Synthesis and Cytotoxicity of Indolopyrrolemaleimides

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A series of indolopyrrolemaleimides have been synthesized and evaluated for their cytotoxicity *in vitro* against human leukemia cell line and four human solid cancer cell lines. Some of the compounds showed high or mediate activity against the lines. 6dc is the most promising compound among them. The inhibition toward topoisomerase I was also studied.

Key words synthesis; cytotoxicity; indolopyrrolemaleimide

Cancer is the second leading cause of death in the world. Currently, there are many different targets in cancer chemotherapy. Among them, DNA topoisomerase I has been shown to be an important target.[1—4] The development of DNA topoisomerase I inhibitors as cancer chemotherapy agents is currently an active area of research.[5] Indolocarbazoles including NB-506 and rebeccamycin exhibit antitumor properties very likely *via* topoisomerase I inhibition. To improve their cytotoxicitives, various modifications were performed.[6—10] In addition, many bisarylmaleimide compounds, which were prepared by replaceing one of the indolo groups with other aryl/heteroarylcycle, exhibited remarkable cancer cells poisons.[11—14] These prompted us to design a new series of bisindolomaleimide (Fig. 1) analogues in order to find more potent cytotoxic compounds and to carry out a structure–activity relationship study. Because pyrrole is a pharmacophore with many biological properties, it was chosen to replace one of the indolo groups of bisindolomaleimides. In this paper, we describe the synthesis of the indolopyrrolemaleimide derivatives **6** and **11** (Fig. 1), the evaluation of these compounds as topoisomerase I inhibitor, and their cytotoxic activity *in vitro* against various human cancer cell lines.

Results and Discussion

The general synthetic approach to the indolopyrrolemaleimides **6** is outlined in Chart 1. Indole adducts **3a**—**d** were prepared starting from appropriate indole.[15,16] The NH of indole adducts **3a**—**d** were protected by di-*tert*-butyl dicarbonate ($(Boc)_2O$) to form the key intermediates **4a**—**d**. **5a**—**d** were prepared by reacting pyrrole with **4a**—**d** using lithium hexamethyldisilazide (LiHMDS) as a base. Deprotection of BOC groups of **5a**—**d** with methylamine or ethylamine afforded **6ac**, **6bc**, **6cc**, **6dc**, **6ad**, **6bd**, **6cd** and **6dd**, respectively.[17] Treatment **5a** with ammonium acetate (NH_4OAc) yielded **6aa** and **6ab** (1 : 1). Similarly, treatment **5b** or **5c** with NH_4OAc got **6ba** and **6bb** or **6ca** and **6cb**, respectively.[17,18]

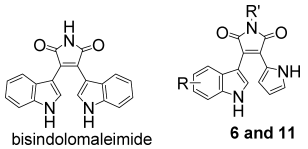


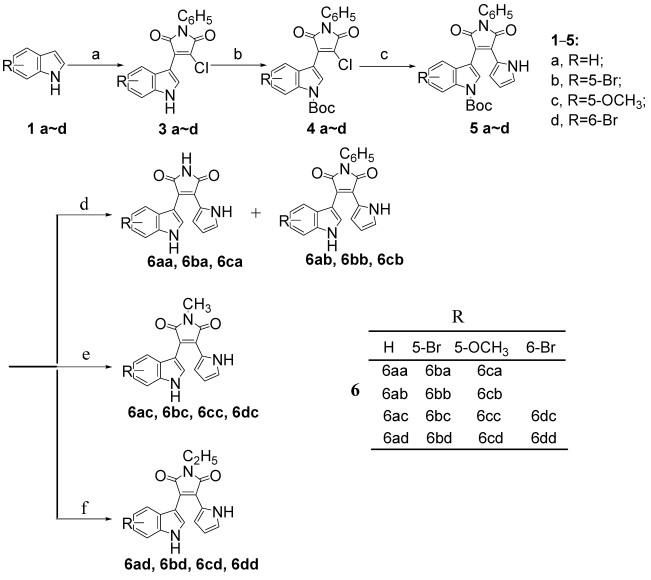
Fig. 1. Structures of Bisindolomaleimide, Indolopyrrolemaleimide Compounds **6** and **11**

Preparation of compounds **11** were performed according to the procedure of Chart 2. Treating **3a** or **3b** with methylamine afforded **7a** or **7b**. **8a** or **8b** was prepared by protecting the NH of **7a** or **7b** with $(Boc)_2O$, and then condensation of **8a** or **8b** with pyrrole obtained **9a** or **9b**.[17] Treating **9a** or **9b** with KOH–dioxane achieved **10a** or **10b**[17—19] which were converted to indolopyrrolemaleimide compounds **11** by treating with various compounds containing–NH_2.[20]

All the prepared compounds were evaluated for their cytotoxicity against cancer cells *in vitro*.

First, compounds **6** were evaluated for their cytotoxic activity *in vitro* against human leukemia cell line (HL60), and four human solid cancer cell lines: human prostate cancer cell line (PC-3), human esophageal cancer cell line (ECA-109), human liver cancer cell line (Bel-7402) and non-small-cell lung cancer cell line (A549) according to the reported methods.[21] The results are summarized in Table 1.

As outlined in Table 1, most tested compounds exhibited cytotoxic activity against HL60 cell, especially **6dc** (IC_{50} is 0.46 μM). Obviously, the cytotoxic potency of **6** against tested



Reactions and conditions: (a) i, ethyl magnesium bromide, Et_2O, benzene; ii, **2** (*N*-phenyl-3,4-dichloromaleimide), THF, rt, overnight; (b) $(Boc)_2O$, DMAP, THF, rt, 2 h; (c) pyrrole, LiHMDS, THF, −20 °C—rt, overnight; (d) NH_4OAc, 150 °C, 1.5 h; (e) 32% CH_3NH_2 in CH_3OH, rt, 1 h; (f) 32% $C_2H_5NH_2$ in CH_3OH, rt, 1 h.

Chart 1. Synthesis and Structures of Compounds **6**

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Table 1. Cytotoxicity of Compounds **6** against Human Cancer Cell Lines *in Vitro*

Compounds	Cytotoxicity (IC_{50}, μM)				
	HL60	PC-3	ECA-109	Bel-7402	A549
6aa	7.22	31.3	>50	>50	41.5
6ab	3.68	12.7	>50	>50	>50
6ac	15.8	10.9	>50	>50	4.12
6ad	0.98	18.9	41.0	>50	27.2
6ba	6.74	6.23	25.0	36.0	9.55
6bb	3.08	19.7	12.5	>50	35.4
6bc	2.32	2.16	6.21	29.7	5.40
6bd	2.71	7.42	15.9	16.1	14.3
6ca	17.6	14.6	38.1	>50	>50
6cb	1.23	31.9	>50	>50	>50
6cc	1.24	14.1	39.2	15.6	21.8
6cd	7.76	9.79	11.6	18.5	10.7
6dc	0.46	7.89	15.7	13.8	9.95
6dd	3.65	2.18	2.24	10.2	6.25

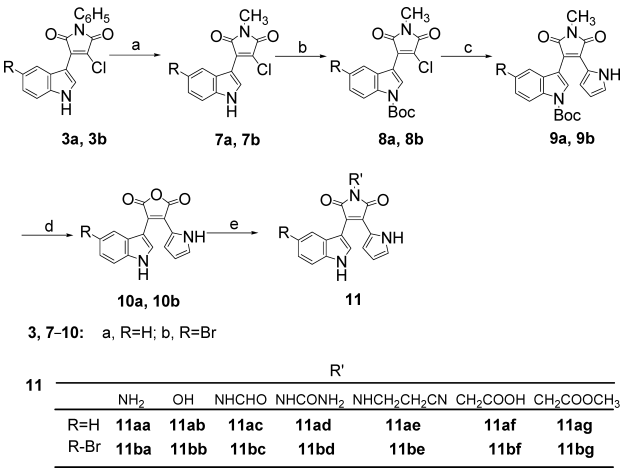
Table 2. Cytotoxicity of Compounds **11** against Human Cancer Cell Lines *in Vitro*

Compounds	Cytotoxicity (IC_{50}, μM)				
	HL60	PC-3	ECA-109	SMMC-7721	A549
11aa	17.0	>50	>50	11.2	27.3
11ab	>50	>50	31.3	48.5	12.8
11ac	50.0	>50	>50	>50	33.9
11ad	>50	>50	>50	>50	>50
11ae	>50	>50	>50	>50	>50
11af	>50	>50	>50	>50	>50
11ag	>50	>50	19.4	22.6	>50
11ba	30.0	>50	16.4	5.1	21.7
11bb	33.6	35.2	15.1	4.3	26.9
11bc	28.0	>50	16.9	36.3	19.0
11bd	33.4	>50	>50	40.2	>50
11be	>50	>50	28.8	25.0	6.6
11bf	>50	>50	>50	>50	>50
11bg	16.8	>50	7.4	5.3	10.4

cancer cells was dependent on substituents of indole ring. Compounds containing bromo substituent at the C-5 (**6ba**—**6bd**) or C-6 (**6dc**, **6dd**) position of indole ring improve cytotoxicity. Compounds bearing OCH_3 at the C-5 position of indole ring seem to have distinct difference of activity toward different cells. For example, **6cb** and **6cc** exhibit remarkable and selective cytotoxicity against HL60 cell. **6cd** has moderate cytotoxicity against all tested cells while **6ca** is less active. When phenyl group is attached to the nitrogen in maleimide, compounds **6ab**, **6bb** and **6cb** exert higher cytotoxicity against HL60 cell while show lower activity against other tested cells. Cytotoxicity of the compounds that the hydrogen of the nitrogen in maleimide is replaced by methyl (**6ac**, **6bc**, **6cc**, **6dc**) or ethyl (**6ad**, **6bd**, **6cd**, **6dd**) group increased.

In order to find the effect of other functional groups in maleimide to the cytotoxicity, a series of compounds **11** that hydrogen atom of the nitrogen in maleimide was replaced by various groups were prepared. The cytotoxicity *in vitro* against HL60, PC-3, ECA-109, SMMC-7721 and A549 cells were also evaluated (Table 2).

Unfortunately, this kind of modification isn't good for improving cytotoxicity. As shown in Table 2, compounds **11** have less cytotoxicity against tested cancer cells compared with compounds **6**. The same conclusion can be drawn from the comparation of cytotoxicity of **6** and **11** that substituent of bromo of indole ring is favor of increasing the activity. **11bg** bearing CH_2COOCH_3 to the nitrogen in maleimide shows moderate cytotoxicity against SMMC-7742, ECA-109 and A549 cells. **11ba** (R′=NH_2) and **11bb** (R′=OH) exerted selective inhibition to SMMC-7721 cells. **11be** (R′=$NHCH_2CH_2CN$) is more sensitive to A549 cells. Although the modification of the nitrogen in maleimide doesn't give satisfactory results, the fact that several compounds exhibit selective inhibition to certain cancer cell lines provide some elicitation for achieving more active compounds.



Reactions and conditions: (a) 32% CH_3NH_2/CH_3OH, 0 °C, 2 h; (b) $(Boc)_2O$, DMAP, THF, rt, 2 h; (c) pyrrole, LiHMDS, THF, −20 °C—rt, overnight; (d) 2 M KOH, dioxane, reflux, 1 h; (e) R′-NH_2, DMF, 80 °C or R′-NH_2·HCl, DMF, Et_3N, 80 °C, 2 h.

Chart 2. Synthesis and Structure of Compounds **11**

The effects of some synthesized indolopyrrolemaleimide compounds on the relaxation of supercoiled DNA by topoisomerase I were investigated. Closed circular DNA was treated with topoisomerase I in the absence and presence of the compound at 200 μM.

As shown in Fig. 2, supercoiled DNA was fully relaxed by the enzyme in the absence of compound. The relaxed DNA migrated slower than the supercoiled plasmid on an agarose gel. In some cases the intensity of the band corresponding to the nicked form of DNA had increased significantly. This effect reflected the stabilization of topoisomerase I–DNA cleavable complexes. An increase of the nicked DNA form was observed with different compounds, in particular compound **6dc** and **6bb** which seemed to be the most active compound. They both seemed to have the same extent inhibition toward topoisomerase I. Compounds **6ba** and **6dd** had mediate activity. **6bd**, **6bc**, **6cd**, **6cc** and **6ac** had less activity toward the enzyme. The result was not very consistent with the rank of their cytotoxicity against human cancer cell lines. This indicated that the tested compounds may have other mechanism against human cancer cell lines except topoisomerase I inhibition.

In conclusion, a novel series of indolopyrrolemaleimide compounds were prepared. From the data in Tables 1 and 2, some of them showed high or mediate cytotoxicity against human cancer cell lines: HL60, PC-3, ECA-109, Bel-7402 or

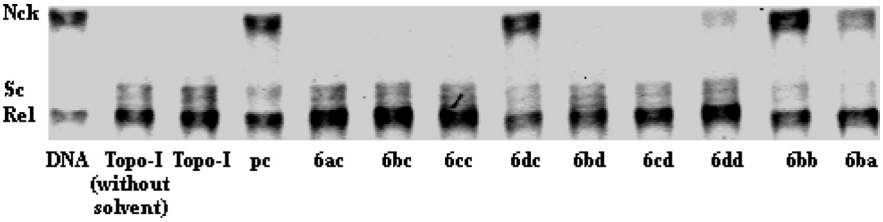


Fig. 2. Inhibition of Topoisomerase I-Mediated Relaxation of DNA by Indolopyrrolemaleimide Compounds

Native supercoiled pBR 322 DNA (0.5 μg) (lane DNA) was incubated for 30 min at 37 °C with 1 unit of calf thymus topoisomerase I in the absence (lane Topo I) or presence of the compound at 200 μM. Reactions were stopped with sodium dodecyl sulfate. The DNA samples were run on an agarose gel followed by ethidium bromide staining. Nck, nicked; Rel, relaxed; Sc, supercoild; pc, positive control. The gel was photographed under UV light.

SMMC-7721 and A549. Among them, the most promising compounds were **6bc** and **6dc**. **6dc** also had remarkable topoisomerase I inhibition.

Experimental

All reagents were obtained from commercial sources and used without further purification unless stated. Et_2O, THF and benzene were distilled from sodium-benzophenone. DMF was distilled from calcium hydride. $(Boc)_2O$ was distilled under pressure. All reactions were detected by thin-layer chromatography (TLC).

Melting points were determined by BÜCHI Melting Point B-450 (Büchi Labortechnik, Flawil, Switzerland). ^{1}H-NMR spectra were recorded in DMSO-d_6 on Bruker Avance DMX 400 using TMS as an internal standard (Bruker, Billerica, MA, U.S.A.), and chemical shifts are expressed as δ ppm. Mass spectral data were obtained on an Esquire-LC-00075 mass spectrometry. Elemental analysis was carried out on ERBA-1110 analyzer.

Production of 3a—d. General Procedure Ethyl magnesium bromide (105 mmol) in Et_2O (40 ml) was added dropwise to a solution of the appropriate indole **1** (100 mmol) in benzene (60 ml) at room temperature over 1 h with stirring. After stirring for 30 min, the mixture was added dropwise to a solution of **2** (70 mmol) in THF (50 ml) over 2 h. The mixture was stirred overnight at room temperature. The dark red mixture was poured into 1 M aqueous hydrochloric acid (50 ml) and extracted with ethyl acetate (30 ml×3). The organic phase was successively washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was recrystallized from ethyl acetate to get **3a**—**d** as a red or orange power.

4-Chloro-3-(1*H*-indol-3-yl)-1-phenyl-1*H*-pyrrole-2,5-dione (**3a**): Yield 82%; mp 203—204 °C; ^{1}H-NMR (DMSO-d_6) δ: 7.19—7.26 (2H, m, H_{phenyl}), 7.45—7.54 (6H, m, H_{phenyl}), 7.97 (1H, d, J=8.0 Hz, 4-H_{indole}), 8.13 (1H, s, 2-H_{indole}), 12.18 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{18}H_{11}ClN_2O_2$: C, 66.99; H, 3.44; N, 8.68. Found: C, 66.87; H, 3.39; N, 8.73.

4-Chloro-3-(5-bromo-1*H*-indol-3-yl)-1-phenyl-1*H*-pyrrole-2,5-dione (**3b**): Yield 80%; mp 212—214 °C; ^{1}H-NMR (DMSO-d_6) δ: 7.37 (1H, dd, J=2.4, 7.6 Hz, 6-H_{indole}), 7.44—7.47 (3H, m, H_{phenyl}), 7.51—7.53 (3H, m, H_{phenyl}), 8.15 (1H, s, H_{phenyl}), 8.20 (1H, d, J=2.8 Hz, 2-H_{indole}), 12.36 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{18}H_{10}BrClN_2O_2$: C, 53.83; H, 2.51; N, 6.97. Found: C, 53.94; H, 2.43; N, 6.90.

4-Chloro-3-(5-methoxy-1*H*-indol-3-yl)-1-phenyl-1*H*-pyrrole-2,5-dione (**3c**): Yield 76%; mp 185—187 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.78 (3H, s, OCH_3), 6.90 (1H, dd, J=2.0, 8.4 Hz, 6-H_{indole}), 7.43—7.47 (5H, m, H_{phenyl}), 7.51—7.55 (2H, m, H_{phenyl}), 8.08 (1H, d, J=3.2 Hz, 2-H_{indole}), 12.10 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{19}H_{13}ClN_2O_3$: C, 64.69; H, 3.71; N, 7.94. Found: C, 64.78; H, 3.63; N, 8.02.

4-Chloro-3-(6-bromo-1*H*-indol-3-yl)-1-phenyl-1*H*-pyrrole-2,5-dione (**3d**): Yield 73%; mp 205—207 °C; ^{1}H-NMR (DMSO-d_6) δ: 7.33 (1H, dd, J=1.6, 8.8 Hz, 6-H_{indole}), 7.44—7.47 (3H, m, H_{phenyl}), 7.51 (2H, d, J=7.2 Hz, H_{phenyl}), 7.73 (1H, d, J=1.6 Hz, H_{phenyl}), 7.91 (1H, d, J=8.8 Hz, H_{phenyl}), 8.15 (1H, d, J=3.2 Hz, 2-H_{indole}), 12.27 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{18}H_{10}BrClN_2O_2$: C, 53.83; H, 2.51; N, 6.97. Found: C, 53.92; H, 2.40; N, 6.92.

Production of 4a—d. General Procedure Di-*tert*-butyl dicarbonate (25 mmol) and catalytic amount of DMAP (0.5%) were added to a solution of **3** (20 mmol) in THF (250 ml) and the mixture was stirred for 2 h at room temperature. After removal of the solvent *in vacuo*, the yellow residue was recrystallized from petroleum ether–ethyl acetate to obtain **4a**—**d** as a yellow crystalline powder.

4-Chloro-3-[1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]-1-phenyl-1*H*-pyrrole-2,5-dione (**4a**): Yield 90%; mp 149—152 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.70 (9H, s, $C(CH_3)_3$), 7.33—7.36 (1H, m, H_{phenyl}), 7.39—7.43 (4H, m, H_{phenyl}), 7.45—7.52 (2H, m, H_{phenyl}), 7.90 (1H, d, J=6.4 Hz, H_{phenyl}), 8.25—8.27 (2H, m, H_{phenyl}, 2-H_{indole}); *Anal.* Calcd for $C_{23}H_{19}ClN_2O_4$: C, 65.33; H, 4.53; N, 6.62. Found: C, 65.21; H, 4.62; N, 6.75.

4-Chloro-3-[5-bromo-1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]-1-phenyl-1*H*-pyrrole-2,5-dione (**4b**): Yield 41%; mp 161—163 °C; ^{1}H-NMR (DMSO-d_6) δ: 7.45—5.47 (3H, m, H_{phenyl}), 7.54—7.55 (2H, m, H_{phenyl}), 7.61—7.64 (2H, m, H_{phenyl}), 8.09 (1H, d, J=8.8, H_{phenyl}), 8.17 (1H, s, 2-H_{indole}); *Anal.* Calcd for $C_{23}H_{18}BrClN_2O_4$: C, 55.06; H, 3.62; N, 5.58. Found: C, 55.13; H, 3.50; N, 5.65.

4-Chloro-3-[5-methoxy-1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]-1-phenyl-1*H*-pyrrole-2,5-dione (**4c**): Yield 45%; mp 176—179 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.65 (9H, s, $C(CH_3)_3$), 3.81 (3H, s, OCH_3), 7.08 (1H, dd, J=2.0, 8.8 Hz, 6-H_{indole}), 7.34 (1H, s, H_{phenyl}), 7.46—7.48 (3H, m, H_{phenyl}), 7.53—7.57 (2H, m, H_{phenyl}), 8.05 (1H, d, J=8.8 Hz, H_{phenyl}), 8.10 (1H, s, 2-H_{indole}); *Anal.* Calcd for $C_{24}H_{21}ClN_2O_5$: C, 63.65; H, 4.67; N, 6.19. Found: C, 63.72; H, 4.59; N, 6.07.

4-Chloro-3-[6-bromo-1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]-1-phenyl-1*H*-pyrrole-2,5-dione (**4d**): Yield 38%; mp 154—156 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.66 (9H, s, $C(CH_3)_3$), 7.45—7.47 (3H, m, H_{phenyl}), 7.53—7.59 (3H, m, H_{phenyl}), 7.82 (1H, d, J=9.2 Hz, H_{phenyl}), 8.15 (1H, s, 2-H_{phenyl}), 8.34 (1H, s, 2-H_{indole}); *Anal.* Calcd for $C_{23}H_{18}BrClN_2O_4$: C, 55.06; H, 3.62; N, 5.58. Found: C, 55.01; H, 3.53; N, 5.48.

Production of 5a—d. General Procedure LiHMDS (1 M in THF, 1.0—1.2 mmol) at −20 °C was added to a solution of 0.6 mmol of the pyrrole in THF (3 ml) under N_2 and stirred for 1 h. A solution of **4** (0.5 mmol) in THF (10 ml) was then added by drip over 40—60 min, followed by stirring at 0 °C overnight. The reaction mixture was poured into 0.5 M aqueous hydrochloric acid (30 ml), and the mixture was extracted with ethyl acetate (15 ml×3). The organic phase was successively washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel using petroleum ether/ethyl acetate as eluent to afford **5a**—**d**.

3-[1-(*tert*-Butyloxycarbonyl)-1*H*-indol-3-yl]-4-(1*H*-pyrrol-2-yl)-1-phenyl-1*H*-pyrrole-2,5-dione (**5a**): Yield 22%; mp 170—172 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.69 (9H, s, $C(CH_3)_3$), 6.18—6.20 (1H, m), 6.29 (1H, s), 6.45 (1H, s), 7.02 (1H, s), 7.18—7.20 (2H, m), 7.35—7.40 (2H, m), 7.45—7.50 (4H, m), 8.00 (1H, s), 8.28 (1H, d, J=8.8 Hz), 10.45 (1H, s, 1-$H_{pyrrole}$); *Anal.* Calcd for $C_{27}H_{23}N_3O_4$: C, 71.51; H, 5.11; N, 9.27. Found: C, 71.39; H, 5.23; N, 9.13.

3-[5-Bromo-[1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]-4-(1*H*-pyrrol-2-yl)-1-phenyl-1*H*-pyrrole-2,5-dione (**5b**): Yield 15%; mp 146—149 °C; *Anal.* Calcd for $C_{27}H_{22}BrN_3O_4$: C, 60.91; H, 4.17; N, 7.89. Found: C, 60.83; H, 4.28; N, 7.76.

3-[5-Methoxy-[1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]-4-(1*H*-pyrrol-2-yl)-1-phenyl-1*H*-pyrrole-2,5-dione (**5c**): Yield 17%; mp 160—162 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.66 (9H, s, $C(CH_3)_3$), 3.50 (3H, s, OCH_3), 6.20—6.22 (m, 1H, H), 6.27—6.28 (1H, m, H), 6.52 (1H, d, J=2.8 Hz), 6.56 (1H, s), 6.96 (1H, dd, J=2.0, 7.2 Hz), 7.04 (1H, s), 7.46—7.48 (2H, m), 7.52—7.56 (2H, m), 7.96 (1H, s), 8.01 (1H, d, J=7.2 Hz), 11.15 (1H, s, 1-$H_{pyrrole}$); *Anal.* Calcd for $C_{28}H_{25}N_3O_5$: C, 69.55; H, 5.21; N, 8.69. Found: C, 69.69; H, 5.23; N, 8.76.

3-[6-Bromo-[1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]-4-(1*H*-pyrrol-2-yl)-1-phenyl-1*H*-pyrrole-2,5-dione (**5d**): Yield 14%; mp 171—174 °C; *Anal.* Calcd for $C_{27}H_{22}BrN_3O_4$: C, 60.91; H, 4.17; N, 7.89. Found: C, 60.97; H, 4.25; N, 7.81.

Production of 3-(1*H*-Indol-3-yl)-4-(1*H*-pyrrol-2-yl-1*H*-pyrrole-2,5-

dione (6aa) and 3-(1*H*-Indol-3-yl)-4-(1*H*-pyrrol-2-yl)-1-phenyl-1*H*-pyrrole-2,5-dione (6ab) Compound **5a** (0.2 mmol) was heated with ammonium acetate (40 mmol) for 1 h at 150 °C (bath temperature). The mixture was cooled and extracted with ethyl acetate after the addition of water. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by chromatography on silica gel using petroleum ether/ethyl acetate as eluent to yield **6aa** and **6ab**, respectively as a red power.

6aa: Yield 39%; mp >250 °C; MS *m/z*: 278 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 6.07—6.09 (1H, m, $H_{pyrrole}$), 6.23—6.25 (1H, m, $H_{pyrrole}$), 6.86—6.95 (3H, m, H_{phenyl}), 7.13 (1H, t, J=8.0 Hz), 7.47 (1H, d, J=8.0 Hz), 7.51 (1H, d, J=3.2 Hz), 10.92 (1H, s, N-H), 11.00 (1H, s, 1-$H_{pyrrole}$), 11.75 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{16}H_{11}N_3O_2$: C, 69.31; H, 4.00; N, 15.15. Found: C, 69.40; H, 4.09; N, 15.06.

6ab: Yield 42%; mp 203 °C; MS *m/z*: 354 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 6.12—6.14 (1H, m, $H_{pyrrole}$), 6.37 (1H, br, $H_{pyrrole}$), 6.94 (2H, d, J=7.2 Hz), 6.99 (1H, s), 7.13—7.17 (1H, m), 7.34—7.54 (6H, m), 7.85 (1H, d, J=2.8 Hz, 2-H_{indole}), 11.10 (1H, s, 1-$H_{pyrrole}$), 11.82 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{22}H_{15}N_3O_2$: C, 74.78; H, 4.28; N, 11.89. Found: C, 74.70; H, 4.19; N, 11.76.

Production of 3-[5-Bromo-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl)-1*H*-pyrrole-2,5-dione (6ba) and 3-[5-Bromo-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl)-1-phenyl-1*H*-pyrrole-2,5-dione (6bb) The same procedure as described above afforded compounds **6ba** and **6bb**, respectively from **5b** after purification by chromatography on silica gel using petroleum ether/ethyl acetate as eluent.

6ba: Yield 48%; mp >250 °C; MS *m/z*: 357(M^+); ^{1}H-NMR (DMSO-d_6) δ: 6.12—6.14 (1H, m, $H_{pyrrole}$), 6.27—6.29 (1H, m, $H_{pyrrole}$), 6.96—6.98 (2H, m), 7.24 (1H, dd, J=2.4, 8.4 Hz), 7.44 (1H, d, J=8.4 Hz), 7.82 (1H, d, J=2.4 Hz, 2-H_{indole}), 10.96 (1H, s, N-H), 11.05 (1H, s, 1-$H_{pyrrole}$), 11.92 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{16}H_{10}BrN_3O_2$: C, 53.95; H, 2.83; N, 11.80. Found: C, 53.89; H, 2.90; N, 11.69.

6bb: Yield 40%; mp >250 °C; MS *m/z*: 433 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 6.17—6.19 (1H, m, $H_{pyrrole}$), 6.41 (1H, br, $H_{pyrrole}$), 7.03 (1H, s), 7.08 (1H, s), 7.27 (1H, dd, J=1.2, 8.4 Hz), 7.15—7.54 (6H, m), 7.89 (1H, d, J=2.8 Hz, 2-H_{indole}), 11.14 (1H, s, 1-$H_{pyrrole}$), 11.98 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{22}H_{14}BrN_3O_2$: C, 61.13; H, 3.26; N, 9.72. Found: C, 61.05; H, 3.37; N, 9.86.

Production of 3-[5-Methoxy-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl)-1*H*-pyrrole-2,5-dione (6ca) and 3-[5-Methoxy-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl)-1-phenyl-1*H*-pyrrole-2,5-dione (6cb) The same procedure as described above afforded compounds **6ca** and **6cb**, respectively from **5c** after purification by chromatography on silica gel using petroleum ether/ethyl acetate as eluent.

6ca: Yield 45%; mp >250 °C; MS *m/z*: 308 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 3.41 (3H, s, OCH_3), 6.10—6.12 (1H, m, $H_{pyrrole}$), 6.21—6.23 (1H, m, $H_{pyrrole}$), 6.72 (1H, dd, J=2.4, 8.4 Hz), 6.94—6.96 (2H, m), 7.36 (1H, d, J=8.4 Hz), 7.54 (1H, d, J=2.0 Hz, 2-H_{indole}), 10.90 (1H, s, N-H), 11.03 (1H, s, 1-$H_{pyrrole}$), 11.67 (s, 1H, 1-H_{indole}); *Anal.* Calcd for $C_{17}H_{13}N_3O_3$: C, 66.44; H, 4.26; N, 13.67. Found: C, 66.51; H, 4.20; N, 13.54.

6cb: Yield 40%; mp 202 °C; MS *m/z*: 384 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 3.44 (3H, s, OCH_3), 6.17—6.19 (1H, m, $H_{pyrrole}$), 6.25 (1H, t, J=2.4 Hz), 6.32 (1H, d, J=2.8 Hz,), 6.34—6.36 (1H, m, $H_{pyrrole}$), 6.76 (1H, dd, J=2.4 Hz, 8.8), 6.99—7.01 (1H, m), 7.06 (1H, t, J=2.4 Hz), 7.32—7.55 (4H, m), 7.85 (1H, d, J=2.8 Hz, 2-H_{indole}), 11.29 (1H, s, 1-$H_{pyrrole}$), 11.74 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{23}H_{17}N_3O_3$: C, 72.05; H, 4.47; N, 10.96. Found: C, 72.17; H, 4.59; N, 10.87.

Production of 6ac, 6bc, 6cc and 6dc. General Procedure 32% methylamine in methanol (3 ml) was added to compounds **5a**—**d** (0.1 mmol) respectively and the mixture was stirred for 1—2 h at room temperature. The solvent was removed *in vacuo* and the residue was purified by chromatography on silica gel using petroleum ether/ethyl acetate as eluent to afford compounds **6ac**, **6bc**, **6cc** and **6dc**, respectively.

3-(1*H*-Indol-3-yl)-4-(1*H*-pyrrol-2-yl)-1-methyl-1*H*-pyrrole-2,5-dione (**6ac**): Yield 88%. MS *m/z*: 292 (M^+); mp 222—224 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.01 (3H, s, CH_3), 6.09—6.11 (1H, m, $H_{pyrrole}$), 6.29 (1H, br, $H_{pyrrole}$), 6.86—6.96 (3H, m), 7.14 (1H, t, J=8.0 Hz), 7.48 (1H, d, J=8.0 Hz), 7.78 (1H, d, J=2.8 Hz, 2-H_{indole}), 11.02 (1H, s, 1-$H_{pyrrole}$), 11.77 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{17}H_{13}N_3O_2$: C, 70.09; H, 4.50; N, 14.42. Found: C, 70.16; H, 4.37; N, 14.54.

3-[5-Bromo-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl)-1-methyl-1*H*-pyrrole-2,5-dione (**6bc**): Yield 81%; MS *m/z*: 371 (M^+); mp 219—220 °C (dec.); ^{1}H-NMR (DMSO-d_6) δ: 3.01 (3H, s, CH_3), 6.13—6.16 (1H, m, $H_{pyrrole}$), 6.33—6.35 (1H, m, $H_{pyrrole}$), 6.98—7.00 (2H, m), 7.25 (1H, dd, J=2.0, 8.0 Hz), 7.44 (1H, d, J=8.0 Hz), 7.83 (1H, d, J=2.4 Hz, 2-H_{indole}), 11.06 (1H, s, 1-$H_{pyrrole}$), 11.94 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{17}H_{12}BrN_3O_2$: C, 55.15; H, 3.27; N, 11.35. Found: C, 55.26; H, 3.17; N, 11.47.

3-[5-Methoxy-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl)-1-methyl-1*H*-pyrrole-2,5-dione (**6cc**): Yield 79%; MS *m/z*: 322 (M^+); mp 227—228 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.02 (3H, s, CH_3), 3.42 (3H, s, OCH_3), 6.13—6.15 (1H, m, $H_{pyrrole}$), 6.25—6.27 (2H, m,), 6.74 (1H, dd, J=2.0, 8.4 Hz), 6.95—6.97 (3H, m), 7.35 (1H, d, J=8.4 Hz), 7.79 (1H, d, J=2.4 Hz, 2-H_{indole}), 11.06 (1H, s, 1-$H_{pyrrole}$), 11.69 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{18}H_{15}N_3O_3$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.20; H, 4.62; N, 13.21.

3-[6-Bromo-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl)-1-methyl-1*H*-pyrrole-2,5-dione (**6dc**): Yield 83%; MS *m/z*: 371 (M^+); mp 197—199 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.02 (3H, s, CH_3), 6.15—6.17 (1H, m, $H_{pyrrole}$), 6.37—6.40 (1H, m, $H_{pyrrole}$), 7.14—7.18 (2H, m), 7.45 (1H, d, J=8.0), 7.59 (1H, s), 8.10 (1H, d, J=2.0, 2-H_{indole}), 11.10 (1H, s, 1-$H_{pyrrole}$), 12.02 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{17}H_{12}BrN_3O_2$: C, 55.15; H, 3.27; N, 11.35. Found: C, 55.23; H, 3.35; N, 11.50.

Production of 6ad, 6bd, 6cd and 6dd The same procedure as described above afforded compounds **6ad**, **6bd**, **6cd** and **6dd**, respectively from **5a**—**d** and 32% ethylamine in methanol after purification by chromatography on silica gel using petroleum ether/ethyl acetate as eluent.

3-(1*H*-indol-3-yl)-4-(1*H*-pyrrol-2-yl)-1-ethyl-1*H*-pyrrole-2,5-dione (**6ad**): Yield 87%; MS *m/z*: 306 (M^+); mp 197—198 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.18 (3H, t, J=7.2, CH_3), 3.55—3.60 (2H, m, CH_2), 6.09—6.11 (1H, m, $H_{pyrrole}$), 6.29 (1H, br, $H_{pyrrole}$), 6.86 (1H, d, J=8.0 Hz), 6.91 (1H, t, J=8.0 Hz), 6.96 (1H, s), 7.14 (1H, t, J=8.0 Hz), 7.48 (1H, d, J=8.0 Hz), 7.80 (1H, d, J=2.4 Hz, 2-H_{indole}), 11.04 (1H, s, 1-$H_{pyrrole}$), 11.78 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{18}H_{15}N_3O_2$: C, 70.81; H, 4.95; N, 13.76. Found: C, 70.94; H, 4.87; N, 13.70.

3-[5-Bromo-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl)-1-ethyl-1*H*-pyrrole-2,5-dione (**6bd**): Yield 77%; MS *m/z*: 385 (M^+); mp 187—190 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.17 (3H, t, J=7.2 Hz, CH_3), 3.54—3.60 (2H, m, CH_2), 6.14—6.16 (1H, m, $H_{pyrrole}$), 6.32—6.39 (1H, m, $H_{pyrrole}$), 6.98—7.00 (2H, m), 7.25 (1H, dd, J=2.4, 8.8 Hz), 7.45 (1H, d, J=8.8 Hz), 7.86 (1H, d, J=4 Hz, 2-H_{indole}), 11.07 (1H, s, 1-$H_{pyrrole}$), 11.95 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{18}H_{14}BrN_3O_2$: C, 56.27; H, 3.67; N, 10.94. Found: C, 56.36; H, 3.57; N, 10.82.

3-[5-Methoxy-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl)-1-ethyl-1*H*-pyrrole-2,5-dione (**6cd**): Yield 80%; MS *m/z*: 336 (M^+); mp 190—193 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.17 (3H, t, J=7.2 Hz, CH_3), 3.42 (3H, s, OCH_3), 3.54—3.59 (2H, m, CH_2), 6.13—6.15 (1H, m, $H_{pyrrole}$), 6.24 (1H, d, J=2.4 Hz), 6.26—6.28 (1H, m, $H_{pyrrole}$), 6.74 (1H, dd, J=2.8, 8.8 Hz), 6.95—6.97 (1H, m), 7.35 (1H, d, J=8.8 Hz), 7.81 (1H, d, J=3.2 Hz, 2-H_{indole}), 11.05 (1H, s, 1-$H_{pyrrole}$), 11.69 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{19}H_{17}N_3O_3$: C, 68.05; H, 5.11; N, 12.53. Found: C, 68.13; H, 5,03; N, 12.40.

3-[6-Bromo-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl)-1-ethyl-1*H*-pyrrole-2,5-dione (**6dd**): Yield 81%; MS *m/z*: 385 (M^+); mp 183—185 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.17 (3H, t, J=7.6 Hz, CH_3), 3.55—3.60 (2H, m, CH_2), 6.14—6.16 (1H, m, $H_{pyrrole}$), 6.36—6.38 (1H, m, $H_{pyrrole}$), 7.13—7.16 (2H, m), 7.40 (1H, d, J=8.4), 7.57 (1H, s), 8.03 (1H, d, J=2.0, 2-H_{indole}), 11.10 (1H, s, 1-$H_{pyrrole}$), 12.00 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{18}H_{14}BrN_3O_2$: C, 56.27; H, 3.67; N, 10.94. Found: C, 56.20; H, 3.74; N, 11.03.

Production of 7a and 7b 32% methylamine in methanol (3 ml) was added to compound **3a** or **3b** (0.2 mmol) and the mixture was stirred for 2—3 h at −5—0 °C. The solvent was removed *in vacuo* and the residue was recrystallized from ethyl acetate to afford compound **7a** or **7b**.

4-Chloro-3-(1*H*-indol-3-yl)-1-methyl-1*H*-pyrrole-2,5-dione (**7a**): Yield 79%; mp 202—202 °C; ^{1}H-NMR (DMSO-d_6) δ: 3.01 (3H, s, CH_3), 7.17 (1H, t, J=8.0 Hz, H_{phenyl}), 7.24 (1H, t, J=8.0 Hz, H_{phenyl}), 7.52 (1H, d, J=8.0 Hz, H_{phenyl}), 7.93 (1H, d, J=8.0 Hz, H_{phenyl}), 8.13 (1H, d, J=2.8 Hz, 2-H_{indole}), 12.16 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{13}H_9ClN_2O_2$: C, 59.90; H, 3.48; N, 10.75. Found: C, 59.81; H, 3.57; N, 10.82.

4-Chloro-3-[5-bromo-(1*H*-indol-3-yl)]-1-methyl-1*H*-pyrrole-2,5-dione (**7b**): Yield 75%; mp 230—231 °C (dec.); ^{1}H-NMR (DMSO-d_6) δ: 3.01 (3H, s, CH_3), 7.35 (1H, dd, J=2.0, 8.8 Hz, 6-H_{phenyl}), 7.49 (1H, d, J=8.8 Hz, H_{phenyl}), 8.12 (1H, d, J=2.0 Hz, H_{phenyl}), 8.14 (1H, d, J=2.8 Hz, 2-H_{indole}), 12.32 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{13}H_8BrClN_2O_2$: C, 45.98; H, 2.37; N, 8.25. Found: C, 45.87; H, 2.46; N, 8.37.

Production of 8a and 8b The same procedure was performed as compounds **4a**—**d** to afford **8a** or **8b** from **7a** or **7b**.

4-Chloro-3-[1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]-1-methyl-1*H*-pyrrole-2,5-dione (**8a**): Yield 77%; mp 161—163 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.66 (9H, s, $C(CH_3)_3$), 2.97 (3H, s, CH_3), 7.34 (1H, t, J=7.2 Hz, H_{phenyl}), 7.41 (1H, t, J=7.2 Hz, H_{phenyl}), 7.95 (1H, d, J=7.2 Hz, H_{phenyl}), 8.10 (1H, s,

2-H_{indole}), 8.13 (1H, d, J=7.2 Hz, H_{phenyl}); *Anal.* Calcd for $C_{18}H_{17}ClN_2O_4$: C, 59.92; H, 4.75; N, 7.76. Found: C, 59.86; H, 4.67; N, 7.87.

4-Chloro-3-[1-(*tert*-butyloxycarbonyl)-5-bromo-(1*H*-indol-3-yl)]-1-methyl-1*H*-pyrrole-2,5-dione (**8b**): Yield 48%; mp 178—180 °C; ^{1}H-NMR (DMSO-d_6) δ: 1.67 (9H, s, $C(CH_3)_3$), 3.03 (3H, s, CH_3), 7.59 (1H, dd, J=2.0, 7.2 Hz, H_{phenyl}), 7.99 (1H, d, J=2.0 Hz, H_{phenyl}), 8.08 (1H, d, J=9.2 Hz, H_{phenyl}), 8.13 (1H, s, indole-2-H); *Anal.* Calcd for $C_{18}H_{16}BrClN_2O_4$: C, 49.17; H, 3.67; N, 6.37. Found: C, 49.29; H, 3.57; N, 6.24.

Production of 9a and 9b The same procedure was performed as compounds **5a**—**d** to afford **9a** or **9b** from **8a** or **8b**.

3-[1-(*tert*-Butyloxycarbonyl)-1*H*-indol-3-yl]-4-(1*H*-pyrrol-2-yl)-1-methyl-1*H*-pyrrole-2,5-dione (**9a**): Yield 58%; mp 180—183 °C; *Anal.* Calcd for $C_{22}H_{21}N_3O_4$: C, 67.51; H, 5.41; N, 10.74. Found: C, 67.64; H, 5.36; N, 10.82.

3-[1-(*tert*-Butyloxycarbonyl)-5-bromo-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl)-1-methyl-1*H*-pyrrole-2,5-dione (**9b**): Yield 54%; mp 169—173 °C; *Anal.* Calcd for $C_{22}H_{20}BrN_3O_4$: C, 56.18; H, 4.29; N, 8.93. Found: C, 56.33; H, 4.35; N, 8.82.

Production of 10a and 10b Compound **9a** or **9b** (0.10 mmol) was dissolved in dioxane (1 ml) and 2 M aqueous potassium hydroxide (2 ml) and refluxed for 1 h. The solution was acidified with 2 M HCl. The mixture was extracted with ethyl acetate (15 ml×3) and the organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by chromatography on silica gel using petroleum ether/ethyl acetate as eluent to yield **10a** or **10b**.

3-(1*H*-Indol-3-yl)-4-(1*H*-pyrrol-2-yl) furan-2,5-dione (**10a**): Yield 72%; mp >250 °C; ^{1}H-NMR (DMSO-d_6) δ: 6.18—6.20 (1H, m, $H_{pyrrole}$), 6.46 (1H, s, $H_{pyrrole}$), 6.91 (1H, d, J=7.6 Hz), 6.94 (1H, t, J=7.6 Hz), 7.05 (1H, s), 7.17 (1H, t, J=7.6 Hz), 7.51 (1H, d, J=7.6 Hz), 7.90 (1H, d, J=3.0 Hz, 2-H_{indole}), 11.21 (1H, s, 1-$H_{pyrrole}$), 12.00 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{16}H_{10}N_2O_3$: C, 69.06; H, 3.62; N, 10.07. Found: C, 69.17; H, 3.57; N, 10.21.

3-[5-Bromo-(1*H*-indol-3-yl)]-4-(1*H*-pyrrol-2-yl) furan-2,5-dione (**10b**): Yield 57%; mp >250 °C; ^{1}H-NMR (DMSO-d_6) δ: 6.22—6.24 (1H, m, $H_{pyrrole}$), 6.51 (1H, br, $H_{pyrrole}$), 7.07 (2H, br), 7.29 (1H, dd, J=2.0, 8.4 Hz), 7.48 (1H, d, J=8.4 Hz), 7.95 (1H, d, J=1.6 Hz, 2-H_{indole}), 11.22 (1H, s, 1-$H_{pyrrole}$), 12.12 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{16}H_9BrN_2O_3$: C, 53.81; H, 2.54; N, 7.84. Found: C, 53.89; H, 2.60; N, 7.75.

Production of 3-(1*H*-Indol-3-yl)-4-(1*H*-pyrrol-2-yl)-1-amino-1*H*-pyrrole-2,5-dione (11aa) To a solution of anhydride **10a** (0.20 mmol) in DMF (5 ml) was added hydrazine hydrate (6 ml). The mixture was stirred at 80 °C for 2 h, and then the solution was extracted with ethyl acetate (30 ml×3) after water (50 ml) was added. The organic phase was washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by chromatography on silica gel using petroleum ether/ethyl acetate as eluent to yield **11aa**. Yield 61%; mp >250 °C; MS *m*/*z*: 293 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 4.79 (2H, s, NH_2), 6.10—6.12 (1H, m, $H_{pyrrole}$), 6.26—6.27 (1H, m, $H_{pyrrole}$), 6.85 (1H, d, J=7.6 Hz, $H_{pyrrole}$), 6.90 (1H, t, J=7.6 Hz, H_{indole}), 6.94 (1H, m, H_{indole}), 7.13 (1H, t, J=7.6 Hz, H_{indole}), 7.47 (1H, d, J=7.6 Hz, H_{indole}), 7.76 (1H, d, J=2.4 Hz, H_{indole}), 11.02 (1H, s, 1-H_{indole}), 11.76 (1H, s, 1-$H_{pyrrole}$); *Anal.* Calcd for $C_{16}H_{12}N_4O_2$: C, 65.75; H, 4.14; N, 19.17. Found: C, 65.65; H, 4.02; N, 19.22.

Production of 11ac, 11ae and 11af The same procedure as described above afforded from **10a** (0.20 mmol) and NH_2NHCHO, $NH_2NHCH_2CH_2CN$ or NH_2CH_2COOH (1.2—2.4 mmol) after purification by chromatography on silica gel using petroleum ether/ethyl acetate as eluent **11ac**, **11ae** and **11af**, respectively.

11ac: Yield 47.1%; mp 230—231 °C; MS *m*/*z*: 321 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 6.13—6.15 (1H, m, $H_{pyrrole}$), 6.35—6.37 (1H, m, $H_{pyrrole}$), 6.80 (1H, d, J=7.6 Hz), 6.91 (1H, t, J=7.6 Hz), 6.98 (1H, s), 7.14 (1H, t, J=7.6 Hz), 7.49 (1H, d, J=7.6 Hz), 7.86 (1H, d, J=2.0 Hz, 2-H_{indole}), 8.33 (1H, s, CHO), 10.63 (1H, s, NH), 11.10 (1H, s, 1-$H_{pyrrole}$), 11.88 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{17}H_{12}N_4O_3$: C, 63.75; H, 3.78; N, 17.49. Found: C, 63.86; H, 3.69; N, 17.42.

11ae: Yield 87%; mp 115—118 °C; MS *m*/*z*: 346 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 2.62 (2H, t, J=6.4 Hz, CH_2), 3.14—3.17 (2H, m, CH_2), 6.01 (1H, t, J=3.6 Hz, NH), 6.09—6.10 (1H, m, $H_{pyrrole}$), 6.28—6.30 (1H, m, $H_{pyrrole}$), 6.86—6.96 (3H, m), 7.13 (1H, t, J=8.4 Hz), 7.47 (1H, d, J=8.4 Hz), 7.77 (1H, d, J=2.8 Hz, 2-H_{indole}), 11.06 (1H, s, 1-$H_{pyrrole}$), 11.78 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{19}H_{15}N_5O_2$: C, 66.08; H, 4.38; N, 20.28. Found: C, 66.05; H, 4.63; N, 20.12.

11af: Yield 20%; mp >250 °C; MS *m*/*z*: 336 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 4.26 (2H, s, CH_2), 6.11—6.13 (1H, m, $H_{pyrrole}$), 6.33—6.35 (1H, m, $H_{pyrrole}$), 6.83 (1H, d, J=7.6 Hz), 6.91 (1H, t, J=7.6 Hz), 6.96 (1H, br), 7.14 (1H, t, J=7.6 Hz), 7.48 (1H, d, J=7.6 Hz), 7.83 (1H, d, J=3.2 Hz, 2-H_{indole}), 11.07 (1H, s, 1-$H_{pyrrole}$), 11.83 (1H, s, 1-H_{indole}), 12.97 (1H, br, COOH); *Anal.* Calcd for $C_{18}H_{13}N_3O_4$: C, 64.47; H, 3.91; N, 12.53. Found: C, 64.45; H, 3.77; N, 12.62.

Production of 11ba, 11bc, 11be and 11bf The same procedure as described above afforded from **10b** and $NH_2NH_2\cdot H_2O$, NH_2NHCHO, $NH_2NHCH_2CH_2CN$ or NH_2CH_2COOH after purification by chromatography compounds **11ba**, **11bc**, **11be** and **11bf**, respectively.

11ba: Yield 40%; mp >250 °C; MS *m*/*z*: 372 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 4.80 (2H, s, NH_2), 6.13—6.15 (1H, m, $H_{pyrrole}$), 6.30—6.34 (1H, m, $H_{pyrrole}$), 6.91 (1H, d, J=8.0 Hz), 7.02—7.03 (1H, m), 7.25 (1H, dd, J=2.0, 8.0 Hz), 7.44 (1H, d, J=8.0 Hz), 7.86 (1H, d, J=2.4 Hz, 2-H_{indole}), 11.08 (1H, s, 1-$H_{pyrrole}$), 11.96 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{16}H_{11}BrN_4O_2$: C, 51.77; H, 2.99; N, 15.09. Found: C, 51.85; H, 3.12; N, 14.99.

11bc: Yield 63%; mp 210 °C (dec.); MS *m*/*z*: 400 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 6.18—6.19 (1H, m, $H_{pyrrole}$), 6.40—6.41 (1H, m, $H_{pyrrole}$), 6.89 (1H, d, J=2.0 Hz), 7.01—7.02 (1H, m), 7.26 (1H, dd, J=2.0, 8.0 Hz), 7.45 (1H, d, J=8.0 Hz), 7.93 (1H, d, J=2.4 Hz, 2-H_{indole}), 8.33 (1H, s, CHO), 10.66 (1H, s, NH), 11.15 (1H, s, 1-$H_{pyrrole}$), 12.06 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{17}H_{11}BrN_4O_3$: C, 51.15; H, 2.78; N, 14.03. Found: C, 51.25; H, 2.72; N, 14.19.

11be: Yield 66%; mp 127—130 °C; MS *m*/*z*: 425 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 2.62 (2H, t, J=5.6 Hz, CH_2), 3.14—3.16 (2H, m, CH_2), 6.02 (1H, t, J=4.0 Hz, NH), 6.14—6.15 (1H, m, $H_{pyrrole}$), 6.32—6.34 (1H, m, $H_{pyrrole}$), 6.97—6.99 (1H, m,), 7.00 (1H, d, J=2.0 Hz), 7.24 (1H, dd, J=2.0, 8.8 Hz), 7.44 (1H, d, J=8.8 Hz), 7.82 (1H, d, J=2.0 Hz, 2-H_{indole}), 11.08 (1H, s, 1-$H_{pyrrole}$), 11.94 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{19}H_{14}BrN_5O_2$: C, 53.79; H, 3.33; N, 16.51. Found: C, 53.85; H, 3.12; N, 16.59.

11bf: Yield 35%; mp >250 °C; MS *m*/*z*: 415 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 4.02 (2H, s, CH_2), 6.14—6.15 (1H, m, $H_{pyrrole}$), 6.33—6.34 (1H, m, $H_{pyrrole}$), 6.93 (1H, s), 6.98 (1H, s), 7.25 (1H, dd, J=2.0, 7.2 Hz), 7.43 (1H, d, J=7.2 Hz), 7.94 (1H, d, J=2.0 Hz, 2-H_{indole}), 11.10 (1H, s, 1-$H_{pyrrole}$), 12.10 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{18}H_{12}BrN_3O_4$: C, 52.19; H, 2.92; N, 10.14. Found: C, 52.25; H, 3.01; N, 10.19.

Production of 3-(1*H*-Indol-3-yl)-4-(1*H*-pyrrol-2-yl)-1-hydroxy-1*H*-pyrrole-2,5-dione (11ab) To a solution of anhydride **10a** (0.10 mmol) in DMF (5 ml) was added hydroxylamine hydrochloride (10 mmol) and then triethylamine (1.8 ml). The mixture was stirred at 80 °C for 2 h, and then the solution was extracted with ethyl acetate (20 ml×3) after water (50 ml) was added. The organic phase was washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by chromatography on silica gel using petroleum ether/ethyl acetate as eluent to yield **11ab**. Yield 33%; mp 244—245 °C; MS *m*/*z*: 294 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 6.16—6.17 (1H, m, $H_{pyrrole}$), 6.36 (1H, br, $H_{pyrrole}$), 6.89 (1H, d, J=8.0 Hz, $H_{pyrrole}$), 6.96 (1H, t, J=8.0 Hz), 7.01 (1H, br), 7.19 (1H, t, J=8.0 Hz), 7.52 (1H, d, J=8.0 Hz), 7.86 (1H, d, J=2.8 Hz, 2-H_{indole}); 10.49 (1H, s, OH), 11.08 (1H, s, 1-$H_{pyrrole}$), 11.87 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{16}H_{11}N_3O_3$: C, 65.53; H, 3.78; N, 14.33. Found: C, 65.45; H, 3.67; N, 14.22.

Production of 11ad and 11ag The same procedure as described above afforded from **10a** (0.10 mmol) and $NH_2NH_2CONH_2\cdot HCl$ or $NH_2CH_2COOCH_3\cdot HCl$ (7—10 mmol) after purification by chromatography compounds **11ad** and **11ag**, respectively.

11ad: Yield 78%; mp >250 °C; MS *m*/*z*: 336 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 6.11—6.12 (1H, m, $H_{pyrrole}$), 6.32 (1H, s, $H_{pyrrole}$), 6.36 (2H, br, NH_2), 6.85 (1H, d, J=7.2 Hz), 6.91 (1H, t, J=7.2 Hz), 6.96 (1H, s), 7.13 (1H, t, J=7.2 Hz), 7.49 (1H, d, J=7.2 Hz), 7.81 (1H, s, 2-H_{indole}), 8.57 (1H, br, NH), 11.07 (1H, s, 1-$H_{pyrrole}$), 11.85 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{17}H_{13}N_5O_3$: C, 60.89; H, 3.91; N, 20.89. Found: C, 60.85; H, 3.82; N, 20.99.

11ag: Yield 81%; mp 185—188 °C; MS *m*/*z*: 350 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 4.40 (2H, s, CH_2), 3.71 (3H, s, CH_3), 6.12—6.14 (1H, m, $H_{pyrrole}$), 6.34—6.36 (1H, m, $H_{pyrrole}$), 6.83 (1H, d, J=8.4 Hz), 6.92 (1H, t, J=8.4 Hz), 6.96—6.98 (1H, m), 7.16 (1H, t, J=8.4 Hz), 7.48 (1H, d, J=8.4 Hz), 7.84 (1H, s, 2-H_{indole}), 11.05 (1H, s, 1-$H_{pyrrole}$), 11.83 (1H, s, 1-H_{indole}); *Anal.* Calcd for $C_{19}H_{15}N_3O_4$: C, 65.32; H, 4.33; N, 12.03. Found: C, 65.41; H, 4.22; N, 12.19.

Production of 11bb, 11bd and 11bg The same procedure as described above afforded from **10b** and $NH_2OH\cdot HCl$, $NH_2NH_2CONH_2\cdot HCl$ or $NH_2CH_2COOCH_3\cdot HCl$ after purification by chromatography compounds **11bb**, **11bd** and **11bg**, respectively.

11bb: Yield 47%; mp 210 °C (dec.); MS *m*/*z*: 373 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 6.6—6.12 (1H, m, pyrrole-H), 6.25—6.26 (1H, m, pyrrole-

H), 6.93 (2H, br), 7.23 (1H, dd, J=2.0, 8.0 Hz), 7.44 (1H, d, J=8.0 Hz), 7.77 (1H, d, J=2.0 Hz, indole-2-H); 11.03 (1H, s, pyrrole-1H), 12.22 (1H, s, indole-1-H); *Anal.* Calcd for $C_{16}H_{10}BrN_3O_3$: C, 51.63; H, 2.71; N, 11.29. Found: C, 51.80; H, 2.82; N, 11.40.

11bd: Yield 80%; mp >250 °C; MS *m/z*: 415 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 6.15—6.17 (1H, m, pyrrole-H), 6.37 (3H, br), 6.95—6.99 (2H, m), 7.25 (1H, dd, J=2.0, 8.8 Hz), 7.44 (1H, d, J=8.8 Hz), 7.87 (1H, s, indole-2-H), 8.51 (1H, br, NH), 11.10 (1H, s, pyrrole-1H), 12.00 (1H, s, indole-1-H); *Anal.* Calcd for $C_{17}H_{12}BrN_5O_3$: C, 49.29; H, 2.92; N, 16.91. Found: C, 49.16; H, 3.00; N, 16.99.

11bg: Yield 74%; mp 205—207 °C (dec.); MS *m/z*: 429 (M^+); ^{1}H-NMR (DMSO-d_6) δ: 3.70 (3H, s, CH_3), 4.40 (2H, s, CH_2), 6.16—6.19 (1H, m, pyrrole-H), 6.38—6.40 (1H, m, pyrrole-H), 6.93 (1H, d, J=2.0 Hz), 6.99—7.01 (1H, m), 7.25 (1H, dd, J=2.0, 8.4 Hz), 7.44 (1H, d, J=8.4 Hz), 7.90 (1H, d, J=2.0 Hz, indole-2-H), 11.11 (1H, s, pyrrole-1H), 12.01 (1H, s, indole-1-H); *Anal.* Calcd for $C_{19}H_{14}BrN_3O_4$: C, 53.29; H, 3.30; N, 9.81. Found: C, 53.22; H, 3.22; N, 9.89.

Pharmacology The cytotoxicity of compounds **6** and **11** were evaluated with human leukemia cell line (HL60), and four human solid cancer cell lines: human prostate cancer cell line (PC-3), human esophageal cancer cell line (ECA-109), human liver cancer cell lines (Bel-7402 or SMMC-7721) and non-small-cell lung cancer cell line (A549) by the MTT method *in vitro*. The cells were seeded in 96-well plate at the concentration of 4000 cells per well in RPM I 1640 medium. After cultured for 24 h at 37 °C in 5% CO_2 atmosphere, cells were incubated with various concentrations of tested compounds for 48 h. MTT was added at a final concentration of 0.25 mg/ml and after 4 h incubation 100 μl of DMSO was added to each well and the optical density was measured at 570 nm. The IC_{50} values were calculated according to Logit method after getting the inhibitory rate.

The inhibition of some indolopyrrolemaleimide compounds on the relaxation of supercoiled DNA by calf thymus topoisomerase I were investigated. Native supercoiled pBR 322 DNA (0.5 μg) was incubated for 30 min at 37 °C with 1 unit of calf thymus topoisomerase I in the absence or presence of the compound at 200 μM. Reactions were stopped with sodium dodecyl sulfate. The DNA samples were run on an agarose gel followed by ethidium bromide (2 mg/ml) staining. The gel was photographed under UV light.

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