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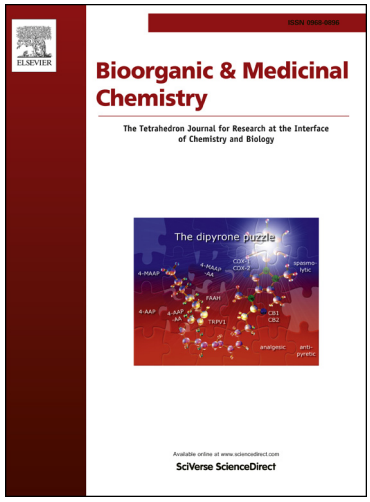

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Design, synthesis and preliminary bioactivity studies of imidazolidine-2,4-dione derivatives as Bcl-2 inhibitors

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Abstract

Anti-apoptotic B-cell lymphoma-2 (Bcl-2) proteins are promising targets for cancer therapy. In the present study, a series of imidazolidine-2,4-dione derivatives were designed and synthesized to test their inhibitory activities against anti-apoptotic Bcl-2 proteins. Among them, compound **8k** had better growth inhibitory effects on K562 and PC-3 cell lines compared to lead compound WL-276.

Keywords: Bcl-2; imidazolidine-2,4-dione; inhibitors; antitumor

1. Introduction

Malignant tumors are major diseases threatening human life. Anti-apoptotic B-cell lymphoma 2 (Bcl-2) proteins, including Bcl-2, Bcl-xL, Mcl-1, Bfl-1/A1, Bcl-B and Bcl-w, are contributors of tumor initiation, progression, and resistance to current anti-tumor treatments.[1-4] Development of Bcl-2 inhibitors to promote cell apoptosis has become one of the important strategies of tumor treatment.[5] So far, many small molecule Bcl-2 inhibitors have been reported as antitumor agents in clinical trials, such as ABT-199, Gossypol, Obatoclax and etc.[6] (**Figure 1**)

Rhodanine (2-thioxo-4-thiazolidinone) has been extensively studied and used as a potential scaffold in drug design to develop potent Bcl-2 inhibitors.[7-10] Among them, BH3I-1 (**Figure 1**) was firstly found to bind the Bcl-2 proteins and induce apoptosis.[10] Later, Xing's group reported a novel rhodanine Bcl-2 inhibitor, WL-276, which showed good antiproliferative activity against PC-3 cell line[11]. Our previous studies focused on the structural modification of WL-276 and found that target compounds with aromatic amino acid side chain and electron withdrawing group in benzenesulfonamide would be favor to enhance binding affinities.[12] In our on-going studies, the new heterocycle, imidazolidine-2,4-dione, which was bioisostere of

rhodanine and had been widely used in drug discovery[13-17], was used as scaffold to replace rhodanine in WL-276 and the structural modification were performed on the different substitutions or side chains. This study will report the synthesis, binding affinities to Bcl-2 proteins and antiproliferative activities against tumor cell lines of imidazolidine-2,4-dione derivatives.

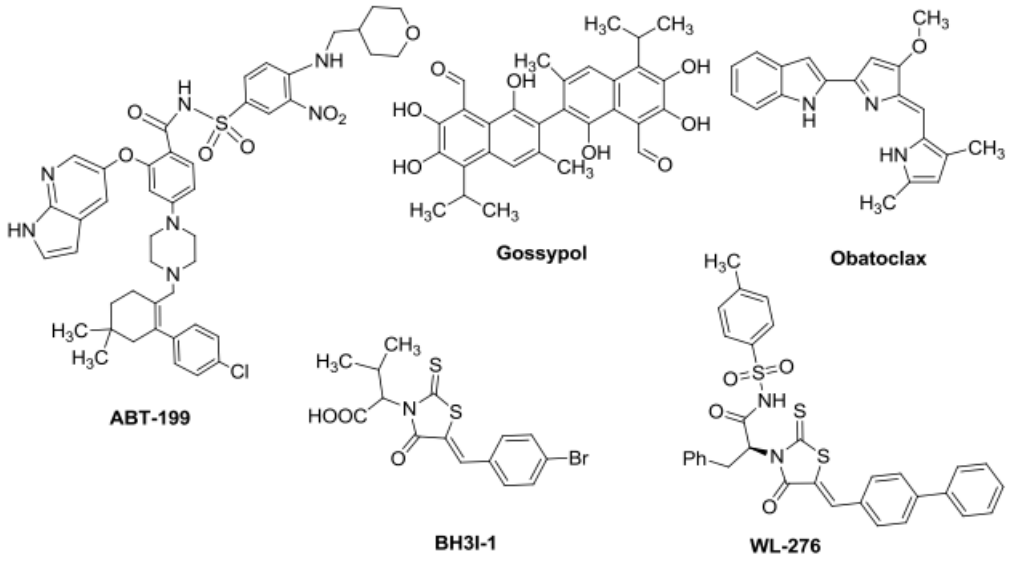
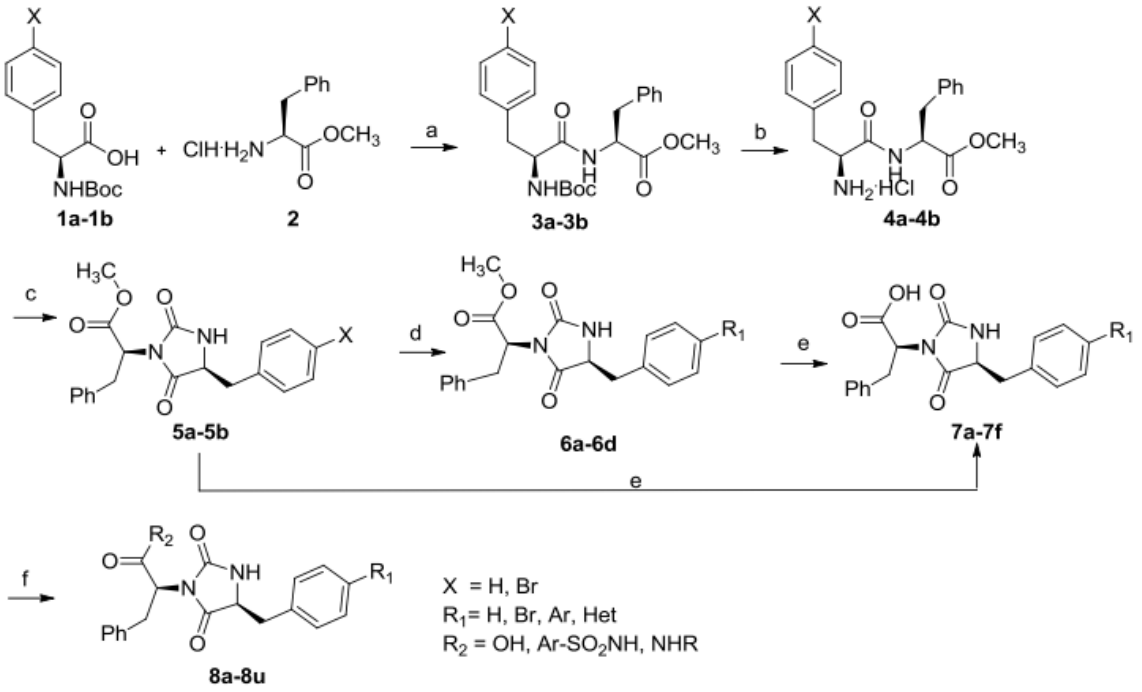


Figure 1. Representative small molecule inhibitors of anti-apoptotic Bcl-2 proteins.

2. Chemistry

Scheme 1 showed the routes of synthesizing imidazolidine-2,4-dione derivatives. The intermediates **4a-4b** were generated from substituted acids **1a-1b** and amine **2** according to reported procedures[18]. Then, **4a-4b** were cyclized to give the key intermediates **5a-5b** by employing triphosgene. Suzuki coupling reaction between **5a-5b** and different arylboronic acids formed biaryl intermediates **6a-6d**. The conversion from methyl esters **5a-5b** or **6a-6d** to acids **7a-7f** was conducted in the presence of hydrochloride. The acids **7a-7f** were reacted with substituted benzenesulfonamides and other amines to yield target compounds **8a-8u** using coupling reagent HATU.



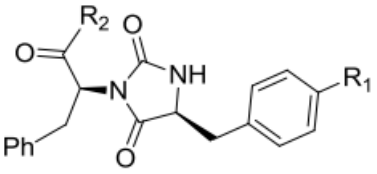
Scheme 1. Reagents and conditions: (a) EDCI, HOBt, TEA, DCM, r.t. , 3 h; (b) HCl gas, DCM, 0~5 °C, 0.5 h; (c) Triphosgene, Py, DCM, reflux, 12 h; (d) $(Ph_3P)_4Pd$, substituted phenylboronic acid, Na_2CO_3, toluene, 80 °C, 2 h; (e) HCl, dioxane, reflux, 16 h; (f) HATU, DIEA, substituted benzsulfamides and other amines, DCM, 20~25 °C, 2 h.

3. Results and discussion

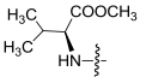
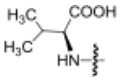
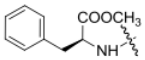
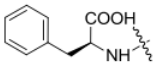
To gain the initial structure-activity relationships of the synthesized imidazolidine-2,4-dione derivatives, all target compounds were evaluated for their binding affinities to Bcl-2 based on competitive binding experiments[19] **(Table 1)**. The results showed that carboxylic acid derivatives (R_2 = OH) and amide derivatives (R_2 = side chain of amino acids), such as compounds **7a-7e** and **8p-8u**, had binding constants (K_i) of higher than 50 μM in the preliminary evaluation. It indicated that carboxylic acid and amide derivatives were unfavorable for the target compounds' affinities to Bcl-2 protein. When the substituted benzenesulfonamide fragments were introduced in R_2 position, compounds **8a-8o** exhibited various inhibitions depending on different structures in R_1 and R_2. For example, hydrogen and bromo substitution in R_1 gave very poor potency. While introducing aromatic ring in R_1 position, most of the derivatives possessed good inhibition against Bcl-2 (**8e-8l**). However, low binding

affinities occurred when aromatic ring was replaced with pyridine ring (**8m-8o**, K_i > 50 μM), which suggested that phenyl group might be critical for binding affinity in R_1 position. In addition, different substituents in aromatic ring of benzenesulfonamide in R_2 position also influenced the binding affinities to Bcl-2. For example, methyl group in para position (**8f**, K_i > 50 μM) is unfavorable for enhancing potency compared with the compound **8e** (K_i = 19 μM) without substitution. On the other hand, substitution of 3-NO_2-4-Cl gave the most potent target compounds (**8g**, K_i = 3.7 μM and **8k**, K_i = 4.4 μM), which indicated that introducing electron withdrawing groups in benzenesulfonamide moiety would be favor to enhance the binding affinities with target protein. The result is in agreement with our previous SAR studies on WL-276 with rhodanine as scaffold.[12]

Table 1. The binding affinities to Bcl-2 protein of imidazolidine-2,4-dione derivatives



Compd	R_1	R_2	K_i (μM)[a]
7a	H	OH	>50
7b	Br	OH	>50
7c	4-Cl-Ph	OH	>50
7d	3-CH_3-Ph	OH	>50
7e	2-F-Py-4-	OH	>50
8a	H	Ph-SO_2NH	>50
8b	H	3-NO_2-4-Cl-Ph-SO_2NH	>50
8c	Br	Ph-SO_2NH	>50
8d	Br	3-NO_2-4-Cl-Ph-SO_2NH	>50
8e	4-Cl-Ph	Ph-SO_2NH	19±1.5
8f	4-Cl-Ph	4-CH_3-Ph-SO_2NH	>50
8g	4-Cl-Ph	3-NO_2-4-Cl-Ph-SO_2NH	3.7±0.44
8h	4-Cl-Ph	3-NO_2-Ph-SO_2NH	15±0.81
8i	3-CH_3-Ph	Ph-SO_2NH	>50
8j	3-CH_3-Ph	4-CH_3-Ph-SO_2NH	13±1.1
8k	3-CH_3-Ph	3-NO_2-4-Cl-Ph-SO_2NH	4.4±0.21
8l	4-CHO-Ph	3-NO_2-4-Cl-Ph-SO_2NH	13±1.2

8m	2-F-Py-4-	Ph-SO_2NH	>50
8n	2-F-Py-4-	4-CH_3-Ph-SO_2NH	>50
8o	2-F-Py-4-	3-NO_2-4-Cl-Ph-SO_2NH	>50
8p	4-Cl-Ph		>50
8q	4-Cl-Ph		>50
8r	4-Cl-Ph	4-AcO-Ph-NH	>50
8s	4-Cl-Ph	4-OH-Ph-NH	>50
8t	4-Cl-Ph		>50
8u	4-Cl-Ph		>50
WL-276			0.62±0.01

[a] Three independent fluorescence polarization assays (FPAs) were conducted and results were expressed as mean ± standard deviations.

To better understanding the interactions of these imidazolidine-2,4-dione compounds to Bcl-2 protein, we docked compound **8g** in the active site of Bcl-2 (PDB entry: 2O2F) using Surflex Dock. (**Figure 2**) The results suggested that **8g** could bind well to Bcl-2 protein. In addition, the acyl, nitro and acyl benzenesulfonamide groups could form several hydrogen bonds with Tyr105, Gly142, Trp141, Asn140 and Arg143. The above interactions might contribute to the affinity of **8g** to Bcl-2.

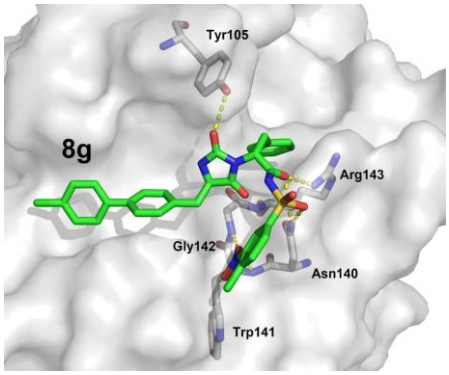


Figure 2. Proposed binding mode of **8g** in Bcl-2.

To confirm if the active target compounds could inhibit other antiapoptotic Bcl-2 proteins, five target compounds (**8g**, **8h**, **8j, 8k** and **8l**) were selected to evaluate their binding abilities to Bcl-X_L and Mcl-1. According to the results in **Table 2**, the most active compounds, **8g** and **8k,** showed similar binding affinities to the three antiapototic Bcl-2 proteins. They exhibited same activity trends to the three Bcl-2 proteins as the control WL-276 due to their structural similarity. Different from them, compounds **8h** and **8j** displayed potent Bcl-2 affinities while had very poor affinities (K_i > 50 μM) on Bcl-X_L and Mcl-1.

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

Compd	K_i (μM)[a]		
	Bcl-X_L	**Bcl-2**	**Mcl-1**
8g	6.1±1.6	3.7±0.44	2.9±0.47
8h	>50	15±0.81	>50
8j	>50	13±1.1	>50
8k	4.7±0.13	4.4±0.21	2.3±0.08
8l	>50	13±1.2	9.3±2.6
WL-276	0.66±0.21	0.62±0.01	0.25±0.08

[a] Three independent fluorescence polarization assays (FPAs) were conducted and results were expressed as mean ± standard deviations.

As Bcl-2 proteins were observed to be overexpressed in many cancer cells, such as myeloma, prostate and acute leukemias,[20] further studies were performed to examine the activities of imidazolidine-2,4-dione compounds at the cellular level. These five compounds were evaluated antiproliferative activities by MTT assay using human K562 (chronic myelogenous leukemia cell), PC-3 (prostatic cancer cell) and MDA-MB-231 (breast cancer cell). As the results in **Table 3**, these five compounds showed obvious inhibition on tumor cell lines' growth. Among them, compound **8g** and **8k** had better antiproliferative activities than the other three compounds.

Especially, compound **8k** showed better inhibitory activities against K562 and PC-3 cell lines compared with WL-276.

Table 3. Antiproliferative activities of representative compounds

Compd	IC_{50} (μM)[a]		
	K562	PC-3	MDA-MB-231
8g	39.4 ± 2.6	51.7 ± 2.0	70.8 ± 4.8
8h	49.0 ± 4.8	73.3 ± 3.0	76.4 ± 2.1
8j	46.9 ± 6.6	56.0 ± 0.4	63.5 ± 3.2
8k	35.1 ± 3.4	28.4 ± 5.0	57.8 ± 3.5
8l	68.4 ± 4.4	63.8 ± 8.7	83.4 ± 5.3
WL-276	44.9 ± 3.2	39.8 ± 3.9	35.2 ± 1.2

[a] Three independent assays were conducted and results were expressed as mean ± standard deviations.

4. Conclusions

In summary, we developed a series of novel imidazolidine-2,4-dione derivatives as potential Bcl-2 inhibitors based on our previous structure-activity studies on WL-276. As a new scaffold for Bcl-2 inhibitiors, imidazolidine-2,4-dione was a good structural motif in drug discovery and had good structural diversity. In our studies, some compounds with biphenyl and benzenesulfonamide showed potent Bcl-2 inhibitory activities. Compounds **8g** and **8k** exhibited most potent binding affinities to Bcl-2 and Mcl-1. Specifically, compound **8k** displayed higher antiproliferative activities against K562 and PC-3 cell lines compared with the positive control WL-276, which would be a good starting point for the development of more potent Bcl-2 inhibitors in the future.

5. Experimental section

5.1. General Chemistry information

Unless otherwise noted, all starting materials, reagents and solvents were

obtained from commercial suppliers and used without further purification. All reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60 GF-254) and visualized with UV light (254 nM or 365 nM) or chromogenic agents. ESI-MS was determined on an Aglient-1100 series LC/MSD trap spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured with a Brucker Avance spectrometer (300 MHz or 400 MHz). The chemical shifts were given in parts per million with tetramethylsilane (TMS) as internal standard and coupling constants (*J* values) were given in hertz (Hz). The splitting patterns were described as s (singlet), d (doublet), dd (doublet doublet), t (triplet), q (quartet), m (multiplet), and brs (broadsinglet). Most products were purified by column chromatography (silica gel 100-200 mesh). Melting points were measured on an electrothermal melting point apparatus without correction. ESI-MS was obtained on an Aglient-1100 series LC/MSD trap spectrometer. HRMS spectrums were conducted on an Agilent 6510 Quadrupole Time-of-Flight LC/MS deliver.

5.1.1. (*S*)-methyl 2-((*S*)-4-benzyl-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanoate (5a)

To the solution of **4a** (1.81 g, 5 mmol) in pyridine (7.5 mL) and DCM (25 mL), was added the solution of triphosgene (0.9 g, 3 mmol) in DCM (10 mL) at 5-10 ℃ and stirred at this temperature for 1.5 h. Reaction mixture was stirred at reflux for 12 h, and then quenched with hydrochloride solution (1N, 7.5 mL) below 10 °C. Organic phase was separated, washed with water and brine, dried over sodium sulfate, concentrated under vacuum to yield yellow oil. This oil was purified by silica gel column (ethyl acetate: petroleum ether, 1:10, v/v) to afford white solid **5a** (1.42 g, 81.0%). ^{1}H-NMR (600 MHz, DMSO-d_6): δ 8.24 (s, 1H), 7.24-7.29 (m, 4H), 7.20-7.22 (m, 2H), 7.13 (d, *J* = 7.8 Hz, 2H), 7.08 (d, *J* = 7.2 Hz, 2H), 4.8 (dd, *J* = 10.8 Hz, 4.8

Hz, 1H), 4.30-4.33 (m, 1H), 3.61 (s, 3H), 3.28 (dd, *J* = 13.8 Hz, 4.8 Hz, 1H), 3.07-3.11 (m, 1H), 2.71 (dd, *J* = 13.8 Hz, 4.8 Hz, 1H), 2.41-2.45 (m, 1H). ESI-MS: m/z 353.3 $(M+H)^+$.

5.1.1.1. (*S*)-methyl 2-((*S*)-4-(4-bromobenzyl)-2,5-dioxoimidazolidin-1-yl)-3-phenyl propanoate (5b)

Compounds **5b** were synthesized following the procedure of **5a**. White solid, yield: 82.0%, mp: 148-150 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.44 (d, *J* = 8.0 Hz, 2H), 7.20-7.33 (m, 5H), 7.00 (d, *J* = 8.0 Hz, 2H), 5.21 (S, 1H), 4.98 (dd, *J* = 6.8 Hz, 10.4 Hz, 1H), 4.05 (dd, *J* = 2.8 Hz, 10.0 Hz, 1H), 3.81 (S, 3H), 3.52 (S, 1H), 3.50 (d, *J* = 4.4 Hz, 1H), 3.02 (dd, *J* = 3.6 Hz, 14.0 Hz, 1H), 2.21 (dd, *J* = 10.0 Hz, 14.0 Hz, 1H). ESI-MS m/z: 430.7 $(M+H)^+$.

5.1.2. (*S*)-methyl 2-((*S*)-4-((4'-chlorobiphenyl-4-yl)methyl)-2,5-dioxoimid azolidin-1-yl)-3-phenylpropanoate (6a)

The mixture of **5b** (2.9 g, 6.7 mmol), 4-chlorophenylboronic acid (1.36 g, 10.1 mmol), tetrakis(triphenylphosphine)palladium (0.7 g, 0.6 mmol) and sodium carbonate (2.8 g, 26.8 mmol) in toluene (29 mL) and water (2.9 mL) was degassed and heated at 80 °C for 2 h. Filter, the filtrate was diluted with EA (50 mL), washed with water, dried over sodium sulfate, concentrated under vacuum to yield yellow oil. The oil was purified by silica gel column (ethyl acetate: petroleum ether, 1:10, v/v) to afford off-white solid **6a** (2.7 g, 87.2%). mp: 150-152 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) : δ 8.22 (S, 1H), 7.06-7.70 (m, 13H), 4.88 (dd, *J* = 4.8 Hz, 11.2 Hz, 1H), 4.32-4.38 (m, 1H), 3.62 (S, 3H), 3.29-3.31 (m, 1H), 3.10-3.16 (m, 1H), 2.76-2.80 (m, 1H), 2.42-2.47 (m, 1H). ESI-MS m/z: 463.0 $(M+H)^+$.

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

5.1.2.1. (*S*)-methyl 2-((*S*)-4-((3'-methylbiphenyl-4-yl)methyl)-2,5-dioxo

imidazolidin-1-yl)-3-phenylpropanoate (6b)

Off-white solid, yield: 90.5%, mp: 137-140°C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.30 (S, 1H), 7.15-7.56 (m, 13H), 4.88 (dd, J = 5.6 Hz, 10.8 Hz, 1H), 4.37 (m, 1H), 3.61 (S, 3H), 3.29-3.33 (m, 1H), 3.10 (dd, J = 11.2 Hz, 13.6 Hz, 1H), 2.79 (dd, J = 4.8 Hz, 14.0 Hz, 1H), 2.47-2.50 (m, 1H), 2.37 (S, 3H). ESI-MS m/z: 443.2 $(M+H)^+$

5.1.2.2. (*S*)-methyl 2-((*S*)-4-(4-(2-fluoropyridin-4-yl)benzyl)-2,5-dioxo imidazolidin-1-yl)-3-phenylpropanoate (6c)

Off-white solid, yield: 80.4%, mp: 142-144 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.24 (d, J = 5.2 Hz, 1H), 7.55 (d, J = 8.0 Hz, 2H), 7.20-7.35 (m, 9H), 5.52 (S, 1H), 4.98 (dd, J = 6.4 Hz, 10.8 Hz, 1H), 4.12 (dd, J = 3.6 Hz, 10.0 Hz, 1H), 3.79 (S, 3H), 3.46-3.50 (m, 2H), 3.09 (dd, J = 8.0 Hz, 14.0 Hz, 1H), 2.30 (dd, J = 10.0 Hz, 14.0 Hz, 1H). ESI-MS m/z: 447.8 $(M+H)^+$.

5.1.2.3. (*S*)-methyl 2-((*S*)-4-((4'-formylbiphenyl-4-yl)methyl)-2,5-dioxo imidazolidin- 1-yl)-3-phenylpropanoate (6d)

Off-white solid, yield: 23.8%, mp: 152-155 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 10.0 (S, 1H), 7.94 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.0 Hz, 2H), 7.20~7.32 (m, 7H), 4.98 (dd, J = 6.4 Hz, 10.4 Hz, 1H), 4.12 (dd, J = 3.6 Hz, 9.6 Hz,, 1H), 3.78 (S, 3H), 3.46-3.49 (m, 2H), 3.10 (dd, J = 4.0 Hz, 14.0 Hz, 1H), 2.28 (dd, J = 10.4 Hz, 14.0 Hz, 1H). ESI-MS m/z: 457.1 $(M+H)^+$.

5.1.3. (*S*)-2-((*S*)-4-benzyl-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanoic acid (7a)

The mixture of **5a** (1.76 g, 5.0 mmol) in dioxane (30 mL) and 3N hydrochloride (60 mL) was refluxed for 16 h, then cooled to room temperature. Filter, the cake was washed with methanol to afford white solid (1.63 g, 96.4%). Mp 204-205 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 13.09 (s, 1H), 8.17 (s, 1H), 7.23-7.29 (m, 4H),

7.18-7.21 (m, 2H), 7.13 (d, *J* = 6.6 Hz, 2H), 7.09 (d, *J* = 6.6 Hz, 2H), 4.72-4.75 (dd, *J* = 11.4 Hz, 5.4 Hz, 1H), 4.26-4.28 (m, 1H), 3.18-3.31 (dd, *J* = 13.8 Hz, 5.4 Hz, 1H), 3.11-3.15 (m, 1H), 2.71-2.74 (dd, *J* = 13.8 Hz, 5.4 Hz, 1H), 2.35-2.40 (m, 1H). HRMS (AP-ESI) Calcd. for $C_{19}H_{18}N_2O_4$: 337.1194 $(M-H)^-$, Found: 337.1206.

Compounds **7b-7f** were synthesized following the procedure described above.

5.1.3.1. (*S*)-2-((*S*)-4-(4-bromobenzyl)-2,5-dioxoimidazolidin-1-yl)-3-phenyl propanoic acid (7b)

White solid, mp: 214-216 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 13.07 (s, 1H), 8.15 (s, 1H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.17-7.29 (m, 3H), 7.10 (d, *J* = 6.6 Hz, 2H), 7.02 (d, *J* = 8.4 Hz, 2H), 4.70 (dd, *J* = 11.1 Hz, 5.1 Hz, 1H), 4.26-4.30 (m, 1H), 3.27-3.30 (m, 1H), 3.12-3.20 (m, 1H), 2.67 (dd, *J* = 16.8 Hz, 5.4 Hz, 1H), 2.37-2.44 (m, 1H). HRMS (AP-ESI) Calcd. for $C_{19}H_{17}BrN_2O_4$: 415.0299 $(M-H)^-$, Found: 415.0302.

5.1.3.2. (*S*)-2-((*S*)-4-((4'-chlorobiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanoic acid (7c)

Off-white solid, yield: 82.5%, mp: 186-188 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.22 (S, 1H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.25-7.29 (m, 2H), 7.14-7.21 (m, 5H), 4.76 (dd, *J* = 5.2 Hz, 11.2 Hz, 1H), 4.31-4.34 (m, 1H), 3.29-3.33 (m, 1H), 3.14-3.20 (m, 1H), 2.77 (dd, *J* = 5.2 Hz, 14.0 Hz, 1H), 2.45 (dd, *J* = 5.2 Hz, 14.0 Hz, 1H). HRMS (AP-ESI) m/z Calcd for $C_{25}H_{22}ClN_2O_4$ $(M+H)^+$: 449.1263, Found: 449.1265.

5.1.3.3. (*S*)-2-((*S*)-4-((3'-methylbiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin-1-yl)-

3-phenylpropanoic acid (7d)

Off-white solid, yield: 59.0%, mp: >250°C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.90 (S, 1H), 7.52 (d, J = 8.0 Hz, 2H), 7.41-7.44 (m, 2H), 7.34 (t, J = 7.6 Hz, 1H), 7.08-7.24 (m, 8H), 4.33 (dd, J =4.4 Hz, 12.0 Hz, 1H), 4.14 (dd, J = 5.6 Hz, 6.8 Hz, 1H), 3.24-3.40 (m, 3H), 2.85 (dd, J = 4.4 Hz, 14.0 Hz, 1H), 2.38 (S, 3H). HRMS (AP-ESI) m/z Calcd for $C_{26}H_{25}N_2O_4$ $(M+H)^+$: 429.1809, Found: 429.1808.

5.1.3.4. (*S*)-2-((*S*)-4-(4-(2-fluoropyridin-4-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanoic acid (7e)

Off-white solid, yield: 87.3%, mp: >250 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.30 (d, J = 5.2 Hz, 1H), 8.00 (d, J = 25.2 Hz, 1H), 7.76 (dd, J = 6.8 Hz, 8.0 Hz, 2H), 7.69 (d, J = 5.2 Hz, 1H), 7.51 (s, 1H), 7.31 (dd, J = 8.0 Hz, 14.8 Hz, 2H), 7.21 (t, J = 7.2 Hz, 1H), 7.05-7.14 (m, 3H), 6.95 (d, J = 7.2 Hz, 1H), 4.24-4.38 (m, 1H), 4.20-4.23 (m, 1H), 3.22-3.31 (m, 2H), 2.85~3.03 (m, 1H), 2.55~2.77 (m, 1H). HRMS (AP-ESI) m/z Calcd for $C_{24}H_{21}FN_3O_4$ $(M+H)^+$: 434.1511, Found: 434.1509.

5.1.3.5. (S)-2-((S)-4-((4'-formylbiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanoic acid (7f)

Off-white solid, yield: 61.9%, mp: 192-193 °C. ^{1}H-NMR (400 MHz, MeOD): δ 7.19-7.29 (m, 5H), 4.93 (t, J = 8.4 Hz, 1H), 3.80 (q, J = 18.0Hz, 2H), 3.46 (d, J = 8.4Hz, 2H). HRMS (AP-ESI) m/z Calcd for $C_{12}H_{13}N_2O_4$ $(M+H)^+$: 249.0870, Found: 249.0871.

5.1.4. (*S*)-2-((*S*)-4-benzyl-2,5-dioxoimidazolidin-1-yl)-3-phenyl-*N*-(phenylsulfonyl) propanamide (8a)

To the mixture of **7a** (0.38 g, 1.11 mmol) in DCM (15 mL), was added DIEA (0.29 g, 2.22 mmol) and HATU (0.5 g, 1.31 mmol) in order below 5 °C. Then another mixture of benzenesulfonamide (0.11 g, 0.67 mmol) in DCM (3 mL) was added

below 10 °C. Reaction mixture was quenched with 1N HCl after stirring for 2 h at 20-25 °C. Organic phase was separated and water phase was extracted with DCM once. Organic phases were combined, washed with water and brine, dried over sodium sulfate, concentrated under vacuum to yield yellow oil. This oil was purified by silica gel column (ethyl acetate: petroleum ether, 1:20, v/v) to afford white powder (0.23 g, 44.0%). Mp: 196-197 °C. ^{1}H-NMR (600 MHz, DMSO-d_6): δ 12.39 (s, 1H), 8.16 (s, 1H), 7.90 (d, J = 7.8 Hz, 2H), 7.71-7.80 (m, 1H), 7.62-7.64 (m, 2H), 7.26-7.28 (m, 4H), 7.18-7.25 (m, 2H), 7.10 (d, J = 6.6 Hz, 2H), 7.07 (d, J = 7.8 Hz, 2H), 4.76 (s, 1H), 4.07-4.09 (m, 1H), 3.23-3.26 (dd, J = 13.8 Hz, 5.4 Hz, 1H), 2.95-2.99 (m, 1H), 2.59-2.61 (m, 1H), 2.12-2.16 (m, 1H). HRMS (AP-ESI) Calcd. for $C_{25}H_{23}N_3O_5S$: 476.1286 $(M-H)^-$, Found: 476.1309.

Compounds **8b-8o** were synthesized following the procedure described above.

5.1.4.1. (*S*)-2-((*S*)-4-benzyl-2,5-dioxoimidazolidin-1-yl)-*N*-((4-chloro-3-nitro phenyl)sulfonyl)-3-phenylpropanamide (8b)

White solid, yield: 39.4%，mp: 214-215 °C。^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.47 (s, 1H), 8.17 (brs, 1H), 8.12 (dd, J = 8.4 Hz, 2.1 Hz, 1H), 8.03(d, J = 8.4 Hz, 1H), 7.17-7.28 (m, 6H), 7.07-7.11 (m, 4H), 4.77 (dd, J = 10.5 Hz, 5.4 Hz, 1H), 4.07-4.11 (m, 1H), 3.19-3.25 (dd, J = 13.8 Hz, 5.1 Hz, 1H), 2.82-2.90 (m, 1H), 2.59-2.65 (dd, J = 13.8 Hz, 5.1 Hz, 1H), 2.12-2.16 (m, 1H). HRMS (AP-ESI) Calcd. for $C_{25}H_{21}ClN_4O_7S$: 555.0447 $(M-H)^-$, Found: 555.0761.

5.1.4.2. (*S*)-2-((*S*)-4-(4-bromobenzyl)-2,5-dioxoimidazolidin-1-yl)-3-phenyl-*N*-(phenyl sulfonyl)propan–amide (8c)

White solid, yield: 32.3%, mp: 209-210 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 12.41 (s, 1H), 8.15 (s, 1H), 7.90-7.93 (m, 2H), 7.71-7.76 (m, 1H), 7.61-7.66 (m, 2H), 7.41 (d, J = 11.4 Hz, 2H), 7.17-7.28 (m, 3H), 7.09 (d, J = 6.6 Hz, 2H), 7.01 (d, J = 8.4

Hz, 2H), 4.74 (dd, *J* = 11.1 Hz, 4.8 Hz, 1H), 4.06-4.11 (m, 1H), 3.22 (dd, *J* = 13.8 Hz, 4.8 Hz, 1H), 2.98-3.06 (m, 1H), 2.55 (dd, *J* = 14.1 Hz, 5.1 Hz, 1H), 2.09-2.16 (m, 1H). HRMS (AP-ESI) Calcd. for $C_{25}H_{22}BrN_3O_5$: 554.0391 $(M-H)^-$, Found: 554.0409.

5.1.4.3. (*S*)-2-((*S*)-4-(4-bromobenzyl)-2,5-dioxoimidazolidin-1-yl)-*N*-((4-chloro-3-nitrophenyl)sulfonyl)-3-phenylpropanamide (8d)

White solid, yield: 31.4%, mp: 199-201 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.47 (d, *J* = 2.1 Hz, 1H), 8.16 (s, 1H), 8.12 (d, *J* = 2.1 Hz, 1H), 8.03-8.06 (m, 1H), 7.42-7.44 (m, 2H), 7.16-7.28 (m, 3H), 7.07-7.09 (m, 2H), 7.02-7.05 (m, 2H), 4.77 (dd, *J* = 10.8 Hz, 5.1 Hz, 1H), 4.08-4.12 (m, 1H), 3.20 (dd, *J* = 13.8 Hz, 5.1 Hz, 1H), 2.87-2.96 (m, 1H), 2.56 (dd, *J* = 13.8 Hz, 5.1 Hz, 1H), 2.11-2.21 (m, 1H). HRMS (AP-ESI) Calcd. for $C_{25}H_{20}BrClN_4O_7S$: 632.9852 $(M-H)^-$, Found: 632.9873.

5.1.4.4. (*S*)-2-((*S*)-4-((4'-chlorobiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin-1-yl)-3-phenyl-*N*-(phenylsulfonyl)propanamide (8e)

Off-white solid, yield: 61.3%, mp: >250 °C. ^{1}H-NMR (400 MHz, MeOD): δ 7.99 (d, *J* = 7.2 Hz, 2H), 7.41-7.58 (m, 9H), 7.16-7.26 (m, 7H), 4.78 (dd, *J* = 5.2 Hz, 11.6 Hz, 1H), 4.16 (dd, *J* = 4.8 Hz, 8.8 Hz, 1H), 3.44 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 3.28-3.34 (m, 1H), 2.87 (dd, *J* = 4.8 Hz, 13.6 Hz, 1H), 2.37 (dd, *J* = 8.8 Hz, 13.6 Hz, 1H). HRMS (AP-ESI) m/z Calcd for $C_{31}H_{27}ClN_3O_5S$ $(M+H)^+$: 588.1354, Found: 588.1355.

5.1.4.5. (*S*)-2-((*S*)-4-((4'-chlorobiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin-1-yl)-3-phenyl-*N*-tosylpropanamide (8f)

Off-white solid, yield: 27.0%, mp: >250 °C. ^{1}H-NMR (400 MHz, PYR-d_5): δ 9.61 (s, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 7.36-7.51 (m, 9H), 7.22-7.26 (m, 4H), 7.13-7.15 (m, 1H), 7.07 (d, *J* = 8.0 Hz, 2H), 5.47 (dd, *J* = 6.0 Hz, 10.8 Hz, 1H), 4.30 (dd, *J* = 2.0 Hz, 10.0 Hz, 1H), 3.90-3.96 (m, 2H), 3.28 (dd, *J* = 3.2 Hz, 14.0 Hz, 1H),

2.54 (dd, *J* = 10.8 Hz, 13.2 Hz, 1H), 2.07 (s, 3H). HRMS (AP-ESI) m/z Calcd for $C_{32}H_{29}ClN_3O_5S$ $(M+H)^+$: 602.1511, Found: 602.1497.

5.1.4.6. (*S*)-*N*-(4-chloro-3-nitrophenylsulfonyl)-2-((*S*)-4-((4'-chlorobiphenyl-4-yl) methyl)-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanamide (8g)

Off-white solid, yield: 19.2%, mp: >250 °C. ^{1}H-NMR (400 MHz, MeOD): δ 8.45 (d, *J* = 2.0 Hz, 1H), 8.12 (dd, *J* = 2.0 Hz, 8.4 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.16-7.24 (m, 7H), 4.73 (dd, *J* = 4.8 Hz, 11.6 Hz, 1H), 4.17 (dd, *J* = 4.8 Hz, 8.8 Hz, 1H), 3.45 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 3.25-3.29 (m, 1H), 2.89 (dd, *J* = 4.4 Hz, 14.0 Hz, 1H), 2.40 (dd, *J* = 8.8 Hz, 14.0 Hz, 1H). HRMS (AP-ESI) m/z Calcd for $C_{31}H_{25}Cl_2N_4O_7S$ $(M+H)^+$: 667.0816, Found: 667.0815.

5.1.4.7. (*S*)-2-((*S*)-4-((4'-chlorobiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin-1-yl)-*N*-(3-nitrophenylsulfonyl)-3-phenylpropanamide (8h)

Off-white solid, yield: 28.5%, mp: 246-248 °C. ^{1}H-NMR (400 MHz, MeOD): δ 8.78 (t, *J* = 2.0 Hz, 1H), 8.30-8.38 (m, 2H), 7.72 (t, *J* = 8.0 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 2H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.16-7.26 (m, 7H), 4.73 (dd, *J* = 4.8 Hz, 12.0 Hz, 1H), 4.16 (dd, *J* = 4.8 Hz, 9.2 Hz, 1H), 3.46 (dd, *J* = 5.2 Hz, 14.0 Hz, 1H), 3.30-3.32 (m, 1H), 2.90 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 2.39 (dd, *J* = 9.2 Hz, 14.0 Hz, 1H). HRMS (AP-ESI) m/z Calcd for $C_{31}H_{26}ClN_4O_7S$ $(M+H)^+$: 633.1205, Found: 633.1209.

5.1.4.8. (*S*)-2-((*S*)-4-((3'-methylbiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin-1-yl)-3-phenyl-*N*-(phenylsulfonyl)propanamide (8i)

Off-white solid, yield: 17.3%, mp: >250 °C. ^{1}H-NMR (400 MHz, MeOD), δ 7.99 (d, *J* = 8.0 Hz, 2H), 7.56 (d, *J* = 7.2 Hz, 1H), 7.48-7.52 (m, 4H), 7.24-7.38 (m, 5H), 7.14~7.19 (m, 6H), 4.81 (dd, *J* = 5.2 Hz, 11.6 Hz, 1H), 4.16 (dd, *J* = 4.4 Hz, 8.8 Hz,

1H), 3.41 (dd, *J* = 5.2 Hz, 14.0 Hz, 1H), 3.26 (dd, *J* = 11.6 Hz, 14.0 Hz 1H), 2.85 (dd, *J* = 4.4 Hz, 14.0 Hz, 1H), 2.39 (s, 3H), 2.34 (dd, *J* = 8.8 Hz, 14.0 Hz, 1H). HRMS (AP-ESI) m/z Calcd for $C_{32}H_{30}N_3O_5S$ (M+H)+: 568.1908, Found: 568.1901.

5.1.4.9. (*S*)-2-((*S*)-4-((3'-methylbiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin-1-yl)-3-phenyl-*N*-tosylpropanamide (8j)

Off-white solid, yield: 16.9%, mp: >250 °C. ^{1}H-NMR (400 MHz, MeOD): δ 7.84 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.14-7.41 (m, 13H), 4.74 (dd, *J* =4.8 Hz, 12.0 Hz, 1H), 4.14 (dd, *J* = 4.4 Hz, 9.2 Hz, 1H), 3.49 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 3.40 (d, *J* = 12.0 Hz, 1H), 2.90 (dd, *J* = 4.0 Hz, 13.6 Hz, 1H), 2.40 (d, *J* = 7.2 Hz, 6H), 2.34 (dd, *J* = 9.2 Hz, 13.6 Hz, 1H). HRMS (AP-ESI) m/z Calcd for $C_{33}H_{32}N_3O_5S$ $(M+H)^+$: 582.2057, Found: 582.2059.

5.1.4.10. (*S*)-*N*-(4-chloro-3-nitrophenylsulfonyl)-2-((*S*)-4-((3'-methylbiphenyl-4-yl) methyl)-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanamide (8k)

Off-white solid, yield: 26.7%, mp: >250 °C. ^{1}H-NMR (400 MHz, MeOD): δ 8.45 (d, *J* = 2.0 Hz, 1H), 8.12 (dd, *J* = 2.4 Hz, 8.4 Hz, 1H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.14-7.40 (m, 11H), 4.72 (dd, *J* = 5.2 Hz, 12.0 Hz, 1H), 4.17 (dd, *J* = 4.4 Hz, 8.8 Hz, 1H), 3.45 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 3.25-3.29 (m, 1H), 2.90 (dd, *J* = 4.4Hz, 14.0 Hz, 1H), 2.41 (s, 3H), 2.37 (dd, *J* = 9.2 Hz, 14.0 Hz, 1H). HRMS (AP-ESI) m/z Calcd for $C_{32}H_{28}ClN_4O_7S$ $(M+H)^+$: 647.1362, Found: 647.1364.

5.1.4.11. (*S*)-*N*-(4-chloro-3-nitrophenylsulfonyl)-2-((*S*)-4-((4'-formylbiphenyl-4-yl) methyl)-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanamide (8l)

Off-white solid, yield: 17.8%, mp: 165-166 °C. ^{1}H-NMR (400 MHz, MeOD): δ 10.04 (S, 1H), 8.45 (d, *J* = 2.0 Hz, 1H), 8.13 (dd, *J* = 2.0 Hz, 8.4 Hz, 1H), 8.00 (d, *J* = 8.4 Hz, 2H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.16-7.27 (m, 7H), 4.72 (dd, *J* = 4.8 Hz, 11.6 Hz, 1H), 4.20 (dd, *J* = 4.8 Hz, 8.4

Hz, 1H), 3.45 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 3.25~3.29 (m, 1H), 2.93 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 2.46 (dd, *J* = 8.8 Hz, 13.6 Hz, 1H). HRMS (AP-ESI) m/z Calcd for $C_{32}H_{26}ClN_4O_8S$ $(M+H)^+$: 661.1154, Found: 661.1166.

5.1.4.12. (*S*)-2-((*S*)-4-(4-(2-fluoropyridin-4-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)-3-phenyl-*N*-(phenylsulfonyl)propanamide (8m)

Off-white solid, yield: 30.1%, mp: >250 °C. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.29 (d, *J* = 5.2 Hz, 1H), 7.95-8.05 (m, 2H), 7.69-7.77 (m, 5H), 6.95-7.50 (m, 10H), 4.27-4.39 (m, 1H), 4.17 (dd, *J* = 1.6 Hz, 10.0 Hz, 1H), 3.16-3.31 (m, 2H), 2.98 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 2.56-2.61 (m, 1H). HRMS (AP-ESI) m/z Calcd for $C_{30}H_{26}FN_4O_5S$ $(M+H)^+$: 573.1602, Found: 573.1605.

5.1.4.13. (*S*)-2-((*S*)-4-(4-(2-fluoropyridin-4-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)-3-phenyl-*N*-tosylpropanamide (8n)

Off-white solid, yield: 29.4%, mp: >250 °C. ^{1}H-NMR (400 MHz, MeOD), δ 8.24 (d, *J* = 5.2Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 2H), 7.67 (d, *J* = 8.4 Hz, 2H), 7.58-7.60 (m, 1H), 7.07-7.33 (m, 10H), 4.67 (dd, *J* = 4.8 Hz, 11.6 Hz, 1H), 4.19 (dd, *J* = 4.8 Hz, 8.0 Hz, 1H), 3.48 (dd, *J* = 4.8 Hz, 14.4 Hz, 1H), 3.38-3.42 (m, 1H), 3.10 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 2.72 (dd, *J* = 8.0 Hz, 14.0 Hz, 1H), 2.40 (S, 3H). HRMS (AP-ESI) m/z Calcd for $C_{31}H_{28}FN_4O_5S$ $(M+H)^+$: 587.1759, Found: 587.1761.

5.1.4.14. (*S*)-*N*-(4-chloro-3-nitrophenylsulfonyl)-2-((*S*)-4-(4-(2-fluoropyridin-4-yl)benzyl)-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanamide (8o)

Off-white solid, yield: 37.5%, mp: >250 °C. ^{1}H-NMR (400 MHz, MeOD): δ 8.43 (dd, *J* = 2.0 Hz, 9.6 Hz, 1H), 8.24 (dd, *J* = 2.8 Hz, 5.2 Hz, 1H), 8.09-8.13 (m, 1H), 7.76 (d, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.05-7.35 (m, 9H), 4.64-4.74 (m, 1H), 4.19 (dd, *J* = 4.8 Hz, 8.0 Hz, 1H), 3.46 (dd, *J* = 4.8 Hz, 14.4 Hz, 1H), 3.11 (dd, *J* = 4.4 Hz, 14.0 Hz, 1H), 2.93 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 2.75 (dd, *J* = 8.0 Hz, 14.0

Hz, 1H). HRMS (AP-ESI) m/z Calcd for $C_{30}H_{24}ClFN_5O_7S$ $(M+H)^+$: 652.1064, Found: 652.1049.

5.1.4.15. (*S*)-methyl 2-((*S*)-2-((*S*)-4-((4'-chlorobiphenyl-4-yl)methyl)-2,5-dioxo imidazolidin-1-yl)-3-phenylpropanamido)-3-methylbutanoate (8p)

To the mixture of **7c** (0.3 g, 0.67 mmol) in DCM (5 mL), was added HOBt (0.11 g, 0.8 mmol) and EDC·HCl (0.14 g, 0.74 mmol) in order below 5 ℃. To another mixture of L-valine methyl ester hydrochloride (0.11 g, 0.67 mmol) in DCM (3 mL), was added TEA (0.07 g, 0.67 mmol) at room temperature. Then this mixture was added into the mixture of **7c** below 10 ℃. Reaction mixture was quenched with water after stirring for 2 h at 20-25 ℃. Organic phase was separated and water phase was extracted with DCM once. Organic phases were combined, washed with water and brine, dried with sodium sulfate, concentrated under vacuum to yield colorless oil. This oil was purified by silica gel column (ethyl acetate: petroleum ether, 1:20, v/v) to afford off-white solid (0.28 g, 74.6%). mp: 70-72 ℃. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.48-7.52 (m, 4H), 7.33-7.43 (m, 4H), 7.17-7.27 (m, 5H), 5.47 (S, 1H), 5.03 (t, J = 8.4 Hz, 1H), 4.58 (dd, J = 4.8 Hz, 8.4 Hz, 1H), 4.10-4.13 (m, 1H), 3.74 (S, 3H), 3.49 (d, J = 8.4 Hz, 2H), 3.07 (dd, J = 3.6 Hz, 14.0 Hz, 1H), 2.17-2.25 (m, 1H), 2.02-2.09 (m, 1H), 0.97 (d, J = 6.8 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H). HRMS (AP-ESI) m/z Calcd for $C_{31}H_{33}ClN_3O_5$ $(M+H)^+$: 562.2103, Found: 562.2100.

5.1.4.16. (*S*)-2-((*S*)-2-((*S*)-4-((4'-chlorobiphenyl-4-yl)methyl)-2,5-dioxoimidazo lidin-1-yl)-3-phenylpropanamido)-3-methylbutanoic acid (8q)

Compound **8q** was synthesized from **8p** following the procedure of **7a**. Off-white solid, yield: 61.6%, mp: 180-182 ℃. ^{1}H-NMR (400 MHz, MeOD): δ 7.53~7.59 (m, 4H), 7.42 (dd, J = 2.0 Hz, 6.8 Hz, 2H), 7.21-7.32 (m, 7H), 4.95 (dd, J = 6.0 Hz, 11.2 Hz, 1H), 4.28 (d, J = 4.4 Hz, 1H), 4.24 (dd, J = 4.8Hz, 8.4 Hz, 1H), 3.37-3.48 (m, 2H),

2.87 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 2.27 (dd, *J* =4.8 Hz, 14.0 Hz, 1H), 2.18 (m, 1H), 0.98 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H). HRMS (AP-ESI) m/z Calcd for $C_{30}H_{31}ClN_3O_5$ $(M+H)^+$: 548.1947, Found: 548.1947.

5.1.4.17. 4-((*S*)-2-((*S*)-4-((4'-chlorobiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanamido)phenyl acetate (8r)

Compound **8r** was synthesized following the procedure of **8p**. Off-white solid, yield: 61.6%, mp: >250 ℃. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.93 (S, 1H), 8.24 (S, 1H), 7.49-7.67 (m, 8H), 7.21-7.33 (m, 7H), 7.09 (d, *J* = 8.8 Hz, 2H), 4.89 (dd, *J* = 5.2 Hz, 10.8 Hz, 1H), 4.24 (dd, *J* = 5.6 Hz, 6.8 Hz, 1H), 3.45 (dd, *J* = 5.2 Hz, 13.6 Hz, 1H), 3.27 (dd, *J* = 11.6 Hz, 13.6 Hz, 1H), 2.79 (dd, *J* = 4.8 Hz, 14.0 Hz, 1H), 2.42 (dd, *J* = 8.0 Hz, 14.0 Hz, 1H), 2.26 (S, 3H). HRMS (AP-ESI) m/z Calcd for $C_{33}H_{29}ClN_3O_5$ $(M+H)^+$: 582.1790, Found: 582.1798.

5.1.4.18. (*S*)-2-((*S*)-4-((4'-chlorobiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin-1-yl)-*N*-(4-hydroxyphenyl)-3-phenylpropanamide (8s)

Compound **8s** was synthesized from **8r** following the procedure of **7a**. Off-white solid, yield: 38.9%, mp: >250 ℃. ^{1}H-NMR (400 MHz, MeOD): δ 7.40-7.59 (m, 8H), 7.20-7.31 (m, 9H), 4.93-4.99 (m, 1H), 4.22-4.28 (m, 1H), 3.40-3.43 (m, 1H), 3.23-3.29 (m, 1H), 2.87-2.95 (m, 1H), 2.44-2.58 (m, 1H). HRMS (AP-ESI) m/z Calcd for $C_{31}H_{27}ClN_3O_4$ $(M+H)^+$: 540.1685, Found: 540.1690.

5.1.4.19. (*S*)-methyl 2-((*S*)-2-((*S*)-4-((4'-chlorobiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin-1-yl)-3-phenylpropanamido)-3-phenylpropanoate (8t)

Compound **8t** was synthesized following the procedure of **8p**. Off-white solid, yield: 74.1%, mp: 72-74 ℃. ^{1}H-NMR (400 MHz, $CDCl_3$), δ 7.47-7.51 (m, 4H), 7.40-7.42 (m, 2H), 7.09-7.31 (m, 12H), 5.55 (S,1H), 4.86-4.96 (m, 2H), 4.03-4.07 (m, 1H), 3.72 (S, 3H), 3.35-3.37 (m, 2H), 3.19 (dd, *J* = 5.6 Hz, 14.0 Hz, 1H), 3.09 (dd, *J* =

6.8 Hz, 14.0 Hz, 1H), 3.01 (dd, *J* = 3.6 Hz, 14.0 Hz, 1H), 2.02 (dd, *J* = 3.2 Hz, 14.0 Hz, 1H). HRMS (AP-ESI) m/z Calcd for $C_{35}H_{33}ClN_3O_5$ $(M+H)^+$: 610.2103, Found: 610.2111.

5.1.4.20. (*S*)-2-((*S*)-2-((*S*)-4-((4'-chlorobiphenyl-4-yl)methyl)-2,5-dioxoimidazolidin- 1-yl)-3-phenylpropanamido)-3-phenylpropanoic acid (8u)

Compound **8u** was synthesized from **8t** following the procedure of **7a**. Off-white solid, yield: 61.5%, mp: 218-220 ℃. ^{1}H-NMR (400 MHz, MeOD): δ 7.55 (dd, *J* = 8.4 Hz, 12.8 Hz, 4H), 7.42 (d, *J* = 8.8 Hz, 2H), 7.16-7.28 (m, 12H), 4.82 (dd, *J* = 7.6 Hz, 9.2 Hz, 1H), 4.52 (t, *J* = 5.2 Hz, 1H), 4.17 (dd, *J* = 4.8 Hz, 8.4 Hz, 1H), 3.26-3.29 (m, 2H), 3.20 (dd, *J* = 4.8 Hz, 13.6 Hz, 1H), 3.02 (dd, *J* = 7.2 Hz, 14.0 Hz, 1H), 2.86 (dd, *J* = 4.4 Hz, 14.0 Hz, 1H), 2.30 (dd, *J* = 8.4 Hz, 14.0 Hz, 1H). HRMS (AP-ESI) m/z Calcd for $C_{34}H_{31}ClN_3O_5$ $(M+H)^+$: 596.1947, Found: 596.1946.

5.2 Binding assay for Bcl-2 proteins based on Fluorescence polarization technique

A 26-reside Bid-BH3 peptide (QEDIIRNIARHLAQVGDSMDRSIPPG) was labeled at the N-terminus by 5-carboxyfluorescein succinimidyl ester (FAM) as the fluorescence labeled peptide (5-FAM-QEDIIRNIARHLAQVGDSMDRSIPPG). The 5-FAM-Bid-BH3 peptide had a K_d value of 58 nM to bind to Bcl-2 protein.

In the competitive binding experiments, Bcl-2 protein and tested compounds were preincubated in the PBS assay solution for 30 min at room temperature. Then the 5-FAM-Bid-BH3 peptide was added into the solution and incubated for 20min. The volume of total solution was 200 μL. Finally, the solutions were transferred into 384-well, black, flat-bottom plates (Corning Inc.) with 60 μL per well and three wells per sample. The polarization values (milipolarization units, mP) were measured under the condition of an excitation wavelength at 485 nm and an emission wavelength at

535 nm using the Tecan GENios-Pro Injector Reader (Tecan Group Ltd). In the binding experiments, the hydantoin derivatives were prepared in DMSO at seven concentrations, which were, 1 nM, 10 nM, 100 nM, 1μM, 10 μM, 50 μM and 100 μM. The final concentrations of Bcl-2 protein and 5-FAM-Bid-BH3 peptide were 290nM and 10nM, respectively.

The procedures of the binding assay for Bcl-X_L and Mcl-1were almost same as that for Bcl-2 except the total concentration of Bcl-X_L as 125 nM and the total concentration of Mcl-1 as 105 nM.

5.3. Molecular docking

Surflex-Dock program in Sybyl 7.3 was used with default values. The structures of **8g** and the Bcl-2 protein were optimized according to reference.[21] The top-scored conformation was selected as the best docking result.

5.4 MTT assay

The antiproliferative activities of 6 representative compounds were evaluated by the MTT (3-[4, 5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) assay. The three cancer cells were incubated in RPMI1640 medium containing 10% FBS in humidified incubator (37 ℃, 5% CO_2). After the cells were seeded at 5000 cells per well in the 96-well plates and incubated for 12 h, the growing cells were treated with the indicated compounds at 15 μM, 20 μM, 30 μM, 40 μM and 50 μM for 48 h. 10 μL MTT (5 mg/mL in PBS) solution was added into every well. The plates were incubated for further 4 h. Finally, the formed formazan in every well was dissolved in 150 μL DMSO and mixed for 15 min. Optical density was read with a microtiter-plate reader at 570 nm.

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Graphical abstract