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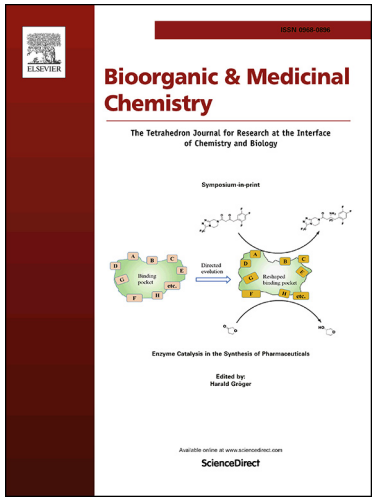


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# Design, synthesis and preliminary bioactivity studies of indomethacin derivatives as Bcl-2/Mcl-1 dual inhibitors

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**Abstract**

Bcl-2 family proteins, which divides into pro-apoptosis proteins and anti-apoptosis proteins, are involved in cell apoptosis progression. As numerous studies illustrated, targeting Bcl-2 family proteins is more and more attractive and practicable to cancer treatment. In this work, we designed and synthesized a series of indomethacin derivatives as new inhibitors for Bcl-2 family proteins. Our results of binding assay to Bcl-2 proteins, MTT assay and apoptotic assay indicated that some compounds had potent binding affinity to Bcl-2/Mcl-1 but not Bcl-$X_L$. Furthermore, compound **8j** showed improved anti-proliferative activity than known Bcl-2 inhibitor WL-276.

Key words: **apoptosis, anticancer, Bcl-2/Mcl-1 dual inhibitor, indomethacin derivatives.**

## 1. Introduction

Cell apoptosis is a process of programmed cell death remove the damaged or unwanted cells, which is strictly controlled by multi-genes. It should be mentioned that one of the causes of tumorigenesis and tumor metastasis is insufficient apoptosis.[1,2] Nowadays, regulating cell apoptosis has become a promising strategy for treatment of cancer.[3] Bcl-2 family of proteins are critical regulators of cell apoptosis, which divides into anti-apoptosis proteins and pro-apoptosis proteins. Anti-apoptosis proteins, including Bcl-2, Bcl-$X_L$, Bcl-W, Mcl-1, and A1, share the BH 1-4 domains. On the other hand, pro-apoptosis proteins can be subdivided into BH3-only proteins and BH1-3 proteins. BH3-only proteins, whose BH3 region is the only homology, include Bad, Bim, Bid, Puma, Noxa, Bmf, Hrk/DP5, Bik/Blk, Beclin-1 and Mule. BH1-3 proteins, which are multi-region proteins, include Bax, Bak and Bok.[4,5] In most cancer cells, the reason why there is resistance to apoptosis is the high expression of anti-apoptosis Bcl-2 proteins.[2,6] Therefore, the inhibition of anti-apoptosis Bcl-2 proteins expression is able to overcome the drug resistance to conventional anticancer agents.

Heretofore, varieties of small molecule inhibitors have been reported for Bcl-2 family proteins (Fig. 1). For example, **WL-276** is a Bcl-2 protein inhibitor, which can overcome the drug resistance due to its pan-inhibition of Bcl-2 family protein.[7] **Obatoclax** (**GX015-070**, pan Bcl-2 family inhibitor) is on phase II clinical trials for previously untreated Acute Myeloid Leukemia (AML).[8] **Navitoclax** (**ABT-263**) is a dual Bcl-$X_L$/Bcl-2 inhibitor which was banned because of transient thrombocytopenia with the inhibition of Bcl-$X_L$, despite the promising phase I/II clinical trial data for various cancers.[3,9] Based on **Navitoclax**, **Venetoclax** (**ABT-199**, Bcl-2-selective inhibitor), which has been approved by FDA for treatment of chronic lymphocytic leukemia (CLL) who harbor a 17p chromosomal deletion, was designed to avoid the side effects.[10–12] Due to the side effects of

Bcl-$X_L$ inhibition and the up-regulation of Mcl-1 and Bcl-$X_L$, the drug resistance to ABT-199 emerged.[13] Therefore, developing a dual inhibitor of Bcl-2 and Mcl-1 is fundamental.

Screening of our "in-house" compound library lead to the identification of compound **4a** (Fig. 2), which shows 94% inhibition against Bcl-2 protein at the concentration of 100μM. Target compounds were designed according to our previous study on the WL-276 derivatives. According previous SAR results, we found that the benzyl group is essential for the inhibitory activities of WL-276 derivatives.[14–16] Thus, we designed new indomethacin derivatives by introducing different side chains ($R_1$) and different substituted benzene sulfonamide ($R_2$) (Fig. 2). In this paper, we will describe the synthesis and the preliminary biological evaluations of these new compounds.

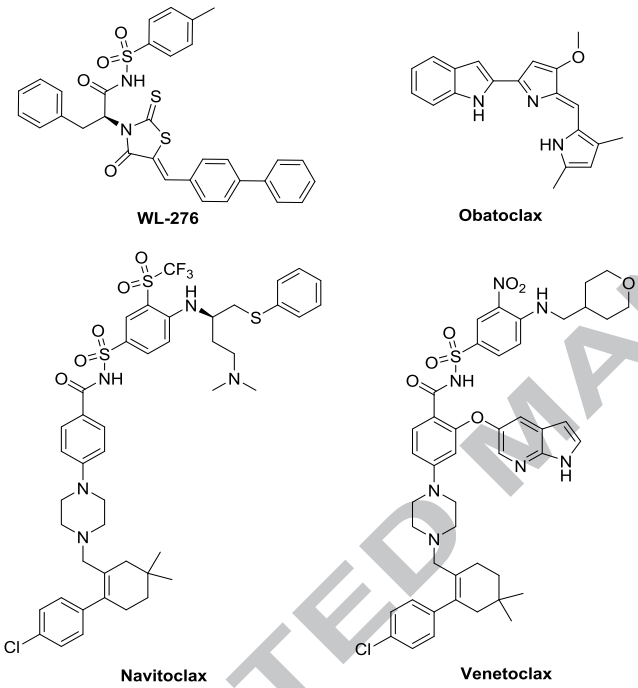



**Fig. 1.** Representative small-molecule inhibitors of anti-apoptotic Bcl-2 proteins.

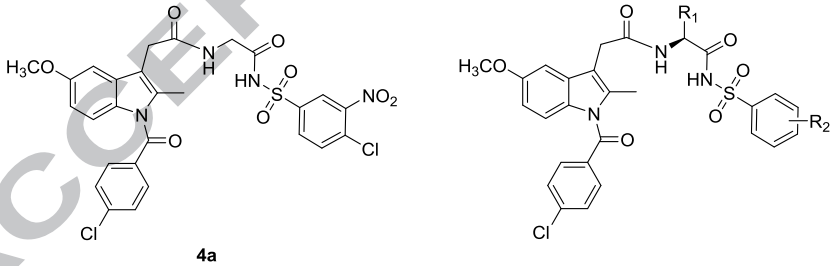
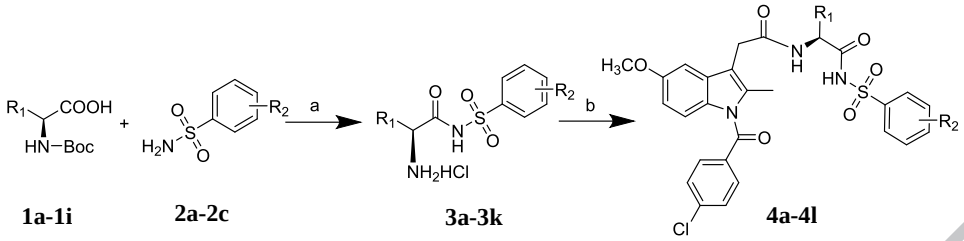



**Fig. 2.** The structure of lead compound **4a** and newly designed indomethacin derivatives.

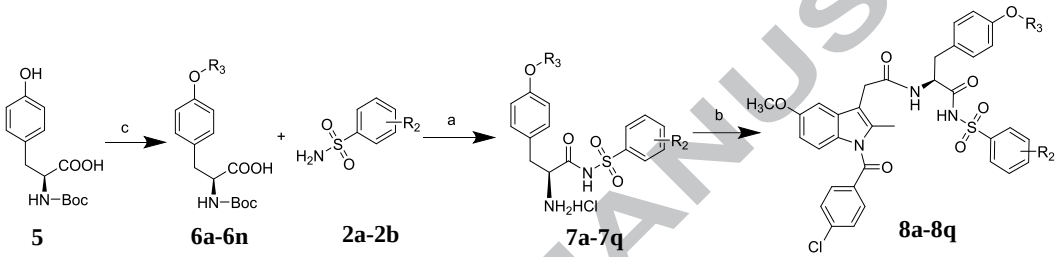
## 2.Chemistry

The synthesis of scaffolds **4** and **8** are listed in **scheme 1** and **2.**[15,17] Briefly, the condensation reaction of N-Boc-amino acid **1a-1i** and benzene sulfonamide derivatives **2a-2c** was performed to obtain the intermediates **3a-3k**, then the Boc group was deprotected in acid condition. Finally

compounds **4a-4l** were gained through coupling reaction. The intermediates **6a-6n** were prepared by Williams reaction. Compounds **8a-8q** were obtained by the same method as what **4a-4l** were prepared.




**Scheme 1.** Synthesis of target compounds **4a-4l**. Reagents and conditions: (a) benzene sulfonamide derivatives, isobutyl chloroformate, NMM, NaH, 0℃ to rt, overnight, ethyl acetate saturated with HCl, rt, overnight; (b) Indomethacin, TBTU, DIEA, $CH_2Cl_2$, 0℃to rt, 8 h.




**Scheme 2.** Synthesis of target compounds **8a-8q**. Reagents and conditions: (a) benzene sulfonamide derivatives, isobutyl chloroformate, NMM, NaH, 0℃ to rt, overnight, ethyl acetate saturated with HCl, rt, overnight; (b) Indomethacin, TBTU, DIEA, $CH_2Cl_2$, 0℃to rt, 8 h. (c) Boc-Tyrosine, Iodide or benzyl bromides, $K_2CO_3$, DMF, 90℃, 4h. NaOH(aq) rt ,6h.

### 3.Results and discussion

We synthesized twenty-nine new indomethacin derivatives. The fluorescence polarization assay (FPA) was performed to evaluate the binding affinities of these target compounds to Bcl-2 protein. Results were listed in **Table 1** as $K_i$ values.

For compounds **4a-4l,** most of them exhibited low binding affinity or even no activity, but surprisingly, compounds **4k** ($K_i$=0.59±0.14μM) showed good binding affinity. This result suggested that the binding affinity gets better when the benzyl group was introduced into $\mathbf{R_1}$. In order to obtain more potent compounds, benzyl derivatives were introduced into $\mathbf{R_1}$ to form compounds **8a-8q**. Compared to **8a-8d**, compounds **8e-8q**, which contained two benzyl group, showed more favorable inhibitory activity and even 2-3 times better than WL-276 and 5-6 times better than UMI-77. For the substitution in the benzyl moiety ($\mathbf{R_3}$), the meta-substitution showed more potent than para- and ortho-substitution (*e.g.* **8l** *vs* **8m** *vs* **8n**). In addition, the introduction of electron withdrawing group or electron donating group did not show obvious impact on the inhibitory activity (*e.g.* **8h** *vs* **8m**). As for the impact of the substitution in benzene sulfonamide moiety ($\mathbf{R_2}$), the 3-$NO_2$-4-Cl substituted exerted higher potency than 4-Br substituted (*e.g.* **8o** *vs* **8p**).

In order to figure out the way that these indomethacin derivatives interact with Bcl-2 protein and, we performed a docking study of **8h**($K_i$=0.43±0.03μM) to Bcl-2 protein (PDB code: 4MAN) by Surflex-Dock software **(Fig. 3A)**. According to our docking result of compound **8h (Fig. 3A)**, the 4-chlorobenzene group of **8h** occupied the p2 pocket in Bcl-2 protein, and the 3-methyl-benzyloxy

group of **8h** occupied the p3 pocket. As the docking result indicates, compound **8h** could bind properly in the active site of Bcl-2 protein **(Fig. 3B)**. The sulfonyl group of compound **8h** could form a hydrogen bond with Arg140 and Arg143 and the nitro-group could form a hydrogen bond with Gly142. The 3-methyl-benzyl group could interact with Tyr199.

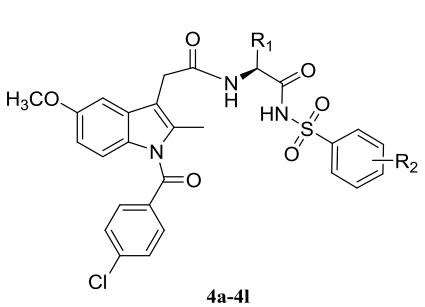
Furthermore, we choose five compounds to evaluate their binding affinities on Bcl-$X_L$ and Mcl-1, aiming to confirm if these compounds could also bind to other anti-apoptotic Bcl-2 family proteins. As the result depicts in **Table 2**, the five compounds had similar binding affinities on Mcl-1 as on Bcl-2, but not on Bcl-$X_L$. It is promising to avoid the transient thrombocytopenia which resulted by the inhibition of Bcl-$X_L$. Based on these data, these compounds can serve as new Bcl-2/Mcl-1 dual inhibitors.

Compounds **8h**, **8i**, **8j**, **8k**, **8l** and **8m** were selected to evaluate their anti-proliferative activities in vitro because of their good inhibition on both Bcl-2 and Mcl-1. Jurkat (Acute T-cell leukemia cell) and K562 (chronic myelogenous leukemia cell), which belong to leukemia cancer cell line, showed high levels of Bcl-2 and Mcl-1 expression and PC-3 (prostatic cancer cell), which belongs to prostate cancer cell line, showed high level of Mcl-1 expression but low level of Bcl-2 expression.[19–22] Then Jurkat, K562 and PC-3 were chosen to be evaluated by MTT assay. As the MTT assay results (**Table 3)** illustrate, compared to WL-276, the anti-proliferative activities to the three tumor cell lines of the most compounds in test are improved. Compound **8j** displayed most potent anti-proliferative activities in PC-3, Jurkat and K562 cell lines. Especially, the anti-proliferative activities to Jurkat cell lines is 2-fold better than WL-276. As shown in **Table 3** and **Figure 4**, the most active compound **8j** (Bcl-2 $K_i$=0.44±0.02μM and Mcl-1 $K_i$=0.44±0.03μM), could significantly inhibit the proliferation and induce apoptosis of PC-3, Jurkat and K562. The good anti-tumor activity of 8j may due to the high expression of Bcl-2 and Mcl-1 protein in these cell lines.

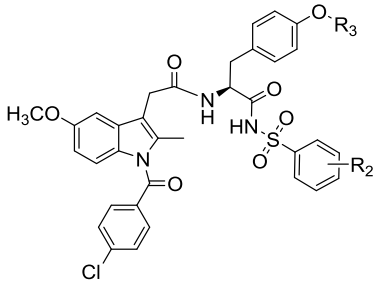
To evaluate the ability to induce apoptosis in Jurkat cell line of compound **8j**, annexin-V and propidium iodide (PI) double staining was performed by flow cytometry (**Fig.4**). As the depicted, it is dose-dependent for compound **8j** to induce apoptosis. Besides, treating Jurkat cell line for 20 μM and 40 μM of **8j** for 48 h leads 43.1% and 56.8% of cell apoptosis (early + late) respectively, compared to 13.3% of cell apoptosis in DMSO control. Compound **8j** showed better ability to induce apoptosis in Jurkat cell line than WL-276, which leads 29.1% and 34.2% of cell apoptosis under 20 μM and 40 μM of WL-276 for 48 h.

**Table 1**

The structures and Bcl-2 inhibitory activities of **4a-4l**, **8a-8q.**




**4a-4l**

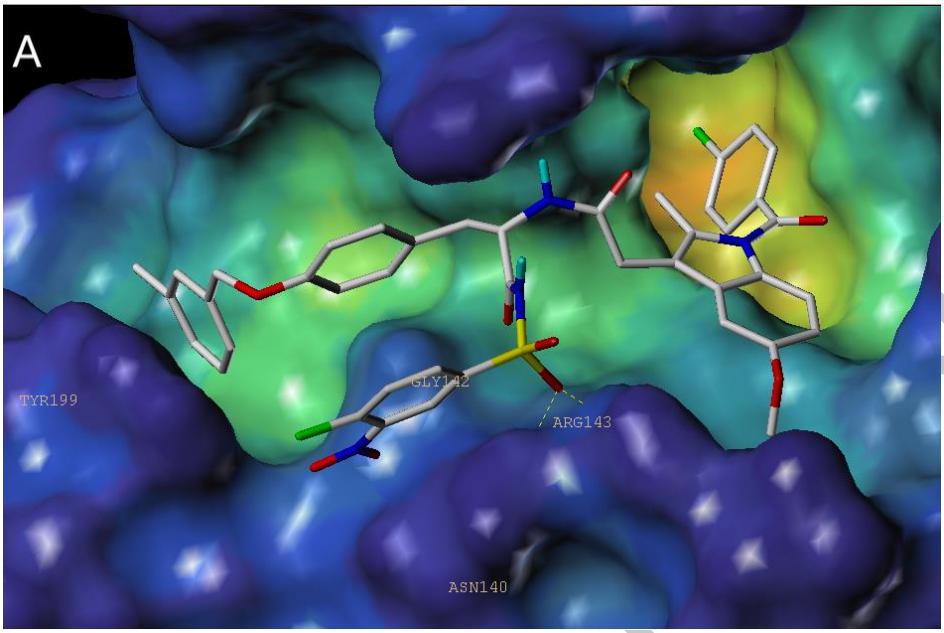



**8a-8q**

| Compound | $R_1$ | $R_2$ | $R_3$ | $K_i$[a](μM) |
|---|---|---|---|---|
| **4a** | H | 3-$NO_2$-4-Cl | — | N.A.[b] |

| | | | | |
|---|---|---|---|---|
| **4b** | H | 4-Br | — | N.A. |
| **4c** | Me | 3-$NO_2$-4-Cl | — | N.A. |
| **4d** | *i*-Pr | 3-$NO_2$-4-Cl | — | N.A. |
| **4e** | *s*-Bu | 3-$NO_2$-4-Cl | — | 4.9±0.4 |
| **4f** | *i*-Bu | 3-$NO_2$-4-Cl | — | ＞5 |
| **4g** | $CH_3S$-$(CH_2)_2$- | 3-$NO_2$-4-Cl | — | ＞5 |
| **4h** | Bn | H | — | N.A. |
| **4i** | Bn | 4-Br | — | ＞5 |
| **4j** | Bn | 3-$NO_2$-4-Cl | — | ＞5 |
| **4k** | 4-Br-Bn- | 3-$NO_2$-4-Cl | — | 0.59±0.14 |
| **4l** | Indolyl-3-$CH_2$- | 3-$NO_2$-4-Cl | — | ＞5 |
| **8a** | — | 4-Br | $CH_3$- | ＞5 |
| **8b** | — | 3-$NO_2$-4-Cl | $CH_3$- | ＞5 |
| **8c** | — | 3-$NO_2$-4-Cl | *i*-Pr- | ＞5 |
| **8d** | — | 3-$NO_2$-4-Cl | *n*-Pr- | 1.3±0.10 |
| **8e** | — | 4-Br | Bn- | 0.53±0.10 |
| **8f** | — | 3-$NO_2$-4-Cl | Bn- | 0.52±0.05 |
| **8g** | — | 3-$NO_2$-4-Cl | 4-$CH_3$-Bn- | 0.56±0.06 |
| **8h** | — | 3-$NO_2$-4-Cl | 3-$CH_3$-Bn- | 0.43±0.03 |
| **8i** | — | 3-$NO_2$-4-Cl | 3-$CH_3O$-Bn- | 0.48±0.04 |
| **8j** | — | 3-$NO_2$-4-Cl | 4-Br-Bn- | 0.44±0.02 |
| **8k** | — | 3-$NO_2$-4-Cl | 2-Br-Bn- | 0.45±0.01 |
| **8l** | — | 3-$NO_2$-4-Cl | 4-CN-Bn- | 0.47±0.04 |
| **8m** | — | 3-$NO_2$-4-Cl | 3-CN-Bn- | 0.44±0.06 |
| **8n** | — | 3-$NO_2$-4-Cl | 2-CN-Bn- | 0.67±0.08 |
| **8o** | — | 4-Br | 4-$NO_2$-Bn- | 0.63±0.15 |
| **8p** | — | 3-$NO_2$-4-Cl | 4-$NO_2$-Bn- | 0.51±0.07 |
| **8q** | — | 3-$NO_2$-4-Cl | Naph-2-$CH_2$- | 0.56±0.01 |
| WL-276 | | | | 1.5±0.25 |
| UMI-77 | | | | 2.90±0.10 |

[a] Each value was expressed with standard deviations and reproduced in three independent assays.
[b] No activity.



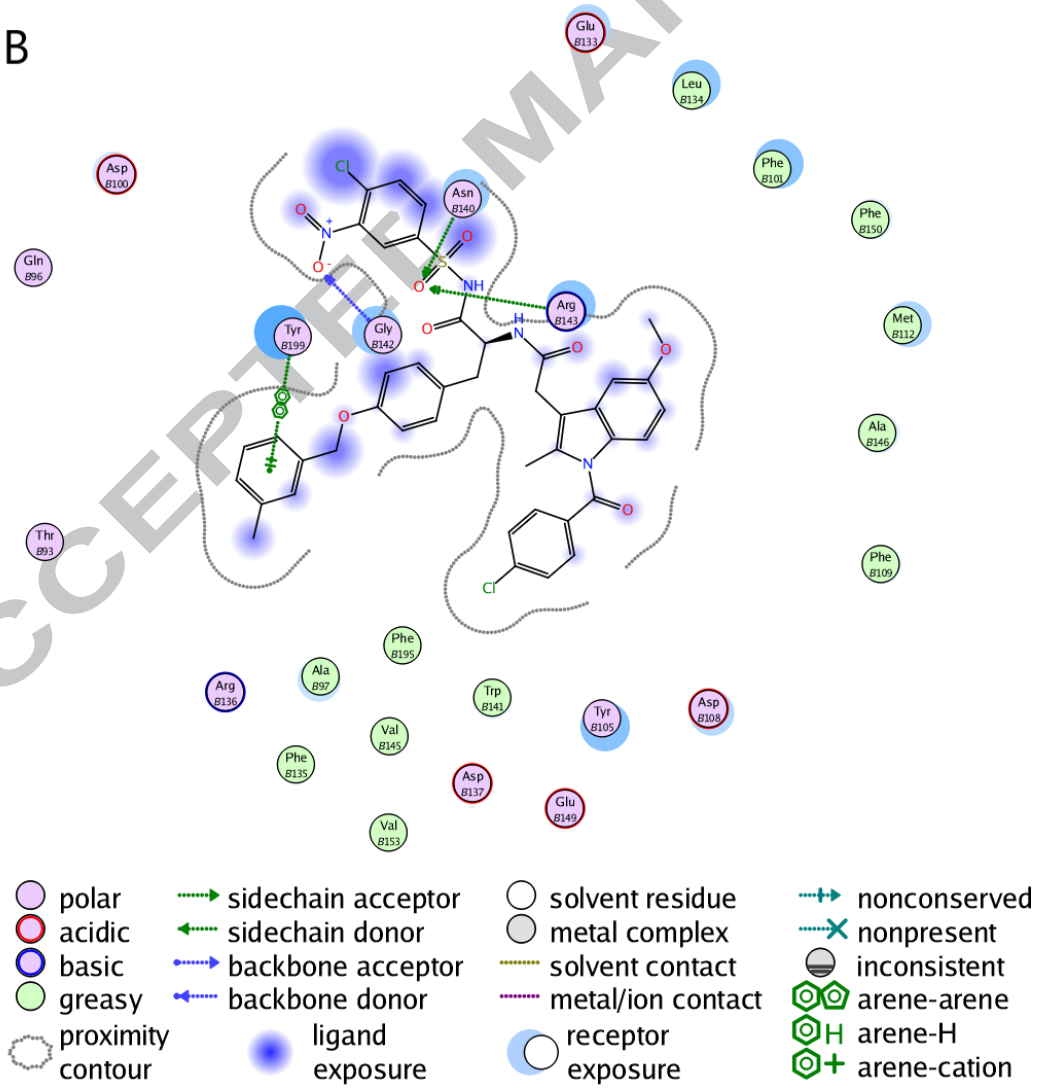



**Fig. 3**. Docking results of **8h** with Bcl-2 protein. (A) Docked compound **8h** with Bcl-2 protein (PDB code: 4MAN). (B) The interactions between compound **8h** and Bcl-2 protein.

**Table 2**

The binding affinities of representative compounds against three Bcl-2 proteins.

| Compound | Bcl-2 $K_i$ [a](μM) | Bcl-$X_L$ $K_i$[a] (μM) | Mcl-1 $K_i$ [a](μM) |
|---|---|---|---|
| **8h** | 0.43±0.03 | N.A.[b] | 0.49±0.08 |
| **8j** | 0.44±0.02 | N.A.[b] | 0.44±0.03 |
| **8k** | 0.45±0.01 | N.A.[b] | 0.52±0.11 |
| **8l** | 0.47±0.04 | N.A.[b] | 0.53 |
| **8m** | 0.44±0.06 | N.A.[b] | 0.48±0.11 |
| WL-276 | 1.50±0.25 | 1.30±0.15 | 1.10±0.02 |
| UMI-77 | 2.90±0.10 | 7.85±2.15 | 0.18±0.11 |

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

[b] No activity.

**Table 3**

Antiproliferative activities of representative compounds.

| Compound | $IC_{50}$ (μM)[a] | | |
|---|---|---|---|
| | PC-3 | Jurkat | K562 |
| **8h** | 28.58±4.16 | 30.32±8.34 | 38.82±0.53 |
| **8i** | 31.70±4.08 | 35.92±0.13 | 36.18±1.99 |
| **8j** | 25.79±0.58 | 24.69±0.76 | 35.90±1.69 |
| **8k** | 36.08±3.18 | 33.98±1.74 | 41.46±2.87 |
| **8l** | 39.31±0.69 | 83.48±0.92 | 49.25±5.78 |
| **8m** | 38.23±0.36 | 55.25±3.39 | 51.62±1.31 |
| WL-276 | 39.62±1.60 | 31.84±4.98 | 42.26±6.70 |

[a] Inhibitory data are means of no fewer than three independent determinations and expressed with standard deviations.

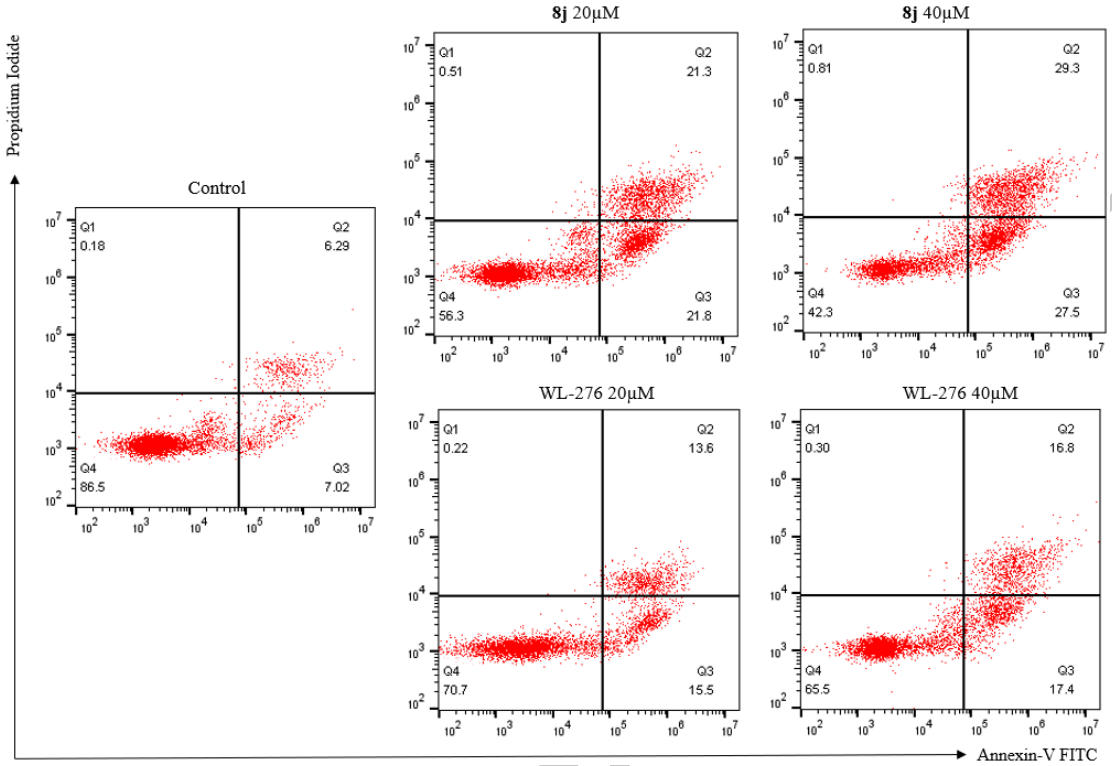



**Fig.4**. Analysis of apoptosis induced by **8j** in the Jurkat cell line.

## 4.Conclusion

In summary, we synthesized a series of new indomethacin derivatives as Bcl-2/Mcl-1 dual inhibitors. Based on the result of binding assay, compounds **8h**, **8i**, **8j**, **8l** and **8m** exhibited favorable inhibitions on Bcl-2/Mcl-1 proteins and no inhibition on Bcl-$X_L$. Compared to WL-276, compound **8j** displayed a better antiproliferative activity. In the future, these new inhibitors can serve as lead compound to develop more potent Bcl-2/Mcl-1 dual inhibitors.

## 5.Experimental Section

### *5.1. General chemistry information*

All solvents and starting materials were purchased without further purification, only if otherwise mentioned. Reactions were all detected by thin-layer chromatography (TLC) on 0.25 mm silica gel plates (60 GF-254) and observed by the UV light (254 nM or 365 nM) or iodine vapor. Most target compounds were purified by silica gel chromatography (200–300mesh) or recrystallization. Proton NMR ($^1$H-NMR) and carbon NMR ($^{13}$C-NMR) spectrums were recorded by a Brucker DRX spectrometer (400 MHz) in the indicated solvent, which using TMS as an internal standard. δ (chemical shift) were given in parts per million and *J* were expressed in Hertz. High-resolution mass spectral (HRMS) was conducted on an Agilent 6510 Quadrupole Time-of-Flight LC/MS deliver and the data were showed as m/z. To measure the melting points, the RY-1 electrothermal melting point apparatus was used and uncorrected.

### *5.1.1. 2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)acetamide hydrochloride* **(3a)**

To a solution of (tert-Butoxycarbonyl)-glycine (1.1 g, 6.5mmol) in 30 mL anhydrous THF at 0℃,

NMM (0.8 ml, 7.1 mmol) and isobutylchloroformate (0.9 ml, 7.1 mmol) were added. Then after stirred for 1 h, the solution of 4-chloro-3-nitrobenzenesulfonamide (1.7 g, 7.1 mmol) in THF, which was pretreated with NaH (0.65 g, 16.1 mmol) for 4 h at room temperature aforehand, was added in drops. This reaction was recovered slowly to room temperature and stirred overnight. Then the solvent was evaporated in vacuum. The resulting residue was neutralized by 1M HCl solution and extracted with EtOAc, then the organic phase was washed by 0.5 M citric acid solution and brine. After dried by $MgSO_4$ and concentrated, use EtOAc which was saturated with HCl gas to dissolve the obtained oil and stirred at room temperature overnight. Then the generated precipitate was filtered and dried. white powder. Yield:13%, mp: 220-223℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.56 (d, *J* = 2.2 Hz, 1H), 8.27 – 8.17 (m, 4H), 8.07 (d, *J* = 8.5 Hz, 1H), 3.68 (t, *J* = 5.5 Hz, 2H).

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

*5.1.1.1. 2-amino-N-((4-bromophenyl)sulfonyl)acetamide hydrochloride* **(3b)** white solid. Yield:85%, mp: 208-211℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.18 (s, 3H), 7.88 (s, 4H), 3.69 (s, 2H).

*5.1.1.2.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)propanamide hydrochloride* **(3c)** light yellow solid. Yield:73%, mp: 218-220℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.55 (d, *J* = 2.1 Hz, 1H), 8.27 (s, 3H), 8.19 (dd, *J* = 8.5, 2.1 Hz, 1H), 8.05 (d, *J* = 8.5 Hz, 1H), 3.90 – 3.82 (m, 1H), 1.35 (d, *J* = 7.1 Hz, 3H).

*5.1.1.3.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-methylbutanamide hydrochloride* **(3d)** white solid. Yield:46%, mp: 234-236℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.51 (d, *J* = 1.7 Hz, 1H), 8.15 (dd, *J* = 8.5, 1.9 Hz, 1H), 8.11 – 8.01 (m, 3H), 7.99 (d, *J* = 8.5 Hz, 1H), 3.61 – 3.51 (m, 1H), 2.15 (dq, *J* = 13.7, 6.6 Hz, 1H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.82 (d, *J* = 6.9 Hz, 3H).

*5.1.1.4.(2S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-methylpentanamide hydrochloride* **(3e)** white solid. Yield:33%, mp: 217-223℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.56 (d, *J* = 2.1 Hz, 1H), 8.32 (d, *J* = 5.6 Hz, 3H), 8.20 (dd, *J* = 8.5, 2.2 Hz, 1H), 8.05 (d, *J* = 8.5 Hz, 1H), 3.75 (t, *J* = 5.3 Hz, 1H), 1.94 – 1.82 (m, 1H), 1.34 (dtd, *J* = 14.7, 7.4, 4.2 Hz, 1H), 1.17 – 1.03 (m, 1H), 0.85 (d, *J* = 6.9 Hz, 3H), 0.81 (t, *J* = 7.4 Hz, 3H).

*5.1.1.5.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-4-methylpentanamide hydrochloride* **(3f)** white solid. Yield:44%, mp: 197-202℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.45 (d, *J* = 4 Hz, 1H), 8.11 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.96 – 7.94 (m, 4H), 3.58 (s, 1H), 1.64 (dt, *J* = 12.8, 6.4 Hz, 1H), 1.60 – 1.52 (m, 1H), 1.45 (ddd, *J* = 13.8, 8.3, 6.0 Hz, 1H), 0.84 (d, *J* = 6.3 Hz, 6H).

*5.1.1.6.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-4-(methylthio)butanamide hydrochloride* **(3g)** white solid. Yield:77%, mp: 204-208℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.52 (d, *J* = 2.0 Hz, 1H), 8.30 (s, 3H), 8.17 (dd, *J* = 8.5, 2.1 Hz, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 3.91 – 3.80 (m, 1H), 2.50 – 2.43 (m, 1H), 2.38 (ddd, *J* = 13.5, 9.4, 5.7 Hz, 1H), 2.09 – 1.91 (m, 5H).

*5.1.1.7.(S)-2-amino-3-phenyl-N-(phenylsulfonyl)propanamide hydrochloride* **(3h)** white solid. Yield:11%, mp: 214-217℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.10 (s, 1H), 8.39 (s, 3H), 7.92 (d, *J* = 7.4 Hz, 2H), 7.77 (t, *J* = 7.4 Hz, 1H), 7.65 (t, *J* = 7.7 Hz, 2H), 7.21 (dt, *J* = 14.0, 6.8 Hz, 3H), 7.05 (d,

*J* = 6.8 Hz, 2H), 4.11 (s, 1H), 3.12 – 2.95 (m, 2H).

*5.1.1.8.(S)-2-amino-N-((4-bromophenyl)sulfonyl)-3-phenylpropanamide hydrochloride* **(3i)** white solid. Yield:94%, mp: 222-223℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.44 (s, 3H), 7.85 (q, *J* = 8.7 Hz, 4H), 7.21 (dt, *J* = 14.1, 6.8 Hz, 3H), 7.07 (d, *J* = 7.0 Hz, 2H), 4.14 (s, 1H), 3.07 (qd, *J* = 14.0, 6.4 Hz, 2H).

*5.1.1.9.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-phenylpropanamide hydrochloride* **(3j)** light yellow solid. Yield:97%, mp: 239-240℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.47 (d, *J* = 1.9 Hz, 1H), 8.16 (s, 3H), 8.11 (dd, *J* = 8.5, 2.0 Hz, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 7.19 (q, *J* = 6.0 Hz, 3H), 7.08 (d, *J* = 6.4 Hz, 2H), 4.00 – 3.91 (m, 1H), 3.03 (qd, *J* = 14.1, 6.4 Hz, 2H).

*5.1.1.10.(S)-2-amino-3-(4-bromophenyl)-N-((4-chloro-3-nitrophenyl)sulfonyl)propanamide hydrochloride* **(3k)** white solid. Yield:70%, mp: 212-217℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.51 (d, *J* = 2.0 Hz, 1H), 8.28 (s, 3H), 8.15 (dd, *J* = 8.5, 2.1 Hz, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.38 (d, *J* = 8.3 Hz, 2H), 7.08 (d, *J* = 8.3 Hz, 2H), 4.08 – 4.00 (m, 1H), 3.05 (ddd, *J* = 42.9, 14.1, 6.2 Hz, 2H).

*5.1.1.11(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(1H-indol-3-yl)propanamide hydrochloride* **(3l)** brown solid. Yield:33%, mp: 235-237℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 11.03 (s, 1H), 8.49 (s, 1H), 8.17 (s, 3H), 8.08 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.13 (d, *J* = 2.0 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.93 (t, *J* = 7.5 Hz, 1H), 4.00 (s, 1H), 3.26 (dd, *J* = 14.7, 5.6 Hz, 1H), 3.11 (dd, *J* = 14.8, 7.6 Hz, 1H).

*5.1.2. N-(2-((4-chloro-3-nitrophenyl) sulfonamido)-2-oxoethyl)-2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl) acetamide* **(4a)**

To the solution of intermediate **3a** (0.2 g, 0.51 mmol) in dry dichloromethane (25 mL), ethyldiisopropylamine (0.26 ml, 1.5 mmol) was added in ice bath. After stirred for 10 min, O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU, 0.22 g, 0.67 mmol) was added and stirred for 30 min in 0℃, then the indomethacin (0.22 g, 0.6 mmol) was added and stirred overnight at room temperature. The solvent was removed in vacuum and then dissolved in EtOAc. 0.5 M citric acid and brine were used to wash the organic phase, then it was concentrated to yield yellow oil. Purification by column chromatography (PE/EA = 10:7) to generate compound **4a**, a white powder. Yield:35%, mp: 156-159℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.54 (d, *J* = 2.2 Hz, 1H), 8.26 (t, *J* = 5.8 Hz, 1H), 8.17 (dd, *J* = 8.5, 2.3 Hz, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.66 (dd, *J* = 8.6 Hz, 4H), 7.07 (d, *J* = 2.5 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.70 (dd, *J* = 9.0, 2.6 Hz, 1H), 3.83 (d, *J* = 5.7 Hz, 2H), 3.75 (s, 3H), 3.54 (s, 2H), 2.19 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 170.80, 170.51, 169.47, 168.29, 156.00, 147.68, 139.72, 138.04, 135.68, 134.66, 133.44, 132.75, 131.64, 131.18, 131.09, 130.69, 129.49, 125.45, 114.95, 114.33, 111.95, 102.19, 60.22, 55.81, 49.07, 42.87, 31.15, 21.23, 14.56, 13.79. HRMS (AP-ESI) *m/z* Calcd for $C_{27}H_{22}Cl_2N_4O_8S$ $[M-H]^-$ 631.04572. Found: 631.0456.

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

*5.1.2.1.N-(2-((4-bromophenyl)sulfonamido)-2-oxoethyl)-2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl) acetamide* **(4b)** yellow solid. Yield:57%, mp: 216-219℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.38 (s, 1H), 8.21 (t, *J* = 5.7 Hz, 1H), 7.83 (s, 4H), 7.66 (q, *J* = 8.6 Hz, 4H), 7.07 (d,

*J* = 2.4 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.70 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.79 (d, *J* = 5.8 Hz, 2H), 3.75 (s, 3H), 3.53 (s, 2H), 2.18 (s, 3H). $^{13}$C NMR (100 MHz, DMSO) δ 170.48, 168.90, 168.29, 156.00, 138.91, 138.03, 135.68, 134.66, 132.74, 131.66, 131.19, 130.68, 129.97, 129.49, 128.26, 114.96, 114.34, 111.96, 102.19, 55.83, 42.65, 31.19, 13.83. HRMS (AP-ESI) *m/z* Calcd for $C_{27}H_{23}BrClN_3O_6S$ $[M-H]^-$ 632.0082. Found: 632.0074.

*5.1.2.2.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl) acetamido) propenamide* **(4c)** light yellow solid. Yield:27%, mp: 188-191℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.75 (s, 1H), 8.61 – 8.49 (m, 2H), 8.13 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 7.75 – 7.61 (m, 4H), 7.05 (d, *J* = 2.3 Hz, 1H), 6.92 (d, *J* = 9.0 Hz, 1H), 6.69 (dd, *J* = 9.0, 2.4 Hz, 1H), 4.23 (p, *J* = 7.0 Hz, 1H), 3.74 (s, 3H), 3.51 (q, *J* = 15.4 Hz, 2H), 2.18 (s, 3H), 1.21 (d, *J* = 7.2 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 172.90, 170.07, 168.25, 155.96, 147.58, 139.57, 138.05, 135.54, 134.65, 133.40, 132.54, 131.60, 131.21, 131.10, 130.61, 129.47, 125.40, 114.94, 114.58, 111.91, 102.11, 55.77, 49.41, 30.93, 17.27, 13.77. HRMS (AP-ESI) *m/z* Calcd for $C_{28}H_{24}Cl_2N_4O_8S$ $[M-H]^-$ 645.0614. Found:645.0611.

*5.1.2.3.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-methylbutanamide* **(4d)** yellow solid. Yield:46%, mp: 154-156℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.80 (s, 1H), 8.55 (d, *J* = 2.2 Hz, 1H), 8.41 (d, *J* = 7.9 Hz, 1H), 8.15 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.72 – 7.60 (m, 4H), 7.10 (d, *J* = 2.5 Hz, 1H), 6.92 (d, *J* = 8.9 Hz, 1H), 6.68 (dd, *J* = 9.0, 2.5 Hz, 1H), 4.18 (t, *J* = 7.1 Hz, 1H), 3.67 – 3.50 (m, 2H), 2.20 (s, 3H), 2.00 (d, *J* = 7.6 Hz, 1H), 0.79 (d, *J* = 6.8 Hz, 6H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 172.04, 170.57, 168.26, 155.98, 147.61, 139.62, 138.04, 135.48, 134.68, 133.39, 132.61, 131.58, 131.20, 131.09, 130.59, 129.49, 125.47, 114.94, 112.06, 101.99, 60.21, 58.61, 55.78, 30.84, 30.45, 19.36, 18.24, 13.72. HRMS (AP-ESI) *m/z* Calcd for $C_{30}H_{28}Cl_2N_4O_8S$ $[M-H]^-$ 673.0927. Found:673.0933.

*5.1.2.4.(2S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-methylpentanamide* **(4e)** yellow solid. Yield:10%, mp: 130-134℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.83 (s, 1H), 8.52 (d, *J* = 2.3 Hz, 1H), 8.41 (d, *J* = 7.7 Hz, 1H), 8.13 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 7.70 – 7.60 (m, 4H), 7.08 (d, *J* = 2.6 Hz, 1H), 6.91 (d, *J* = 8.9 Hz, 1H), 6.68 (dd, *J* = 9.0, 2.6 Hz, 1H), 4.17 (t, *J* = 7.4 Hz, 1H), 3.73 (s, 3H), 3.66 – 3.45 (m, 2H), 2.18 (s, 3H), 1.72 (d, *J* = 8.1 Hz, 1H), 1.28 (s, 1H), 1.07 (dq, *J* = 14.8, 7.7 Hz, 1H), 0.73 (dt, *J* = 7.6, 4.0 Hz, 6H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 172.29, 170.50, 168.27, 155.99, 147.60, 138.02, 135.47, 134.70, 133.35, 132.59, 131.58, 131.18, 130.97, 130.59, 129.50, 125.41, 114.95, 114.91, 112.09, 101.98, 57.83, 55.78, 36.63, 31.43, 30.85, 24.69, 22.53, 15.59, 13.71, 11.14. HRMS (AP-ESI) *m/z* Calcd for $C_{31}H_{30}Cl_2N_4O_8S$ $[M-H]^-$ 687.1083. Found:687.1093.

*5.1.2.5.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-4-methylpentanamide* **(4f)** white solid. Yield:13%, mp: 168-174℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.82 (s, 1H), 8.51 (d, *J* = 2.2 Hz, 1H), 8.46 (d, *J* = 7.2 Hz, 1H), 8.13 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.93 (d, *J* = 8.5 Hz, 1H), 7.66 (t, *J* = 6.3 Hz, 4H), 7.05 (d, *J* = 2.5 Hz, 1H), 6.93 (d, *J* = 9.0 Hz, 1H), 6.68 (dd, *J* = 9.0, 2.5 Hz, 1H), 4.26 (s, 1H), 3.73 (s, 3H), 3.59 – 3.44 (m, 2H), 2.17 (s, 3H), 1.58 (p, *J* = 6.6 Hz, 1H), 1.41 (dqd, *J* = 13.7, 9.0, 5.2 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.77 (d, *J* = 6.5 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ172.99, 170.37, 168.27, 155.99, 147.63, 138.02,

135.48, 134.70, 133.40, 132.53, 131.59, 131.18, 131.02, 130.61, 129.49, 125.35, 114.94, 114.71, 112.05, 101.99, 55.77, 52.21, 30.95, 24.75, 23.36, 21.52, 13.72. HRMS (AP-ESI) *m/z* Calcd for $C_{31}H_{30}Cl_2N_4O_8S$ $[M-H]^-$ 687.1083. Found:687.1090.

*5.1.2.6.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-4-(methylthio)butanamide* **(4g)** white solid. Yield:51%, mp: 184-186℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.73(s, 1H), 8.56 – 8.46 (m, 2H), 8.14 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.71 – 7.61 (m, 4H), 7.07 (d, *J* = 2.6 Hz, 1H), 6.93 (d, *J* = 9.0 Hz, 1H), 6.69 (dd, *J* = 9.0, 2.5 Hz, 1H), 4.30 (ddd, *J* = 9.6, 7.3, 4.3 Hz, 1H), 3.74 (s, 3H), 3.57 (dd, *J* = 15.3 Hz, 2H), 2.35 (dt, *J* = 8.7, 5.8 Hz, 2H), 2.18 (s, 3H), 1.95 (s, 3H), 1.74 (dtd, *J* = 14.2, 9.0, 5.6 Hz, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.90, 170.44, 168.27, 156.01, 147.62, 139.47, 138.04, 135.56, 134.67, 133.44, 132.64, 131.60, 131.16, 130.61, 129.50, 125.48, 114.99, 114.59, 112.04, 101.96, 55.81, 53.02, 31.04, 30.84, 29.91, 15.00, 13.78. HRMS (AP-ESI) *m/z* Calcd for $C_{30}H_{28}Cl_2N_4O_8S_2$ $[M-H]^-$ 705.0647. Found:706.0655.

*5.1.2.7.(S)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-phenyl-N-(phenylsulfonyl)propanamide* **(4h)** white solid. Yield:54%, mp: 193-195℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.53 (s, 1H), 8.33 (d, *J* = 7.2 Hz, 1H), 7.90 (d, *J* = 7.5 Hz, 2H), 7.77 – 7.55 (m, 7H), 7.11 (s, 5H), 7.03 (d, *J* = 2.4 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.69 (dd, *J* = 9.0, 2.4 Hz, 1H), 4.50 (td, *J* = 9.5, 4.4 Hz, 1H), 3.73 (s, 3H), 3.52 – 3.41 (m, 2H), 2.91 (dd, *J* = 13.6, 4.3 Hz, 1H), 2.67 (dd, *J* = 13.6, 9.9 Hz, 1H), 2.03 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.13, 170.10, 168.25, 156.01, 139.57, 137.99, 137.15, 135.47, 134.67, 134.18, 131.57, 131.25, 130.57, 129.59, 129.49, 128.43, 127.87, 126.88, 114.89, 114.49, 111.97, 102.16, 55.85, 54.87, 36.90, 30.97, 13.71. HRMS (AP-ESI) *m/z* Calcd for $C_{34}H_{30}ClN_3O_6S$ $[M-H]^-$ 642.1466. Found:642.1481.

*5.1.2.8.(S)-N-((4-bromophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-phenylpropanamide* **(4i)** white solid. Yield:50%, mp: 198-200℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.64 (s, 1H), 8.38 (d, *J* = 7.7 Hz, 1H), 7.81 (s, 4H), 7.63 (s, 4H), 7.12 (s, 5H), 7.03 (d, *J* = 2.2 Hz, 1H), 6.93 (d, *J* = 9.0 Hz, 1H), 6.69 (dd, *J* = 9.0, 2.2 Hz, 1H), 4.49 (td, *J* = 9.1, 4.7 Hz, 1H), 3.74 (s, 3H), 3.53 – 3.41 (m, 2H), 2.92 (dd, *J* = 13.5, 4.4 Hz, 1H), 2.70 (dd, *J* = 13.5, 9.8 Hz, 1H), 2.05 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$). δ 171.29, 170.14, 168.26, 156.00, 138.00, 137.09, 135.50, 134.67, 132.69, 131.57, 131.23, 130.57, 129.93, 129.59, 129.50, 128.42, 126.91, 114.89, 114.48, 111.97, 102.16, 55.85, 54.92, 36.85, 30.95, 13.71. HRMS (AP-ESI) *m/z* Calcd for $C_{34}H_{29}BrClN_3O_6S$ $[M-H]^-$ 722.0553. Found:722.0550.

*5.1.2.9.(S)-N-((4-chloro-3-nitrophenyl) sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-phenylpropanamide* **(4j)** light yellow solid. Yield:9%, mp: 196-200℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.94 (s, 1H), 8.51 (d, *J* = 2.0 Hz, 1H), 8.44 (d, *J* = 7.0 Hz, 1H), 8.11 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.64 (s, 4H), 7.12 (s, 5H), 7.03 (d, *J* = 2.3 Hz, 1H), 6.92 (d, *J* = 9.0 Hz, 1H), 6.69 (dd, *J* = 9.0, 2.4 Hz, 1H), 4.53 – 4.43 (m, 1H), 3.73 (s, 3H), 3.55 – 3.42 (m, 2H), 2.95 (dd, *J* = 13.6, 4.7 Hz, 1H), 2.74 (dd, *J* = 14.0, 9.0 Hz, 1H), 2.07 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$). δ 172.03, 170.18, 168.25, 155.99, 147.54, 139.88, 138.02, 137.08, 135.52, 134.65, 133.34, 132.67, 131.57, 131.21, 130.91, 130.58, 129.61, 129.48, 128.37, 126.87, 125.43, 114.90, 114.50, 111.97, 102.10, 55.81, 55.16, 36.86, 30.94, 13.70. HRMS (AP-ESI) *m/z* Calcd for

$C_{34}H_{30}Cl_2N_4O_8S$ $[M-H]^-$ 721.0927. Found:721.0926.

*5.1.2.10.(S)-3-(4-bromophenyl)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)propenamide* **(4k)** light yellow solid. Yield:30%, mp: 137-141℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.68 (s, 1H), 8.54 (d, *J* = 2.2 Hz, 1H), 8.43 (d, *J* = 7.7 Hz, 1H), 8.13 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.98 (d, *J* = 8.5 Hz, 1H), 7.64 (s, 4H), 7.27 (d, *J* = 7.9 Hz, 2H), 7.08 (d, *J* = 8.0 Hz, 2H), 7.02 (d, *J* = 2.5 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.70 (dd, *J* = 9.0, 2.5 Hz, 1H), 4.47 (td, *J* = 8.7, 4.7 Hz, 1H), 3.74 (s, 3H), 2.94 (dd, *J* = 13.8, 4.5 Hz, 1H), 2.70 (dd, *J* = 13.7, 9.7 Hz, 1H), 2.08 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$). δ 171.60, 170.23, 168.24, 155.98, 147.62, 138.04, 136.52, 135.56, 134.67, 133.42, 132.66, 131.80, 131.57, 131.24, 131.18, 131.11, 130.60, 129.49, 125.48, 120.22, 114.90, 114.41, 111.83, 102.22, 55.84, 54.83, 36.13, 30.95, 19.11, 13.71. HRMS (AP-ESI) *m/z* Calcd for $C_{34}H_{27}BrCl_2N_4O_8S$ $[M-H]^-$ 801.0012. Found:801.0013.

*5.1.2.11.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(1H-indol-3-yl)propenamide* **(4l)** light yellow solid. Yield:37%, mp: 140-144℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.95 (s, 1H), 10.84 (s, 1H), 8.51 – 8.44 (m, 2H), 8.00 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.82 (d, *J* = 8.5 Hz, 1H), 7.68 – 7.60 (m, 5H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.11 – 7.08 (m, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.99 (d, *J* = 2.3 Hz, 1H), 6.96 – 6.88 (m, 2H), 6.67 (dd, *J* = 9.0, 2.4 Hz, 1H), 4.55 (q, *J* = 7.3 Hz, 1H), 3.69 (s, 3H), 3.47 (d, *J* = 16.6 Hz, 3H), 3.38 (q, *J* = 7.0 Hz, 2H), 3.09 (dd, *J* = 14.5, 5.5 Hz, 1H), 2.91 (dd, *J* = 14.5, 8.8 Hz, 1H), 2.05 (s, 3H), 1.09 (t, *J* = 7.0 Hz, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 172.34, 170.27, 168.23, 155.95, 147.41, 139.40, 138.01, 136.40, 135.48, 134.66, 133.37, 132.36, 131.58, 131.24, 131.16, 130.56, 129.48, 127.41, 125.43, 124.50, 121.38, 118.84, 118.70, 114.93, 114.57, 112.06, 111.75, 109.01, 101.92, 55.74, 54.52, 30.85, 27.26, 13.60. HRMS (AP-ESI) *m/z* Calcd for $C_{36}H_{29}Cl_2N_5O_8S$ $[M-H]^-$ 760.1036. Found:760.1044.

*5.1.3.(S)-2-((tert-butoxycarbonyl)amino)-3-(4-methoxyphenyl)propanoic acid* **(6a)**

L – tyrosine (2.8 g, 10 mmol) was dissolved in 50 mL DMF, then $K_2CO_3$(4.15 g, 30 mmol) and iodomethane (1.37 mL, 22mmol) were added in a row. Stirred at room temperature till the reactant was run out, detected by TLC. Then, 150 mLwater was added into the solution and extracted with EtOAc. After dried by $MgSO_4$ and concentrated, the obtained oil was dissolved in 30mL methanol and NaOH(1.3mmol/mL) was added. After stirred for 5 h, the solution was neutralized by 1M HCl solution and extracted with EtOAc. 0.5 M citric acid and brine were used to wash the organic phase, and concentrated to colorless oil. Finally, purification by column chromatography (PE/EA = 10:3) to generate compound **6a**, colorless oil. Yield:13%. $^1$H NMR (400 MHz, Chloroform-d) δ 7.12 (d, *J* = 8.2 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 4.68 – 4.49 (m, 1H), 3.81 (s, 3H), 3.11 (ddd, *J* = 34.8, 13.6, 5.3 Hz, 2H), 1.45 (s, 9H).

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

*5.1.3.1.(S)-2-((tert-butoxycarbonyl)amino)-3-(4-isopropoxyphenyl)propanoic acid* **(6b)** white solid. Yield:27%, mp: 120-124℃. $^1$H NMR (400 MHz, Chloroform-d) δ 7.10 (d, *J* = 8.0 Hz, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 4.55 (tq, *J* = 12.0, 6.2 Hz, 2H), 3.10 (ddd, *J* = 37.7, 13.9, 5.6 Hz, 2H), 1.44 (s, 6H), 1.34 (d, *J* = 6.1 Hz, 9H).

*5.1.3.2.(S)-2-((tert-butoxycarbonyl)amino)-3-(4-propoxyphenyl)propanoic acid* **(6c)** colorless oil. Yield:60%. $^1$H NMR (400 MHz, Chloroform-d) δ 7.08 (d, *J* = 8.1 Hz, 2H), 6.84 (d, *J* = 8.4 Hz, 2H), 4.64 – 4.51 (m, 1H), 3.90 (t, *J* = 6.6 Hz, 2H), 3.24 – 2.93 (m, 2H), 1.79 (h, *J* = 7.1 Hz, 2H), 1.38 (d, *J* = 36.4 Hz, 9H), 1.03 (t, *J* = 7.4 Hz, 3H).

*5.1.3.3.(S)-3-(4-(benzyloxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid* **(6d)** white solid. Yield:34%, mp: 110-113℃. $^1$H NMR (400 MHz, Chloroform-*d*) δ 10.69 (s, 1H), 7.34 (m, 5H), 7.09 (d, *J* = 8.2 Hz, 2H), 6.90 (d, *J* = 8.1 Hz, 2H), 5.00 (s, 2H), 4.58 (q, *J* = 6.6 Hz, 1H), 3.13 (dd, *J* = 14.3, 5.6 Hz, 1H), 3.02 (dd, *J* = 14.4, 6.2 Hz, 1H), 1.30 (s, 9H).

*5.1.3.4.(S)-2-((tert-butoxycarbonyl)amino)-3-(4-((4-methylbenzyl)oxy)phenyl)propanoic acid* **(6e)** yellow oil. Yield: 41%. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.54 (s, 1H), 7.31 (d, *J* = 7.8 Hz, 2H), 7.16 (dd, *J* = 14.4, 7.9 Hz, 4H), 7.04 (d, *J* = 8.3 Hz, 1H), 6.90 (d, *J* = 8.2 Hz, 2H), 5.00 (s, 2H), 4.08 – 3.98 (m, 1H), 2.93 (dd, *J* = 13.9, 4.6 Hz, 1H), 2.75 (dd, *J* = 13.9, 10.2 Hz, 1H), 2.30 (s, 3H), 1.32 (s, 9H).

*5.1.3.5.(S)-2-((tert-butoxycarbonyl)amino)-3-(4-((3-methylbenzyl)oxy)phenyl)propanoic acid* **(6f)** yellow oil. Yield: 38%. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.48 (s, 1H), 7.29 – 7.19 (m, 3H), 7.14 (m, 3H), 7.05 (d, *J* = 8.3 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 2H), 5.01 (s, 2H), 4.07 – 3.98 (m, 1H), 2.93 (dd, *J* = 13.8, 4.6 Hz, 1H), 2.75 (dd, *J* = 13.9, 10.2 Hz, 1H), 2.31 (s, 3H), 1.32 (s, 9H).

*5.1.3.6.(S)-2-((tert-butoxycarbonyl)amino)-3-(4-((3-methoxybenzyl)oxy)phenyl)propanoic acid* **(6g)** yellow oil. Yield: 35%. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.54 (s, 1H), 7.30 (t, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 2H), 7.04 – 6.96 (m, 3H), 6.90 (t, *J* = 8.5 Hz, 3H), 5.04 (s, 2H), 4.02 (td, *J* = 8.8, 8.0, 4.5 Hz, 1H), 3.76 (s, 3H), 2.94 (dd, *J* = 13.7, 4.6 Hz, 1H), 2.76 (dd, *J* = 13.8, 10.0 Hz, 1H), 1.33 (s, 9H).

*5.1.3.7.(S)-3-(4-((4-bromobenzyl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid* **(6h)** yellowy oil. Yield: 37%. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.54 (s, 1H), 7.60 – 7.55 (m, 2H), 7.39 (d, *J* = 8.3 Hz, 2H), 7.16 (d, *J* = 8.2 Hz, 2H), 7.05 (d, *J* = 8.4 Hz, 1H), 6.94 – 6.87 (m, 2H), 5.05 (s, 2H), 4.03 (ddd, *J* = 10.1, 8.3, 4.5 Hz, 1H), 2.94 (dd, *J* = 13.9, 4.6 Hz, 1H), 2.75 (dd, *J* = 13.9, 10.3 Hz, 1H), 1.32 (s, 9H).

*5.1.3.8.(S)-3-(4-((2-bromobenzyl)oxy)phenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid* **(6i)** yellowy oil. Yield: 34%. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.47 (s, 1H), 7.67 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.57 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.46 – 7.38 (m, 1H), 7.31 (td, *J* = 7.7, 1.7 Hz, 1H), 7.17 (d, *J* = 8.1 Hz, 2H), 6.92 (d, *J* = 8.3 Hz, 2H), 5.08 (s, 2H), 4.03 (d, *J* = 9.5 Hz, 1H), 2.97 (d, *J* = 13.6 Hz, 1H), 2.83 – 2.71 (m, 1H), 1.33 (s, 9H).

*5.1.3.9.(S)-2-((tert-butoxycarbonyl)amino)-3-(4-((4-cyanobenzyl)oxy)phenyl)propanoic acid* **(6j)** green solid. Yield: 45%, mp: 127-130℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.58 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.63 (d, *J* = 8.1 Hz, 2H), 7.18 (d, *J* = 8.3 Hz, 2H), 7.05 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 8.2 Hz, 2H), 5.19 (s, 2H), 4.11 – 4.01 (m, 1H), 2.96 (dd, *J* = 13.9, 4.7 Hz, 1H), 2.77 (dd, *J* = 13.9, 10.2 Hz, 1H), 1.32 (s, 9H).

*5.1.3.10.(S)-2-((tert-butoxycarbonyl)amino)-3-(4-((3-cyanobenzyl)oxy)phenyl)propanoic acid* **(6k)**

yellowy oil. Yield: 32%. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.90 (q, *J* = 2.5, 1.7 Hz, 1H), 7.79 (t, *J* = 7.8 Hz, 2H), 7.61 (t, *J* = 7.8 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 2H), 6.95 (dd, *J* = 13.5, 8.2 Hz, 3H), 5.13 (s, 2H), 4.02 (td, *J* = 9.1, 4.4 Hz, 1H), 2.99 – 2.91 (m, 1H), 2.76 (dd, *J* = 13.7, 9.8 Hz, 1H), 1.32 (s, 9H).

*5.1.3.11.(S)-2-((tert-butoxycarbonyl)amino)-3-(4-((2-cyanobenzyl)oxy)phenyl)propanoic acid* **(6l)** white solid. Yield: 46%. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.59 (s, 1H), 7.91 (d, *J* = 7.6 Hz, 1H), 7.74 (q, *J* = 7.8 Hz, 2H), 7.57 (t, *J* = 8.1 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.3 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 2H), 5.21 (s, 2H), 4.05 (t, *J* = 9.2 Hz, 1H), 2.96 (dd, *J* = 13.8, 4.4 Hz, 1H), 2.77 (dd, *J* = 13.7, 10.4 Hz, 1H), 1.32 (s, 9H).

*5.1.3.12.(S)-2-((tert-butoxycarbonyl)amino)-3-(4-((4-nitrobenzyl)oxy)phenyl)propanoic acid* **(6m)** brown oil. Yield: 30%. $^1$H NMR (400 MHz, Chloroform-*d*) δ 8.23 (d, *J* = 8.6 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 8.0 Hz, 2H), 5.14 (s, 2H), 4.66 – 4.46 (m, 1H), 3.09 (ddd, *J* = 43.4, 13.8, 5.3 Hz, 2H), 1.38 (d, *J* = 30.5 Hz, 9H).

*5.1.3.13.(S)-2-((tert-butoxycarbonyl)amino)-3-(4-(naphthalen-2-ylmethoxy)phenyl)propanoic acid* **(6n)**. white solid. Yield: 23%, mp: 145-147℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.57 (s, 1H), 8.01 – 7.88 (m, 4H), 7.60 – 7.48 (m, 3H), 7.17 (d, *J* = 8.1 Hz, 2H), 7.06 (d, *J* = 8.3 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 2H), 5.24 (s, 2H), 4.03 (ddd, *J* = 10.2, 8.2, 4.6 Hz, 1H), 2.94 (dd, *J* = 13.8, 4.6 Hz, 1H), 2.75 (dd, *J* = 13.9, 10.3 Hz, 1H), 1.31 (s, 9H).

*5.1.4.(S)-2-amino-N-((4-bromophenyl)sulfonyl)-3-(4-methoxyphenyl)propanamide hydrochloride* **(7a)**

To a solution of **6a** (1.1 g, 6.5mmol) in 30 mL anhydrous THF at 0℃, NMM (0.3 ml, 2.5 mmol) and isobutylchloroformate (0.32 ml, 7.3 mmol) in THF, which was pretreated with NaH (0.23 g, 6.4 mmol) for 4 h at room temperature aforehand, were added in drops. This reaction was recovered slowly to room temperature and stirred overnight. Then the solvent was evaporated in vacuum. The resulting residue was neutralized by 1M HCl solution and extracted with EtOAc, then the organic phase was washed by 0.5 M citric acid solution and brine. After dried by $MgSO_4$ and concentrated, use EtOAc which was saturated with HCl gas to dissolve the obtained oil and stirred at room temperature overnight. Then the generated precipitate was filtered and dried. white solid. Yield:66%, mp: 227-229℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.61 (s, 1H), 8.42 (s, 3H), 7.87 (q, *J* = 8.7 Hz, 4H), 6.95 (d, *J* = 8.5 Hz, 2H), 6.72 (d, *J* = 8.5 Hz, 2H), 4.10 (s, 1H), 3.73 (s, 3H), 3.08 – 2.94 (m, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 169.88, 158.79, 140.79, 133.02, 131.06, 130.32, 126.46, 125.62, 114.16, 55.44, 54.95, 35.58. ESI-MS *m/z*: 411.4 $[M-H]^-$.

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

*5.1.4.1.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(4-methoxyphenyl)propanamide hydrochloride* **(7b)** white solid. Yield:65%, mp: 214-216℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.86 (s, 1H), 8.52 (s, 1H), 8.27 (s, 3H), 8.15 (dd, *J* = 8.5, 1.6 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.72 (d, *J* = 8.5 Hz, 2H), 4.06 – 3.96 (m, 1H), 3.71 (s, 3H), 3.01 (ddq, *J* = 20.3, 14.0, 6.6 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 169.90, 158.82, 147.45, 140.98, 133.07, 133.00, 131.07, 130.26, 126.42, 125.55, 114.13, 55.44, 54.95, 35.58. ESI-MS *m/z*: 412.4 $[M-H]^-$.

*5.1.4.2.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(4-isopropoxyphenyl)propanamide hydrochloride* **(7c)** white solid. Yield:34%, mp: 210-212℃. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 10.53 (s, 1H), 8.53 (d, *J* = 2.0 Hz, 1H), 8.28 (s, 3H), 8.17 (dd, *J* = 8.5, 2.0 Hz, 1H), 8.05 (d, *J* = 8.5 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 2H), 6.69 (d, *J* = 8.5 Hz, 2H), 4.53 (hept, *J* = 6.0 Hz, 1H), 4.07 – 3.96 (m, 1H), 3.00 (ddt, *J* = 20.7, 14.2, 6.1 Hz, 2H), 1.25 (d, *J* = 2.6 Hz, 3H), 1.23 (d, *J* = 2.6 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 169.84, 157.06, 147.48, 140.86, 133.10, 131.13, 130.34, 126.09, 125.59, 115.72, 69.45, 54.91, 35.55, 22.30. ESI-MS *m/z*: 440.5 [M-H]$^-$.

*5.1.4.3.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(4-propoxyphenyl)propenamide hydrochloride* **(7d)** white solid. Yield:49%, mp: 210-214℃. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 11.82 (s, 1H), 8.53 (s, 1H), 8.35 (s, 3H), 8.17 (dd, *J* = 8.5, 2.0 Hz, 1H), 8.06 (d, *J* = 8.5 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 2H), 6.71 (d, *J* = 8.5 Hz, 2H), 4.04 (dd, *J* = 9.0, 5.2 Hz, 1H), 3.87 (t, *J* = 6.5 Hz, 2H), 3.02 (qd, *J* = 14.2, 6.2 Hz, 2H), 1.71 (h, *J* = 7.1 Hz, 2H), 0.98 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 169.60, 158.27, 147.48, 140.46, 133.20, 133.04, 131.06, 130.56, 126.13, 125.61, 114.61, 69.27, 54.78, 35.50, 22.50, 10.88. ESI-MS *m/z*: 440.5 [M-H]$^-$.

*5.1.4.4.(S)-2-amino-3-(4-(benzyloxy) phenyl)-N-((4-bromophenyl) sulfonyl) propenamide hydrochloride* **(7e)** white powder. Yield:55%, mp: 228-230℃. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 13.09 (s, 1H), 8.35 (s, 3H), 7.91 – 7.81 (m, 4H), 7.49 – 7.31 (m, 6H), 6.96 (d, *J* = 8.5 Hz, 2H), 6.82 (d, *J* = 8.6 Hz, 2H), 5.07 (s, 2H), 4.10 – 4.02 (m, 1H), 3.07 – 2.92 (m, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 168.58, 157.99, 138.74, 137.51, 132.66, 131.11, 130.29, 128.93, 128.33, 128.12, 126.34, 115.09, 69.58, 54.46, 35.46. ESI-MS *m/z*: 487.4 [M-H]$^-$.

*5.1.4.5.(S)-2-amino-3-(4-(benzyloxy)phenyl)-N-((4-chloro-3-nitrophenyl)sulfonyl)propanamide hydrochloride* **(7f)** gray powder. Yield:43%, mp: 219-222℃. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 8.54 (d, *J* = 2.1 Hz, 1H), 8.38 – 8.25 (m, 3H), 8.16 (dd, *J* = 8.5, 2.2 Hz, 1H), 8.04 (d, *J* = 8.5 Hz, 1H), 7.47 – 7.32 (m, 5H), 7.01 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 8.3 Hz, 2H), 5.06 (s, 2H), 3.02 (qd, *J* = 14.2, 6.2 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 168.58, 157.99, 137.51, 132.66, 131.11, 130.29, 128.93, 128.33, 128.12, 126.34, 115.09, 69.58, 54.46, 35.46. ESI-MS *m/z*: 488.4 [M-H]$^-$.

*5.1.4.6.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(4-((4-methylbenzyl)oxy)phenyl) propanamide hydrochloride* **(7g)** white solid. Yield:71%, mp: 184-186℃. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 8.50 (d, *J* = 2.1 Hz, 1H), 8.13 (h, *J* = 4.4 Hz, 3H), 8.00 (d, *J* = 8.5 Hz, 1H), 7.32 (d, *J* = 7.8 Hz, 2H), 7.20 (d, *J* = 7.8 Hz, 2H), 6.97 (d, *J* = 8.3 Hz, 2H), 6.79 (d, *J* = 8.4 Hz, 2H), 5.00 (s, 2H), 2.97 (qd, *J* = 14.2, 6.1 Hz, 2H), 2.31 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 168.56, 157.99, 147.46, 140.46, 137.45, 136.86, 133.16, 133.05, 131.10, 130.54, 128.87, 128.09, 126.32 125.60, 115.06, 69.56, 54.43, 35.46, 20.88. ESI-MS *m/z*: 502.4 [M-H]$^-$.

*5.1.4.7.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(4-((3-methylbenzyl)oxy)phenyl) propanamide hydrochloride* **(7h)** white solid. Yield:94%, mp: 208-210℃. $^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 8.52 (d, *J* = 2.1 Hz, 1H), 8.23 (s, 3H), 8.14 (dd, *J* = 8.5, 2.2 Hz, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.31 – 7.19 (m, 3H), 7.14 (d, *J* = 7.4 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 2H), 6.81 (d, *J* = 8.3 Hz, 2H), 5.01 (s, 2H), 3.99 (q, *J* = 6.1 Hz, 1H), 3.00 (qd, *J* = 14.3, 6.2 Hz, 2H), 2.32 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 168.60, 157.98, 147.51, 140.49, 138.15, 137.48, 133.22, 133.08, 131.16, 130.56,

128.43, 128.36, 126.35, 125.68, 125.31, 115.08, 69.58, 54.46, 35.51, 20.86. ESI-MS *m/z*: 502.1 [M-H]$^-$.

*5.1.4.8.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(4-((3-methoxybenzyl)oxy)phenyl) propanamide hydrochloride* **(7i)** pink solid. Yield:13%, mp: 200-202℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.51 (d, *J* = 2.2 Hz, 1H), 8.18 (d, *J* = 5.1 Hz, 3H), 8.13 (dd, *J* = 8.4, 2.2 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.04 – 6.97 (m, 4H), 6.93 – 6.87 (m, 1H), 6.85 – 6.78 (m, 2H), 5.03 (s, 2H), 3.96 (q, *J* = 5.4, 5.0 Hz, 1H), 2.99 (qd, *J* = 14.2, 6.2 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 168.60, 159.68, 157.99, 139.51, 132.66, 131.14, 130.32, 129.46, 126.38, 121.62, 115.12, 113.45, 113.16, 69.63, 55.24, 54.52, 35.48. ESI-MS *m/z*: 518.1 [M-H]$^-$.

*5.1.4.9.(S)-2-amino-3-(4-((4-bromobenzyl)oxy)phenyl)-N-((4-chloro-3-nitrophenyl)sulfonyl) propanamide hydrochloride* **(7j)** white solid. Yield:61%, mp: 230-232℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.50 (d, *J* = 2.7 Hz, 1H), 8.24 – 8.10 (m, 3H), 8.01 (dd, *J* = 8.5, 4.0 Hz, 1H), 7.60 (d, *J* = 8.1 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.00 (dd, *J* = 8.6, 2.4 Hz, 2H), 6.81 (d, *J* = 8.3 Hz, 2H), 5.04 (s, 2H), 2.99 (tdd, *J* = 20.2, 13.9, 5.4 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 168.58, 157.96, 132.66, 131.53, 131.14, 130.32, 129.64, 126.36, 122.80, 115.12, 69.60, 54.51, 35.48. ESI-MS *m/z*: 568.0 [M-H]$^-$.

*5.1.4.10.(S)-2-amino-3-(4-((2-bromobenzyl)oxy)phenyl)-N-((4-chloro-3-nitrophenyl)sulfonyl) propanamide hydrochloride* **(7k)** white solid. Yield:41%, mp: 202-204℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.41 (s, 1H), 8.06 (d, *J* = 10.2 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.76 (s, 3H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.57 (d, *J* = 7.5 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 5.06 (s, 2H), 3.73 – 3.61 (m, 1H), 3.02 – 2.84 (m, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 168.59, 157.94, 136.18, 132.71, 131.14, 130.84, 130.55, 130.31, 127.82, 127.25, 126.32, 115.14, 69.54, 54.43, 35.45. ESI-MS *m/z*: 568.1 [M-H]$^-$.

*5.1.4.11.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(4-((4-cyanobenzyl)oxy)phenyl) propanamide hydrochloride* **(7l)** white powder. Yield:55.2%, mp: 210-212℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.52 (d, *J* = 2.1 Hz, 1H), 8.27 (d, *J* = 5.4 Hz, 3H), 8.14 (dd, *J* = 8.5, 2.2 Hz, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.64 (d, *J* = 8.1 Hz, 2H), 7.02 (d, *J* = 8.3 Hz, 2H), 6.83 (d, *J* = 8.3 Hz, 2H), 5.18 (s, 2H), 4.01 (s, 1H), 3.01 (qd, *J* = 14.2, 6.2 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 168.60, 158.02, 144.31, 132.63, 132.46, 131.15, 130.26, 129.67, 126.33, 119.34, 115.14, 111.18, 69.55, 54.41, 35.52. ESI-MS *m/z*: 513.2 [M-H]$^-$.

*5.1.4.12.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(4-((3-cyanobenzyl)oxy)phenyl) propanamide hydrochloride* **(7m)** white solid. Yield:52%, mp: 182-184℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.47 (d, *J* = 2.1 Hz, 1H), 8.10 (dd, *J* = 8.5, 2.1 Hz, 1H), 8.07 – 7.94 (m, 4H), 7.90 (d, *J* = 1.8 Hz, 1H), 7.85 – 7.76 (m, 2H), 7.63 (t, *J* = 7.8 Hz, 1H), 6.99 (d, *J* = 8.5 Hz, 2H), 6.88 – 6.80 (m, 2H), 5.13 (s, 2H), 3.04 – 2.87 (m, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 168.56, 157.94, 138.11, 132.59, 131.04, 130.74, 130.23, 130.06, 129.06, 128.08, 126.29, 118.53, 115.07, 111.93, 69.52, 54.46, 35.46. ESI-MS *m/z*: 513.3 [M-H]$^-$.

*5.1.4.13.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(4-((2-cyanobenzyl)oxy)phenyl) propanamide hydrochloride* **(7n)** white solid. Yield:72%, mp: 210-212℃. $^1$H NMR (400 MHz,

DMSO-$d_6$) δ 8.41 (s, 1H), 8.06 (d, *J* = 10.2 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.76 (s, 3H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.57 (d, *J* = 7.5 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 5.06 (s, 2H), 3.71 – 3.64 (m, 1H), 3.00 – 2.86 (m, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 168.64, 157.97, 139.66, 132.62, 131.86, 131.09, 130.25, 128.67, 128.34, 128.31, 126.33, 117.21, 115.06, 112.58, 69.55, 54.43, 35.41. ESI-MS *m/z*: 513.3 $[M-H]^-$.

*5.1.4.14.(S)-2-amino-N-((4-bromophenyl)sulfonyl)-3-(4-((4-nitrobenzyl)oxy)phenyl)propanamide hydrochloride* **(7o)** light yellow solid. Yield:47%, mp: 232-234℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.36 (s, 3H), 8.28 (d, *J* = 8.7 Hz, 2H), 7.85 (q, *J* = 8.8 Hz, 4H), 7.73 (d, *J* = 8.6 Hz, 2H), 6.99 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 5.26 (s, 2H), 4.13 – 4.04 (m, 1H), 3.11 – 2.93 (m, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 168.60, 157.54, 147.45, 145.48, 139.04, 138.80, 132.63, 131.19, 130.27, 128.63, 128.28, 126.76, 124.10, 115.17, 68.42, 54.48, 35.53. ESI-MS *m/z*: 532.2 $[M-H]^-$.

*5.1.4.15.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(4-((4-nitrobenzyl)oxy)phenyl) propanamide hydrochloride* **(7p)** light yellow solid. Yield:54%, mp: 218-220℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.50 (d, *J* = 2.7 Hz, 1H), 8.24 – 8.11 (m, 3H), 8.01 (dd, *J* = 8.5, 4.0 Hz, 1H), 7.60 (d, *J* = 8.1 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 7.00 (dd, *J* = 8.6, 2.4 Hz, 2H), 6.81 (d, *J* = 8.3 Hz, 2H), 5.04 (s, 2H), 2.99 (tdd, *J* = 20.2, 13.9, 5.4 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 168.56, 157.52, 147.48, 147.43, 145.44, 140.47, 138.77, 133.22, 133.06, 131.16, 130.55, 128.62, 126.72, 125.59, 124.08, 115.16, 68.41, 54.44, 35.47. ESI-MS *m/z*: 533.2 $[M-H]^-$.

*5.1.4.16.(S)-2-amino-N-((4-chloro-3-nitrophenyl)sulfonyl)-3-(4-(naphthalen-2-ylmethoxy)phenyl) propanamide hydrochloride* **(7q)** light yellow solid. Yield:92%, mp: 201-204℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.49 (d, *J* = 2.1 Hz, 1H), 8.16 – 8.05 (m, 4H), 8.02 – 7.90 (m, 6H), 7.60 – 7.51 (m, 3H), 6.99 (d, *J* = 8.3 Hz, 2H), 6.87 (d, *J* = 8.3 Hz, 2H), 5.23 (s, 2H), 3.05 – 2.89 (m, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 168.60, 157.91, 136.43, 133.64, 133.32, 132.74, 131.19, 130.35, 128.87, 128.68, 127.93, 127.47, 126.39, 126.36, 126.42, 126.17, 115.19, 69.66, 54.57, 35.53. ESI-MS *m/z*: 538.5 $[M-H]^-$.

*5.1.5.(S)-N-((4-bromophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-methoxyphenyl)propenamide* **(8a)**

To the solution intermediate **7a** (0.76 g, 1.8 mmol) in dry dichloromethane (25 mL), ethyldiisopropylamine (0.82 ml, 7.4 mmol) was added in ice bath. After stirred for 10 min, O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTO, 0.77 g, 2.4 mmol) was added and stirred for 30 min in 0℃, then the indomethacin (0.79 g, 2.2 mmol) was added and stirred overnight at room temperature. The solvent was removed in vacuum and then dissolved in EtOAc. 0.5 M citric acid and brine were used to wash the organic phase, then it was concentrated to yield yellow oil. Purification by column chromatography (PE/EA = 10:7) to generate compound **8a**, a white solid. Yield:54%, mp: 217-220℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.62 (s, 1H), 8.31 (d, *J* = 7.7 Hz, 1H), 7.82 (s, 4H), 7.63 (s, 4H), 7.06 (d, *J* = 2.2 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.70 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.63 (d, *J* = 8.4 Hz, 2H), 4.44 (td, *J* = 8.8, 4.8 Hz, 1H), 3.74 (s, 3H), 3.65 (s, 3H), 3.55 – 3.42 (m, 2H), 2.85 (dd, *J* = 13.6, 4.3 Hz, 1H), 2.63 (dd, *J* = 13.6, 9.6 Hz, 1H), 2.07 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.27, 170.08, 168.26, 158.29, 156.00, 138.78, 138.01, 135.53, 134.66, 132.69, 131.57, 131.23, 130.60, 129.96, 129.48, 128.80, 128.24, 114.90, 114.50, 113.77, 111.91, 102.23, 55.85, 55.29, 55.10, 36.07, 30.97, 13.75. HRMS (AP-ESI) *m/z*

Calcd for $C_{35}H_{31}BrClN_3O_7S$ $[M-H]^-$ 752.0659. Found:752.0664.

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

*5.1.5.1.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-methoxyphenyl)propenamide* **(8b)** white solid. Yield:42%, mp: 190-194℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.90 (s, 1H), 8.53 (d, *J* = 2.1 Hz, 1H), 8.38 (d, *J* = 7.3 Hz, 1H), 8.13 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.64 (s, 4H), 7.07 – 7.00 (m, 3H), 6.93 (d, *J* = 9.0 Hz, 1H), 6.69 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.64 (d, *J* = 8.5 Hz, 2H), 4.49 – 4.39 (m, 1H), 3.74 (s, 3H), 3.65 (s, 3H), 3.49 (q, *J* = 15.4 Hz, 2H), 2.88 (dd, *J* = 13.7, 4.7 Hz, 1H), 2.67 (dd, *J* = 13.6, 9.4 Hz, 1H), 2.08 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.88, 170.17, 168.26, 158.32, 155.98, 147.58, 138.02, 135.55, 134.66, 133.41, 132.67, 131.57, 131.20, 131.08, 130.61, 130.59, 129.48, 128.76, 125.48, 114.90, 114.49, 113.74, 111.91, 102.17, 55.81, 55.28, 35.99, 30.93, 13.72. HRMS (AP-ESI) *m/z* Calcd for $C_{35}H_{30}Cl_2N_4O_9S$ $[M-H]^-$ 751.1032. Found:751.1033.

*5.1.5.2.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-isopropoxyphenyl)propenamide* **(8c)** white solid. Yield:52%, mp: 122-126℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.92 (s, 1H), 8.57 – 8.53 (m, 1H), 8.42 (d, *J* = 7.4 Hz, 1H), 8.17 – 8.11 (m, 1H), 7.98 (d, *J* = 8.5 Hz, 1H), 7.64 (s, 4H), 7.09 – 7.05 (m, 1H), 7.01 (d, *J* = 8.3 Hz, 2H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.69 (dd, *J* = 9.0, 1.9 Hz, 1H), 6.62 (d, *J* = 8.3 Hz, 2H), 4.50 – 4.40 (m, 2H), 3.75 (s, 3H), 3.49 (q, *J* = 15.4 Hz, 2H), 2.87 (dd, *J* = 13.6, 4.2 Hz, 1H), 2.69 – 2.61 (m, 1H), 2.08 (s, 3H), 1.19 (t, *J* = 6.7 Hz, 7H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.80, 170.24, 168.22, 156.56, 155.98, 147.60, 139.41, 138.05, 135.53, 134.63, 133.45, 132.68, 131.57, 131.21, 130.68, 130.59, 129.47, 128.49, 125.53, 115.43, 114.89, 114.48, 111.90, 102.16, 69.37, 55.80, 55.27, 35.96, 31.45, 30.93, 22.56, 22.23, 14.43, 13.71. HRMS (AP-ESI) *m/z* Calcd for $C_{37}H_{34}Cl_2N_4O_9S$ $[M-H]^-$ 779.1345. Found:779.1346.

*5.1.5.3.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-propoxyphenyl)propenamide* **(8d)** white solid. Yield:39%, mp: 134-138℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.90 (s, 1H), 8.54 (d, *J* = 2.0 Hz, 1H), 8.40 (d, *J* = 7.6 Hz, 1H), 8.13 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.63 (s, 4H), 7.09 – 6.98 (m, 3H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.69 (dd, *J* = 9.0, 2.3 Hz, 1H), 6.62 (d, *J* = 8.5 Hz, 2H), 4.44 (q, *J* = 8.5 Hz, 1H), 3.83 – 3.71 (m, 5H), 3.49 (q, *J* = 15.4 Hz, 2H), 2.87 (dd, *J* = 13.7, 4.4 Hz, 1H), 2.68 – 2.62 (m, 1H), 2.09 (s, 3H), 1.67 (h, *J* = 7.0 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.77, 170.20, 168.24, 157.78, 155.98, 147.59, 139.44, 138.03, 135.54, 134.64, 133.45, 132.68, 131.58, 131.19, 130.60, 129.47, 128.57, 125.51, 114.89, 114.47, 114.25, 111.91, 102.18, 69.16, 55.81, 55.25, 38.71, 35.99, 30.94, 22.51, 13.72, 10.86. HRMS (AP-ESI) *m/z* Calcd for $C_{37}H_{34}Cl_2N_4O_9S$ $[M-H]^-$ 779.1345. Found:779.1350.

*5.1.5.4.(S)-3-(4-(benzyloxy)phenyl)-N-((4-bromophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)propenamide* **(8e)** white powder. Yield:72%, mp: 196-199℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.64 (s, 1H), 8.33 (d, *J* = 7.7 Hz, 1H), 7.81 (s, 4H), 7.67 – 7.58 (m, 4H), 7.44 – 7.30 (m, 5H), 7.07 (d, *J* = 2.4 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.93 (d, *J* = 9.0 Hz, 1H), 6.70 (td, *J* = 6.8, 3.2 Hz, 3H), 4.97 (s, 2H), 4.44 (td, *J* = 8.8, 4.8 Hz, 1H), 3.74 (s, 3H), 3.56 – 3.41 (m, 2H), 2.85 (dd, *J* = 13.6, 4.4 Hz, 1H), 2.63 (dd, *J* = 13.6, 9.7 Hz, 1H), 2.08 (s, 3H). $^{13}$C NMR (100

MHz, DMSO-$d_6$) δ 171.28, 170.09, 168.26, 157.45, 156.02, 138.80, 138.02, 137.54, 135.56, 134.65, 132.68, 131.58, 131.25, 130.66, 130.61, 129.95, 129.48, 129.16, 128.87, 128.25, 128.04, 114.87, 114.63, 114.54, 111.95, 102.29, 69.54, 55.90, 55.17, 36.05, 31.02, 13.76. HRMS (AP-ESI) *m/z* Calcd for $C_{41}H_{35}BrClN_3O_7S$ $[M-H]^-$ 828.0973. Found:828.0993.

*5.1.5.5.(S)-3-(4-(benzyloxy)phenyl)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)propenamide* **(8f)** gray solid. Yield:13%, mp: 132-135℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.94 (s, 1H), 8.55 (d, *J* = 2.2 Hz, 1H), 8.43 (d, *J* = 7.6 Hz, 1H), 8.14 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.67 – 7.59 (m, 4H), 7.43 – 7.32 (m, 5H), 7.10 – 7.02 (m, 3H), 6.93 (d, *J* = 8.9 Hz, 1H), 6.74 (d, *J* = 8.3 Hz, 2H), 6.70 (dd, *J* = 9.0, 2.5 Hz, 1H), 4.98 (s, 2H), 4.47 (td, *J* = 8.1, 7.5, 4.9 Hz, 1H), 3.50 (q, *J* = 15.4 Hz, 2H), 2.90 (dd, *J* = 13.9, 4.8 Hz, 1H), 2.68 (dd, *J* = 13.8, 9.4 Hz, 1H), 2.10 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.77, 170.24, 168.25, 157.49, 155.98, 147.60, 139.39, 138.05, 137.51, 135.59, 134.63, 133.46, 132.67, 131.58, 131.21, 130.65, 129.47, 129.04, 128.88, 128.27, 128.06, 125.54, 114.90, 114.64, 114.47, 111.92, 102.18, 69.52, 55.82, 55.23, 35.97, 30.95, 13.71. HRMS (AP-ESI) *m/z* Calcd for $C_{41}H_{34}Cl_2N_4O_9S$ $[M-H]^-$ 827.1345. Found:827.1355.

*5.1.5.6.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-((4-methylbenzyl)oxy)phenyl)propenamide* **(8g)** light yellow solid. Yield:25%, mp: 131-136℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.89 (s, 1H), 8.54 (d, *J* = 2.2 Hz, 1H), 8.38 (d, *J* = 7.6 Hz, 1H), 8.13 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.96 (d, *J* = 8.5 Hz, 1H), 7.70 – 7.55 (m, 4H), 7.28 (d, *J* = 7.7 Hz, 2H), 7.18 (d, *J* = 7.7 Hz, 2H), 7.09 – 6.98 (m, 3H), 6.92 (d, *J* = 9.0 Hz, 1H), 6.70 (dd, *J* = 8.9, 6.8 Hz, 3H), 4.92 (s, 2H), 4.45 (td, *J* = 8.4, 4.8 Hz, 1H), 3.74 (s, 3H), 3.49 (q, *J* = 15.4 Hz, 2H), 2.88 (dd, *J* = 14.0, 4.8 Hz, 1H), 2.66 (dd, *J* = 14.0, 4.8 Hz , 1H), 2.30 (s, 3H), 2.10 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.80, 170.20, 168.25, 157.52, 155.99, 147.60, 139.50, 138.05, 137.48, 135.58, 134.65, 134.48, 133.44, 132.66, 131.57, 131.21, 131.16, 130.61, 129.48, 129.41, 128.97, 128.14, 125.50, 114.88, 114.65, 114.48, 111.92, 102.22, 69.44, 55.84, 55.23, 38.71, 35.99, 30.96, 21.23, 13.70. $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ HRMS (AP-ESI) *m/z* Calcd for $C_{42}H_{36}Cl_2N_4O_9S$ $[M-H]^-$ 841.1502. Found:841.1507.

*5.1.5.7.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-((3-methylbenzyl)oxy)phenyl)propenamide* **(8h)** light yellow solid. Yield:30%, mp: 134-138℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.89 (s, 1H), 8.55 (d, *J* = 2.2 Hz, 1H), 8.40 (d, *J* = 7.6 Hz, 1H), 8.13 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.70 – 7.59 (m, 4H), 7.31 – 7.11 (m, 4H), 7.10 – 7.00 (m, 3H), 6.93 (d, *J* = 8.9 Hz, 1H), 6.77 – 6.66 (m, 3H), 4.93 (s, 2H), 4.45 (td, *J* = 8.3, 4.8 Hz, 1H), 3.74 (s, 3H), 3.49 (q, *J* = 15.4 Hz, 2H), 2.89 (dd, *J* = 13.8, 4.8 Hz, 1H), 2.73 – 2.64 (m, 1H), 2.32 (s, 3H), 2.10 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.79, 170.20, 168.25, 157.53, 155.98, 147.61, 139.47, 138.04, 137.42, 135.58, 134.64, 133.45, 132.66, 131.58, 131.21, 131.17, 130.64, 129.48, 129.00, 128.90, 128.77, 128.63, 125.51, 125.16, 114.88, 114.61, 114.47, 111.93, 102.20, 69.57, 55.83, 55.22, 35.99, 30.95, 21.46, 13.71. HRMS (AP-ESI) *m/z* Calcd for $C_{42}H_{36}Cl_2N_4O_9S$ $[M-H]^-$ 841.1502. Found:841.1521.

*5.1.5.8.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-((3-methoxybenzyl)oxy)phenyl)propenamide* **(8i)** light yellow solid.

Yield:32%, mp: 157-160℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.92 (s, 1H), 8.54 (d, *J* = 2.2 Hz, 1H), 8.40 (d, *J* = 7.6 Hz, 1H), 8.13 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.97 (d, *J* = 8.6 Hz, 1H), 7.69 – 7.58 (m, 4H), 7.30 (t, *J* = 8.0 Hz, 1H), 7.09 – 7.00 (m, 3H), 6.97 (dd, *J* = 4.3, 2.3 Hz, 2H), 6.93 (d, *J* = 9.0 Hz, 1H), 6.89 (dd, *J* = 8.2, 2.5 Hz, 1H), 6.71 (dd, *J* = 12.4, 7.5 Hz, 3H), 4.96 (s, 2H), 4.45 (td, *J* = 8.3, 4.9 Hz, 1H), 3.75 (d, *J* = 6.9 Hz, 6H), 3.49 (q, *J* = 15.4 Hz, 2H), 2.89 (dd, *J* = 13.8, 4.8 Hz, 1H), 2.73 – 2.64 (m, 1H), 2.10 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.82, 170.20, 168.25, 159.78, 157.44, 155.98, 147.60, 139.51, 139.11, 138.04, 135.58, 134.64, 133.43, 132.66, 131.57, 131.21, 131.14, 130.60, 129.99, 129.48, 129.07, 125.51, 120.08, 114.88, 114.64, 114.48, 113.66, 113.49, 111.93, 102.20, 69.39, 55.83, 55.49, 55.23, 36.00, 30.95, 13.71. HRMS (AP-ESI) *m/z* Calcd for $C_{42}H_{36}Cl_2N_4O_{10}S$ [M-H]$^-$ 857.1456. Found:857.1450.

*5.1.5.9.(S)-3-(4-((4-bromobenzyl)oxy)phenyl)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)propenamide* **(8j)** light yellow solid. Yield:28%, mp: 164-170℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.89 (s, 1H), 8.54 (d, *J* = 2.2 Hz, 1H), 8.39 (d, *J* = 7.5 Hz, 1H), 8.13 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.61 (dt, *J* = 12.1, 8.3 Hz, 6H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.10 – 6.99 (m, 3H), 6.92 (d, *J* = 9.0 Hz, 1H), 6.78 – 6.65 (m, 3H), 4.97 (s, 2H), 4.44 (td, *J* = 8.6, 4.9 Hz, 1H), 3.74 (s, 3H), 3.48 (q, *J* = 15.4 Hz, 2H), 2.89 (dd, *J* = 13.9, 4.7 Hz, 1H), 2.67 (dd, *J* = 13.7, 9.3 Hz, 1H), 2.10 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.84, 170.18, 168.25, 157.25, 155.97, 147.60, 138.04, 137.01, 135.57, 134.63, 133.42, 132.66, 131.81, 131.58, 131.20, 131.11, 131.09, 130.66, 130.59, 130.11, 129.48, 129.25, 125.49, 121.35, 114.88, 114.67, 114.47, 111.93, 102.19, 68.71, 55.83, 55.23, 35.98, 30.95, 13.70. HRMS (AP-ESI) *m/z* Calcd for $C_{41}H_{33}BrCl_2N_4O_9S$ [M-H]$^-$ 907.0432. Found:907.0425.

*5.1.5.10.(S)-3-(4-((2-bromobenzyl)oxy)phenyl)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)propenamide* **(8k)** light yellow solid. Yield:18%, mp: 162-167℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.81 (s, 1H), 8.55 (d, *J* = 2.2 Hz, 1H), 8.40 (d, *J* = 7.6 Hz, 1H), 8.14 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.98 (d, *J* = 8.5 Hz, 1H), 7.73 – 7.58 (m, 5H), 7.53 (d, *J* = 7.7, 1H), 7.42 (t, *J* = 7.5, 1H), 7.31 (t, *J* = 7.7, 1H), 7.11 – 7.02 (m, 3H), 6.92 (d, *J* = 9.0 Hz, 1H), 6.74 (d, *J* = 8.3 Hz, 2H), 6.69 (dd, *J* = 9.0, 2.6 Hz, 1H), 5.00 (s, 2H), 4.45 (td, *J* = 8.6, 4.9 Hz, 1H), 3.74 (s, 3H), 3.49 (q, *J* = 15.2 Hz, 2H), 2.90 (dd, *J* = 13.8, 4.7 Hz, 1H), 2.69 (dd, *J* = 13.7, 9.4 Hz, 1H), 2.10 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.83, 170.20, 168.24, 157.26, 155.98, 147.61, 138.04, 136.35, 135.58, 134.63, 133.43, 133.07, 132.66, 131.58, 131.20, 131.11, 130.74, 130.59, 130.51, 130.45, 129.48, 129.44, 128.35, 125.51, 123.11, 114.88, 114.59, 114.47, 111.92, 102.21, 69.35, 55.85, 55.22, 35.97, 30.96, 13.71. HRMS (AP-ESI) *m/z* Calcd for $C_{41}H_{33}BrCl_2N_4O_9S$ [M-H]$^-$ 907.0432. Found:907.0418.

*5.1.5.11.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-((4-cyanobenzyl)oxy)phenyl)propenamide* **(8l)** light yellow solid. Yield:30%, mp: 172-174℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.87 (s, 1H), 8.53 (d, *J* = 2.2 Hz, 1H), 8.39 (d, *J* = 7.6 Hz, 1H), 8.13 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.86 (d, *J* = 8.0 Hz, 2H), 7.61 (dd, *J* = 11.3, 5.5 Hz, 6H), 7.05 (dd, *J* = 5.6, 3.0 Hz, 3H), 6.91 (d, *J* = 9.0 Hz, 1H), 6.79 – 6.66 (m, 3H), 5.11 (s, 2H), 4.44 (td, *J* = 8.5, 4.9 Hz, 1H), 3.73 (s, 3H), 3.48 (q, *J* = 15.4 Hz, 2H), 2.89 (dd, *J* = 13.8, 4.8 Hz, 1H), 2.68 (dd, *J* = 13.7, 9.4 Hz, 1H), $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 2.09 (s, 3H). 171.84, 170.19, 168.24, 157.07, 155.97, 147.60, 143.38, 139.57, 138.04, 135.57, 134.62, 133.42, 132.87,

132.66, 131.58, 131.19, 131.11, 130.71, 130.58, 129.47, 128.37, 125.48, 119.22, 114.88, 114.67, 114.46, 111.92, 110.91, 102.19, 68.58, 55.83, 55.22, 35.97, 30.96, 13.69. HRMS (AP-ESI) *m/z* Calcd for $C_{42}H_{33}Cl_2N_5O_9S$ $[M-H]^-$ 852.1298. Found:852.1315.

*5.1.5.12.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-((3-cyanobenzyl)oxy)phenyl)propenamide* **(8m)** light yellow solid. Yield:17.18%, mp: 182-186℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.91 (s, 1H), 8.53 (d, *J* = 2.1 Hz, 1H), 8.39 (d, *J* = 7.3 Hz, 1H), 8.12 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.87 (s, 1H), 7.81 (d, *J* = 7.7 Hz, 1H), 7.75 (d, *J* = 7.9 Hz, 1H), 7.68 – 7.58 (m, 5H), 7.04 (dd, *J* = 5.5, 2.8 Hz, 3H), 6.91 (d, *J* = 9.0 Hz, 1H), 6.74 (d, *J* = 8.5 Hz, 2H), 6.69 (dd, *J* = 9.0, 2.4 Hz, 1H), 5.05 (s, 2H), 4.44 (q, *J* = 8.4 Hz, 1H), 3.73 (s, 3H), 2.89 (dd, *J* = 13.7, 4.6 Hz, 1H), 2.71 – 2.63 (m, 1H), 2.09 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.79, 170.23, 168.24, 157.11, 155.96, 147.60, 139.42, 139.26, 138.04, 135.57, 134.61, 133.45, 132.73, 132.67, 132.08, 131.58, 131.34, 131.19, 130.71, 130.57, 130.18, 129.47, 129.41, 125.52, 119.15, 114.89, 114.67, 114.46, 111.90, 102.16, 68.34, 55.82, 55.21, 35.96, 30.95, 13.71. HRMS (AP-ESI) *m/z* Calcd for $C_{42}H_{33}Cl_2N_5O_9S$ $[M-H]^-$ 852.1298. Found:852.1292.

*5.1.5.13.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-((2-cyanobenzyl)oxy)phenyl)propenamide* **(8n)** light yellow solid. Yield:55%, mp: 150-155℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.94 (s, 1H), 8.54 (s, 1H), 8.44 (d, *J* = 6.8 Hz, 1H), 8.13 (d, *J* = 8.0 Hz, 1H), 7.94 (dd, *J* = 21.7, 8.0 Hz, 2H), 7.65 (dtt, *J* = 26.8, 18.2, 7.3 Hz, 7H), 7.17 – 6.97 (m, 3H), 6.92 (d, *J* = 8.9 Hz, 1H), 6.79 (d, *J* = 8.0 Hz, 2H), 6.69 (d, *J* = 8.2 Hz, 1H), 5.14 (s, 2H), 4.45 (s, 1H), 3.74 (s, 3H), 3.49 (q, *J* = 15.0 Hz, 2H), 2.90 (d, *J* = 10.0 Hz, 1H), 2.76 – 2.63 (m, 1H), 2.10 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.78, 170.27, 168.24, 157.17, 155.97, 147.60, 140.48, 139.42, 138.03, 135.58, 134.63, 133.89, 133.74, 133.45, 132.65, 131.58, 131.20, 130.75, 130.58, 129.87, 129.67, 129.46, 125.53, 117.63, 114.90, 114.69, 114.48, 111.93, 111.61, 102.17, 67.83, 55.82, 55.23, 35.95, 30.93, 13.71. HRMS (AP-ESI) *m/z* Calcd for $C_{42}H_{33}Cl_2N_5O_9S$ $[M-H]^-$ 852.1298. Found:852.1311.

*5.1.5.14.(S)-N-((4-bromophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-((4-nitrobenzyl)oxy)phenyl)propenamide* **(8o)** pink solid. Yield:18%, mp: 228-230℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.58 (s, 1H), 8.25 (d, *J* = 8.6 Hz, 3H), 7.80 (s, 4H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.62 (d, *J* = 9.1 Hz, 4H), 7.03 (dd, *J* = 12.3, 5.1 Hz, 3H), 6.92 (d, *J* = 9.0 Hz, 1H), 6.78 – 6.66 (m, 3H), 5.16 (s, 2H), 4.49 – 4.40 (m, 1H), 3.73 (s, 3H), 3.53 – 3.41 (m, 2H), 2.85 (dd, *J* = 13.6, 4.4 Hz, 1H), 2.64 (dd, *J* = 13.5, 9.8 Hz, 1H), 2.07 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.78, 170.78, 170.24, 168.23, 157.05, 155.98, 147.58, 147.41, 145.48, 139.48, 138.07, 135.58, 134.61, 133.44, 132.67, 131.56, 131.20, 130.74, 130.60, 129.45, 128.48, 125.50, 124.02, 114.87, 114.71, 114.45, 111.90, 102.20, 68.37, 60.22, 55.82, 55.22, 35.97, 30.98, 21.20, 14.53, 13.68. HRMS (AP-ESI) *m/z* Calcd for $C_{41}H_{34}BrClN_4O_9S$ $[M-H]^-$ 873.0824. Found:873.0824.

*5.1.5.15.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-((4-nitrobenzyl)oxy)phenyl)propenamide* **(8p)** light yellow solid. Yield:13%, mp: 182-186℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.94 (s, 1H), 8.55 (d, *J* = 2.2 Hz, 1H), 8.43 (d, *J* = 7.6 Hz, 1H), 8.14 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.69 – 7.58 (m, 4H), 7.39 (q, *J* = 7.3 Hz, 4H), 7.10 – 7.01 (m, 3H), 6.93 (d, *J* = 8.9 Hz, 1H), 6.74 (d, *J* = 8.3 Hz, 2H), 6.70 (dd,

*J* = 9.0, 2.5 Hz, 1H), 4.98 (s, 2H), 4.47 (td, *J* = 8.1, 7.5, 4.9 Hz, 1H), 3.50 (q, *J* = 15.4 Hz, 2H), 2.90 (dd, *J* = 13.9, 4.8 Hz, 1H), 2.68 (dd, *J* = 13.8, 9.4 Hz, 1H), 2.10 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.77, 170.24, 168.25, 157.49, 155.98, 147.60, 139.39, 138.05, 137.51, 135.59, 134.63, 133.46, 132.67, 131.58, 131.21, 130.65, 130.59, 129.47, 129.04, 128.88, 128.27, 128.06, 125.54, 114.90, 114.64, 114.47, 111.92, 102.18, 69.52, 55.82, 55.23, 35.97, 30.95, 13.71. HRMS (AP-ESI) *m/z* Calcd for $C_{41}H_{33}Cl_2N_5O_{11}S$ [M-H]$^-$ 872.1196. Found:872.1194.

*5.1.5.16.(S)-N-((4-chloro-3-nitrophenyl)sulfonyl)-2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetamido)-3-(4-(naphthalen-2-ylmethoxy)phenyl)propenamide* **(8q)** light yellow solid. Yield:34%, mp: 197-199℃. $^1$H NMR (400 MHz, DMSO-$d_6$ ) δ 12.95 (s, 1H), 8.54 (d, *J* = 2.1 Hz, 1H), 8.41 (d, *J* = 7.2 Hz, 1H), 8.13 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.94 (td, *J* = 11.4, 10.4, 6.6 Hz, 5H), 7.67 – 7.58 (m, 4H), 7.53 (dd, *J* = 8.5, 3.5 Hz, 3H), 7.10 – 7.00 (m, 3H), 6.91 (d, *J* = 9.0 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 2H), 6.69 (dd, *J* = 9.0, 2.4 Hz, 1H), 5.15 (s, 2H), 4.44 (t, *J* = 10.7 Hz, 1H), 3.73 (s, 3H), 3.56 – 3.41 (m, 2H), 2.88 (dd, *J* = 13.6, 4.6 Hz, 1H), 2.74 – 2.62 (m, 1H), 2.09 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 171.83, 170.21, 168.25, 157.50, 155.98, 147.60, 138.04, 135.58, 135.16, 134.63, 133.44, 133.23, 132.99, 132.67, 131.58, 131.21, 131.15, 130.68, 130.59, 129.47, 129.13, 128.52, 128.23, 128.08, 126.79, 126.59, 126.08, 125.52, 114.89, 114.73, 114.48, 111.93, 102.19, 69.66, 55.83, 55.23, 35.99, 30.95, 13.72. HRMS (AP-ESI) *m/z* Calcd for $C_{45}H_{36}Cl_2N_4O_9S$ [M-H]$^-$ 877.1502. Found: 877.1509.

*5.2. In vitro binding assay for Bcl-2 proteins*

A Bid-BH3 peptide marked with 5-carboxyfluorescein succinimidyl ester (5-FAM-QEDIIRNIARHLAQVGDSMDRSIPPG) could bind to Bcl-2 family proteins and, meanwhile, generate high polarization values (milipolarization units, mP). When the test compounds compete with the marked Bid-BH3 peptide to bind the Bcl-2 family protein, the mP values decrease. The $IC_{50}$ values are calculated by a formula related to mP values and corresponding concentrations. The $K_i$ values are calculated by a formula related to the concentrations of Bid-BH3 peptide and Bcl-2 protein, the $IC_{50}$ values and the $K_d$ values.[23,24]

*5.3. MTT assay*

The three cancer cells (PC-3, Jurkat and K562 cells) were cultured in RPMI 1640 medium which contains 10% FBS at 37℃ in 5% $CO_2$ humidified incubator. Cells were plated in a 96-well plate at 2000-4000 cells per well. After culturing for 8h, different concentrations of the compounds were added and cultured for 48h. Then 10 μL of 0.5% MTT solution was added to each well and incubated for another 4h. After aspirating the culture medium out, the generated formazan was extracted by 200 μL DMSO. After mixing and dissolving for 15 min, the absorbance was read by a microtiter-plate reader at 570 nm and the $IC_{50}$ values were calculated according to the corresponding concentrations and the inhibitory ratios.

*5.4. Molecular docking*

Molecular docking studies of compound **8h** were performed using Surflex-Dock program in Sybyl-X2.1. Compound **8h** were assigned with Gasteiger-Hückel charges and other parameters were set as default values. The Bcl-2 protein was downloaded from the Protein Data Bank (PDB code: 4MAN). The top-scored docking poses of **8h** were selected for analysis.

*5.5. Analysis of cell apoptosis*

Jurkat cells were plated in 12-well plate at $2 \times 10^5$ cells per well and treated with 20 μM and 40 μM of **8j** for 48 h. After washing with PBS, cells harvested and treated with annexin-V FITC and propidium iodide (PI) using Annexin V-FITC Apoptosis Detection Kit (Beyotime). The percentage of cells apoptosis was evaluated by flow cytometry.

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**Reference**

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## Graphic abstract

**Design, synthesis and preliminary bioactivity studies of indomethacin derivatives as Bcl-2/Mcl-1 dual inhibitors**

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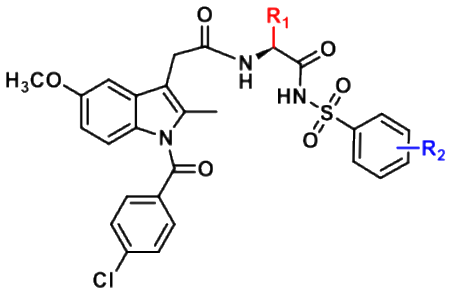


**8j**: $R_1$=4-Br-Bn-O-Bn-

$R_2$=3-$NO_2$-4-Cl-

| Ki (μM) | | |
|---|---|---|
| | Bcl-2 | 0.44±0.02 |
| | Mcl-1 | 0.44±0.03 |
| | Bcl-XL | N.A. |