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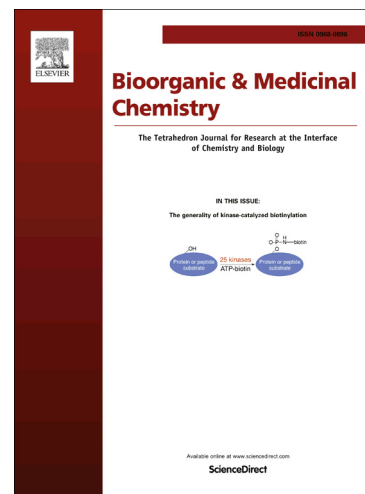
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**Design, synthesis and preliminary biological evaluation of
indole-3-carboxylic acid-based skeleton of Bcl-2/Mcl-1 dual
inhibitors**

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Abstract

The B-cell lymphoma-2 (Bcl-2) family proteins are attractive targets for cancer therapy. In our previous work, the structure-activity relationship of WL-276 was studied. According to the results, rhodanine derivatives show potent binding affinity for Bcl-2 and Mcl-1 protein and show weaker activity against Bcl-X_L protein. Based on the previous results, a new class of indole-3-carboxylic acid-based derivatives were designed and synthesized as Bcl-2/Mcl-1 dual inhibitors. Among them, compound **17** has a K_i value of 0.26 μM for Bcl-2 protein and is better than WL-276. Further more, it inhibits the myeloid cell leukemia sequence 1 (Mcl-1) protein with a K_i value of 72 nM. Especially, compound **31** can selectively acting on Bcl-2 and Mcl-1 protein but not Bcl-X_L protein, which has great significance for developing dual inhibitors targeting Bcl-2 and Mcl-1 protein, as well as specific antitumor abilities in cells.

Keywords: Bcl-2/Mcl-1; indole-3-carboxylic acid-based derivatives; anti-tumor; inhibitors

1. Introduction

Apoptosis is considered as a major type of programmed cell death (PCD)¹, which can maintain the homeostasis in normal organisms. In addition, it is related to embryogenesis and development of the immune system². The existent phenomenon suggests that elevating anti-apoptotic Bcl-2 proteins expression levels can be observed in many cancer lines and primary tumor biopsy samples, which may be a critical reason for tumor cells to evade apoptosis and subsequently acquire drug resistance for chemotherapy³.

The Bcl-2 proteins family contains at least 17 members. They all have the BH homology domains which mean B-cell lymphoma-2 homology domains. Structural studies have established that they can regulate intrinsic apoptosis and form the interaction sites of the Bcl-2 family⁴. According to the difference of the structure and function, it is divided into three groups: (1) anti-apoptosis (pro-survival or anti-apoptotic) family includes Bcl-2, Bcl-X_L, Bcl-w, Mcl-1 and A1 which having three or four BH domains (BH1-3 or BH1-4); (2) more BH domain pro-apoptotic family, including Bax and Bak, contains three BH domains; (3) only BH3 domain pro-apoptotic family contains only the BH3 domain of Bcl-2 homology, such as Noxa, Bid, Bik, Bim, Bmf, Hrk, Bad, PUMA and Beclin-1⁵. Only BH3 domain proteins are apoptotic effector molecules⁶. The fate of cells is related to the relative ratio of the three groups mentioned above. As an important regulator in apoptosis, Bcl-2 proteins can suppress the apoptosis to affect the chemotherapy. Therefore, down-regulating anti-apoptotic Bcl-2 proteins can be effective for the treatment of cancers. Along this line, numbers of Bcl-2 family inhibitors have been reported in the past few years, which can be classed into BH3 peptides(Liu et al designed a BH3-only peptide sequence TATDV3-(PU-MA) BH3)⁷, the Bcl-2 antisense oligonucleotides(Genasense)⁸ and some non-peptide small molecular inhibitors⁹.

Compared to the BH3 peptides, the non-peptide anti-apoptotic Bcl-2 inhibitors are more potential in the tumor treatment. In recent years, researchers have reported various small molecular anti-apoptotic proteins inhibitors through the advanced technologies, such as virtual screening and structure-based design. Based on their structures, they are grouped into five classes, BH3Is derivatives (BH3I-1)¹⁰, polyphenol derivatives (Gossypol)¹¹, chromene derivatives

(HA14-1)¹², acylsulfonamide derivatives (ABT-263)¹³ and others (GX15-070)¹⁴. Recent researches revealed that Bcl-X_L protein can lead to the reduction of platelet¹⁵. Therefore, there is more and more interest in the development of novel selective Bcl-2 inhibitors. Fortunately, ABT-199 as an effective Bcl-2 inhibitor has been listed recently. However, it shows no binding affinity for Mcl-1 protein, so it has no effect on carcinoma cells which exhibit overexpressed Mcl-1. Besides, the relationship between Mcl-1 and Bcl-2 was studied¹⁶ and the results showed that Mcl-1 is responsible for the resistance to the Bcl-2 inhibitor ABT-737. Furthermore, Mcl-1-mediated resistance could be a very common phenomenon in patients. In other words, the combination of Bcl-2 inhibitor and an agent which inhibits Mcl-1 function is a rational therapeutic strategy for tumor patients who have high levels of Mcl-1 expression. Thus, it is urgent to find new small molecule inhibitors of anti-apoptotic protein with Bcl-2/Mcl-1 dual inhibition.

In our recent work, several thiazolidinone derivatives, thiadiazole derivatives and pyrrolidine derivatives were designed and synthesized¹⁷⁻²⁰. The binding affinities demonstrate that thiazolidinone derivatives and pyrrolidine derivatives show potent inhibitory activities against Bcl-2 and Mcl-1 protein. In these studies, SAR of thiazolidinone derivatives were investigated based on the lead compound structure of WL-276 (Figure 1) which showed a similar repressive activity against Bcl-2 and Mcl-1 protein compared with (-)-gossypol^{21,22}. The results suggest that some compounds show poor affinity with Bcl-X_L protein^{15,16,18}, which is good for developing selective Bcl-2/Mcl-1 dual inhibitors. However, thiazolidinone derivatives showed poor water solubility. So we attempted to find a new aromatic ring as the scaffold of Bcl-2/Mcl-1 dual inhibitor and found the derivatives based on indole scaffold displayed much more advantages. In our on-going studies, a series of indole-3-carboxylic acid-based derivatives have been developed and their binding affinities to Bcl-2 and Mcl-1 protein have been studied.

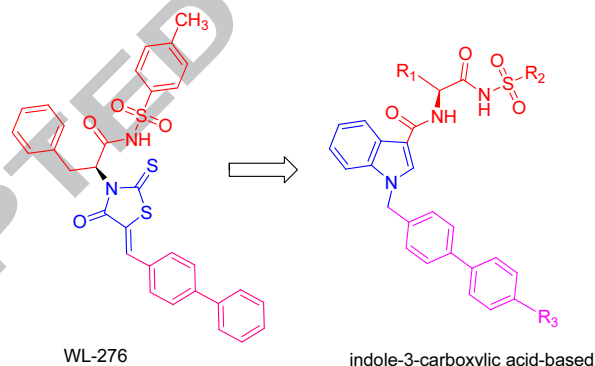


Figure 1. Representative Bcl-2/Mcl-1 dual inhibitors and design of our indole-3-carboxylic acid-based derivatives.

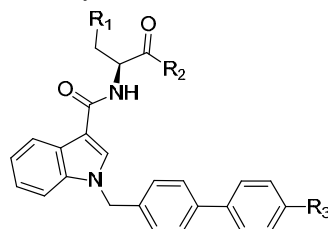
2. Chemistry

Synthesis of indole-3-carboxylic acid-based derivatives is described in **Scheme 1**.

The intermediate **2** was generated by a nucleophilic substitution reaction of 3-indolyl carboxylate **1** with benzyl bromide. The intermediate **2** was treated with different substituted phenylboronic acid to obtain intermediates **3** by Suzuki reaction. Then, the methyl groups of intermediates **3** are removed to give intermediates **4**. The different substituted Boc protected amino acids react with different sulfonamides to yield intermediates **7** by amide condensation reaction. Sequentially, the Boc groups of intermediates **7** are removed to generate intermediates **8** and react with intermediates **4** to obtain the target compounds **9-32**. The methyl group of **3C** is

In our recent work, twenty-five target compounds were synthesized. Using fluorescence polarization assays (FPAs), the inhibitory activities of all the target compounds against Bcl-2 protein were examined. The binding affinities were tabulated as K_i values and the results were displayed in **Table 1**. Based on the results in **Table 1**, some target compounds exhibited better activities than WL-276.

Table 1. The binding affinities of indole-3-carboxylic acid-based derivatives to Bcl-2 protein



Compd	R ₁	R ₂	R ₃	Bcl-2 K _i (μM) ^a
9	4-propoxy-Ph-	4-NO ₂ -Ph-SO ₂ NH-	4-Cl-	>10
10	4-propoxy-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-Cl-	>10
11	4-propoxy-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-methoxy-	>10
12	4-propoxy-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	H-	5.6±0.40
13	Ph-	Ph-SO ₂ NH-	4-Cl-	0.74±0.10
14	Ph-	Ph-SO ₂ NH-	H-	>10
15	Ph-	Ph-SO ₂ NH-	4-methoxy-	1.3±0.10
16	Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-methoxy-	>10
17	Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-Cl-	0.26±0.051
18	Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	H-	2.6±0.64
19	4-Br-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-Cl-	>10
20	4-Br-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-methoxy-	>10
21	4-Br-Ph-	Ph-SO ₂ NH-	4-Cl-	0.91±0.06
22	4-Br-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	H-	>10
23	4-OBzl-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-Cl-	>10
24	4-OBzl-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-methoxy-	>10
25	4-OBzl-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	H-	>10
26	4-OBzl-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-CH ₃ -	>10
27	4-NO ₂ -4-OBzl-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-methoxy-	>10
28	4-NO ₂ -4-OBzl-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	H-	>10
29	4-NO ₂ -4-OBzl-Ph-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-Cl-	>10
30	1 <i>H</i> -Indole-3-	3-NO ₂ -4-Cl-Ph-SO ₂ NH-	4-methoxy-	>10
31	1 <i>H</i> -Indole-3-	4-CH ₃ -Ph-SO ₂ NH-	4-methoxy-	0.75±0.01
32	Isopropyl-	Ph-SO ₂ NH-	4-Cl-	0.92±0.07
34	Ph-	OH-	4-methoxy-	>10
WL-276				0.76±0.08

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

In this work, the target compounds were mainly modified by three parts, amino acid side chain (R₁), sulfonamide moiety (R₂) and the substituent of biphenyl part (R₃). For the modification on R₁, different aromatic and aliphatic amino acid were introduced such as isoleucine, phenylalanine, different substituted phenylalanine, tryptophan and tyrosine. The binding affinities indicated that most of the substituted phenylalanine derivatives (eg. compound **9-12**, **22-29**) showed poor binding affinities except compound **21** with small substituent. While the target compounds with

unsubstituted phenylalanine side chain (eg. compound **13** and **17**) show much better binding affinities than others. Some compounds with tyrosine side chain (compound **31**) and aliphatic side chain (eg. compound **32**) exhibited the similar binding abilities compared with positive control.

Sulfonamide moiety (R_2) was also important for binding affinities. For example, this fragment was introduced to inactive compound **34** and yields the compound **15** with K_i value of 1.3 μ M. Electron-donating group attaching to the phenyl ring would enhance the potency (eg. compound **13** with methyl substitution) and the compounds with electron withdrawing group in this position (eg. compound **19**) usually have poor binding affinities compared with unsubstituted derivatives (eg. compound **21**).

In addition, the substituents the biphenyl group (R_3) could influence interaction between the compounds and target protein. Some compounds with electron withdrawing substitution, such as chloro (compound **13** and **17**), exhibited better binding affinities than that of electron donating substitution (eg. compound **15** and **16**) or unsubstitution (eg. compound **18**).

In order to understand the interaction between these indole-based compounds and Bcl-2 protein, docking studies were performed using the most active target compound **17** and inactive compound **16** using with Surflex-Dock software (Figure 2). The results suggested that biphenyl fragment of compound **17** could insert into the pocket formed by the residues of Met112, Glu133, Arg136 and Leu134 (Fig.2a). But compound **16** showed another binding mode that the biphenyl did not occupy the pocket formed by the above mentioned four amino acids (Fig.2b)

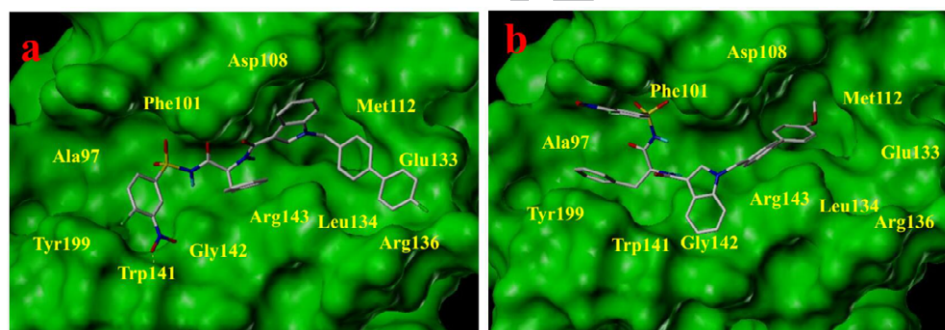


Figure 2. (a) The proposed mode between compound **17** and Bcl-2 protein.
(b) The proposed mode between compound **16** and Bcl-2 protein.

To further examine if these target compounds also have good inhibitory activities for other anti-apoptotic Bcl-2 proteins, four compounds were chose to test the inhibitory activities against Bcl-X_L, Mcl-1 protein by FPAs and the results were listed as K_i values in **Table 2**. As shown in **Table 2**, the binding affinities of most of the compounds for Mcl-1 are almost the same as Bcl-2 except compound **17**. However, these compounds exhibit weaker potency in inhibiting the Bcl-X_L protein compared to Bcl-2 and Mcl-1, especially compound **31** shows no activity for Bcl-X_L and it shows better antiproliferative activity, which may have important significance for the cancer treatment.

Table 2. The binding affinities of seven representative indole-3-carboxylic acid-based derivatives to three Bcl-2 proteins

Compd	Bcl-2	Bcl-X _L	Mcl-1
	K _i (μM) ^a	K _i (μM) ^a	K _i (μM) ^a
13	0.74±0.10	2.1±0.40	0.49±0.06
17	0.26±0.051	0.71±0.15	0.072±0.023
31	0.75±0.01	N.A.	0.53±0.10
32	0.92±0.07	6.2±1.5	0.60±0.03
WL-276	0.76±0.08	0.81±0.16	0.44±0.06

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

According to the previous reports, Bcl-2 proteins are overexpressed in most of cancer cells, such as prostate, myeloma and acute leukemias²³. Therefore, compound **13**, **17** and **31** were chosen as the representatives to test their antiproliferative activities *in vitro* against three kinds of human cancer cell lines PC-3 (prostatic cancer cell), K562 (chronic myelogenous leukemia cell) and MDA-MB-231 (breast cancer cell) by using MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazoliumbromide) assay. (R)-Gossypol, which was reported as a potent Bcl-2 inhibitor²⁴, was also evaluated as the positive control under the same condition. The results of activities were listed as IC₅₀ values in **Table 3**. According to the results listed in **Table 3**, all the selected compounds except **13** display slightly better activities against K562 than PC-3 and MDA-MB-231. Moreover, compound **31** shows similar antiproliferative activity towards K562 compared to (R)-Gossypol. These three compounds show much poorer inhibitory activities towards PC-3 and MDA-MB-231 than (R)-Gossypol.

Table 3. Antiproliferative activities of representative compounds

Compd	IC ₅₀ (μM) ^a		
	MDA-MB-231	PC-3	K562
31	11.51±0.92	18.27±0.70	10.07±1.23
13	29.62±1.17	23.58±1.68	32.44±2.27
17	29.48±0.44	28.81±1.39	19.70±0.90
(R)-Gossypol	8.54±0.44	8.58±0.98	8.28±1.89

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

4. Conclusions

In conclusion, a series of indole-3-carboxylic acid-based derivatives as Bcl-2/Mcl-1 inhibitors based on the structure of WL-276 were designed and synthesized. We mainly modified the target compounds by three parts that is the amino acid side chain, biphenyl moiety and sulfonamide moiety. Some compounds exhibit better Bcl-2/Mcl-1 inhibitory activities than WL-276, such as compounds **17**. And compound **31** shows considerably potent antiproliferative activity against

K562. These results could lay a firmer structural foundation to find much more potent Bcl-2/Mcl-1 dual inhibitors.

5. Experiment section

5.1. General chemistry information

In this work, unless otherwise mentioned, all the starting materials, solvents and chemical reagents (analytical grade) were purchased from commercial suppliers and used directly. Moreover, all reactions were detected on 0.25 mm silica gel plates (60 GF-254) by thin-layer chromatography (TLC) and then visualized with UV light (365nm or 254nm), ninhydrin solution or iodine vapor. ¹H-NMR (Proton nuclear magnetic resonance) spectra were gained on a Bruker Avance spectrometer (300 MHz or 400 MHz). Chemical shifts are showed in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. The analysis of spectrums was referenced to the solvent (2.5 ppm for ¹H (DMSO) or 7.26 ppm for ¹H (CDCl₃)). Coupling constants are expressed in hertz (Hz). The splitting patterns were described in the following: multiplicity(s singlet, d doublet, t triplet, q quartet, m multiplet, dd doublet doublet and brs broadsinglet) number of protons. HRMS spectrums were conducted on an Agilent 6510 Quadrupole Time-of-Flight LC/MS deliver. Products were purified by column chromatography (silica gel 200-300 mesh) or recrystallization. Melting points were monitored on an electrothermal melting point apparatus without correction. ElectroSpray Ionization-Mass Spectrometry (ESI-MS) was determined on an Agilent-1100 series LC/MSD trap spectrometer.

5.1.1. Methyl 1-(4-bromobenzyl)-1*H*-indole-3-carboxylate (**2**)

The solution of **1** (8.76 g, 50 mmol) in DMSO (100 mL) was mixed with K₂CO₃ (13.8 g, 100 mmol), then 4-Bromobenzyl bromide (23.46 g, 150 mmol) was added step by step and then the reaction mixture was heated in 30°C overnight. Subsequently, the mixture was poured into cool water (5 times) and extracted with a large amount of EtOAc. Combining the organic phases and washing it three times with 1M citric acid, brine and drying it over MgSO₄ for 0.5 h, then it was filtered and concentrated to give the crude product compound **2**. The compound **2** was purified by silica gel chromatography (ethyl acetate: petroleum ether: =1:30-1:40) to obtain White solid **2**. Yield: 72.6%, mp: 117-119 °C; ¹H-NMR (400 MHz, DMSO-*d*₆), δ 8.58 (s, 1H), 8.03-8.01 (m, 1H), 7.72-7.41 (m, 3H), 7.24-7.20 (m, 4H), 5.50 (s, 2H), 3.81 (s, 3H).

5.1.2. Methyl 1-((4'-chloro-[1, 1'-biphenyl]-4-yl) methyl)-1*H*-indole-3-carboxylate (**3B**)

First, compound **2** (1.72 g, 5 mmol), 2M Na₂CO₃ aqueous solution (3 mL, 6 mmol), palladium acetate (0.011 g, 0.05 mmol), triphenylphosphine (0.039 g, 0.15 mmol), 4-chlorophenyl boronic acid (0.82 g, 5.25 mmol) were dissolved in 30 mL DMSO in a 50 mL round bottom flask under nitrogen. The mixture was heated at 80°C later on under stirring conditions overnight. A lot of distilled water (5 times) was poured to the reaction mixture and the reaction liquid was extracted with a half amount of ethyl acetate by twice times. Then the ethyl acetate layer was washed twice times with 2M citric acid, brine and dried, filtered and concentrated to obtain crude product compound **3B**. The crude product **3B** was recrystallized from ethanol to obtain the white solid **3B**. Yield: 100.0%, mp: 130-132°C; ¹H-NMR (400 MHz, DMSO-*d*₆), δ 8.38 (s, 1H), 8.05-8.02 (m, 1H), 7.66-7.57 (m, 5H), 7.51-7.47 (m, 2H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.26-7.20 (m, 2H), 5.56 (s, 2H), 3.82 (s, 3H).

Compounds **3C–3E** were synthesized following the procedure described above.

5.1.2.1. Methyl 1-((4'-methoxy-[1, 1'-biphenyl]-4-yl) methyl)-1*H*-indole-3-carboxylate (3C).

Red solid. Yield: 57%, mp: 145-146°C. ¹H-NMR (400 MHz, DMSO-*d*₆), δ 8.37 (s, 1H), 8.04 (dd, $J_1 = 8.0$ Hz, $J_2 = 4.0$ Hz, 1H), 7.59-7.55 (m, 5H), 7.35 (d, $J = 4.0$ Hz, 2H), 7.51-7.47 (m, 2H), 7.24 (d, $J = 8.0$ Hz, 2H), 7.00 (d, $J = 8.0$ Hz), 5.54 (s, 2H), 3.82 (s, 3H), 3.77 (s, 3H).

5.1.2.2. Methyl 1-([1, 1'-biphenyl]-4-ylmethyl)-1*H*-indole-3-carboxylate (3D).

White solid. Yield: 80%, mp: 137-139°C. ¹H-NMR (400 MHz, DMSO-*d*₆), δ 8.61 (s, 1H), 8.04 (dd, $J_1 = 8.0$ Hz, $J_2 = 4.0$ Hz, 1H), 7.63-7.60 (m, 5H), 7.44-7.23 (m, 7H), 5.56 (s, 2H), 3.82 (s, 3H).

5.1.2.3. Methyl 1-((4'-methyl-[1, 1'-biphenyl]-4-yl) methyl)-1*H*-indole-3-carboxylate (3E)

Grey solid. Yield: 28%, mp: 136-138°C. ¹H-NMR (400 MHz, DMSO-*d*₆), δ 8.22 (d, $J = 8.0$ Hz, 1H), 7.88 (s, 1H), 7.53 (d, $J = 8.0$ Hz, 2H), 7.45 (d, $J = 8.0$ Hz, 2H), 7.36 (d, $J = 8.0$ Hz, 1H), 7.31-7.20 (m, 6H), 5.37 (s, 2H), 3.91 (s, 3H), 2.38 (s, 3H).

5.1.3. (S)-tert-butyl (1-oxo-3-phenyl-1-(phenylsulfonamido) propan-2-yl) carbamate (7A)

The solution of Boc-protected phenylalanine (1.33 g, 5 mmol) in dry dichloromethane (40 mL) was treated with ethyldiisopropylamine (1.29 g, 10 mmol) under ice bath condition, after stirred for 10 min, 2-(7-Aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (2.28 g, 6 mmol) was added in and the cloudy reaction solution was stirred for 30 min, then the benzene sulfonamide (0.86 g, 5.5 mmol) was added. After stirred for about 6 h at room temperature. The solvent was removed and the concentrated solution was dissolved in EtOAc. The overlying phases were washed with 2M citric acid, brine and dried, concentrated to yield yellow oil. Finally, the oil was purified by silica gel chromatography (Ethyl acetate: Petroleum ether = 1:3-1:5) to generate compound **7A** as a white powder-like solid. Yield: 100%, mp: 133-136°C. ¹H-NMR (300 MHz, CDCl₃): δ 9.34 (s, 1H), 8.05 (d, $J = 7.5$ Hz, 2H), 7.57-7.66 (m, 1H), 7.52-7.57 (m, 2H), 7.04-7.23 (m, 3H), 7.02-7.04 (m, 2H), 4.90 (s, 1H), 4.30 (s, 1H), 3.05 (dd, $J_1 = 14.0$ Hz, $J_2 = 6.3$ Hz, 1H), 2.93 (dd, $J = 14.0$ Hz, $J_2 = 6.9$ Hz, 1H), 1.38 (s, 9H).

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

5.1.3.1. (S)-tert-butyl (3-(4-bromophenyl)-1-oxo-1-(phenylsulfonamido) propan-2-yl) carbamate (7B)

White solid. Yield: 70%, mp: 119-122°C. ¹H-NMR (300 MHz, CDCl₃): δ 12.43 (s, 1H), 7.88-7.95 (m, 2H), 7.70 (d, $J = 7.2$ Hz, 1H), 7.58-7.66 (m, 2H), 7.42 (d, $J = 8.1$ Hz, 2H), 7.16 (d, $J = 8.1$ Hz, 2H), 7.07 (s, 1H), 4.06-4.13 (m, 1H), 2.79-2.85 (m, 1H), 2.57-2.61 (m, 1H), 1.26 (s, 9H).

5.1.3.2. (S)-tert-butyl(3-(4-bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxoprop an-2-yl) carbamate (7C)

Yellow solid. Yield: 71%, mp: 116-118°C. ¹H-NMR (300 MHz, CDCl₃): δ 8.32 (s, 1H), 7.98 (d, $J = 8.4$ Hz, 1H), 7.82 (d, $J = 8.1$ Hz, 1H), 7.28 (d, $J = 8.1$ Hz, 2H), 6.94 (d, $J = 8.1$ Hz, 2H), 6.01 (d, $J = 8.1$ Hz, 1H), 3.82-3.89 (m, 1H), 2.91-2.98 (m, 1H), 2.68-2.75 (m, 1H), 1.31 (s, 9H).

5.1.3.3. (S)-tert-butyl(1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-phenylpropan-2-yl) carbamate(7D)

Yellow solid. Yield: 75%, mp: 147-150°C. ¹H-NMR (300 MHz, CDCl₃): δ 9.50 (s, 1H), 8.48 (d, J = 2.1 Hz, 1H), 8.19 (dd, J_1 = 8.4 Hz, J_2 = 2.1 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.26-7.28(m, 3H), 7.07-7.10 (m, 2H), 4.81(d, J = 6.6 Hz, 1H), 4.20-4.27 (m, 1H), 3.07 (dd, J_1 = 14.1 Hz, J_2 = 6.3 Hz, 1H), 2.96 (dd, J_1 = 14.1 Hz, J_2 = 7.8 Hz, 1H), 1.40(s, 9H).

5.1.3.4. (S)-tert-butyl(1-(4-nitrophenylsulfonamido)-1-oxo-3-(4-propoxyphenyl)propan-2-yl) carbamate(7E)

Yellow solid. Yield: 80%, mp: 234-236°C. ¹H-NMR (400 MHz, CDCl₃), δ 9.49 (s, 1H), 8.38 (d, J = 8.0 Hz, 2H), 8.23(d, J = 8.0 Hz, 2H), 6.98(d, J = 8.0 Hz, 2H), 6.78 (d, J = 8.0 Hz, 2H), 4.83 (d, J = 6.8 Hz, 1H), 4.20 (d, J = 6.8 Hz, 1H), 3.89 (t, J = 6.8 Hz, 2H), 3.00-2.88 (m, 2H), 1.82-1.77 (m, 2H), 1.40 (s, 9H), 1.05 (t, J = 8.0 Hz, 3H).

5.1.3.5. (S)-tert-butyl(1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-(4-propoxyphenyl)propan-2-yl) carbamate (7F)

Yellow solid. Yield: 76%, mp: 122-124°C. ¹H-NMR (400 MHz, CDCl₃), δ 9.58 (s, 1H), 8.50 (d, J = 2.0 Hz, 1H), 8.20 (dd, J_1 = 8.0 Hz, J_2 = 2.1 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 6.98 (d, J = 8.0 Hz, 2H), 6.79 (d, J = 8.0 Hz, 2H), 4.87 (d, J = 6.9 Hz, 1H), 4.24-4.17 (m, 1H), 3.90 (t, J = 6.9 Hz, 2H), 3.02-2.87 (m, 2H), 1.86-1.74 (m, 2H), 1.41 (s, 9H), 1.06 (t, J = 7.2 Hz, 3H).

5.1.3.6. (S)-tert-butyl (3-(4-(benzyloxy) phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl) carbamate (7G)

Yellow solid. Yield: 77%, mp: 112-114°C. ¹H-NMR (300 MHz, DMSO-*d*₆), δ 8.32 (d, J = 1.8 Hz, 1H), 8.00 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.45-7.29 (m, 5H), 6.87 (d, J = 8.0 Hz, 2H), 6.74 (d, J = 8.0 Hz, 2H), 5.85 (d, J = 8.0 Hz, 1H), 5.02 (s, 2H), 3.85-3.79 (m, 1H), 2.92-2.86 (m, 1H), 2.73-2.66 (m, 1H), 1.31 (s, 9H).

5.1.3.7. (S)-tert-butyl(1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-((4-nitrobenzyl)oxy)phenyl)-1-oxopropan-2-yl)carbamate(7H)

Yellow solid. Yield: 98%, mp: 224-226°C. ¹H-NMR (400 MHz, CDCl₃), δ 9.53 (s, 1H), 8.49 (d, J = 2.0 Hz, 1H), 8.26-8.20 (m, 3H), 7.76 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.01 (d, J = 8.0 Hz, 2H), 5.15 (s, 2H), 4.83 (d, J = 6.8 Hz, 1H), 4.23-4.18 (m, 1H), 3.04-2.91 (m, 2H), 1.40 (s, 9H).

5.1.3.8. (S)-tert-butyl (1-(4-chloro-3-nitrophenylsulfonamido)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl) carbamate (7I)

Yellow solid, yield 38%, mp: 96-99°C. ¹H-NMR (400 MHz, DMSO-*d*₆), δ 12.82 (s, 1H), 10.38 (s, 1H), 8.51-8.46 (m, 1H), 8.10-7.96 (m, 2H), 7.76-7.60 (m, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.11-6.94 (m, 4H), 4.24-4.11 (m, 1H), 3.01(dd, J_1 = 12.0 Hz, J_2 = 4Hz, 1H), 2.85 (dd, J_1 = 12.0 Hz, J_2 = 8.0 Hz, 1H), 1.22-1.08 (m, 9H).

5.1.3.9. (S)-tert-butyl(3-(1*H*-indol-3-yl)-1-(4-methylphenylsulfonamido)-1-oxopropan-2-yl) carbamate (7J)

White solid. Yield about 30%, mp: 90-93°C. ¹H-NMR (400 MHz, DMSO-*d*₆), δ 12.37 (s, 1H), 10.81 (s, 1H), 7.83-7.65 (m, 3H), 7.43-7.27 (m, 3H), 7.15-6.91 (m, 4H), 4.24-4.14 (m, 1H), 2.97 (dd, $J_1 = 16.0$ Hz, $J_2 = 4.0$ Hz, 1H), 2.78 (dd, $J_1 = 16.0$ Hz, $J_2 = 4.0$ Hz, 1H), 2.39 (s, 3H), 1.33-1.04 (m, 9H).

5.1.3.10. (S)-tert-butyl (4-methyl-1-oxo-1-(phenylsulfonamido) pentan-2-yl)carbamate(7K)

White solid. Yield 68%, mp: 151-153°C. ¹H-NMR (400 MHz, DMSO-*d*₆), δ 12.26 (s, 1H), 7.96-7.89(m, 2H), 7.72 (t, $J = 8.0$ Hz, 1H), 7.63 (t, $J = 8.0$ Hz, 1H), 7.05 (d, $J = 8.0$ Hz, 1H), 4.04-3.97 (m, 1H), 1.52-1.49 (m, 1H), 1.38-1.12 (m, 11H), 0.82-0.79 (m, 6H).

5.1.4. (S)-methyl2-(1-((4'-methoxy-[1, 1'-biphenyl]-4-yl) methyl)-1H-indole-3-carboxamido)-3-phenylpropanoate (33)

To a 50 mL round-bottomed flask, intermediate **3C** (1.11 g, 3 mmol) was added in with 15 mL THF, then potassiumhydroxide (1.2 g, 30 mmol), distilled water(10 mL) and methanol(5 mL) was added in and the mixed solution was refluxed for 4h. Then, the solvent THF and were removed by distillation and 5M hydrochloric acid was added in to keep the PH value is 1 of the reaction mixture, and the white solid was isolated by filtration to give intermediate **4C** without purification.

The solution of **4C** (0.72 g, 2 mmol) in dry dichloromethane (50 mL) was treated with ethyldiisopropylamine (1.29 g, 10 mmol) and stirred under an ice bath for 10 min, then, 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate(0.91 g, 2.4 mmol) was added, subsequently the cloudy mixture was stirred for 30min, phenylalanine methyl ester hydrochloride (0.47 g, 2.2 mmol) was added and stirred for next 6h at room temperature, the resulted solution was clear. Then the solvent of the reaction was removed and EtOAc was added to the concentrated residue. The phases of EtOAc were washed with 2M citric acid, saturated brine and dried over MgSO₄ and the organic solvent was concentrated to yield yellow oil. Finally, the oil was further purified by silica gel chromatography (P: E =5:1-3:1) to generate compound **33** as a white solid. Yield: 35%, mp: 160-162°C. ¹H-NMR (400 MHz, DMSO-*d*₆), δ 8.33 (d, $J = 8.0$ Hz), 8.22 (s, 1H), 8.07 (d, $J = 8.0$ Hz), 7.60-7.56 (m, 5H), 7.31-7.10 (m, 9H), 7.01 (d, $J = 8.0$ Hz), 5.54-5.46 (m, 2H), 4.71-4.65 (m, 1H), 3.78 (s, 3H), 3.62 (s, 3H), 3.18-3.05 (s, 2H).

Compounds **4C-4E** were synthesized following the procedure described above.

5.1.5. (R)-N-(1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-phenylpropan-2-yl)-1-((4'-chloro-[1, 1'-biphenyl]-4-yl) methyl)-1H-indole-3-carboxamide (17)

Intermediate **7D** (0.48 g, 1 mmol) mixed with saturated HCl ethyl acetate (15 mL) at a normal temperature and the final solution was stirred overnight. Then the key intermediate **8D** was obtained by filtering without purification. Then, the key intermediate **4B** (0.18 g, 0.5 mmol) was dissolved in 15 mL of DCM dried by CaCl₂ and cooled to 0°C, 5 min latter, ethyldiisopropylamine(0.19 g, 1.5 mmol) was added, 10min latter, 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate(0.23 g, 0.6 mmol) was added, after half an hour, intermediate **8D** (0.24 g, 0.55 mmol) was added and the resulted cloudy solution was stirred at ambient temperature overnight. The next day, the reaction solvent was removed and then extracted with EtOAc. The EtOAc phases were washed using 1M citric acid, 1M sodium bicarbonate, brine and dried over MgSO₄, filtered and concentrated to give the yellow product **17**.

The yellow solid was further purified by silica gel chromatography (P: E = 4:1-3:1 with 0.2% HOAc) to generate pure product **17**. White solid. Yield: 95%, mp: 136-139°C. ¹H-NMR: (300 MHz, DMSO-*d*₆), δ 12.89(s, 1H), 8.53 (d, *J* = 2.1 Hz, 1H), 8.18-8.12(m, 3H), 8.05 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 7.2 Hz, 1H), 7.67-7.62 (m, 4H), 7.56-7.48 (m, 3H), 7.33-7.09 (m, 10H), 5.61-5.46 (m, 2H), 4.69-4.62 (m, 1H), 3.06-2.86 (m, 2H). ¹³C-NMR (100 MHz, DMSO-*d*₆), δ 172.44, 164.84, 147.62, 139.62, 138.94, 138.69, 137.59, 137.49, 136.61, 133.51, 132.90, 132.81, 132.22, 131.20, 129.65, 129.34, 128.86, 128.55, 128.30, 127.44, 127.03, 126.94, 125.49, 122.78, 121.68, 121.43, 111.14, 109.78, 55.35, 49.57, 36.56. HRMS (AP-ESI) *m/z* Calcd for C₃₇H₂₈Cl₂N₄O₆S [M-H]⁺ 725.1028. Found: 725.1010.

Compounds **9–32** were synthesized following the procedure described above.

5.1.5.1. (R)-1-((4'-chloro-[1,1'-biphenyl]-4-yl)methyl)-N-(1-(4-nitrophenylsulfonamido)-1-oxo-3-(4-propoxyphenyl)propan-2-yl)-1*H*-indole-3-carboxamide(9)

Yellow solid. Yield: 27%, mp: 170-172°C. ¹H-NMR (400 MHz, DMSO-*d*₆), δ 12.84 (s, 1H), 8.42 (d, *J* = 8.0 Hz, 2H), 8.18 (m, 4H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 4H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 4.63-4.60 (m, 1H), 3.83 (t, *J* = 8.0 Hz, 2H), 2.96 (dd, *J*₁ = 14.0 Hz, *J*₂ = 4.4 Hz, 1H), 2.84 (dd, *J*₁ = 14.0 Hz, *J*₂ = 8.0 Hz, 1H), 1.67(m, 2H), 0.95 (t, *J* = 8.0 Hz, 3H). ¹³C-NMR (100 MHz, DMSO-*d*₆), δ 172.38, 164.75, 157.82, 150.65, 145.02, 138.93, 138.69, 137.51, 136.61, 132.90, 132.16, 130.66, 129.67, 129.33, 128.85, 128.31, 127.43, 127.01, 124.83, 122.78, 121.64, 121.37, 114.49, 111.16, 109.88, 69.24, 55.49, 49.55, 35.82, 22.52, 10.83. HRMS (AP-ESI) *m/z* Calcd for C₄₀H₃₅ClN₄O₇S [M-H]⁺ 749.1837. Found: 749.1821.

5.1.5.2. (R)-N-(1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-(4-propoxyphenyl)propan-2-yl)-1-((4'-chloro-[1,1'-biphenyl]-4-yl)methyl)-1*H*-indole-3-carboxamide(10)

White solid. Yield: 6%, mp: 130-134°C. ¹H-NMR(400 MHz, DMSO-*d*₆), δ 12.63 (s, 1H), 8.55 (d, *J* = 2.0 Hz, 1H), 8.18-7.96 (m, 5H), 7.67 (t, *J* = 9.6 Hz, 4H), 7.57-7.48 (m, 3H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.20-7.10 (m, 4H), 6.75 (d, *J* = 8.8 Hz, 2H), 5.56-5.47 (m, 2H), 4.62-4.56 (m, 1H), 3.84 (t, *J* = 8.4 Hz, 2H), 2.97 (dd, *J*₁ = 13.6 Hz, *J*₂ = 5.2 Hz, 1H), 2.90 (dd, *J*₁ = 13.8 Hz, *J*₂ = 4.0 Hz, 1H), 1.69-1.64 (m, 2H), 0.98-0.77 (m, 3H). ¹³C-NMR (100 MHz, DMSO-*d*₆), δ 172.60, 164.77, 157.82, 147.57, 139.81, 138.93, 138.69, 137.49, 136.59, 133.44, 132.89, 132.80, 132.19, 131.07, 130.66, 129.33, 129.23, 128.86, 128.32, 127.43, 127.00, 125.48, 122.78, 121.65, 121.42, 114.45, 111.14, 109.83, 69.24, 55.62, 49.56, 35.81, 22.52, 10.85. HRMS (AP-ESI) *m/z* Calcd for C₄₀H₃₄Cl₂N₄O₇S [M-H]⁺ 783.1447. Found: 783.1437.

5.1.5.3. (R)-N-(1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-(4-propoxyphenyl)propan-2-yl)-1-((4'-methoxy-[1,1'-biphenyl]-4-yl)methyl)-1*H*-indole-3-carboxamide (11)

Yellow solid. Yield: 8%, mp: 154-156°C. ¹H-NMR(400 MHz, DMSO-*d*₆), δ 12.98 (s, 1H), 8.55 (d, *J* = 1.6 Hz, 1H), 8.18-8.16 (m, 3H), 8.04 (d, *J* = 8.8 Hz, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.59 (t, *J* = 5.6 Hz, 5H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.20-7.10 (m, 4H), 7.00 (d, *J* = 8.8 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 2H), 5.53-5.45 (m, 2H), 4.61-4.56 (m, 1H), 3.84-3.78 (m, 5H), 2.97 (dd, *J*₁ = 13.6 Hz, *J*₂ = 5.2 Hz, 1H), 2.90 (dd, *J*₁ = 13.8 Hz, *J*₂ = 5.2 Hz, 1H), 1.71-1.63 (m, 2H), 0.95 (t, *J* = 5.8 Hz, 3H). ¹³C-NMR (100 MHz, DMSO-*d*₆), δ 172.63, 164.79, 159.42, 157.81, 147.57, 139.73, 136.59, 136.27, 133.45, 132.81, 132.47, 132.17, 131.09, 130.67, 129.24, 128.22, 128.19, 127.01, 126.92,

125.50, 122.75, 121.64, 121.40, 114.81, 114.43, 111.16, 109.74, 69.22, 55.62, 49.60, 35.79, 22.52, 10.86. HRMS (AP-ESI) m/z Calcd for $C_{41}H_{37}ClN_4O_8S$ $[M-H]^-$ 779.1942. Found: 779.1945.

5.1.5.4. (R)-1-([1,1'-biphenyl]-4-ylmethyl)-N-(1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-(4-propoxyphenyl)propan-2-yl)-1H-indole-3-carboxamide (12)

White solid. Yield: 9%, mp: 166-168°C. 1H -NMR (400 MHz, DMSO- d_6): δ 12.89 (s, 1H), 8.54 (d, J = 1.6 Hz, 1H), 8.19-8.13 (m, 3H), 8.04 (d, J = 8.8 Hz, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.64-7.56 (m, 5H), 7.44-7.30 (m, 5H), 7.20-7.10 (m, 4H), 6.75 (d, J = 8.4 Hz, 2H), 5.56-5.47 (m, 2H), 4.61-4.56 (m, 1H), 3.84 (t, J = 7.2 Hz, 2H), 2.98 (dd, J_1 = 13.6 Hz, J_2 = 5.2 Hz, 1H), 2.80 (dd, J_1 = 13.8 Hz, J_2 = 9.6 Hz, 1H), 1.71-1.62 (m, 2H), 0.95-0.85 (m, 3H). ^{13}C -NMR (100 MHz, DMSO- d_6): δ 172.61, 164.79, 157.83, 147.58, 140.14, 140.07, 139.80, 137.08, 136.61, 133.45, 132.81, 132.20, 131.09, 130.67, 129.38, 129.24, 128.24, 127.98, 127.47, 127.10, 127.01, 125.48, 122.78, 121.65, 121.42, 114.45, 111.15, 109.80, 69.24, 55.63, 49.59, 35.81, 22.52, 10.85. HRMS (AP-ESI) m/z Calcd for $C_{40}H_{35}ClN_4O_7S$ $[M-H]^-$ 749.1837. Found: 749.1830.

5.1.5.5. (R)-1-((4'-chloro-[1,1'-biphenyl]-4-yl)methyl)-N-(1-oxo-3-phenyl-1-(phenylsulfonamido)propan-2-yl)-1H-indole-3-carboxamide (13)

White solid. Yield: 31%, mp: 136-138°C. 1H -NMR (400 MHz, DMSO- d_6): δ 12.55 (s, 1H), 8.18-8.12 (m, 2H), 8.01 (d, J = 7.6 Hz, 1H), 7.94 (d, J = 7.6 Hz, 1H), 7.73-7.49 (m, 10H), 7.42 (d, J = 8.0 Hz, 2H), 7.31-7.10 (m, 9H), 5.56-5.47 (m, 2H), 4.73-4.68 (m, 1H), 3.02 (dd, J_1 = 13.2 Hz, J_2 = 4.0 Hz, 1H), 2.86 (dd, J_1 = 13.8 Hz, J_2 = 4.8 Hz, 1H). ^{13}C -NMR (100 MHz, DMSO- d_6): δ 171.83, 164.73, 139.74, 138.94, 138.69, 137.85, 137.51, 136.61, 134.13, 132.90, 132.15, 129.66, 129.59, 129.35, 128.87, 128.59, 128.29, 127.89, 127.44, 126.99, 126.91, 122.76, 121.68, 121.40, 111.15, 109.97, 55.10, 49.55, 36.65. HRMS (AP-ESI) m/z Calcd for $C_{37}H_{30}ClN_3O_4S$ $[M-H]^-$ 646.1567. Found: 646.1553.

5.1.5.6. (R)-1-([1,1'-biphenyl]-4-ylmethyl)-N-(1-oxo-3-phenyl-1-(phenylsulfonamido)propan-2-yl)-1H-indole-3-carboxamide (14)

White solid. Yield: 49%, mp: 120-122°C. 1H -NMR (400 MHz, DMSO- d_6): δ 12.55 (s, 1H), 8.18 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.73-7.55 (m, 8H), 7.46 (t, J = 7.2 Hz, 2H), 7.37-7.10 (m, 12H), 5.56-5.46 (m, 2H), 4.73-4.67 (m, 1H), 3.03 (dd, J_1 = 13.6 Hz, J_2 = 4.4 Hz, 1H), 2.86 (dd, J_1 = 13.8 Hz, J_2 = 10.4 Hz, 1H). ^{13}C -NMR (100 MHz, DMSO- d_6): δ 171.88, 164.76, 140.14, 140.06, 139.73, 137.86, 137.10, 136.61, 134.14, 132.16, 129.67, 129.60, 129.40, 128.60, 128.22, 127.99, 127.90, 127.48, 127.12, 126.99, 126.93, 122.78, 121.67, 121.42, 111.17, 109.93, 55.13, 49.57, 36.65. HRMS (AP-ESI) m/z Calcd for $C_{37}H_{31}N_3O_4S$ $[M-H]^-$ 612.1957. Found: 612.1954.

5.1.5.7. (R)-1-((4'-methoxy-[1,1'-biphenyl]-4-yl)methyl)-N-(1-oxo-3-phenyl-1-(phenylsulfonamido)propan-2-yl)-1H-indole-3-carboxamide (15)

White solid. Yield: 31%, mp: 204-206°C. 1H -NMR (400 MHz, DMSO- d_6): δ 12.54 (s, 1H), 8.18-8.11 (m, 2H), 8.00 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.73-7.54 (m, 8H), 7.30-7.11 (m, 9H), 7.01 (d, J = 8.8 Hz, 2H), 5.53-5.43 (m, 2H), 4.73-4.67 (m, 1H), 3.78 (s, 3H), 3.02 (dd, J_1 = 13.6 Hz, J_2 = 4.4 Hz, 1H), 2.90 (dd, J_1 = 13.8 Hz, J_2 = 10.4 Hz, 1H). ^{13}C -NMR (100 MHz, DMSO- d_6): δ 171.85, 164.77, 159.45, 139.75, 137.85, 136.63, 136.27, 134.13, 132.50, 132.15,

129.67, 129.59, 128.60, 128.21, 127.91, 127.01, 126.93, 122.75, 121.68, 121.39, 114.84, 111.17, 109.93, 55.63, 55.12, 49.62, 36.67. HRMS (AP-ESI) m/z Calcd for $C_{38}H_{33}N_3O_5S$ [M-H]⁻ 642.2063. Found: 642.2062.

5.1.5.8. (R)-N-(1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-phenylpropan-2-yl)-1-((4'-methoxy-[1,1'-biphenyl]-4-yl)methyl)-1H-indole-3-carboxamide(16)

Yellow solid. Yield: 21%, mp: 120-123°C. ¹H-NMR(400 MHz, DMSO-*d*₆), δ 12.84 (s, 1H), 8.75 (s, 1H), 8.20-8.13 (m, 3H), 8.05 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.59-7.48 (m, 5H), 7.29-7.10 (m, 9H), 7.01 (d, J = 8.8 Hz, 2H), 5.53-5.32 (m, 2H), 4.68-4.62 (m, 1H), 3.77 (s, 3H), 3.05 (dd, J_1 = 13.6 Hz, J_2 = 5.2 Hz, 1H), 2.90 (dd, J_1 = 13.8 Hz, J_2 = 4.0 Hz, 1H). ¹³C NMR (100MHz, MHz, DMSO-*d*₆), δ 172.49, 164.86, 159.42, 147.61, 139.73, 139.59, 137.59, 136.59, 136.27, 133.50, 132.82, 132.47, 132.20, 131.21, 129.65, 128.56, 128.20, 127.02, 126.93, 125.50, 122.76, 121.65, 121.42, 114.82, 111.16, 109.68, 55.62, 55.37, 49.61, 36.53. HRMS (AP-ESI) m/z Calcd for $C_{38}H_{31}ClN_4O_7S$ [M-H]⁻ 721.1524. Found: 721.1506.

5.1.5.9. (R)-1-([1,1'-biphenyl]-4-ylmethyl)-N-(1-(4-chloro-3-nitrophenylsulfonamido)-1-oxo-3-phenylpropan-2-yl)-1H-indole-3-carboxamide(18)

White solid. Yield: 29%, mp: 150-153°C. ¹H-NMR(400 MHz, DMSO-*d*₆), δ 12.86 (s, 1H), 8.54 (d, J = 2.0 Hz, 1H), 8.21-8.15 (m, 3H), 8.05 (d, J = 8.8 Hz, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.64-7.55 (m, 5H), 7.46 (t, J = 8.0 Hz, 2H), 7.37-7.10 (m, 10H), 5.56-5.47 (m, 2H), 4.68-4.63 (m, 1H), 3.05 (dd, J_1 = 13.6 Hz, J_2 = 5.2 Hz, 1H), 2.90 (dd, J_1 = 13.8 Hz, J_2 = 10.0 Hz, 1H). ¹³C-NMR (100 MHz, DMSO-*d*₆), δ 172.51, 164.86, 147.61, 140.14, 140.07, 139.63, 137.60, 137.09, 136.61, 133.50, 132.83, 132.23, 131.19, 129.66, 129.39, 128.57, 128.22, 127.98, 127.48, 127.12, 127.03, 126.95, 125.51, 122.79, 121.67, 121.44, 111.16, 109.74, 55.39, 49.59, 36.55. HRMS (AP-ESI) m/z Calcd for $C_{37}H_{29}ClN_4O_6S$ [M-H]⁻ 691.1418. Found: 691.1408.

5.1.5.10. (R)-N-(3-(4-bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)-1-((4'-chloro-[1,1'-biphenyl]-4-yl)methyl)-1H-indole-3-carboxamide(19)

Yellow solid. Yield:12%, mp: 184-185°C. ¹H-NMR (400 MHz, DMSO-*d*₆), δ 12.78 (s, 1H), 8.55 (s, 1H), 8.18-8.16 (m, 3H), 8.05 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.67 (t, J = 7.2 Hz, 4H), 7.57-7.49 (m, 3H), 7.42 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.20-7.10 (m, 4H), 5.56-5.47 (m, 2H), 4.04-4.02 (m, 1H), 3.03 (dd, J_1 = 13.6 Hz, J_2 = 4.8 Hz, 1H), 2.90 (dd, J_1 = 13.8 Hz, J_2 = 4.8 Hz, 1H). ¹³C-NMR (100 MHz, DMSO-*d*₆), δ 172.26, 164.82, 147.63, 139.67, 138.93, 138.70, 137.49, 137.16, 136.61, 133.49, 132.90, 132.79, 132.18, 131.91, 131.43, 131.18, 129.34, 128.87, 128.31, 127.44, 127.01, 125.51, 122.80, 121.66, 121.44, 120.23, 111.15, 109.75, 55.14, 49.56, 35.89. HRMS (AP-ESI) m/z Calcd for $C_{37}H_{27}BrCl_2N_4O_6S$ [M-H]⁻ 805.0113. Found: 805.0102.

5.1.5.11. (R)-N-(3-(4-bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)-1-((4'-methoxy-[1,1'-biphenyl]-4-yl)methyl)-1H-indole-3-carboxamide(20)

Yellow solid. Yield: 25%, mp: 180-182°C. ¹H-NMR(400 MHz, DMSO-*d*₆), δ 12.81 (s, 1H), 8.78 (s, 1H), 8.23-8.11 (m, 3H), 8.05 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.59 (t, J = 8.0 Hz, 5H), 7.43 (d, J = 8.4 Hz, 2H), 7.29-7.12 (m, 6H), 7.01 (d, J = 8.8 Hz, 2H), 5.54-5.45 (m, 2H), 4.66-4.60 (m, 1H), 3.77 (s, 1H), 3.03 (dd, J_1 = 13.6 Hz, J_2 = 4.8 Hz, 1H), 2.90 (dd, J_1 = 13.8 Hz, J_2

= 10.5 Hz, 1H). ^{13}C -NMR (100 MHz, $\text{DMSO}-d_6$), δ 172.23, 164.87, 159.44, 147.62, 139.76, 139.57, 137.14, 136.62, 136.24, 133.51, 132.80, 132.47, 132.18, 131.91, 131.44, 131.26, 128.22, 128.19, 127.03, 126.93, 125.53, 122.79, 121.67, 121.44, 120.26, 114.83, 111.16, 109.68, 55.62, 55.15, 49.64, 35.87. HRMS (AP-ESI) m/z Calcd for $\text{C}_{38}\text{H}_{30}\text{BrClN}_4\text{O}_7\text{S}$ $[\text{M}-\text{H}]^-$ 799.0629. Found: 799.0651.

5.1.4.12. (R)-N-(3-(4-bromophenyl)-1-oxo-1-(phenylsulfonamido)propan-2-yl)-1-((4'-chloro-[1,1'-biphenyl]-4-yl)methyl)-1H-indole-3-carboxamide(21)

White solid. Yield: 9%, mp: 134-136°C. ^1H -NMR(400 MHz, $\text{DMSO}-d_6$), δ 12.55 (s, 1H), 8.16 (s, 2H), 8.01 (d, $J = 3.2$ Hz, 1H), 7.94 (d, $J = 3.2$ Hz, 1H), 7.81 (d, $J = 3.6$ Hz, 1H), 7.71 (d, $J = 3.6$ Hz, 1H), 7.71-7.12 (m, 11H), 5.56-5.47 (m, 2H), 4.67 (s, 1H), 3.00 (d, $J = 11.6$ Hz, 1H), 2.84 (m, 1H). ^{13}C -NMR (100 MHz, $\text{DMSO}-d_6$), δ 171.56, 164.71, 138.93, 138.69, 137.52, 137.33, 136.61, 134.17, 132.88, 132.10, 131.90, 131.44, 129.60, 129.35, 128.88, 128.31, 127.88, 127.44, 126.97, 122.78, 121.66, 121.42, 120.17, 111.16, 109.88, 56.51, 54.83, 35.99. HRMS (AP-ESI) m/z Calcd for $\text{C}_{37}\text{H}_{29}\text{BrClN}_3\text{O}_4\text{S}$ $[\text{M}-\text{H}]^-$ 726.0652. Found: 726.0611.

5.1.5.13. (R)-1-([1,1'-biphenyl]-4-ylmethyl)-N-(3-(4-bromophenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)-1H-indole-3-carboxamide(22)

White solid. Yield: 32%, mp: 146-149°C. ^1H -NMR(400 MHz, $\text{DMSO}-d_6$), δ 12.67 (s, 1H), 8.55 (s, 1H), 8.21-8.17 (m, 3H), 8.05 (d, $J = 8.8$ Hz, 1H), 7.98 (d, $J = 8.8$ Hz, 1H), 7.64-7.56 (m, 5H), 7.46-7.10 (m, 11H), 5.56-5.47 (m, 2H), 4.63 (s, 1H), 3.04 (dd, $J_1 = 13.6$ Hz, $J_2 = 4.8$ Hz, 1H), 2.90 (dd, $J_1 = 13.8$ Hz, $J_2 = 10.0$ Hz, 1H). ^{13}C -NMR (100 MHz, $\text{DMSO}-d_6$), δ 172.29, 164.83, 147.62, 140.13, 140.08, 139.74, 137.16, 137.07, 136.62, 133.47, 132.78, 132.18, 131.91, 131.42, 131.14, 129.39, 128.23, 127.98, 127.47, 127.11, 127.00, 125.49, 122.80, 121.64, 121.44, 120.22, 111.16, 109.73, 55.15, 49.59, 35.90. HRMS (AP-ESI) m/z Calcd for $\text{C}_{37}\text{H}_{28}\text{ClN}_4\text{O}_6\text{S}$ $[\text{M}-\text{H}]^-$ 771.0503. Found: 771.0512.

5.1.5.14. (R)-N-(3-(4-(benzyloxy)phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)-1-((4'-chloro-[1,1'-biphenyl]-4-yl)methyl)-1H-indole-3-carboxamide(23)

White solid. Yield: 12%, mp: 120-123°C. ^1H -NMR(400 MHz, $\text{DMSO}-d_6$), δ 12.93 (s, 1H), 8.52 (s, 1H), 8.18-8.13 (m, 2H), 8.00-7.97 (m, 3H), 7.65-7.10 (m, 17H), 7.83 (d, $J = 8.0$ Hz, 2H), 5.55-5.47 (m, 2H), 5.00 (s, 2H), 4.58-4.57 (m, 1H), 2.99 (dd, $J_1 = 10.0$ Hz, $J_2 = 8.8$ Hz, 1H), 2.90 (dd, $J_1 = 10.0$ Hz, $J_2 = 9.6$ Hz, 1H). ^{13}C -NMR (100 MHz, $\text{DMSO}-d_6$), δ 173.23, 164.64, 157.48, 147.49, 138.92, 138.68, 137.58, 137.50, 136.61, 133.20, 132.88, 132.78, 132.16, 130.73, 129.94, 129.33, 128.84, 128.32, 128.23, 128.12, 127.43, 126.98, 125.40, 122.75, 121.62, 121.41, 114.77, 111.16, 110.05, 69.61, 55.73, 49.56, 36.07. HRMS (AP-ESI) m/z Calcd for $\text{C}_{44}\text{H}_{34}\text{Cl}_2\text{N}_4\text{O}_7\text{S}$ $[\text{M}-\text{H}]^-$ 831.1447. Found: 831.1436.

5.1.5.15. (R)-N-(3-(4-(benzyloxy)phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)-1-((4'-methoxy-[1,1'-biphenyl]-4-yl)methyl)-1H-indole-3-carboxamide(24)

Yellow solid. Yield: 18%, mp: 119-121°C. ^1H -NMR(400 MHz, $\text{DMSO}-d_6$), δ 12.91 (s, 1H), 8.55 (s, 1H), 8.18-8.15 (m, 3H), 8.04 (d, $J = 8.4$ Hz, 1H), 7.98 (d, $J = 8.4$ Hz, 1H), 7.58 (t, $J = 8.0$ Hz, 5H), 7.41-7.12 (m, 11H), 6.99 (d, $J = 8.8$ Hz, 2H), 6.86 (d, $J = 8.8$ Hz, 2H), 5.53-5.45 (m, 2H), 5.01 (s, 1H), 4.60-4.59 (m, 1H), 3.77 (s, 3H), 2.98 (dd, $J_1 = 13.6$ Hz, $J_2 = 4.8$ Hz, 1H), 2.86 (dd, J_1

= 13.8 Hz, J_2 = 9.6 Hz, 1H). ^{13}C -NMR (100 MHz, DMSO- d_6), δ 172.64, 164.83, 159.43, 157.56, 147.59, 139.75, 137.58, 136.62, 136.26, 133.46, 132.81, 132.48, 132.19, 131.12, 130.72, 129.74, 128.85, 128.22, 128.18, 128.13, 127.04, 126.93, 125.53, 122.77, 121.67, 121.42, 114.82, 111.16, 109.79, 69.62, 55.61, 49.64, 35.80, 19.03. HRMS (AP-ESI) m/z Calcd for $\text{C}_{45}\text{H}_{37}\text{ClN}_4\text{O}_8\text{S}$ $[\text{M}-\text{H}]^-$ 827.1942. Found: 827.1932.

5.1.5.16. (R)-1-([1,1'-biphenyl]-4-ylmethyl)-N-(3-(4-(benzyloxy)phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)-1H-indole-3-carboxamide(25)

White solid. Yield: 19%, mp: 134-136°C. ^1H -NMR(400 MHz, DMSO- d_6), δ 12.88 (s, 1H), 8.56 (d, J = 2.0 Hz, 1H), 8.20-8.16 (m, 3H), 8.05 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 8.4 Hz, 1H), 7.64-7.56 (m, 5H), 7.43-7.05 (m, 14H), 6.87 (d, J = 8.4 Hz, 2H), 5.56-5.47 (m, 2H), 5.02 (s, 2H), 4.68-4.58 (m, 1H), 2.99 (dd, J_1 = 14.0 Hz, J_2 = 5.2 Hz, 1H), 2.86 (dd, J_1 = 14.0 Hz, J_2 = 10.0 Hz, 1H). ^{13}C -NMR (100 MHz, DMSO- d_6), δ 172.56, 164.87, 157.55, 147.60, 140.13, 140.07, 139.59, 137.56, 137.09, 136.61, 133.50, 132.82, 132.22, 131.22, 130.72, 129.70, 129.39, 128.86, 128.23, 128.13, 127.97, 127.48, 127.11, 127.04, 125.55, 122.80, 121.69, 121.45, 114.84, 111.16, 109.76, 69.60, 55.62, 49.60, 35.73. HRMS (AP-ESI) m/z Calcd for $\text{C}_{44}\text{H}_{35}\text{ClN}_4\text{O}_7\text{S}$ $[\text{M}-\text{H}]^-$ 797.1837. Found: 797.1835.

5.1.5.17. (R)-N-(3-(4-(benzyloxy)phenyl)-1-(4-chloro-3-nitrophenylsulfonamido)-1-oxopropan-2-yl)-1-((4'-methyl-[1,1'-biphenyl]-4-yl)methyl)-1H-indole-3-carboxamide(26)

White solid. Yield: 31%, mp: 145-147°C. ^1H -NMR(400 MHz, DMSO- d_6), δ 12.90 (s, 1H), 8.56 (d, J = 2.0 Hz, 1H), 8.19-8.15 (m, 3H), 8.04-7.97 (m, 3H), 7.61-7.50 (m, 5H), 7.42-7.12 (m, 13H), 7.46 (t, J = 8.0 Hz, 2H), 6.87 (d, J = 8.4 Hz, 2H), 5.54-5.46 (m, 2H), 5.01 (s, 2H), 4.63-4.57 (m, 1H), 2.98 (dd, J_1 = 13.6 Hz, J_2 = 5.2 Hz, 1H), 2.86 (dd, J_1 = 13.8 Hz, J_2 = 9.6 Hz, 1H), 2.15 (s, 3H). ^{13}C -NMR (100 MHz, DMSO- d_6), δ 172.55, 164.84, 157.56, 147.61, 139.99, 139.65, 137.58, 137.30, 137.23, 136.73, 136.62, 133.49, 132.81, 132.20, 131.19, 130.72, 129.98, 129.71, 128.85, 128.22, 128.13, 127.19, 127.04, 126.91, 125.54, 122.77, 121.68, 121.43, 114.84, 111.15, 109.78, 69.62, 55.60, 49.62, 35.75. HRMS (AP-ESI) m/z Calcd for $\text{C}_{45}\text{H}_{37}\text{ClN}_4\text{O}_7\text{S}$ $[\text{M}-\text{H}]^-$ 811.1993. Found: 811.2004.

5.1.5.18. (R)-N-(1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-((4-nitrobenzyl)oxy)phenyl)-1-oxopropan-2-yl)-1-((4'-methoxy-[1,1'-biphenyl]-4-yl)methyl)-1H-indole-3-carboxamide(27)

Yellow solid. Yield: 29%, mp: 164-166°C. ^1H -NMR(400 MHz, DMSO- d_6), δ 12.94 (s, 1H), 8.55 (d, J = 2.0 Hz, 1H), 8.24-8.13 (m, 5H), 8.04 (d, J = 8.8 Hz, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.67 (d, J = 8.8 Hz, 2H), 7.58 (t, J = 8.8 Hz, 5H), 7.29 (d, J = 8.0 Hz, 2H), 7.58-7.54 (m, 5H), 7.30 (d, J = 8.0 Hz, 2H), 7.22-7.10 (m, 4H), 7.00 (d, J = 8.8 Hz, 2H), 6.70 (d, J = 8.8 Hz, 2H), 5.53-5.44 (m, 2H), 5.19 (s, 1H), 4.63-4.57 (m, 1H), 3.03 (s, 3H), 3.00 (dd, J_1 = 13.6 Hz, J_2 = 4.8 Hz, 1H), 2.86 (dd, J_1 = 13.6 Hz, J_2 = 9.6 Hz, 1H). ^{13}C -NMR (100 MHz, DMSO- d_6), δ 172.58, 164.84, 159.41, 157.10, 147.58, 147.41, 145.52, 139.74, 139.66, 136.59, 136.26, 133.48, 132.82, 132.45, 132.18, 131.19, 130.82, 130.17, 128.62, 128.22, 128.17, 127.02, 126.92, 125.53, 124.01, 122.76, 121.65, 121.42, 114.88, 114.79, 111.16, 109.74, 68.41, 55.58, 49.62, 35.74. HRMS (AP-ESI) m/z Calcd for $\text{C}_{45}\text{H}_{36}\text{ClN}_5\text{O}_{10}\text{S}$ $[\text{M}-\text{H}]^-$ 872.1793. Found: 872.1793.

5.1.5.19. (R)-1-([1,1'-biphenyl]-4-ylmethyl)-N-(1-(4-chloro-3-nitrophenylsulfonamido)-3-

(4-((4-nitrobenzyl)oxy)phenyl)-1-oxopropan-2-yl)-1*H*-indole-3-carboxamide(28)

White solid. Yield: 24%, mp: 174-176°C. ¹H-NMR(400 MHz, DMSO-*d*₆), δ 12.91 (s, 1H), 8.56 (d, *J* = 2.0 Hz, 1H), 8.24-8.16 (m, 5H), 8.05 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 8.4 Hz, 1H), 7.69-7.56 (m, 7H), 7.45 (t, *J* = 7.2 Hz, 2H), 7.36-7.12 (m, 7H), 6.90 (d, *J* = 8.8 Hz, 2H), 5.56-5.32 (m, 2H), 5.01 (s, 2H), 4.63-4.58 (m, 1H), 3.00 (dd, *J*₁ = 13.6 Hz, *J*₂ = 4.8 Hz, 1H), 2.87 (dd, *J*₁ = 13.8 Hz, *J*₂ = 4.0 Hz, 1H). ¹³C-NMR (100 MHz, DMSO-*d*₆), δ 172.55, 164.85, 157.10, 147.59, 147.42, 145.54, 140.12, 140.07, 139.61, 137.08, 136.60, 133.49, 132.82, 132.21, 131.21, 130.82, 130.16, 129.38, 128.62, 128.23, 127.97, 127.48, 127.10, 127.02, 125.54, 124.03, 122.79, 121.66, 121.44, 114.90, 111.16, 109.77, 68.41, 55.58, 49.60, 35.72. HRMS (AP-ESI) *m/z* Calcd for C₄₄H₃₄ClN₅O₉S [M-H]⁺ 842.1688. Found: 842.1673.

5.1.5.20. (R)-N-(1-(4-chloro-3-nitrophenylsulfonamido)-3-(4-((4-nitrobenzyl)oxy)phenyl)-1-oxopropan-2-yl)-1-((4'-chloro-[1,1'-biphenyl]-4-yl)methyl)-1*H*-indole-3-carboxamide(29)

Yellow solid. Yield: 34%, mp: 179-181°C. ¹H-NMR(400 MHz, DMSO-*d*₆), δ 12.99 (s, 1H), 8.55 (d, *J* = 1.6 Hz, 1H), 8.24-8.16 (m, 5H), 8.04 (d, *J* = 8.8 Hz, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.68-7.62 (m, 6H), 7.57 (t, *J* = 8.8 Hz, 1H), 7.53-7.46 (m, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.22-7.10 (m, 6H), 6.89 (d, *J* = 8.8 Hz, 2H), 5.56-5.47 (m, 2H), 5.19 (s, 2H), 4.63-4.57 (m, 1H), 3.00 (dd, *J*₁ = 13.6 Hz, *J*₂ = 5.2 Hz, 1H), 2.87 (dd, *J*₁ = 13.8 Hz, *J*₂ = 10.0 Hz, 1H). ¹³C-NMR (100 MHz, DMSO-*d*₆), δ 172.53, 164.82, 157.11, 147.59, 147.43, 145.52, 139.67, 138.90, 138.70, 137.48, 136.60, 133.48, 132.89, 132.81, 132.20, 131.18, 130.81, 130.16, 129.32, 128.84, 128.63, 128.31, 127.43, 127.01, 125.52, 124.01, 122.79, 121.66, 121.44, 114.90, 111.14, 109.82, 68.44, 55.56, 49.57, 35.74. HRMS (AP-ESI) *m/z* Calcd for C₄₄H₃₃Cl₂N₅O₉S [M-H]⁺ 876.1298. Found: 876.1283.

5.1.5.21. (S)-N-(1-(4-chloro-3-nitrophenylsulfonamido)-3-(1*H*-indol-3-yl)-1-oxopropan-2-yl)-1-((4'-methoxy-[1,1'-biphenyl]-4-yl)methyl)-1*H*-indole-3-carboxamide(30)

Yellow solid. Yield 33%, mp: 148-150°C. ¹H-NMR(400 MHz, DMSO-*d*₆), δ 12.91 (brs, 1H), 10.85 (s, 1H), 8.54 (s, 1H), 8.19 (s, 1H), 8.12 (dd, *J*₁ = 8.0 Hz, *J*₂ = 4.0 Hz, 2H), 7.97 (dd, *J*₁ = 8.0 Hz, *J*₂ = 4.0 Hz, 2H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.62-7.48 (m, 5H), 7.32-6.94 (m, 10H), 5.53-5.44 (m, 2H), 4.74-4.68 (m, 1H), 3.78 (s, 3H), 3.20 (dd, *J*₁ = 12.0 Hz, *J*₂ = 4.0 Hz, 1H), 3.07 (dd, *J*₁ = 12.0 Hz, *J*₂ = 8.0 Hz, 1H). ¹³C-NMR (100 MHz, DMSO-*d*₆), δ 173.02, 164.78, 159.44, 147.47, 139.74, 136.59, 136.46, 136.26, 133.44, 132.64, 132.50, 132.24, 131.15, 128.20, 127.56, 127.01, 126.94, 125.50, 124.52, 122.72, 121.64, 121.64, 121.42, 121.39, 118.98, 118.75, 114.84, 111.81, 111.14, 109.76, 109.64, 55.64, 54.76, 49.64, 27.01. HRMS (AP-ESI) *m/z* Calcd for C₄₀H₃₂ClN₅O₇S [M-H]⁺ 760.1633. Found: 760.1612.

5.1.5.22. (S)-N-(3-(1*H*-indol-3-yl)-1-(4-methylphenylsulfonamido)-1-oxopropan-2-yl)-1-((4'-methoxy-[1,1'-biphenyl]-4-yl)methyl)-1*H*-indole-3-carboxamide(31)

White solid. Yield 22%, mp: 138-140°C. ¹H-NMR(400 MHz, DMSO-*d*₆), δ 12.47 (s, 1H), 10.81 (s, 1H), 8.26 (s, 1H), 7.99-7.96 (m, 1H), 7.81 (d, *J* = 8.0 Hz, 2H), 7.74 (d, *J* = 8.0 Hz, 2H), 7.59-7.54 (m, 5H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.32-7.26 (m, 3H), 7.17-6.97 (m, 7H), 5.52-5.43 (m, 2H), 4.79-4.74 (m, 1H), 3.79 (s, 3H), 3.16 (dd, *J*₁ = 16.0 Hz, *J*₂ = 4.0 Hz, 1H), 3.01 (dd, *J*₁ = 16.0 Hz, *J*₂ = 8.0 Hz, 1H), 2.38 (s, 3H). ¹³C-NMR (100 MHz, DMSO-*d*₆), δ 172.18, 164.63, 159.43, 144.59, 139.73, 136.95, 136.59, 136.51, 136.27, 132.50, 132.16, 129.94, 128.20, 127.93, 127.65,

126.93, 124.43, 122.69, 121.62, 121.40, 121.31, 119.15, 118.73, 114.84, 111.76, 111.14, 109.95, 55.63, 54.34, 49.61, 27.24, 21.53. HRMS (AP-ESI) m/z Calcd for $C_{41}H_{36}N_4O_5S$ $[M-H]^-$ 695.2328. Found: 695.2312.

5.1.5.23. (S)-1-((4'-chloro-[1,1'-biphenyl]-4-yl)methyl)-N-(4-methyl-1-oxo-1-(phenylsulfonamido)pentan-2-yl)-1H-indole-3-carboxamide(32)

White solid. Yield 8%, mp: 200-203°C. 1H -NMR(400 MHz, DMSO- d_6), δ 12.39 (s, 1H), 8.26 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.72-7.49 (m, 10H), 7.34 (d, J = 8.0 Hz, 2H), 7.21-7.13 (m, 2H), 4.54-4.48 (m, 1H), 1.64-1.38 (m, 2H), 0.90 (dd, J_1 = 16.0 Hz, J_2 = 8.0 Hz, 6H). ^{13}C -NMR (100 MHz, DMSO- d_6), δ 172.94, 164.81, 139.87, 138.93, 138.67, 137.56, 136.63, 134.04, 132.88, 132.19, 129.51, 129.34, 128.87, 128.30, 127.82, 127.44, 127.19, 122.75, 121.80, 121.39, 111.11, 109.97, 52.04, 49.57, 24.83, 23.44, 21.65. HRMS (AP-ESI) m/z Calcd for $C_{34}H_{32}ClN_3O_4S$ $[M-H]^-$ 612.1724. Found: 612.1734.

5.1.6. (S)-2-(1-((4'-methoxy-[1,1'-biphenyl]-4-yl)methyl)-1H-indole-3-carboxamido)-3-phenylpropanoic acid(34)

Compound **33** (0.35 g, 0.7 mmol) was dissolved in 10 ml THF, potassiumhydroxide (0.11 g, 4 mmol), distilled water (10 mL) and methanol (5 mL) was added and the final mixed solution was refluxed for 8 hours. After reaction, the solvent of the reaction were removed and the reaction mixture was basified to PH 1 with 5M hydrochloric acid, then, the generated white solid was filtered from the solution to obtain target compound **33**. White solid. Yield 57%, mp: 152-154°C. 1H -NMR(400 MHz, DMSO- d_6), δ 12.68 (s, 1H), 8.23 (s, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.60-7.56 (m, 5H), 7.38-7.10 (m, 9H), 7.02 (d, J = 12.0 Hz, 2H), 5.55-5.33 (m, 2H), 4.68-4.63 (m, 1H), 3.78 (s, 3H), 3.20 (dd, J_1 = 12.0 Hz, J_2 = 4.0 Hz, 1H), 3.08 (dd, J_1 = 12.0 Hz, J_2 = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO- d_6), δ 174.18, 164.63, 159.43, 139.73, 138.71, 136.63, 136.34, 132.50, 131.95, 129.58, 128.65, 128.25, 128.20, 127.09, 126.93, 126.79, 114.84, 111.14, 110.28, 55.64, 53.99, 49.61, 37.11. HRMS (AP-ESI) m/z Calcd for $C_{32}H_{28}N_2O_4$ $[M-H]^-$ 503.1971. Found: 503.1967.

5.2 Fluorescence polarization(FP)-based binding assay for measuring Bcl-2 Protein

A 26-residue Bid-BH3 peptide (QEDIIRNIARHLAQVGDSMDRSIPPG) derived from its BH3 domain and labeled at the N-terminus with 5-carboxyfluorescein succinimidyl ester (FAM) as a fluorescence labeled peptide (5-FAM-QEDIIRNIARHLAQVGDSMDRSIPPG). A large number of experiments determined that the labeled Bid-BH3 peptide can bind to Bcl-2 protein and the k_d value of it is 58 nM.²⁵

For the competitive binding experiments, these tested compounds and Bcl-2 protein were pre-cultured in the assay buffer (PBS assay solution) for 30 min at room temperature. After the 5-FAM-Bid-BH3 peptide was added and incubated for 20 min. The final volume of the solution was 200 μ L. Finally, the total solutions were moved into black, Corning 384-well, flat-bottom plates (Corning Inc.) with three wells per sample and 60 μ L per well. Under the condition of an excitation wavelength (485 nm) and an emission wavelength (535 nm), the polarization values (milipolarization units) were monitored by the Tecan GENios-Pro Injector Reader (Tecan Group Ltd). In the binding experiments, the indole-based derivatives were prepared in dimethylsulfoxide (DMSO) at seven concentrations(1 nM, 10 nM, 100 nM, 1 μ M, 10 μ M, 50 μ M, 100 μ M). The

final concentrations of 5-FAM-Bid-BH3 peptide and Bcl-2 protein were 10nM and 290nM, respectively.

The FP-based binding assay for Bcl-X_L was same to that for Bcl-2 except that the final concentration of Bcl-X_L was 125 nM. The FP-based binding assay for Mcl-1 was parallel to the assay for Bcl-2 except that the final concentration of Mcl-1 was 105 nM.

All the FP-based assays were tested in triplicate and the K_i value of all compounds were calculated by the equation developed by Wang's team.

5.3. MTT Assay

The antiproliferative activities of the three representative target compounds were measured by using the MTT (3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium bromide) assay. Briefly, the three cancer cells (MDA-MB-231, PC-3 and K562) were cultured in RPMI1640 medium, which contains 10% FBS, in humidified incubator (37 °C, 5% CO₂). After the cell lines were seeded in 96-well plates with a density of 4000 cells per well and incubated for 12 h, then the growing cells were mixed with the indicated compounds at 5 μM, 10 μM, 20 μM, 40 μM and 80 μM for 48 h. Then 15 μL MTT solution (5 mg/mL in PBS) was added into every well and the plates were incubated for a further 4 h. Finally, 150 μL DMSO was added into every well and mixed with the formed formazan for 10-15 min and the final optical density was monitored by a microtiter-plate reader at 570 nm.

5.4. Docking study

By Surflex-Dock program, the molecular of **17** docking was performed. The Gasteiger-Hückel charges were added to the structure of compound **17** and other parameters were set at default values. The Bcl-2 protein and Mcl-1 protein were down loaded from the Protein Data Bank (PDB code: 2O2F and 2PQK).

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