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Original article

Synthesis of *N*-methyl-bisindolylmaleimide amino acid methyl ester conjugates and cytotoxicity study

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ABSTRACT

A novel series of *N*-methylbisindolylmaleimides derivatives bearing 2-acetamino acid moieties were synthesized. The cytotoxic activities of these compounds were tested in six tumor cell lines. The most potent compound **8d** displayed cytotoxicity against six human tumor cell lines in the micromolar range.

1. Introduction

Staurosporine is a well-known natural product that displays a wide range of potent biological activities, including antimicrobial, hypotensive, cytotoxic and protein kinase inhibitory activities. The greatest interest of staurosporine was due to its potent antitumor activity. However, the poor selectivity for kinase family limits its value as a tool for searching new anticancer drugs [1–5]. So far, many derivatives of bisindolymaleimides, which are structurally related to staurosporine, have been synthesized and biologically evaluated [6–8]. So far, several staurosporine derivatives, such as ruboxistaurin (LY333531) mesylate, enzastaurin (LY317615), and Ro 31-7453, are in clinical trials [9–13]. (Fig. 1)

It is well-known that amino acids, which have structurally diverse side chains, are fundamental building blocks of biological systems and many natural products [14]. In order to increase the water solubility and improve the binding capacity to the cellular targets, amino acids have been incorporated into indolocarbazole derivatives [15,16]. In this paper, a series of *N*-methyl-bisindolymaleimide derivatives bearing modified 2-acetamino acid moieties were synthesized, and their antiproliferative activities against six tumor cell lines were evaluated.

2. Results and discussion

2.1. Chemistry

Bisindolylmaleimides nucleus was synthesized from *N*-methyl pyrrole **1**. Bromination and oxidation of *N*-methyl pyrrole gave *N*-methyl-dibromomaleimide **2** [17]. Indolyllithium was coupled with *N*-methyl-dibromomaleimide **2** to afford the bisindolylmaleimide **3** [18]. Treatment of the intermediate with 2 equivalent of di-tert-butyl dicarbonate, followed by selective mono-deprotection gave compound **5** [19]. Alkylation of the bisindolylmaleimide **5** with ethyl bromoacetate in the presence of sodium hydride gave ester **6** [20]. Treatment of **6** with 1 M aqueous potassium hydroxide readily afforded the acid **7**. Finally, compound **7** was coupled with various amino acid methyl esters in the presence of 1-(3-dimethylamino-propyl)-3-ethyl-carbodiimide hydrochloride (EDC) and 4-(dimethylamino) pyridine (DMAP) to give the target compound **8** in a reasonable yield. (Scheme 1)

2.2. Pharmacology

By the MTT assay, cytotoxicity of these derivatives was evaluated against six human cancer cell lines: human intestinal adenocarcinoma (HCT-8), human hepatoma cell (BEL-7402), human ovarian carcinoma (A2780), human breast carcinoma (MCF-7), non-small cell lung carcinoma (A549), human gastric carcinoma (BGC-823). The results of cytotoxicity studies were summarized in Table 1.

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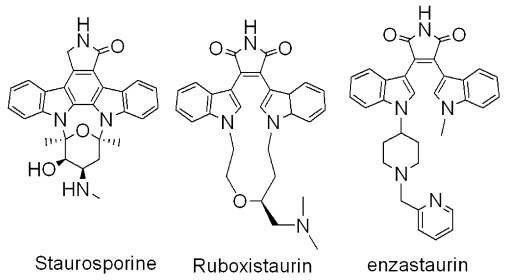
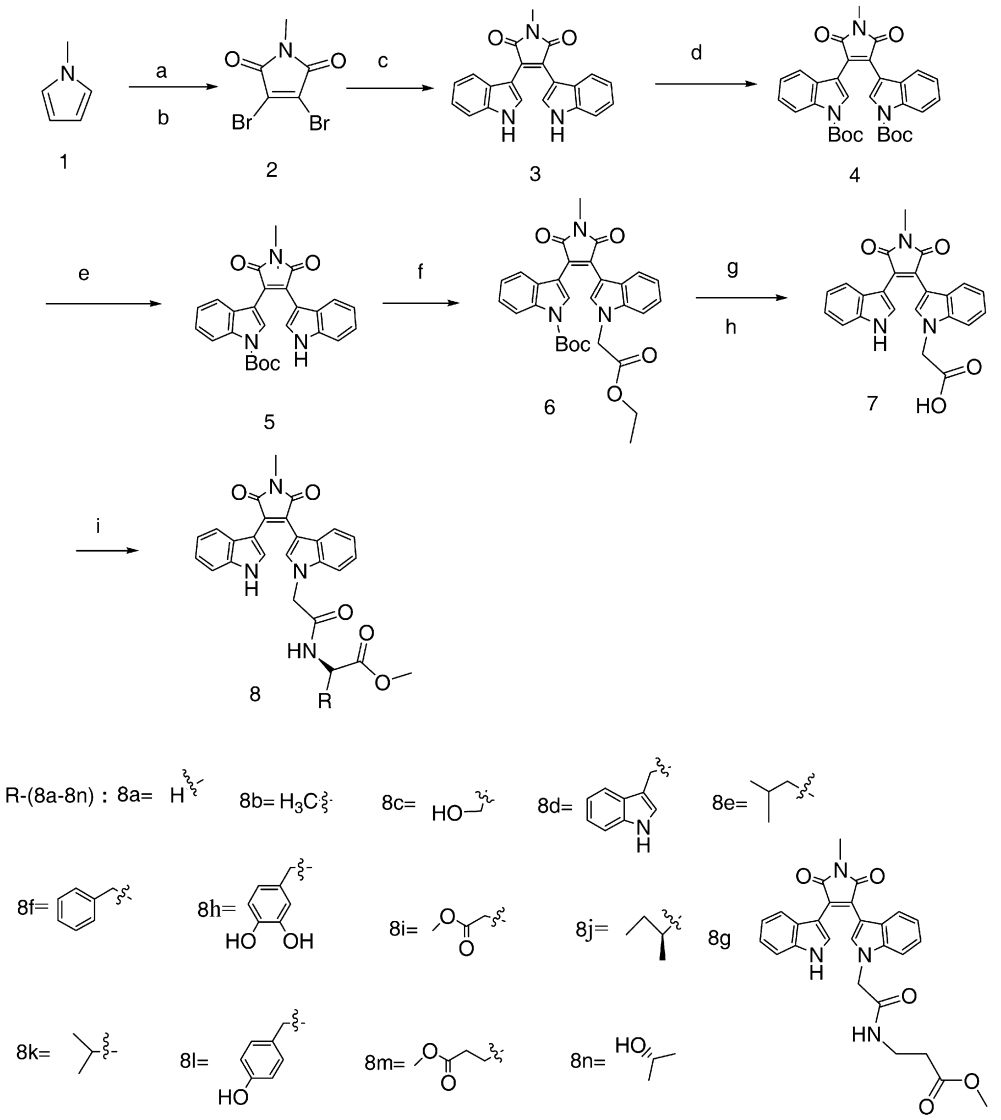


Fig. 1. Structures of staurosporine and its derivatives.

Among the four aromatic amino acids, compound **8d**, which was derived from L-tryptophane, exhibited the most potent inhibitory activity against HCT-8, BEL-7402, A2780, MCF-7, A549, BGC-823 cell lines, with the inhibitory concentration (IC_{50}) values of 9.19, 4.11, 5.39, 4.83, 45.18, 3.73 μM, respectively. Compound **8f**, which was derived from L-phenylalanine, showed moderate to weak cytotoxic activities against all tested human tumor cell lines with IC_{50} values of 50–100 μM. However, compound **8h**, which was derived from L-DOPA, exhibited no cytotoxicity against all the tested tumor cell lines.

Among the ten derivatives of aliphatic amino acids, compound **8j**, which was derived from L-isoleucine, was the only one that was inactive to all tumor cell lines. However, compound **8e**, which was derived from L-leucine, exhibited inhibitory activities against the six tested cell lines, with IC_{50} values of 30–50 μM (except for A549 cell line, $IC_{50} > 100$ μM). Ala and Val conjugates (**8b**, **8k**), which share a similar hydrophobic residue, exhibited some similar pattern in cytotoxic activity against the tested cell lines. Similarly, when comparing Ser with Thr conjugate (**8c**, **8n**), it was apparent that they exhibited similar cytotoxic activity against the five tested cell lines. Bearing one more carbonyl group on the side chain, Glu and Asp conjugates (**8m**, **8i**) exhibited similar antiproliferative activity in five tested cell lines. The IC_{50} values of the two achiral derivatives **8a** and **8g**, which were synthesized from glycine and β-amino-propionic acid respectively, were 20–90 μM.



Scheme 1. Preparation of compounds **8a**–**n.** Reagents and conditions: (a) NBS,THF, (b) HNO_3, 46% for 2 steps; (c) indole, LiHMDS, toluene, 73%; (d) $(Boc)_2O$, DMAP, Et_3N, CH_2Cl_2, 95%; (e) Bu_4NF,THF, reflux, 90%; (f) NaH, $BrCH_2COOC_2H_5$, 0 °C, 88%; (g) CH_3OH, KOH, 85%; (h) 1 M HCl, 87%; (i) EDC, DMAP, CH_2Cl_2, amino acid methyl ester. 60–90%.

Table 1
Cytotoxicity of compounds **8a**–**n**.

Compound	In vitro cytotoxicity (IC_{50}, μM)					
	BCT-8	BEL-7402	A2780	MCF-7	A549	BGC-823
8a	22.91	28.36	33.90	35.97	78.32	88.36
8b	21.40	9.34	15.83	15.30	30.47	60.43
8c	22.93	67.23	23.15	24.46	37.94	28.90
8d	9.19	4.11	5.39	4.83	45.18	3.73
8e	37.81	42.44	38.47	37.41	>100	45.49
8f	72.42	96.66	77.90	95.27	88.14	98.04
8g	50.94	28.38	44.51	47.75	37.58	44.92
8h	>100	>100	>100	>100	>100	>100
8i	23.37	9.36	15.36	25.80	74.22	24.60
8j	>100	>100	>100	>100	>100	>100
8k	25.83	4.01	23.77	16.01	30.62	48.19
8l	93.18	28.14	>100	51.72	>100	>100
8m	29.15	47.43	32.33	23.40	73.66	25.02
8n	23.35	>100	21.42	22.86	25.67	23.26
Cisplatin	3.08	7.39	4.83	3.36	1.75	1.02

The IC_{50} values represent the compound concentration (μM) required to inhibit tumour cell proliferation by 50%.

3. Conclusions

In conclusion, a series of novel *N*-methyl-bisindolylmaleimide amino acid conjugates were prepared. Most of the compounds showed moderate antiproliferative activity against human cancer cell lines including BCT-8, BEL-7402, A2780, MCF-7, A549 and BGC-823. Compound **8d**, which was derived from L-tryptophane, was the most potent one. This study demonstrated that this type of bisindolylmaleimide derivatives could be promising lead compounds for the discovery of novel antitumor drugs. Further studies of the antiproliferative mechanism of this series of compounds are in progress.

4. Experimental protocols

Starting materials, reagents and solvents were purchased from commercial suppliers and purified before use. Melting points were recorded on Yanaco Mp-500D melting point apparatus and were uncorrected. Optical rotations were measured on a Perkin–Elmer Polarimeter 341LC using 10 cm cells and the sodium D line (589 nm) at 20 °C and concentration indicated. NMR spectra were recorded on a Varian Oxford 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz) or Varian Oxford 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), chemical shifts (δ) are expressed in ppm, and the following abbreviations are used: singlet (s), doublet (d), triplet (t), doubled doublet (dd), multiplet (m). HRMS were carried out by Agilent LC/MSD TOF.

4.1. General procedure for the preparation of compounds *8a–n*

A mixture of acid **7** (1 eq), DMAP (1.2 eq), EDC (1.2 eq), and anhydrous CH_2Cl_2 was stirred at room temperature, and then L-amino acid ester (1.2 eq) was added and the mixture was stirred at room temperature for 4–6 h. After completion of the reaction as indicated by TLC, the CH_2Cl_2 was removed on the rotary evaporator to give a red solid. The solid obtained was purified by silica column chromatography with ethyl acetate/petroleum ether as the eluent to give compounds **8a**–**n**. Yield: 60–90%.

4.1.1. Ethyl 2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} acetyl) amino] acetate (*8a*)

Yield 82%, red solid, mp: 146–148 °C; HRMS (ES) calcd for $C_{27}H_{25}N_4O_5$ $[M + H]^+$: 485.1819, found 485.1838. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.98 (s, 1H), 7.72 (t, 1H, J = 2.7 Hz), 7.49 (s, 1H), 7.28 (m, 2H), 7.17 (m, 2H), 7.04 (t, 1H, J = 7.2 Hz), 6.89 (t, 2H, J = 7.8 Hz), 6.71 (t, 1H, J = 7.2 Hz), 5.92 (t, 1H, J = 5.4 Hz), 4.78 (s, 2H), 4.11 (q, 2H, J = 7.2 Hz), 3.88 (d, 2H, J = 5.7 Hz), 3.19 (s, 3 H), 1.21 (t, 3H, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.2, 168.9, 167.9, 136.3, 136.0, 131.3, 130.9, 128.9, 128.8, 126.4, 125.0, 123.3, 122.5, 122.4, 121.6, 121.1, 120.1, 111.6, 109.4, 107.9, 106.6, 61.5, 49.8, 41.1, 24.2, 14.0.

4.1.2. Methyl (2S)-2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} acetyl) amino] propanoate (*8b*)

Yield 78%, red solid, mp: 112–114 °C; $[\alpha]_D^{20}$: −28.2 (c = 0.002, CH_2Cl_2); HRMS (ES) calcd. for $C_{27}H_{25}N_4O_5$ $[M + H]^+$: 485.1819, found 485.1842. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.95 (s, 1H), 7.69 (d, 1H, J = 2.7 Hz), 7.65 (s, 1H), 7.24 (m, 2H), 7.03 (m, 4H), 6.74 (m, 2H), 6.13 (d, 1H, J = 7.8 Hz), 4.78 (s, 2H), 4.57 (m, 1H), 3.66 (s, 3H), 3.19 (s, 3H), 1.30 (d, 3H, J = 7.5 Hz). ^{13}C NMR (75 Hz, $CDCl_3$): δ = 172.5, 172.2, 167.0, 136.2, 135.9, 131.6, 128.7, 128.5, 126.4, 125.3, 123.0, 122.4, 122.3, 121.6, 120.8, 120.1, 111.4, 109.3, 107.7, 106.7, 60.3, 50.0, 48.0, 24.1, 17.9.

4.1.3. Methyl (2S)-3-hydroxy-2-{[1-({3-[4-(1H-3-indoyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} methyl) acetyl]amino} propanoate (*8c*)

Yield 67%, red solid, mp: 143–145 °C; $[\alpha]_D^{20}$: −22.0 (c = 0.002, CH_2Cl_2); HRMS (ES) calcd for $C_{27}H_{25}N_4O_6$ $[M + H]^+$: 501.1774, found 501.1750. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.68 (s, 1H), 7.73 (d, 1H, J = 2.7 Hz), 7.69 (s, 1H), 7.29 (m, 2H), 7.11 (d, 1H, J = 7.2 Hz), 7.06 (m, 2H), 6.88 (d, 1H, J = 7.8 Hz), 6.77 (m, 2H), 6.35 (d, 1H, J = 7.5 Hz), 4.86 (s, 2H), 4.63 (m, 1H), 3.85 (m, 2H), 3.67 (s, 3H), 3.17 (s, 3H), 2.49 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.5, 172.2, 170.1, 167.8, 136.3, 135.9, 131.4, 130.9, 128.8, 128.7, 126.4, 125.5, 123.1, 122.6, 122.4, 121.7, 120.9, 120.4, 111.3, 109.2, 107.8, 106.7, 62.6, 54.5, 52.7, 50.0, 24.2.

4.1.4. Methyl (2S)-3-(1H-3-indolyl)-2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} acetyl) amino] propanoate (*8d*)

Yield 72%, red solid, mp: 103–105 °C; $[\alpha]_D^{20}$: −141.0 (c = 0.002, CH_2Cl_2); HRMS (ES) calcd for $C_{35}H_{30}N_5O_5$ $[M + H]^+$: 600.2241, found 600.2226. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.56 (s, 1H), 8.33 (s, 1H), 7.71 (d, 1H, J = 2.7 Hz), 7.49 (d, 1H, J = 8.4 Hz), 7.28 (m, 5H), 7.06 (m, 5H), 6.67 (d, 2H, J = 2.7H), 5.73 (d, 2H, J = 8.1 Hz). 4.69 (m, 3H), 3.58 (s, 3H), 3.26 (m, 4H), 3.00 (dd, 1H, J_1 = 14.7 Hz, J_2 = 5.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 172.73, 171.97, 171.44, 166.78, 136.16, 136.12, 136.01, 131.13, 129.62, 129.16, 126.75, 126.14, 124.35, 123.44, 123.17, 122.80, 122.44, 122.05, 121.98, 121.35, 120.52, 119.34, 118.25, 111.36, 109.61, 107.88, 107.62, 106.63, 52.47, 51.11, 50.10, 26.99, 24.28.

4.1.5. Methyl (2S)-2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} acetyl) amino]-4-methylpentanoate (*8e*)

Yield 77%, red solid, mp: 149–151 °C; $[\alpha]_D^{20}$:-10.0 (c = 0.002, CH_2Cl_2); HRMS (ES) calcd for $C_{30}H_{31}N_4O_5$ $[M + H]^+$: 527.2289, found 527.2266. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.61(s, 1H), 7.80 (d, 1H, J = 3.0 Hz), 7.69 (s, 1H), 7.33 (d, 1H, J = 8.1 Hz), 7.22(m, 1H), 7.09 (m, 2H), 6.99 (dd, 2H, J_1 = 8.1 Hz, J_2 = 2.7 Hz), 6.76 (m, 2H), 5.74 (d, 1H, J = 8.4 Hz), 4.85 (s, 2H), 4.62 (m, 1H), 3.65 (s, 3H), 3.20 (s, 3H), 1.35 (m, 3H), 0.84 (d, 3H, J = 6.3 Hz), 0.80 (d, 3H, J = 6.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.30, 172.23, 171.50, 167.40, 136.10, 135.81, 131.64, 128.53, 128.34, 126.45, 126.39, 125.61, 123.06, 122.46, 122.38, 121.51, 120.84, 120.25, 111.30, 109.23, 107.73, 106.90, 57.08, 52.19, 50.07, 30.85, 24.16, 18.81, 17.43.

4.1.6. Methyl (2S)-2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} acetyl) amino]-3-phenylpropanoate (*8f*)

Yield 83%, red solid, mp: 100–102 °C; $[\alpha]_D^{20}$:+10.5 (c = 0.002, CH_2Cl_2); HRMS (ES) calcd for $C_{33}H_{29}N_4O_5$ $[M + H]^+$: 561.2137, found

561.2137. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.81 (s, 1H), 7.60 (d, 1H, J = 2.7 Hz), 7.57 (s, 1H), 7.29 (m, 1H), 7.02 (m, 8H), 6.78 (m, 3H), 6.62 (t, 1H, J = 7.8 Hz), 5.92 (d, 1H, J = 7.8 Hz), 4.83 (q, J = 6.0 Hz), 4.74 (s, 2H), 3.74 (m, 1H), 3.65 (s, 3H), 3.20 (s, 3H), 3.02 (dd, 2H, J_1 = 5.1 Hz, J_2 = 13.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.25, 171.09, 167.11, 136.11, 135.80, 135.08, 131.47, 129.08, 128.95, 128.56, 128.31, 127.08, 126.58, 126.34, 125.40, 123.15, 122.59, 122.43, 121.67, 120.93, 120.39, 111.28, 109.26, 107.76, 106.89, 52.95, 52.43, 49.89, 37.48, 24.19.

4.1.7. Methyl 3-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2.5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} acetyl) amino] propanoate (**8g**)

Yield 62%, red solid, mp: 103–104 °C; HRMS (ES) calcd for $C_{27}H_{25}N_4O_5$ [M + H]$^+$: 485.1819, found 485.1804. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.70 (s, 1H), 7.77 (d, 1H, J = 2.7 Hz), 7.53 (s, 1H), 7.33 (d, 1H, J = 8.1 Hz), 7.12 (m, 4H), 6.99 (d, 1H, J = 7.8 Hz), 6.85 (m, 2H), 5.91 (t, 1H, J = 6.0 Hz), 4.76 (s, 2H), 3.42 (m, 5H), 3.20 (s, 3H), 2.44 (t, 2H, J = 6.3 Hz). ^{13}C NMR (300 MHz, $CDCl_3$): δ = 172.15, 172.04, 136.20, 135.95, 131.40, 128.66, 126.41, 125.16, 123.29, 122.73, 122.54, 121.73, 121.07, 120.36, 111.44, 109.22, 107.82, 106.96, 51.74, 50.29, 34.93, 33.59, 24.22.

4.1.8. Methyl (2S)-3-(3,4-dihydroxyphenyl)-2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} acetyl) amino] propanoate (**8h**)

Yield 73%, red solid, mp: 154–155 °C; $[\alpha]_D^{20}$: −133.0 (0.002, CH_2Cl_2); HRMS (ES) calcd for $C_{33}H_{29}N_4O_7$ [M + H]$^+$: 593.2036, found 593.2033. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.74 (s, 1H), 7.69 (d, 1H, J = 2.7 Hz), 7.49 (s, 1H), 7.30 (d, 1H, J = 8.1 Hz), 7.13 (m, 2H), 7.07 (t, 2H, J = 7.5 Hz), 6.89 (m, 2H), 6.70 (t, 2H, J = 7.5 Hz), 6.59 (d, 1H, J = 7.8 Hz), 6.19 (d, 1H, J = 1.8 Hz), 5.75 (d, 1H, J = 7.8 Hz), 5.67 (s, 1H), 4.71 (m, 3H), 3.64 (s, 3H), 3.22 (s, 3H), 2.87 (dd, 1H, J_1 = 4.5 Hz, J_2 = 13.5 Hz), 2.69 (dd, 1H, J_1 = 7.2 Hz, J_2 = 14.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.25, 172.04, 171.06, 167.25, 143.51, 136.13, 135.95, 131.31, 129.04, 127.05, 126.18, 125.17, 123.33, 122.76, 122.40, 121.81, 121.28, 121.06, 120.52, 115.68, 115.43, 111.38, 109.44, 107.59, 106.83, 52.98, 52.38, 49.93, 36.74, 24.29.

4.1.9. Dimethyl (2S)-2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} acetyl) amino] butanedioate (**8i**)

Yield 70%, red solid, mp: 97–99 °C; $[\alpha]_D^{20}$: +19.2 (0.002, CH_2Cl_2); HRMS (ES) calcd for $C_{29}H_{27}N_4O_7$ [M + H]$^+$: 543.1874, found 543.1852. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.75 (s, 1H), 7.70 (s, 1H), 7.66 (d, 1H, J = 2.7 Hz), 7.28 (d, 1H, J = 8.4 Hz), 7.18 (m, 2H), 7.07 (m, 2H), 6.89 (d, 1H, J = 8.1 Hz), 6.83 (t, 1H, J = 7.8 Hz), 6.74 (t, 1H, 6.6 Hz), 6.50 (d, 1H, J = 8.1 Hz), 4.82 (m, 3H), 3.64 (s, 3H), 3.43 (s, 3H), 3.19 (s, 3H), 2.94 (dd, 1H, J_1 = 4.5 Hz, J_2 = 17.1 Hz), 2.81 (dd, 1H, J_1 = 4.5 Hz, J_2 = 17.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.20, 170.81, 170.32, 167.28, 136.22, 135.84, 131.59, 128.66, 128.57, 126.62, 126.29, 125.57, 122.96, 122.53, 122.43, 121.81, 120.80, 120.47, 111.24, 109.26, 107.68, 106.94, 52.83, 52.00, 50.07, 48.45, 35.68, 24.15.

4.1.10. Methyl (2S,3R)-2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indoyl} acetyl) amino]-3-methylpentanoate (**8j**)

Yield 76%, red solid, mp: 118–120 °C; $[\alpha]_D^{20}$: −3.0 (0.002, CH_2Cl_2); HRMS (ES) calcd for $C_{30}H_{31}N_4O_5$ [M + H]$^+$: 527.2289, found 527.2288. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.94 (s, 1H), 7.75 (s, 1H), 7.67 (d, 1H, J = 2.7 Hz), 7.23 (m, 2H), 7.04 (m, 3H), 6.86 (d, 1H, J = 8.1 Hz), 6.72 (m, 2H), 6.00 (d, 1H, J = 8.7 Hz), 4.83 (s, 2H), 4.57 (dd, 1H, J_1 = 4.8 Hz, J_2 = 8.4 Hz), 3.64 (s, 3H), 3.19 (s, 3H), 1.79 (m, 1H), 1.19 (m, 2H), 0.90 (m, 3H), 0.77 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.30, 172.22, 171.47, 167.25, 136.11, 135.83, 131.66, 128.58, 128.34, 126.40, 126.36, 125.58, 122.99, 122.40, 122.36, 121.49, 120.80, 120.21, 111.32, 109.23, 107.66, 106.83, 56.39, 52.11, 50.04, 37.54, 24.89, 24.13, 15.25, 11.27.

4.1.11. Metyl(2S)-2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indoyl}acetyl)amino]-3-methylbutanoate (**8k**)

Yield 65%, red solid, mp: 123–125 °C; $[\alpha]_D^{20}$: −5.0 (0.002, CH_2Cl_2); HRMS (ES) calcd for $C_{29}H_{29}N_4O_5$ [M + H]$^+$: 513.2132, found 513.2143. ^{1}HNMR (300 MHz, $CDCl_3$): δ = 8.90 (s, 1H), 7.76 (s, 1H), 7.68 (d, 1H, J = 2.7 Hz), 7.24 (m, 2H), 7.06 (m, 3H), 6.86 (d, 1H, J = 7.8 Hz), 6.72 (m, 2H), 5.97 (d, 1H, J = 8.7 Hz), 4.85 (s, 2H), 4.52 (dd, 1H, J_1 = 4.8 Hz, J_2 = 8.4 Hz), 3.64 (s, 3H), 3.19 (s, 3H), 2.08 (m, 1H), 0.83 (d, 3H, J = 7.2 Hz), 0.68 (d, 3H, J = 6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.30, 172.23, 171.50, 167.40, 136.10, 135.81, 131.64, 128.53, 128.34, 126.45, 126.39, 125.61, 123.06, 122.46, 122.38, 121.51, 120.84, 120.25, 111.30, 109.23, 107.73, 106.90, 57.08, 52.19, 50.07, 30.85, 24.16, 18.81, 17.43.

4.1.12. Methyl (2S)-3-(4-hydroxyphenyl)-2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} acetyl) amino] propanoate (**8l**)

Yield 63%, red solid, mp: 153–155 °C; $[\alpha]_D^{20}$: −25.0 (0.002, CH_2Cl_2); HRMS (ES) calcd for $C_{33}H_{29}N_4O_6$ [M + H]$^+$: 577.2082, found 577.2063. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.09 (d, 1H, J = 1.5 Hz), 7.45 (s, 1H), 7.24 (m, 2H), 7.12 (m, 3H), 7.02 (t, 1H, J = 7.2 Hz), 6.90 (d, 1H, J = 1H), 6.79 (m, 2 H), 6.65 (s, 1H), 6.47 (d, 2H, J = 8.4 Hz), 6.38 (d, 2H, J = 8.4 Hz), 6.00 (d, 1H, J = 8.1 Hz), 4.83 (dd, J_1 = 5.4 Hz, J_2 = 13.5 Hz), 4.63 (dd, J_1 = 17.1 Hz, J_2 = 13.1 Hz), 3.64 (s, 3H), 3.15 (s, 3H), 2.89 (d, 2H, J = 5.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.41, 172.17, 171.13, 167.13, 154.94, 136.29, 136.00, 131.68, 130.04, 128.96, 128.82, 126.60, 126.29, 125.94, 125.23, 123.23, 122.79, 122.64, 121.94, 121.05, 120.46, 115.66, 111.37, 109.39, 107.61, 106.72, 60.38, 52.43, 49.96, 36.57, 24.18.

4.1.13. Dimethyl (2S)-2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} acetyl) amino] pentanedioate (**8m**)

Yield 83%, red solid, mp: 108–110 °C; $[\alpha]_D^{20}$: −2.5 (0.002, CH_2Cl_2); HRMS (ES) calcd for $C_{30}H_{29}N_4O_7$ [M + H]$^+$: 557.2031, found 557.2037. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.65 (s, 1H), 7.23 (m, 3H), 7.53 (m, 1H), 7.33 (d, 1H, J = 8.1 Hz), 7.22 (s, 1H), 7.11 (m, 2H), 6.95 (d, 1H, J = 8.1 Hz), 6.79 (t, 2H, J = 7.2 Hz), 6.19 (d, 1H, J = 8.1 Hz), 4.85 (s, 2H), 4.59 (m, 1H), 3.66 (s, 3H), 3.58 (s, 3H), 3.20 (s, 3H), 2.27 (m, 2H), 1.74 (m, 2H).

4.1.14. Methyl (2S,3S)-3-hydroxy-2-[(2-{3-[4-(1H-3-indolyl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-3-pyrrolyl]-1H-1-indolyl} acetyl) amino] butanoate (**8n**)

Yield 67%, red solid, mp: 158–160 °C; $[\alpha]_D^{20}$: −12.0 (0.002, CH_2Cl_2); HRMS (ES) calcd for $C_{28}H_{27}N_4O_6$ [M + H]$^+$: 516.1956, found 516.1961. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.03 (d, 1H, 2.1 Hz), 7.64 (s, 1H), 7.57 (d, 1H, J = 2.7 Hz), 7.24 (m, 2H), 7.03 (m, 3H), 6.82 (d, 1H, J = 7.8 Hz), 6.71 (m, 2H), 6.37 (d, 1H, J = 9.0 Hz), 4.81 (s, 2H), 4.53 (dd, J_1 = 2.4 Hz, J_2 = 9.0 Hz), 4.30 (d, 1H, J = 5.1 Hz), 3.63 (s, 3H), 3.12 (s, 3H), 2.70 (s, 1H), 1.08 (d, 3H, J = 6.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.44, 172.23, 170.63, 168.21, 136.30, 136.78, 131.44, 128.62, 126.41, 125.59, 123.16, 122.66, 122.35, 121.76, 120.95, 120.53, 111.24, 109.33, 107.84, 107.05, 67.59, 57.23, 52.64, 50.04, 30.90, 20.01.

5. Pharmacology

The tumor cell lines panel consisted of BCT-8, BEL-7402, A2780, MCF-7, A549, BGC-823. Human cancer cells were cultured in PRMI1640 or DMEM/F12 supplemented with 10% fetal bovine serum, containing penicillin streptomycin at 37 °C and humidified at

5% CO_2. Briefly, cells were plated in the appropriate media on 96-well plates in a 100 μl total volume at a density of $1–2.5 \times 10^4$ cells/ml and were allowed to adhere for 24 h before treatment with tested drugs in DMSO solution (10^{-5}, 10^{-6}, 10^{-7} mol/l final concentration). Triplicate wells were treated with media and agents. Cell viability was assayed after 96 h continuous drug exposure with a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt [MTT (0.5 mg/mL, 100 μL), Ameresco Corp] in fresh medium. After the medium was removed, 150 μL of DMSO was added to each well. The plates were gently agitated until the color reaction was uniform and the OD_{570} was determined using microplate reader (Wellscan MK3, Labsystems Dragon). Microsoft Excel 2003 was used for data analysis. Media-only treated cells served as the indicator of 100% cell viability. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of vehicle in the MTT assay. Assays were performed in triplicate on three independent experiments.

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