.84 mmol) was added. The

resulting mixture was stirred at −20 °C for 45 min and then added dropwise to the prepared solution of benzenesulfonamide salt. The mixture was stirred at room temperature for overnight. EtOAc was added and the mixture was washed with 5% citric acid solution and brine, then dried over $MgSO_4$. The solvent was evaporated in vacuum. Purification with silica gel column chromatography gave 0.59 g (60%) of **1** as white powder. mp: 157–158 °C. HPLC purity: 99.9%, t_R = 6.8 min, MeOH/water = 83/17 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.43 (s, 1H), 8.05 (d, J = 7.7 Hz, 2H), 7.69–7.57 (m, 5H), 7.56–7.50 (m, 2H), 7.51–7.40 (m, 4H), 7.39–7.32 (m, 1H), 6.94 (d, J = 8.5 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 5.05 (s, 2H), 4.94 (s, 1H), 4.31 (s, 1H), 2.97 (dd, J = 14.2, 6.2 Hz, 1H), 2.89 (dd, J = 14.2, 7.1 Hz, 1H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.67, 158.02, 155.92, 141.05, 140.74, 138.40, 135.87, 134.03, 130.35, 128.95, 128.84, 128.51, 127.99, 127.44, 127.42, 127.15, 115.21, 81.38, 69.78, 55.97, 36.19, 28.20. HRMS (AP-ESI) m/z Calcd for $C_{33}H_{34}N_2O_6S$ $[M-H]^-$ 585.2065, found: 585.2049.

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

*5.1.8. Tert-butyl (S)-(3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-1-((4-chloro-3-nitrophenyl) sulfonamide)-1-oxopropan-2-yl)carbamate **(4a)***

Yellowish powder, yield: 87%, mp: 104–106 °C. HPLC purity: 91.3%, t_R = 8.3 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1H), 8.49 (d, J = 2.1 Hz, 1H), 8.16 (dd, J = 8.5, 1.9 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.64–7.54 (m, 4H), 7.54–7.39 (m, 4H), 7.38–7.29 (m, 1H), 6.97 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 5.05 (s, 2H), 4.98 (s, 1H), 4.26 (s, 1H), 2.97 (dd, J = 14.2, 6.4 Hz, 1H), 2.88 (dd, J = 14.2, 7.5 Hz, 1H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.02, 158.17, 156.19, 147.64, 141.05, 140.71, 138.36, 135.79, 133.04, 132.70, 130.28, 128.82, 127.97, 127.44, 127.39, 127.11, 125.89, 115.27, 81.98, 69.80, 56.23, 36.05, 28.16. HRMS (AP-ESI) m/z Calcd for $C_{33}H_{32}ClN_3O_8S$ $[M-H]^-$ 664.1526, found: 664.1500.

*5.1.9. Tert-butyl (S)-(1-((4-chloro-3-nitrophenyl)sulfonamido)-3-(4-((4′-chloro-[1,1′-biphenyl]-4-yl) methoxy)phenyl)-1-oxopropan-2-yl)carbamate **(4b)***

White powder, yield: 53%, mp: 219–220 °C. HPLC purity: 99.2%, t_R = 6.4 min, MeOH/water = 83/17 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H), 8.50 (d, J = 2.1 Hz, 1H), 8.18 (dd, J = 8.5, 2.1 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.57 (d, J = 8.2 Hz, 2H), 7.54–7.45 (m, 4H), 7.41 (d, J = 8.5 Hz, 2H), 7.00 (d, J = 8.2 Hz, 2H), 6.89 (d, J = 8.5 Hz, 2H), 5.07 (s, 2H), 4.93–4.80 (m, 1H), 4.28–4.14 (m, 1H), 3.00 (dd, J = 14.3, 6.5 Hz, 1H), 2.92 (dd, J = 14.2, 7.3 Hz, 1H), 1.41 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.58, 157.50, 155.87, 147.53, 139.49, 139.09, 138.75, 137.28, 133.39, 132.86, 132.83, 131.15, 130.73, 129.60, 129.36, 128.89, 128.72, 127.17, 125.59, 114.85, 78.92, 69.22, 57.10, 35.50, 28.42. HRMS (AP-ESI) m/z Calcd for $C_{27}H_{28}ClN_3O_8S$ $[M-H]^-$ 588.1213, found: 588.1198.

*5.1.10. Tert-butyl (S)-(1-((4-chloro-3-nitrophenyl)sulfonamido)-3-(4-((4′-methyl-[1,1′-biphenyl]-4-yl) methoxy)phenyl)-1-oxopropan-2-yl) carbamate **(4c)***

Yellowish powder, yield: 88%, mp: 118–119 °C. HPLC purity: 98.7%, t_R = 10.1 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.55 (s, 1H), 8.50 (d, J = 2.1 Hz, 1H), 8.18 (dd, J = 8.5, 2.1 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.60 (d, J = 8.2 Hz, 2H), 7.48 (dd, J = 8.0, 5.4 Hz, 4H), 7.24 (s, 1H), 7.00 (d, J = 8.2 Hz, 2H), 6.90 (d, J = 8.6 Hz, 2H), 5.07 (s, 2H), 4.84 (d, J = 6.9 Hz, 1H), 4.20 (d, J = 6.6 Hz, 1H), 3.00 (dd, J = 14.3, 6.4 Hz, 1H), 2.92 (dd, J = 14.3, 7.4 Hz, 1H), 2.40 (s, 3H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.03, 158.19, 147.63, 140.98, 138.34, 137.81, 137.25, 135.46, 133.04, 132.71, 130.27, 129.55, 127.97, 127.18, 126.94, 125.90, 115.25, 81.98, 69.83, 56.21, 36.10, 28.17, 21.13. HRMS (AP-ESI) m/z Calcd for $C_{34}H_{34}ClN_3O_8S$ $[M-H]^-$ 678.1682, found: 678.1669.

*5.1.11. Tert-butyl (S)-(1-((4-chloro-3-nitrophenyl)sulfonamido)-3-(4-((4′-chloro-[1,1′-biphenyl]-4-yl) methoxy)phenyl)-1-oxopropan-2-yl) carbamate **(4d)***

White powder, yield: 53%, mp: 219–220 °C. HPLC purity: 94.0%, t_R = 10.6 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H), 8.50 (d, J = 2.1 Hz, 1H), 8.18 (dd, J = 8.5, 2.1 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.57 (d, J = 8.2 Hz, 2H), 7.54–7.45 (m, 4H), 7.41 (d, J = 8.5 Hz, 2H), 7.00 (d, J = 8.2 Hz, 2H), 6.89 (d, J = 8.5 Hz, 2H), 5.07 (s, 2H), 4.93–4.80 (m, 1H), 4.28–4.14 (m, 1H), 3.00 (dd, J = 14.3, 6.5 Hz, 1H), 2.92 (dd, J = 14.2, 7.3 Hz, 1H), 1.41 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.58, 157.50, 155.87, 147.53, 139.49, 139.09, 138.75, 137.28, 133.39, 132.86, 132.83, 131.15, 130.73, 129.60, 129.36, 128.89, 128.72, 127.17, 125.59, 114.85, 78.92, 69.22, 57.10, 35.50, 28.42. HRMS (AP-ESI) m/z Calcd for $C_{33}H_{31}Cl_2N_3O_8S$ $[M-H]^-$ 698.1136, found: 698.1118.

*5.1.12. Tert-butyl (S)-(1-((4-chloro-3-nitrophenyl)sulfonamido)-1-oxo-3-(4-((4-phenoxybenzyl)oxy) phenyl)propan-2-yl)carbamate **(4e)***

White powder, yield: 77%, mp: 91–93 °C. HPLC purity: 88.1%, t_R = 7.5 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.61 (s, 1H), 8.53–8.47 (m, 1H), 8.18 (dd, J = 8.5, 1.9 Hz, 1H), 7.73 (d, J = 8.5 Hz, 1H), 7.42–7.35 (m, 2H), 7.35–7.31 (m, 1H), 7.11 (t, J = 7.4 Hz, 1H), 7.08–6.96 (m, 5H), 6.91–6.81 (m, 2H), 4.99 (s, 2H), 4.88 (d, J = 5.9 Hz, 1H), 4.22 (d, J = 6.3 Hz, 1H), 2.99 (dd, J = 14.2, 6.4 Hz, 1H), 2.91 (dd, J = 14.2, 7.4 Hz, 1H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.12, 158.14, 157.27, 156.97, 147.63, 138.36, 133.04, 132.72, 131.44, 130.27, 129.82, 129.27, 127.07, 125.88, 123.50, 119.08, 118.82, 115.20, 81.92, 69.64, 56.20, 36.14, 28.17. HRMS (AP-ESI) m/z Calcd for $C_{33}H_{32}ClN_3O_9S$ $[M-H]^-$ 680.1475, found: 680.1450.

*5.1.13. Tert-butyl (S)-(3-(4-(benzyloxy)phenyl)-1-((3-nitro-4-((3-phenylpropyl)amino)phenyl) sulfonamido)-1-oxopropan-2-yl)carbamate **(4f)***

Yellow powder, yield: 63%, mp: 144–146 °C. HPLC purity: 92.1%, t_R = 12.6 min, MeOH/water = 80/20 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, J = 2.2 Hz, 1H), 8.47 (t, J = 5.2 Hz, 1H), 8.00 (d, J = 8.7 Hz, 1H), 7.44–7.34 (m, 4H), 7.34–7.27 (m, 3H), 7.23 (d, J = 7.3 Hz, 1H), 7.19 (d, J = 7.1 Hz, 2H), 6.97 (d, J = 8.5 Hz, 2H), 6.88–6.76 (m, 3H), 5.00 (s, 2H), 4.94 (s, 1H), 4.28 (s, 1H), 3.35 (dd, J = 12.6, 6.8 Hz, 2H), 2.97 (dd, J = 14.2, 6.2 Hz, 1H), 2.89 (dd, J = 14.2, 7.3 Hz, 1H), 2.77 (t, J = 7.4 Hz, 2H), 2.13–2.01 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.89, 158.06, 155.89, 147.99, 140.31, 136.85, 135.16, 130.68, 130.33, 129.25, 128.70, 128.62, 128.38, 128.04, 127.51, 127.38, 126.43, 124.05, 115.16, 113.92, 81.45, 69.99, 56.02, 42.49, 36.25, 32.96, 30.05, 28.18. HRMS (AP-ESI) m/z Calcd for $C_{36}H_{40}N_4O_8S$ $[M-H]^-$ 687.2494, found: 687.2478.

*5.1.14. Tert-butyl (S)-(3-(4-((4-cyanobenzyl)oxy)phenyl)-1-((3-nitro-4-((3-phenylpropyl)amino)phenyl) sulfonamido)-1-oxopropan-2-yl)carbamate **(4g)***

Yellow powder, yield: 86%, mp: 161–162 °C. HPLC purity: 95.2%, t_R = 5.3 min, MeOH/water = 83/17 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.78 (d, J = 2.2 Hz, 1H), 8.48 (t, J = 5.2 Hz, 1H), 8.02 (d, J = 8.7 Hz, 1H), 7.66 (d, J = 8.3 Hz, 2H), 7.52 (d, J = 8.3 Hz, 2H), 7.31 (t, J = 7.3 Hz, 2H), 7.25–7.21 (m, 1H), 7.21–7.16 (m, 2H), 7.01 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 9.4 Hz, 1H), 6.82 (d, J = 8.6 Hz, 2H), 5.07 (s, 2H), 4.94 (s, 1H), 4.27 (s, 1H), 3.37 (dd, J = 12.6, 6.9 Hz, 2H), 2.98 (dd, J = 14.1, 6.3 Hz, 1H), 2.91 (dd, J = 14.1, 7.2 Hz, 1H), 2.78 (t, J = 7.4 Hz, 2H), 2.16–2.00 (m, 2H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.74, 157.49, 155.79, 148.01, 142.37, 140.26, 135.23, 132.43, 130.71, 130.48, 129.18, 128.71, 128.36, 128.01, 127.59, 126.46, 124.03, 118.70, 115.13, 113.89, 111.73, 81.50, 68.90, 56.02, 42.49, 36.26, 32.95, 30.05, 28.16. HRMS (AP-ESI) m/z Calcd for $C_{37}H_{39}N_5O_8S$ $[M-H]^-$ 712.2447, found: 712.2423.

*5.1.15. Tert-butyl (S)-(3-(4-((4-cyanobenzyl)oxy)phenyl)-1-((4-(cyclopentylamino)-3-nitrophenyl) sulfonamido)-1-oxopropan-2-yl)carbamate (**4h**)*

Yellow powder, yield: 82%, mp: 177–178 °C. HPLC purity: 97.8%, t_R = 6.7 min, MeOH/water = 80/20 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (s, 1H), 8.78 (d, J = 2.2 Hz, 1H), 8.51 (d, J = 6.3 Hz, 1H), 8.03 (d, J = 8.7 Hz, 1H), 7.68 (d, J = 8.2 Hz, 2H), 7.53 (d, J = 8.2 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 9.3 Hz, 1H), 6.83 (d, J = 8.6 Hz, 2H), 5.07 (s, 2H), 4.95 (s, 1H), 4.29 (s, 1H), 4.08–3.97 (m, 1H), 2.99 (dd, J = 14.1, 6.3 Hz, 1H), 2.92 (dd, J = 14.1, 7.1 Hz, 1H), 2.24–2.06 (m, 2H), 1.88–1.78 (m, 2H), 1.78–1.70 (m, 2H), 1.70–1.60 (m, 2H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.74, 157.47, 155.77, 147.62, 142.39, 134.99, 132.43, 130.61, 130.50, 129.27, 128.00, 127.59, 123.70, 118.72, 115.10, 114.72, 111.71, 81.46, 68.88, 55.98, 54.53, 36.29, 33.45, 28.17, 24.00. HRMS (AP-ESI) m/z Calcd for $C_{33}H_{37}N_5O_8S$ $[M-H]^-$ 662.2290, found: 662.2260.

*5.1.16. Tert-butyl (S)-(3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-1-((4-(cyclohexylamino)-3- nitrophenyl)sulfonamido)-1-oxopropan-2-yl)carbamate (**4i**)*

Cyclohexanamine (0.57 mL, 5 mmol) was added to a solution of **4b** (666 mg, 1 mmol) in DMF (10 mL). Then the mixture was stirred at 120 °C for 8 h. EtOAc was added and the mixture was washed with 5% citric acid solution and brine, then dried over $MgSO_4$. The solvent was evaporated in vacuum. Purification with silica gel column chromatography gave 510 mg (70%) of **4i** as yellow powder. mp: 171–173 °C. HPLC purity: 95.9%, t_R = 9.8 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1H), 8.81 (d, J = 2.3 Hz, 1H), 8.52 (d, J = 7.5 Hz, 1H), 8.01 (dd, J = 9.2, 1.9 Hz, 1H), 7.60 (t, J = 8.2 Hz, 4H), 7.53–7.40 (m, 4H), 7.39–7.32 (m, 1H), 7.01 (d, J = 8.4 Hz, 2H), 6.94 (d, J = 9.4 Hz, 1H), 6.91–6.84 (m, 2H), 5.06 (s, 2H), 4.82 (d, J = 7.4 Hz, 1H), 4.24 (s, 1H), 3.58 (s, 1H), 3.06–2.88 (m, 2H), 2.12–2.00 (m, 2H), 1.88–1.75 (m, 2H), 1.71–1.64 (m, 1H), 1.52–1.25 (m, 14H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.86, 158.08, 147.20, 141.00, 140.76, 135.88, 134.99, 130.48, 130.36, 129.53, 128.81, 128.01, 127.41, 127.36, 127.12, 123.58, 115.17, 114.26, 81.45, 69.78, 56.08, 51.60, 36.39, 32.45, 28.18, 25.35, 24.36. HRMS (AP-ESI) m/z Calcd for $C_{39}H_{44}N_4O_8S$ $[M-H]^-$ 727.2807, found: 727.2790.

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

*5.1.17. Tert-butyl (S)-(3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-1-((3-nitro-4-((3-phenylpropyl) amino)phenyl)sulfonamido)-1-oxopropan-2-yl) carbamate (**4j**)*

Yellow powder, yield: 55%, mp: 83–85 °C. HPLC purity: 92.2%, t_R = 9.0 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.42 (s, 1H), 8.80 (d, J = 2.2 Hz, 1H), 8.46 (t, J = 5.1 Hz, 1H), 8.00 (d, J = 8.2 Hz, 1H), 7.58 (t, J = 7.6 Hz, 4H), 7.49–7.39 (m, 4H), 7.38–7.32 (m, 1H), 7.29 (t, J = 7.3 Hz, 2H), 7.24–7.20 (m, 1H), 7.17 (d, J = 7.0 Hz, 2H), 6.99 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 6.83–6.79 (m, 1H), 5.03 (s, 2H), 4.97 (s, 1H), 4.31 (s, 1H), 3.39–3.29 (m, 2H), 2.98 (dd, J = 14.1, 6.2 Hz, 1H), 2.89 (dd, J = 14.1, 7.3 Hz, 1H), 2.76 (t, J = 7.4 Hz, 2H), 2.13–2.00 (m, 2H), 1.38 (s, 9H).^{13}C NMR (100 MHz, $CDCl_3$) δ 169.88, 158.06, 155.80, 147.96, 140.98, 140.71, 140.27, 135.85, 135.14, 130.70, 130.33, 129.19, 128.80, 128.68, 128.34, 127.98, 127.39, 127.34, 127.09, 126.40, 124.11, 115.17, 113.87, 81.41, 69.76, 56.06, 42.47, 36.32, 32.94, 30.03, 28.16. HRMS (AP-ESI) m/z Calcd for $C_{42}H_{44}N_4O_8S$ $[M-H]^-$ 763.2807, found: 763.2789.

*5.1.18. Tert-butyl (S)-(1-((4-((cyclohexylmethyl)amino)-3-nitrophenyl) sulfonamido)-3-(4-((4′-methyl- [1,1′-biphenyl]-4-yl)methoxy)phenyl)-1-oxopropan-2-yl)carbamate (**4k**)*

Yellow powder, yield: 41%, mp: 181–183 °C. HPLC purity: 98.0%, t_R = 6.4 min, MeOH/water = 92/8 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 8.81 (d, J = 2.2 Hz, 1H), 8.57 (t, J = 5.2 Hz, 1H), 8.02 (dd, J = 9.2, 2.0 Hz, 1H), 7.60 (d, J = 8.2 Hz, 2H), 7.54–7.44 (m, 4H), 7.25 (d, J = 7.3 Hz, 2H), 7.01 (d, J = 8.5 Hz, 2H), 6.96–6.83 (m, 3H), 5.05 (s, 2H), 4.81 (d, J = 7.1 Hz, 1H), 4.24 (s, 1H), 3.26–3.15 (m, 2H), 3.05–2.88 (m, 2H), 2.40 (s, 3H), 1.89–1.74 (m, 4H), 1.74–1.64 (m, 2H), 1.40 (s, 9H), 1.35–1.16 (m, 3H), 1.11–0.98 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.83, 158.11, 148.26, 140.95, 137.86, 137.21, 135.54, 135.09, 130.61, 130.34, 129.54, 129.30, 128.00, 127.40, 127.16, 126.95, 123.81, 115.19, 114.03, 81.52, 69.82, 56.04, 49.83, 37.26, 36.35, 31.05, 28.19, 26.21, 25.73, 21.12. HRMS (AP-ESI) m/z Calcd for $C_{41}H_{48}N_4O_8S$ $[M-H]^-$ 755.3120, found: 755.3098.

*5.1.19. Tert-butyl (S)-(3-(4-((4′-methyl-[1,1′-biphenyl]-4-yl)methoxy) phenyl)-1-((3-nitro-4-((2- phenoxyethyl)amino)phenyl)sulfonamido)-1-oxopropan-2-yl)carbamate (**4l**)*

Yellow powder, yield: 70%, mp: 140–142 °C. HPLC purity: 92.8%, t_R = 8.7 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (s, 1H), 8.83 (d, J = 2.3 Hz, 1H), 8.79 (t, J = 5.3 Hz, 1H), 8.07 (dd, J = 9.2, 2.0 Hz, 1H), 7.59 (d, J = 8.3 Hz, 2H), 7.53–7.43 (m, 4H), 7.34–7.27 (m, 2H), 7.26–7.19 (m, 2H), 7.09–6.96 (m, 4H), 6.96–6.82 (m, 4H), 5.06 (s, 2H), 4.82 (d, J = 7.2 Hz, 1H), 4.33–4.16 (m, 3H), 3.78 (dd, J = 10.5, 5.3 Hz, 2H), 2.99–2.86 (m, 2H), 2.40 (s, 3H), 1.39 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.88, 158.09, 158.04, 147.99, 140.93, 137.82, 137.23, 135.55, 135.21, 131.10, 130.34, 129.68, 129.55, 129.17, 128.01, 127.44, 127.15, 126.94, 124.57, 121.71, 115.19, 114.68, 113.98, 81.55, 69.82, 65.53, 56.07, 42.63, 36.31, 28.18, 21.13. HRMS (AP-ESI) m/z Calcd for $C_{42}H_{44}N_4O_9S$ $[M-H]^-$ 779.2756, found: 779.2733.

*5.1.20. Tert-butyl (S)-(1-((4-(cyclohexylamino)-3-nitrophenyl)sulfonamido)-1-oxo-3-(4-((4-phenoxy benzyl)oxy)phenyl)propan-2-yl)carbamate (**4m**)*

Yellow powder, yield: 61%, mp: 160–162 °C. HPLC purity: 88.8%, t_R = 9.6 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.81 (d, J = 2.2 Hz, 1H), 8.52 (d, J = 7.4 Hz, 1H), 8.01 (d, J = 9.2 Hz, 1H), 7.45–7.30 (m, 4H), 7.11 (t, J = 7.4 Hz, 1H), 7.05–6.96 (m, 5H), 6.94 (d, J = 9.4 Hz, 1H), 6.86 (d, J = 8.4 Hz, 2H), 4.98 (s, 2H), 4.84 (d, J = 7.3 Hz, 1H), 4.24 (s, 1H), 3.58 (s, 1H), 3.04–2.86 (m, 2H), 2.13–1.98 (m, 2H), 1.88–1.76 (m, 2H), 1.74–1.65 (m, 1H), 1.52–1.30 (m, 14H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.81, 158.07, 157.22, 157.01, 147.20, 135.00, 131.54, 130.48, 130.34, 129.80, 129.52, 129.41, 129.29, 127.44, 123.57, 123.46, 119.05, 118.82, 115.15, 114.25, 81.46, 69.63, 56.02, 51.60, 36.31, 32.45, 28.18, 25.36, 24.36. HRMS (AP-ESI) m/z Calcd for $C_{39}H_{44}N_4O_9S$ $[M-H]^-$ 743.2756, found: 743.2743.

*5.1.21. (S)-3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl) propanamide hydrochloride (**5a**)*

4a (1.5 g, 2.25 mmol) was dissolved in 30 mL EtOAc saturated with HCl gas and stirred overnight at room temperature. The precipitate was filtered and washed with EtOAc to afford 1.3 g (96%) of **5a** as yellowish powder. mp: 196–198 °C. HPLC purity: 98.9%, t_R = 5.7 min, MeOH/water = 80/20 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (d, J = 2.1 Hz, 1H), 8.27 (s, 3H), 8.15 (dd, J = 8.5, 2.1 Hz, 1H), 8.04 (d, J = 8.5 Hz, 1H), 7.69 (t, J = 7.6 Hz, 4H), 7.54 (d, J = 8.2 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.38 (t, J = 7.3 Hz, 1H), 7.01 (d, J = 8.6 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 5.11 (s, 2H), 4.04–3.97 (m, 1H), 3.05 (dd, J = 14.2, 5.7 Hz, 1H), 2.98 (dd, J = 14.2, 6.6 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.83, 157.96, 147.49, 140.82, 140.26, 140.19, 136.74, 133.14, 133.02, 131.16, 130.40, 129.44, 128.72, 127.99, 127.24, 127.14, 126.74, 125.61, 115.04, 69.26, 54.88, 35.59. HRMS (AP-ESI) m/z Calcd for $C_{28}H_{24}ClN_3O_6S$ $[M-H]^-$ 564.1002, found: 564.0982.

Compounds **5b-5f** were synthesized following the procedure described above.

*5.1.22. (S)-2-amino-3-(4-(benzyloxy)phenyl)-N-((3-nitro-4-((3-phenylpropyl)amino)phenyl) sulfonyl)propanamide hydrochloride (**5b**)*

Yellow powder, yield: 94%, mp: 216–218 °C. HPLC purity: 99.6%, t_R = 7.6 min, MeOH/water = 74/26 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (t, J = 5.8 Hz, 1H), 8.57 (d, J = 2.3 Hz, 1H), 8.42 (s, 3H), 7.86 (dd, J = 9.2, 2.2 Hz, 1H), 7.48–7.36 (m, 4H), 7.36–7.30 (m, 1H), 7.30–7.13 (m, 6H), 6.94 (d, J = 8.6 Hz, 2H), 6.77 (d, J = 8.7 Hz, 2H), 5.01 (s, 2H), 4.07 (s, 1H), 3.54–3.40 (m, 2H), 3.11–2.94 (m, 2H), 2.75–2.60 (m, 2H), 2.04–1.84 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.32, 158.01, 147.91, 141.68, 137.47, 134.47, 131.15, 130.19, 128.90, 128.81, 128.71, 128.32, 128.12, 126.35, 126.31, 124.08, 115.64, 114.97, 69.61, 54.26, 42.75, 35.49, 32.81, 30.18. HRMS (AP-ESI) m/z Calcd for $C_{31}H_{32}N_4O_6S$ $[M-H]^-$ 587.1970, found: 587.1955.

*5.1.23. (S)-2-amino-3-(4-((4-cyanobenzyl)oxy)phenyl)-N-((3-nitro-4-((3-phenylpropyl)amino) phenyl)sulfonyl)propanamide hydrochloride (**5c**)*

Yellow powder, yield: 81%, mp: 222–224 °C. HPLC purity: 99.2%, t_R = 7.7 min, MeOH/water = 70/30 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (t, J = 5.7 Hz, 1H), 8.56 (d, J = 2.3 Hz, 1H), 8.44 (s, 3H), 7.93–7.78 (m, 3H), 7.62 (d, J = 8.3 Hz, 2H), 7.36–7.11 (m, 6H), 6.98 (d, J = 8.6 Hz, 2H), 6.79 (d, J = 8.7 Hz, 2H), 5.14 (s, 2H), 4.09 (s, 1H), 3.49 (dd, J = 13.4, 6.7 Hz, 2H), 3.14–2.91 (m, 2H), 2.78–2.60 (m, 2H), 2.04–1.83 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.30, 157.62, 147.92, 143.31, 141.67, 134.47, 132.87, 131.23, 130.19, 128.81, 128.75, 128.70, 128.49, 126.71, 126.36, 124.06, 119.20, 115.64, 115.01, 110.97, 68.66, 54.21, 42.74, 35.48, 32.80, 30.17. HRMS (AP-ESI) m/z Calcd for $C_{32}H_{31}N_5O_6S$ $[M-H]^-$ 612.1922, found: 612.1901.

*5.1.24. (S)-2-amino-3-(4-((4-cyanobenzyl)oxy)phenyl)-N-((4-(cyclopentylamino)-3-nitrophenyl) sulfonyl)propanamide hydrochloride (**5d**)*

Yellow powder, yield: 82%, mp: 224–226 °C. HPLC purity: 94.2%, t_R = 5.9 min, MeOH/water = 70/30 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (d, J = 2.3 Hz, 1H), 8.44 (s, 3H), 8.37 (d, J = 6.8 Hz, 1H), 7.97—7.80 (m, 3H), 7.64 (d, J = 8.3 Hz, 2H), 7.30 (d, J = 9.4 Hz, 1H), 6.99 (d, J = 8.6 Hz, 2H), 6.80 (d, J = 8.6 Hz, 2H), 5.16 (s, 2H), 4.22–4.02 (m, 2H), 3.12–2.89 (m, 2H), 2.17–1.96 (m, 2H), 1.85–1.67 (m, 2H), 1.67–1.47 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.32, 157.64, 147.49, 143.33, 134.55, 132.89, 131.23, 130.23, 128.66, 128.50, 126.73, 124.44, 119.20, 116.33, 115.03, 110.98, 68.70, 54.58, 54.23, 35.47, 33.02, 24.05. HRMS (AP-ESI) m/z Calcd for $C_{28}H_{29}N_5O_6S$ $[M-H]^-$ 562.1766, found: 562.1753.

*5.1.25. (S)-3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-2-amino-N-((4-(cyclohexylamino)-3-nitro phenyl)sulfonyl)propanamide hydrochloride (**5e**)*

Yellow powder, yield: 85%, mp: 204–206 °C. HPLC purity: 99.4%, t_R = 6.0 min, MeOH/water = 83/17 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 2.3 Hz, 1H), 8.47–8.24 (m, 4H), 7.87 (dd, J = 9.3, 2.2 Hz, 1H), 7.69 (t, J = 8.1 Hz, 4H), 7.58–7.43 (m, 4H), 7.39 (d, J = 7.4 Hz, 1H), 7.37–7.30 (m, 1H), 6.95 (d, J = 8.6 Hz, 2H), 6.80 (d, J = 8.7 Hz, 2H), 5.08 (s, 2H), 4.07 (s, 1H), 3.81–3.67 (m, 1H), 3.12–2.93 (m, 2H), 1.99–1.87 (m, 2H), 1.76–1.62 (m, 2H), 1.62–1.51 (m, 1H), 1.49–1.30 (m, 4H), 1.28–1.18 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.18, 165.46, 157.52, 147.83, 141.67, 140.28, 140.13, 136.77, 134.81, 134.22, 131.99, 130.66, 130.16, 130.09, 129.66, 129.39, 128.78, 128.71, 128.46, 127.94, 127.16, 127.11, 126.31, 124.48, 124.34, 123.04, 115.66, 114.87, 69.26, 56.03, 42.69, 35.82, 32.81, 30.10. HRMS (AP-ESI) m/z Calcd for $C_{34}H_{36}N_4O_6S$ $[M-H]^-$ 627.2283, found: 627.2272.

*5.1.26. (S)-3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-2-amino-N-((3-nitro-4-((3-phenylpropyl) amino)phenyl)sulfonyl)propanamide hydrochloride (**5f**)*

Yellow powder, yield: 65%, mp: 217–219 °C. HPLC purity: 97.2%, t_R = 5.5 min, MeOH/water = 83/17 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (t, J = 5.7 Hz, 1H), 8.58 (d, J = 2.3 Hz, 1H), 8.44 (s, 3H), 7.86 (dd, J = 9.2, 2.2 Hz, 1H), 7.73–7.63 (m, 4H), 7.56–7.45 (m, 4H), 7.41–7.34 (m, 1H), 7.30–7.14 (m, 6H), 6.95 (d, J = 8.6 Hz, 2H), 6.80 (d, J = 8.7 Hz, 2H), 5.07 (s, 2H), 4.09 (s, 1H), 3.49 (dd, J = 13.4, 6.7 Hz, 2H), 3.10–2.94 (m, 2H), 2.72–2.62 (m, 2H), 2.00–1.87 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.22, 156.91, 146.85, 140.60, 139.16, 139.13, 135.59, 133.39, 130.10, 129.08, 128.36, 127.73, 127.66, 127.63, 126.93, 126.14, 126.06, 125.27, 125.21, 122.89, 114.60, 113.90, 68.18, 53.15, 41.68, 34.40, 31.73, 29.10. HRMS (AP-ESI) m/z Calcd for $C_{37}H_{36}N_4O_6S$ $[M-H]^-$ 663.2283, found: 663.2261.

*5.1.27. (S)-N-(3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-1-((4-chloro-3-nitrophenyl)sulfonamido)-1- oxopropan-2-yl)hexanamide (**6a**)*

TBTU (320 mg, 1.0 mmol), NMM (0.28 mL, 2.49 mmol) and **5a** (500 mg, 0.83 mmol) was added to a solution of hexanoic acid (0.1 mL, 0.83 mmol) in anhydrous DMF (15 mL) at 0 °C. The reaction mixture was stirred at room temperature for overnight. EtOAc was added and the mixture was washed with 5% citric acid solution and brine, then dried over $MgSO_4$. The solvent was evaporated in vacuum. Purification with silica gel column chromatography gave 160 mg (29%) of **6a** as yellowish powder. mp: 148–149 °C. HPLC purity: 98.9%, t_R = 8.9 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (d, J = 2.2 Hz, 1H), 8.13 (dd, J = 8.5, 2.2 Hz, 1H), 8.08 (d, J = 7.4 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H), 7.72–7.63 (m, 4H), 7.56–7.43 (m, 4H), 7.41–7.33 (m, 1H), 7.09 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.09 (s, 2H), 4.47–4.35 (m, 1H), 2.86 (dd, J = 13.7, 5.1 Hz, 1H), 2.62 (dd, J = 13.7, 9.5 Hz, 1H), 1.98 (t, J = 7.3 Hz, 2H), 1.32 (dd, J = 15.0, 7.5 Hz, 2H), 1.17 (dd, J = 14.7, 7.4 Hz, 2H), 1.09–0.96 (m, 2H), 0.78 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 171.18, 165.46, 157.52, 147.83, 141.67, 140.28, 140.13, 136.77, 134.81, 134.22, 131.99, 130.66, 130.16, 130.09, 129.66, 129.39, 128.78, 128.71, 128.46, 127.94, 127.16, 127.11, 126.31, 124.48, 124.34, 123.04, 115.66, 114.87, 69.26, 56.03, 42.69, 35.82, 32.81, 30.10. HRMS (AP-ESI) m/z Calcd for $C_{34}H_{34}ClN_3O_7S$ $[M-H]^-$ 662.1733, found: 662.1718.

Compounds **6b-6i** were synthesized following the procedure described above.

*5.1.28. (S)-N-(3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-1-((4-chloro-3-nitrophenyl)sulfonamido)-1- oxopropan-2-yl)nicotinamide (**6b**)*

White powder, yield: 61%, mp: 102–104 °C. HPLC purity: 98.0%, t_R = 8.4 min, MeOH/water = 80/20 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (d, J = 7.4 Hz, 2H), 8.75 (s, 1H), 8.57 (d, J = 2.1 Hz, 1H), 8.18 (dd, J = 8.5, 2.1 Hz, 1H), 8.13 (d, J = 7.9 Hz, 1H), 8.06 (d, J = 8.5 Hz, 1H), 7.72–7.61 (m, 4H), 7.58–7.42 (m, 5H), 7.37 (t, J = 7.3 Hz, 1H), 7.22 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 8.6 Hz, 2H), 5.09 (s, 2H), 4.70–4.57 (m, 1H), 3.04 (dd, J = 13.7, 4.6 Hz, 1H), 2.85 (dd, J = 13.5, 10.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.76, 164.48, 156.47, 151.32, 147.69, 146.61, 139.21, 139.06, 138.56, 135.71, 134.84, 132.42, 131.70, 130.04, 129.64, 128.50, 128.34, 127.67, 126.89, 126.11, 126.06, 124.46, 123.10, 113.84, 68.16, 55.16, 34.51. HRMS (AP-ESI) m/z Calcd for $C_{34}H_{27}ClN_4O_7S$ $[M-H]^-$ 669.1216, found: 669.1200.

*5.1.29. (S)-3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2- (2-(p-tolyl)acetamido)propanamide (**6c**)*

Yellowish powder, yield: 79%, mp: 180–181 °C. HPLC purity: 91.9%, t_R = 9.4 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 (d, J = 2.2 Hz, 1H), 8.37 (d, J = 7.4 Hz, 1H), 8.13 (dd, J = 8.5, 2.2 Hz, 1H), 8.01 (d, J = 8.5 Hz, 1H), 7.73–7.63 (m, 4H), 7.54 (d, J = 8.2 Hz, 2H), 7.47 (t, J = 7.6 Hz, 2H), 7.37 (t, J = 7.3 Hz, 1H), 7.05 (d, J = 8.6 Hz, 2H), 7.01 (d, J = 7.9 Hz, 2H), 6.93 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 5.10 (s, 2H), 4.46–4.38 (m, 1H), 3.32–3.25 (m, 2H), 2.88 (dd, J = 13.7, 5.0 Hz, 1H), 2.65 (dd,

J = 13.7, 9.3 Hz, 1H), 2.23 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.18, 165.46, 157.52, 147.83, 141.67, 140.28, 140.13, 136.77, 134.81, 134.22, 131.99, 130.66, 130.16, 130.09, 129.66, 129.39, 128.78, 128.71, 128.46, 127.94, 127.16, 127.11, 126.31, 124.48, 124.34, 123.04, 115.66, 114.87, 69.26, 56.03, 42.69, 35.82, 32.81, 30.10. HRMS (AP-ESI) m/z Calcd for $C_{37}H_{32}ClN_3O_7S$ $[M-H]^-$ 696.1577, found: 696.1548.

5.1.30. (S)-N-(3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-1-((4-chloro-3-nitrophenyl)sulfonamido)-1- oxopropan-2-yl)-2-naphthamide ***(6d)***

Yellowish powder, yield: 60%, mp: 182–184 °C. HPLC purity: 91.6%, t_R = 10.8 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (d, J = 7.2 Hz, 1H), 8.57 (d, J = 2.2 Hz, 1H), 8.38 (s, 1H), 8.18 (dd, J = 8.5, 2.2 Hz, 1H), 8.06 (d, J = 8.5 Hz, 1H), 8.04–7.92 (m, 3H), 7.87–7.80 (m, 1H), 7.68–7.56 (m, 6H), 7.54–7.42 (m, 4H), 7.36 (t, J = 7.3 Hz, 1H), 7.24 (d, J = 8.6 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H), 5.08 (s, 2H), 4.70–4.61 (m, 1H), 3.04 (dd, J = 13.6, 4.8 Hz, 1H), 2.97–2.85 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.18, 165.46, 157.52, 147.83, 141.67, 140.28, 140.13, 136.77, 134.81, 134.22, 131.99, 130.66, 130.16, 130.09, 129.66, 129.39, 128.78, 128.71, 128.46, 127.94, 127.16, 127.11, 126.31, 124.48, 124.34, 123.04, 115.66, 114.87, 69.26, 56.03, 42.69, 35.82, 32.81, 30.10. HRMS (AP-ESI) m/z Calcd for $C_{39}H_{30}ClN_3O_7S$ $[M-H]^-$ 718.1420, found: 718.1406.

5.1.31. (S)-3-(4-(benzyloxy)phenyl)-N-((3-nitro-4-((3-phenylpropyl) amino)phenyl)sulfonyl)-2-(2- (p-tolyl)acetamido)propanamide ***(6e)***

Yellow powder, yield: 83%, mp: 152–154 °C. HPLC purity: 96.9%, t_R = 5.8 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.39 (s, 1H), 8.79 (d, J = 2.3 Hz, 1H), 8.46 (t, J = 5.2 Hz, 1H), 7.92 (dd, J = 9.2, 2.2 Hz, 1H), 7.43–7.34 (m, 4H), 7.34–7.31 (m, 1H), 7.29 (d, J = 7.5 Hz, 2H), 7.24–7.21 (m, 1H), 7.21–7.15 (m, 2H), 7.10 (d, J = 7.8 Hz, 2H), 6.98 (d, J = 8.0 Hz, 2H), 6.81 (d, J = 9.3 Hz, 1H), 6.74 (d, J = 8.8 Hz, 2H), 6.69 (d, J = 8.8 Hz, 1H), 5.95 (d, J = 7.8 Hz, 1H), 4.97 (s, 2H), 4.85–4.71 (m, 1H), 3.51 (s, 2H), 3.39–3.29 (m, 2H), 2.86 (dd, J = 14.2, 6.1 Hz, 1H), 2.81–2.69 (m, 3H), 2.33 (s, 3H), 2.14–1.98 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.88, 175.49, 162.28, 152.59, 146.44, 142.36, 140.39, 138.96, 138.12, 135.45, 134.88, 133.97, 133.89, 133.80, 133.64, 133.55, 133.47, 133.25, 133.03, 132.84, 131.08, 129.03, 120.41, 119.45, 74.35, 59.61, 47.46, 46.59, 41.11, 37.57, 34.89, 25.83. HRMS (AP-ESI) m/z Calcd for $C_{40}H_{40}N_4O_7S$ $[M-H]^-$ 719.2545, found: 719.2529.

5.1.32. (S)-N-(3-(4-((4-cyanobenzyl)oxy)phenyl)-1-((3-nitro-4-((3-phenylpropyl)amino)phenyl) sulfonamido)-1-oxopropan-2-yl)-2-naphthamide ***(6f)***

Yellow powder, yield: 67%, mp: 112–114 °C. HPLC purity: 97.7%, t_R = 9.5 min, MeOH/water = 80/20 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.52 (s, 1H), 8.80 (d, J = 7.8 Hz, 1H), 8.68 (t, J = 5.7 Hz, 1H), 8.58 (d, J = 2.3 Hz, 1H), 8.39 (s, 1H), 8.04–7.92 (m, 3H), 7.88 (dd, J = 9.2, 2.1 Hz, 1H), 7.86–7.78 (m, 3H), 7.66–7.55 (m, 4H), 7.29–7.22 (m, 4H), 7.22–7.12 (m, 4H), 6.88 (d, J = 8.6 Hz, 2H), 5.13 (s, 2H), 4.75–4.63 (m, 1H), 3.45 (dd, J = 13.3, 6.6 Hz, 2H), 3.01 (dd, J = 13.6, 4.5 Hz, 1H), 2.89 (dd, J = 13.4, 10.4 Hz, 1H), 2.67 (t, J = 7.6 Hz, 2H), 1.99–1.86 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.41, 166.90, 157.12, 147.83, 143.42, 141.68, 134.70, 134.24, 132.82, 132.45, 131.25, 130.77, 130.17, 130.14, 129.32, 128.79, 128.71, 128.49, 128.30, 128.24, 128.09, 127.27, 126.32, 124.60, 124.35, 119.21, 115.68, 114.86, 110.88, 68.60, 56.02, 42.68, 35.80, 32.80, 30.10. HRMS (AP-ESI) m/z Calcd for $C_{43}H_{37}N_5O_7S$ $[M-H]^-$ 766.2341, found: 766.2312.

5.1.33. (S)-2-(2-([1,1′-biphenyl]-4-yl)acetamido)-3-(4-((4-cyanobenzyl) oxy)phenyl)-N-((4- (cyclopentylamino)-3-nitrophenyl)sulfonyl)propanamide ***(6g)***

Yellow powder, yield: 64%, mp: 143–145 °C. HPLC purity: 97.0%, t_R = 11.3 min, MeOH/water = 80/20 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.45 (s, 1H), 8.58 (d, J = 2.3 Hz, 1H), 8.42 (d, J = 8.0 Hz, 1H), 8.35 (d, J = 6.7 Hz, 1H), 7.89 (dd, J = 9.2, 2.2 Hz, 1H), 7.84 (d, J = 8.3 Hz, 2H), 7.65–7.55 (m, 4H), 7.49 (d, J = 8.2 Hz, 2H), 7.45–7.38 (m, 2H), 7.37–7.31 (m, 1H), 7.27 (d, J = 9.5 Hz, 1H), 7.12 (d, J = 8.2 Hz, 2H), 7.06 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 5.11 (s, 2H), 4.53–4.42 (m, 1H), 4.09 (dd, J = 12.4, 6.1 Hz, 1H), 3.47–3.36 (m, 2H), 2.89 (dd, J = 13.6, 4.6 Hz, 1H), 2.64 (dd, J = 13.6, 9.7 Hz, 1H), 2.14–1.97 (m, 2H), 1.77–1.64 (m, 2H), 1.64–1.48 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.18, 170.53, 157.15, 147.43, 143.38, 140.41, 138.62, 135.76, 134.30, 132.84, 130.80, 130.18, 129.92, 129.58, 129.35, 128.41, 127.74, 126.93, 126.78, 124.61, 119.23, 116.37, 114.76, 110.89, 68.63, 54.89, 54.53, 41.90, 36.36, 32.97, 24.03. HRMS (AP-ESI) m/z Calcd for $C_{42}H_{39}N_5O_7S$ $[M-H]^-$ 756.2497, found: 756.2466.

5.1.34. (S)-N-(3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-1-((4-(cyclohexylamino)-3-nitrophenyl) sulfonamido)-1-oxopropan-2-yl)-2-naphthamide ***(6h)***

Yellow powder, yield: 63%, mp: 118–120 °C. HPLC purity: 95.3%, t_R = 12.7 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.51 (s, 1H), 8.78 (d, J = 7.7 Hz, 1H), 8.59 (d, J = 2.2 Hz, 1H), 8.39 (s, 1H), 8.35 (d, J = 7.7 Hz, 1H), 8.00 (d, J = 8.7 Hz, 1H), 7.96 (d, J = 8.6 Hz, 2H), 7.92–7.80 (m, 2H), 7.69–7.55 (m, 6H), 7.53–7.42 (m, 4H), 7.40–7.34 (m, 1H), 7.31 (d, J = 9.5 Hz, 1H), 7.23 (d, J = 8.5 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 5.06 (s, 2H), 4.75–4.63 (m, 1H), 3.71 (s, 1H), 3.00 (dd, J = 13.6, 4.7 Hz, 1H), 2.93–2.81 (m, 1H), 1.98–1.85 (m, 2H), 1.77–1.63 (m, 2H), 1.61–1.52 (m, 1H), 1.47–1.33 (m, 4H), 1.27–1.19 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.18, 165.46, 157.52, 147.83, 141.67, 140.28, 140.13, 136.77, 134.81, 134.22, 131.99, 130.66, 130.16, 130.09, 129.66, 129.39, 128.78, 128.71, 128.46, 127.94, 127.16, 127.11, 126.31, 124.48, 124.34, 123.04, 115.66, 114.87, 69.26, 56.03, 42.69, 35.82, 32.81, 30.10. HRMS (AP-ESI) m/z Calcd for $C_{45}H_{42}N_4O_7S$ $[M-H]^-$ 781.2702, found: 781.2688.

5.1.35. (S)-3-(4-([1,1′-biphenyl]-4-ylmethoxy)phenyl)-N-((3-nitro-4-((3-phenylpropyl)amino)phenyl) sulfonyl)-2-(2-(p-tolyl)acetamido)propanamide ***(6i)***

Yellow powder, yield: 44%, mp: 146–148 °C. HPLC purity: 95.5%, t_R = 10.2 min, MeOH/water = 87/13 (0.1% HCOOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (s, 1H), 8.70 (t, J = 5.7 Hz, 1H), 8.55 (d, J = 2.3 Hz, 1H), 8.27 (d, J = 7.8 Hz, 1H), 7.83 (dd, J = 9.2, 2.2 Hz, 1H), 7.72–7.64 (m, 4H), 7.56–7.43 (m, 4H), 7.37 (t, J = 7.3 Hz, 1H), 7.29–7.23 (m, 2H), 7.23–7.13 (m, 4H), 7.05–6.97 (m, 4H), 6.92 (d, J = 8.0 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 5.07 (s, 2H), 4.49–4.37 (m, 1H), 3.46 (dd, J = 13.3, 6.7 Hz, 2H), 3.32–3.23 (m, 2H), 2.83 (dd, J = 13.7, 4.9 Hz, 1H), 2.71–2.65 (m, 2H), 2.65–2.58 (m, 1H), 2.22 (s, 3H), 1.99–1.88 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.14, 170.75, 157.52, 147.85, 141.68, 140.28, 140.16, 136.83, 135.65, 134.22, 133.37, 130.72, 130.14, 129.42, 129.23, 129.17, 129.06, 128.79, 128.72, 128.68, 128.50, 127.97, 127.21, 127.13, 126.33, 124.31, 115.66, 114.74, 69.28, 54.87, 42.72, 41.85, 36.38, 32.83, 30.14, 21.08. HRMS (AP-ESI) m/z Calcd for $C_{46}H_{44}N_4O_7S$ $[M-H]^-$ 795.2858, found: 795.2839.

5.2. In vitro binding assay for Bcl-2 proteins

A labeled Bid-BH3 peptide (5-FAM-QEDIIRNIARHLAQVGDSMDRS-IPPG) can bind to Bcl-2, Bcl-X_L and Mcl-1 protein with K_d values of 30–60 nM. And when it binds to these proteins, high polarization values (milipolarization units, mP) is generated. However, test compounds can compete with this Bid-BH3 peptide to bind to these proteins, which will lead to the decrease of mP values. The IC_{50} values of test compounds are calculated according to mP values and corresponding concentrations. Then the K_i values are calculated based on concentrations of the Bid-

BH3 peptide and Bcl-2 proteins, the K_d value and the IC_{50} values.[18,19]

5.3. Docking study

Surflex-dock program in Sybyl-X 2.1.1 was used with default values for the docking study of **6i** and Bcl-2. Compound **6i** was optimized using concord method and assigned with Gasteiger-Hückel charges. The Bcl-2 protein used was downloaded from the Protein Data Bank (PDB code: 4LVT). The proposed interactions between **6i** and Bcl-2 in the binding groove were performed by Molecular Operating Environment.

5.4. MTT assay

Cancer cells were all cultured in RPMI1640 medium (10% FBS, 1% penicillin-streptomycin solution) at 37 °C in a 5% CO_2 humidified incubator. Cells were plated at 2000–4000 cells/100 μL/well in 96-well plates. After 8 h, cells were treated with different concentrations of compounds (100 μL/well) for 72 h. Then 0.5% MTT solution (20 μL/well) was added and cells were incubation for another 4 h. After removal of culture medium, DMSO (150 μL/well) was added. Absorbance was measured by a microtiter-plate reader at 570 nm. The IC_{50} values were calculated according to the inhibition ratios and corresponding concentrations.

5.5. Analysis of apoptosis

Jurkat cells were plated in 6-well plates at 2×10^5 cells/well and treated with 10 and 20 μM of compound **6i**. After 48 h, cells were harvested, washed with PBS, and treated with annexin-V FITC and propidium iodide (PI) using Annexin V-FITC Apoptosis Detection Kit (Beyotime). The percentage of cells undergoing apoptosis was assessed by flow cytometry.

5.6. Determination of Caspase-3 activity

Jurkat cells were treated with 10 and 20 μM of compound **6i**. After 48 h, cells (1×10^6) were harvested and washed with PBS. Caspase-3 activity was determined using the Caspase-3 fluorometric assay kit (BioVision) following the protocol provided by the manufacturer. Caspase-3 activity was reported as the fold change relative to control cells (DMSO treated).

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