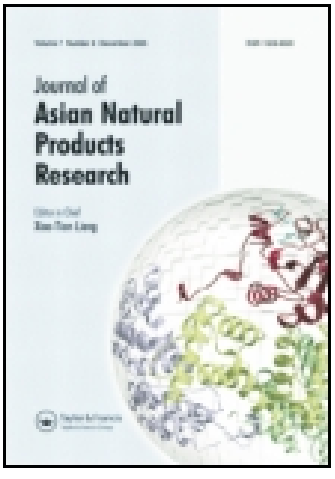

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Synthesis and cytotoxic activities of a series of novel N-methyl-bisindolylmaleimide amide derivatives

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Synthesis and cytotoxic activities of a series of novel *N*-methyl-bisindolylmaleimide amide derivatives

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A novel series of *N*-methyl-bisindolylmaleimides were synthesized and evaluated for their inhibitory activities against nine tumor cell lines. Some of the compounds showed an interesting activity against the tested cell lines. The most potent compounds **5e** and **5j** displayed antiproliferative activity with 50% inhibitory concentration values in the μM range against some tested cell lines.

Keywords: bisindolylmaleimide; conjugate; cytotoxic activity

1. Introduction

The family of bisindolylmaleimides natural products is extremely interesting owing to the broad spectrum of biological properties that they possess, including antimicrobial, hypotensive, cell cytotoxicity, and inhibition of platelet aggregation [1–4]. Furthermore, bisindolylmaleimide derivatives [5–7], such as enzastaurin (LY317615), have entered clinical trials for the treatment of cancer [8,9] (Figure 1).

Previously, we reported the synthesis and cytotoxic evaluation of a series of novel *N*-methyl-bisindolylmaleimide amino acid ester conjugates [10–12]. In this article, as part of our ongoing studies concerning the preparation of potential antitumor compounds, we designed and synthesized a series of novel *N*-methyl-bisindolylmaleimides derivatives. The antiproliferative activities of these compounds against nine tumor cell lines were evaluated.

2. Results and discussion

2.1 Chemistry

Bisindolylmaleimides **5a**–**5m** were prepared as shown in Scheme 1. Compound **2** was synthesized following the method described in the literature [13,14]. Indolyllithium was coupled with *N*-methyldibromomaleimide **2** to afford the bisindolylmaleimide **3**. Alkylation of bisindolylmaleimide **3** with 2-bromoethylamine in the presence of NaH gave amine **4** [15]. Compound **4** was coupled with various acids in the presence of 4-(dimethylamino) pyridine (DMAP) and 1-(3-dimethylaminopropyl)-3-ethylcarbodi-imide hydrochloride (EDC) to give the target compound **5** (Scheme 1).

2.2 Pharmacology

By the MTT assay, cytotoxicity of these derivatives was evaluated against nine human cancer cell lines: human intestinal

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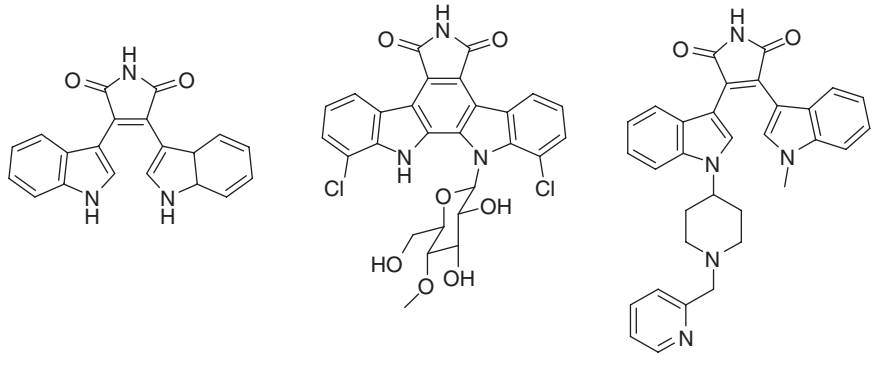
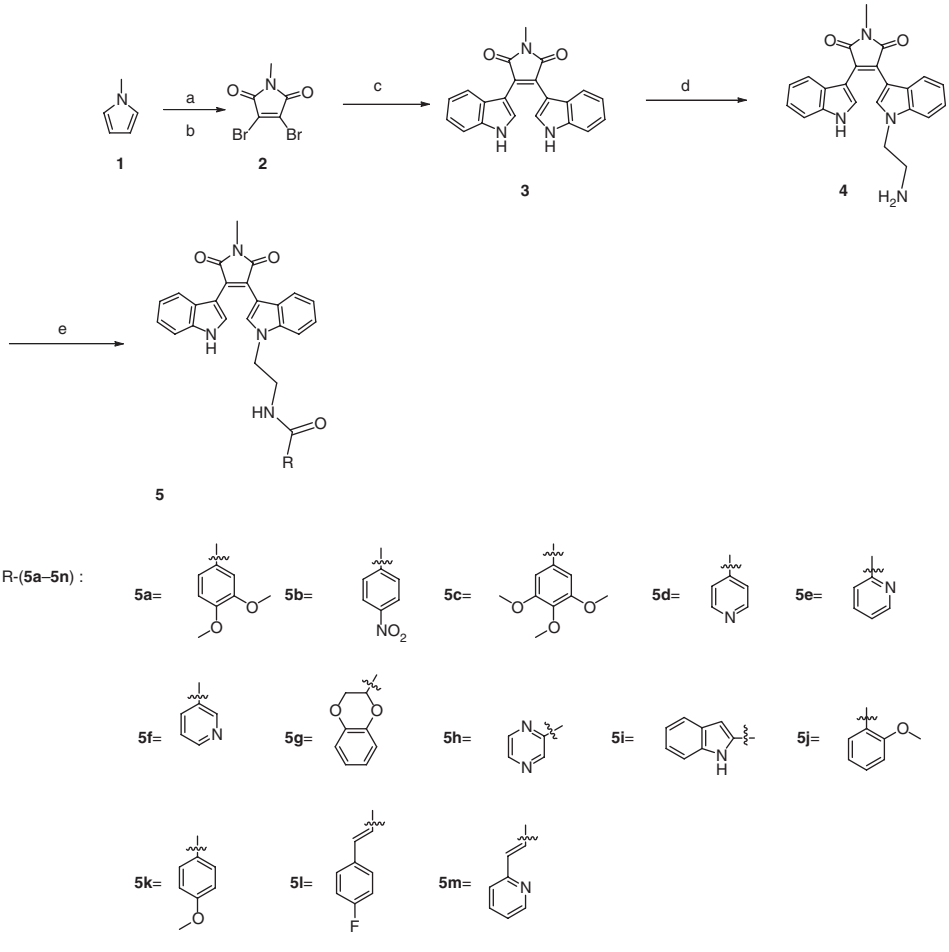


Figure 1. Structures of typical bisindolylmaleimides.



Scheme 1. Reagents and conditions: (a) NBS, THF, (b) HNO_3, 46%; (c) indole, LiHMDS, toluene, 73%; (d) $BrCH_2CH_2NH_2$, NaH, DMF, 80°C, 48%; (e) EDC, DMAP, CH_2Cl_2, 50–90%.

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), human kidney carcinoma (Ketr3), oral squamous cell carcinoma (KB), and human cervical carcinoma cell (HeLa). The results of cytotoxicity studies are summarized in Table 1.

The data in Table 1 indicate that some compounds exhibited moderate to higher antiproliferative activity against the tested cancer cells than the control compound. Bearing one methoxyl group on the aromatic derivatives, compound **5j** exhibited the most potent inhibitory activity against HCT-8, MCF-7, A549, KB, and HeLa cell lines, with the 50% inhibitory concentration (IC_{50}) values of 0–10 μM. Compound **5j** showed moderate activity against BEL-7402, A2780, BGC-823, and Ketr3 cell lines, with IC_{50} values of 15–25 μM (Table 1). Changing the methoxyl group from the *ortho* (**5j**) to the *para* (**5k**) position reduced cytotoxicity to an IC_{50} value of higher than 100 μM. Bearing two or three methoxyl groups on the aromatic derivatives, compound **5a** was inactive to all tumor cell lines. Compound **5c** only exhibited cytotoxicity against Ketr3 cell line with IC_{50} value of 59.43 μM. Compound **5b** bearing a nitro group exhibited inhibitory activities against KB cell line with an IC_{50} value of 4.26 μM, while compound **5b** was inactive to all the other tumor cell lines.

As for the six heterocyclic derivatives, compound **5e** with 2-pyridyl group exhibited broad-spectrum inhibitory activities against the tested cell lines, with IC_{50} values of 0–35 μM except for A2780 and BGC-823. Compound **5d** with 4-pyridyl group and compound **5f** with 3-pyridyl group exhibited inhibitory activities against all the tested cell lines, with IC_{50} values of 10–35 μM. Compound **5h** with 2-pyrazinyl group displayed cytotoxic activities against HCT-8, MCF-7, Ketr3, and KB cell lines, with IC_{50} values of 10.98, 14.62, 24.34, and 8.32 μM, but it showed moderate or weak activity against the other cell lines with IC_{50} values of 40–100 μM. Compound **5i** with 2-indolyl group displayed cytotoxic activities against MCF-7 and KB cell lines, with IC_{50} values of 12.95 and less than 10 μM, but it showed moderate cytotoxic activities against A549, BGC-823, Ketr3, and HeLa cell lines with IC_{50} values of 38–72 μM.

In conclusion, a series of novel aryl- and heteroaryl-*N*-methyl-bisindolylmaleimides were prepared. Some compounds showed antiproliferative activity and selectivity against nine human cancer cell lines. Further studies on the mechanism of action and the structure–activity relationship of these compounds are in progress and will be reported in due course.

3. Experimental

3.1 General experimental procedures

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) (Varian, Palo Alto, CA, USA), chemical shifts (δ) are expressed in ppm, and the following abbreviations are used: singlet (s), doublet (d), triplet (t), doubled doublet (dd), and multiplet (m). HR-MS were carried out by using Agilent LC/MSD TOF (Santa Clara, CA, USA). Starting materials, reagents, and solvents were purchased from commercial suppliers and purified before use.

3.2 Cytotoxicity assay

The tumor cell lines panel consisted of BCT-8, BEL-7402, A2780, MCF-7, A549, BGC-823, Ketr3, KB, and HeLa. Human cancer cells were cultured in PRMI1640 or DMEM/F12 supplemented with 10% fetal bovine serum, containing penicillin and 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

Table 1. *In vitro* cytotoxicity of compounds **5a**–**5m** (IC_{50}[a], μM).

Compounds	HCT-8	BEL-7402	A2780	MCF-7	A549	BGC-823	Ketr3	KB	HeLa
5a	>100	>100	>100	>100	>100	>100	>100	>100	>100
5b	>100	>100	>100	>100	>100	>100	>100	**4.26**[b]	>100
5c	>100	>100	>100	>100	>100	>100	59.43	>100	>100
5d	13.32	26.08	21.00	13.09	20.21	32.22	19.51	14.46	16.18
5e	17.70	33.78	79.48	**3.32**	12.47	>100	19.74	**9.19**	**6.52**
5f	12.35	30.14	24.18	12.76	23.36	32.44	19.35	23.27	16.00
5g	>100	>100	>100	>100	88.06	>100	66.07	38.94	>100
5h	10.98	56.54	88.51	14.62	44.21	>100	24.34	**8.32**	42.40
5i	>100	>100	>100	12.95	71.22	42.92	58.09	$1 < IC_{50} < 10$	38.05
5j	**6.66**	16.18	16.60	**5.65**	**5.64**	21.75	23.91	10.82	**6.13**
5k	>100	>100	>100	>100	>100	>100	>100	>100	>100
5l	>100	>100	>100	>100	>100	>100	>100	47.74	>100
5m	>100	>100	>100	69.58	74.84	>100	57.90	21.81	31.15
Cisplatin	3.88	4.52	8.10	1.94	4.20	0.63	1.49	0.47	1.81

[a] The IC_{50} values represent the compound concentration (μM) required to inhibit tumor cell proliferation by 50%. [b]Boldface: The IC_{50} values at the level of 10^{-6} mol/L.

adhere for 24 h before treatment with tested drugs in dimethyl sulfoxide (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 of 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., Solon, OH, USA] 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 of the reaction mixture was uniform, and OD_{570} was determined using a microplate reader (Wellscan MK3, Labsystems Dragon, Helsinki, Finland). Microsoft Excel 2003 was used for data analysis. Media-only treated cells served as the indicator of 100% cell viability. 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.

3.3 *Preparation of compounds 5a–5m*

A mixture of amine **4** (1 equiv.), DMAP (1.2 equiv.), EDC (1.2 equiv.), and anhydrous CH_2Cl_2 was stirred at room temperature, and then aromatic acid (1.2 equiv.) was added and the mixture was stirred at room temperature for 4–6 h. After completion of the reaction as indicated by TLC, CH_2Cl_2 was removed on the rotary evaporator to give a red solid. The solid obtained was purified by silica gel column chromatography with ethyl acetate/petroleum ether as the eluent to give compounds **5a**–**5m**. Yields: 50–90%.

3.3.1 *N-(2-{3-[4-(1H-Indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-3,4-dimethoxy-benzamide (**5a**)*

Yield 78.4%; red solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.67 (s, 1H), 8.49 (t, 1H, $J = 5.4$ Hz), 7.82 (s, 1H), 7.43 (d, 1H, $J = 2.7$ Hz), 7.55 (d, 1H, $J = 8.4$ Hz), 7.37 (m, 3H), 6.97 (m, 3H), 6.77 (dd, 2H, $J = 7.8$, 3.9 Hz), 6.63 (m, 2H), 4.43 (t, 2H, $J = 5.4$ Hz), 3.78 (s, 3H), 3.74 (s, 3H), 3.61 (m, 2H), 3.04 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 171.7 (×2), 166.3, 151.2, 148.1, 135.9 (×2), 132.3, 129.2, 127.1, 126.6, 126.2, 125.9, 125.3, 121.7, 121.6, 121.2, 121.1, 120.3, 119.5, 119.4, 111.7, 110.8, 110.6, 110.1, 105.6, 105.1, 55.5, 55.4, 45.1, 38.9, 23.9. HR-ESI-MS: *m/z* 549.2141 $[M + H]^+$ (calcd for $C_{32}H_{29}N_4O_5$, 549.2132).

3.3.2 *N-(2-{3-[4-(1H-Indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-4-nitro-benzamide (**5b**)*

Yield 89.9%; red solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.66 (s, 1H), 8.54 (t, 1H, $J = 5.1$ Hz), 8.26 (d, 2H, $J = 9.0$ Hz), 7.97 (d, 2H, $J = 9.0$ Hz), 7.86 (s, 1H), 7.75 (d, 1H, $J = 2.7$ Hz), 7.53 (d, 1H, $J = 8.1$ Hz), 7.34 (d, 1H, $J = 8.4$ Hz), 7.01 (t, 1H, $J = 7.5$ Hz), 6.95 (t, 1H, $J = 7.5$ Hz), 6.77 (t, 2H, $J = 8.4$ Hz), 6.61 (m, 2H), 4.48 (t, 2H, $J = 5.7$ Hz), 3.67 (m, 2H), 3.04 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 171.7, 171.6, 165.1, 149.0, 139.9, 135.9 (×2), 132.2, 129.2, 128.6 (×2), 127.2, 126.2, 125.9, 125.3, 123.5 (×2), 121.7, 121.6, 121.2, 121.0, 119.5, 119.4, 111.7, 110.0, 105.6, 105.2, 99.3, 44.9, 38.9, 23.9. HR-ESI-MS: *m/z* 534.1756 $[M + H]^+$ (calcd for $C_{30}H_{24}N_5O_5$, 534.1772).

3.3.3 *N-(2-{3-[4-(1H-Indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-3,4,5-trimethoxy-benzamide (**5c**)*

Yield 72.0%; red solid. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.65 (s, 1H), 8.56 (m, 1H), 7.83 (s, 1H), 7.72 (d, 1H, $J = 2.7$ Hz), 7.55 (d, 1H, $J = 8.1$ Hz), 7.33 (d, 1H, $J = 8.1$ Hz), 7.08 (s, 2H), 7.02 (t, 1H, $J = 7.5$ Hz), 6.94 (t, 1H, $J = 7.5$ Hz), 6.76

(m, 2H), 6.58 (m, 2H), 4.43 (m, 2H), 3.75 (s, 6H), 3.62 (m, 5H), 3.02 (s, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 171.7 (×2), 166.3, 152.5 (×2), 139.9, 135.9, 135.9, 132.3, 129.6, 129.2, 127.0, 126.2, 125.8, 125.3, 121.8, 121.6, 121.3, 121.1, 119.5, 119.4, 111.7, 110.1, 105.6, 105.1, 104.8 (×2), 60.0, 55.9 (×2), 45.1, 40.1, 23.9. HR-ESI-MS: *m/z* 579.2237 $[M + H]^+$ (calcd for $C_{33}H_{31}N_4O_6$, 579.2238).

*3.3.4 N-(2-{3-[4-(1H-Indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-isonicotinamide (**5d**)*

Yield 67.4%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.29 (s, 1H), 8.61 (s, 1H), 8.54 (d, 1H, $J = 2.4$ Hz), 7.87 (d, 1H, $J = 7.8$ Hz), 7.59 (d, 1H, $J = 2.1$ Hz), 7.51 (s, 1H), 7.27 (m, 1H), 7.21 (m, 2H), 7.03 (m, 2H), 6.95 (t, 1H, $J = 6.0$ Hz), 6.68 (m, 4H), 4.30 (t, 2H, $J = 5.1$ Hz), 3.63 (m, 2H), 3.10 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.4, 172.4, 166.0, 151.4, 147.4, 136.3, 136.0, 135.7, 131.3, 130.0, 128.7, 127.8, 127.1, 126.2, 125.1, 123.6, 122.6, 122.4, 122.3, 121.7, 120.4, 120.0, 111.5, 109.4, 106.7, 106.5, 45.5, 40.3, 24.1. HR-ESI-MS: *m/z* 490.1863 $[M + H]^+$ (calcd for $C_{29}H_{24}N_5O_3$, 490.1874).

*3.3.5 Pyridine-2-carboxylic acid (2-{3-[4-(1H-Indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-amide (**5e**)*

Yield 71.7%; red solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.86 (s, 1H), 8.45 (d, 1H, $J = 1.8$ Hz), 8.20 (d, 2H, $J = 7.5$ Hz), 7.82 (t, 1H, $J = 7.5$ Hz), 7.71 (s, 1H), 7.61 (s, 1H), 7.39 (m, 2H), 7.27 (d, 1H, $J = 7.5$ Hz), 7.02 (m, 4H), 7.75 (m, 2H), 4.38 (t, 2H, $J = 6.0$ Hz), 3.79 (q, 2H, $J = 6.0$ Hz), 3.16 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.5, 172.4, 164.9, 149.2, 148.2, 137.4, 136.1, 135.9, 131.6, 128.3, 127.5, 127.4, 126.4, 126.4, 125.3, 122.5, 122.4, 122.2, 122.1, 121.8, 120.3, 120.2, 111.2, 109.5, 107.1, 106.4, 46.0, 39.3, 24.1. HR-ESI-MS: *m/z* 490.1877 $[M + H]^+$ (calcd for $C_{29}H_{24}N_5O_3$, 490.1874).

*3.3.6 N-(2-{3-[4-(1H-Indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-nicotinamide (**5f**)*

Yield 73.5%; red solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.87 (s, 1H), 8.58 (s, 2H), 7.70 (s, 1H), 7.55 (s, 1H), 7.24 (m, 3H), 7.07 (t, 1H, $J = 7.5$ Hz), 7.00 (t, 1H, $J = 7.5$ Hz), 6.90 (t, 1H, $J = 7.5$ Hz), 6.81 (m, 3H), 6.64 (t, 1H, $J = 7.5$ Hz), 6.40 (t, 1H, $J = 6.0$ Hz), 4.38 (t, 2H, $J = 6.0$ Hz), 3.74 (m, 2H), 3.17 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.5, 172.4, 166.1, 156.0, 150.0, 141.4, 136.3, 135.9, 130.9, 129.6, 128.7, 126.4, 125.2, 122.8, 122.6, 122.3, 121.7, 121.2, 120.6, 120.4, 120.1, 115.3, 111.5, 109.3, 107.0, 106.8, 45.5, 40.5, 24.2. HR-ESI-MS: *m/z* 490.1866 $[M + H]^+$ (calcd for $C_{29}H_{24}N_5O_3$, 490.1874).

*3.3.7 2,3-Dihydro-benzo[1,4]dioxine-2-carboxylic acid (2-{3-[4-(1H-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-amide (**5g**)*

Yield 66.2%; red solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.92 (s, 1H), 7.62 (d, 1H, $J = 2.4$ Hz), 7.46 (s, 1H), 7.25 (m, 2H), 7.04 (m, 3H), 6.88 (m, 3H), 6.74 (m, 5H), 4.62 (dd, 1H, $J = 6.6$, 2.7 Hz), 4.42 (dd, 1H, $J = 11.4$, 2.7 Hz), 4.23 (t, 2H, $J = 5.7$ Hz), 4.07 (m, 1H), 3.57 (m, 2H), 3.15 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.4, 172.3, 167.9, 143.1, 141.4, 136.1, 135.9, 131.3, 128.5, 127.6, 127.1, 126.2, 125.1, 122.5, 122.4, 122.3, 122.3, 121.8, 121.7, 120.4, 120.0, 117.5, 117.0, 111.35, 109.2, 106.8, 106.5, 64.9, 60.3, 45.3, 39.4, 24.1. HR-ESI-MS: *m/z* 547.1954 $[M + H]^+$ (calcd for $C_{32}H_{27}N_4O_5$, 547.1976).

*3.3.8 Pyrazine-2-carboxylic acid(2-{3-[4-(1H-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-amide (**5h**)*

Yield 76.2%; red solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.40 (s, 1H), 8.76 (s, 1H),

8.70 (s, 1H), 8.40 (s, 1H), 7.89 (t, 1H, $J = 6.0$ Hz), 7.75 (s, 1H), 7.60 (s, 1H), 7.26 (m, 2H), 7.02 (m, 3H), 6.91 (d, 1H, $J = 8.4$ Hz), 6.75 (m, 2H), 4.40 (s, 2H), 3.81 (q, 2H, $J = 6.0$ Hz), 3.16 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.4, 172.3, 163.6, 147.5, 144.2, 143.9, 142.6, 136.1, 135.9, 131.4, 128.3, 127.5, 127.4, 126.5, 125.3, 122.6, 122.5, 122.3, 121.8, 120.4, 120.1, 111.3, 109.4, 107.2, 106.6, 45.8, 39.4, 24.1. HR-ESI-MS: *m/z* 491.1819 $[M + H]^+$ (calcd for $C_{28}H_{23}N_6O_3$, 491.1826).

*3.3.9 1H-Indole-2-carboxylic acid (2-{3-[4-(1H-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-amide (**5i**)*

Yield 80.2%; red solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.64 (s, 1H), 8.74 (s, 1H), 7.53 (m, 3H), 7.35 (d, 1H, $J = 8.4$ Hz), 7.24 (m, 3H), 7.05 (m, 4H), 6.89 (d, 1H, $J = 8.4$ Hz), 6.74 (m, 2H), 6.59 (s, 1H), 6.47 (t, 1H, $J = 6.0$ Hz), 4.26 (t, 2H, $J = 4.8$ Hz), 3.66 (m, 2H), 3.14 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.5, 172.3, 161.9, 136.4, 136.3, 135.9, 131.2, 130.1, 128.4, 127.9, 127.6, 127.4, 126.5, 125.3, 124.8, 122.7, 122.3, 122.1, 121.9, 120.8, 120.6, 120.2, 111.9, 111.3, 109.4, 107.3, 106.7, 103.3, 46.2, 37.0, 24.2. HR-ESI-MS: *m/z* 528.2016 $[M + H]^+$ (calcd for $C_{32}H_{25}N_5O_3$, 528.2030).

*3.3.10 N-(2-{3-[4-(1H-Indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-2-methoxy-benzamide (**5j**)*

Yield 75.7%; red solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.74 (s, 1H), 8.19 (dd, 1H, $J = 8.1$, 1.8 Hz), 7.92 (t, 1H, $J = 5.4$ Hz), 7.76 (d, 1H, $J = 2.4$ Hz), 7.58 (s, 1H), 7.38 (m, 3H), 7.16 (d, 1H, $J = 8.1$ Hz), 7.04 (m, 3H), 6.84 (m, 3H), 6.70 (t, 1H, $J = 7.5$ Hz), 4.36 (t, 2H, $J = 5.7$ Hz), 3.76 (q, 2H, $J = 5.7$ Hz), 3.46 (s, 3H), 3.19 (s, 3H). HR-ESI-MS: *m/z* 519.2019 $[M + H]^+$ (calcd for $C_{31}H_{27}N_4O_4$, 519.2027).

*3.3.11 N-(2-{3-[4-(1H-Indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-4-methoxy-benzamide (**5k**)*

Yield 73.6%; red solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.72 (s, 1H), 7.70 (d, 1H, $J = 2.4$ Hz), 7.55 (m, 3H), 7.31 (m, 2H), 7.05 (m, 3H), 6.84 (m, 4H), 6.69 (t, 1H, $J = 8.1$ Hz), 6.08 (t, 1H, $J = 6.0$ Hz), 4.35 (t, 2H, $J = 5.7$ Hz), 3.81 (s, 3H), 3.70 (q, 2H, $J = 6.0$ Hz), 3.18 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.4, 172.4, 167.5, 162.3, 136.4, 135.9, 131.3, 128.8 ($\times 2$), 128.4, 127.8, 127.5, 126.4, 126.2, 125.2, 122.6 ($\times 2$), 122.3, 121.8, 120.5, 120.1, 113.8 ($\times 2$), 111.4, 109.6, 107.2, 106.6, 55.4, 46.0, 40.4, 24.2. HR-ESI-MS: *m/z* 519.2025 $[M + H]^+$ (calcd for $C_{31}H_{27}N_4O_4$, 519.2027).

*3.3.12 3-(4-Fluoro-phenyl)-N-(2-{3-[4-(1H-indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-acrylamide (**5l**)*

Yield 63.4%; red solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.86 (s, 1H), 7.69 (d, 1H, $J = 2.7$ Hz), 7.47 (d, 1H, $J = 15.6$ Hz), 7.40 (m, 5H), 7.18 (d, 1H, $J = 7.8$ Hz), 7.13 (d, 1H, $J = 7.5$ Hz), 7.05 (m, 3H), 6.84 (m, 2H), 6.74 (t, 1H, $J = 8.1$ Hz), 5.97 (d, 1H, $J = 15.6$ Hz), 5.61 (t, 1H, $J = 5.7$ Hz), 4.27 (t, 2H, $J = 5.4$ Hz), 3.58 (q, 2H, $J = 5.7$ Hz), 3.16 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.4, 172.3, 166.3, 162.6, 140.3, 136.3, 136.1, 131.3, 131.0, 130.9, 129.7, 129.7, 128.8, 128.0, 127.4, 126.5, 125.1, 122.7, 122.3, 122.1, 120.6, 120.1, 119.9, 116.0, 115.8, 111.5, 109.5, 107.1, 106.5, 45.9, 40.1, 24.2. HR-ESI-MS: *m/z* 533.1993 $[M + H]^+$ (calcd for $C_{32}H_{26}FN_4O_3$, 533.1983).

*3.3.13 N-(2-{3-[4-(1H-Indol-3-yl)-1-methyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indol-1-yl}-ethyl)-3-pyridin-2-yl-acrylamide (**5m**)*

Yield 67.0%; red solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.65 (s, 1H), 8.65 (d, 1H,

$J = 3.9$ Hz), 7.69 (m, 2H), 7.48 (d, 1H, $J = 15.3$ Hz), 7.43 (d, 1H, $J = 8.4$ Hz), 7.32 (m, 4H), 7.16 (t, 1H, $J = 7.5$ Hz), 7.08 (t, 1H, $J = 7.5$ Hz), 6.97 (t, 1H, $J = 7.2$ Hz), 6.78 (m, 3H), 6.59 (d, 1H, $J = 15.3$ Hz), 5.37 (t, 1H, $J = 6.0$ Hz), 4.15 (m, 2H), 3.43 (m, 2H), 3.15 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.4, 172.3, 166.0, 153.2, 149.9, 139.8, 137.3, 136.5, 136.4, 131.4, 129.7, 129.0, 128.8, 128.4, 126.5, 125.3, 124.6, 124.3, 122.7, 122.5, 122.1, 120.9, 120.4, 115.6, 112.2, 109.5, 106.9, 106.6, 45.6, 40.4, 24.3. HR-ESI-MS: *m/z* 516.2048 $[M + H]^+$ (calcd for $C_{31}H_{26}N_5O_3$, 516.2030).

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