Synthesis and cytotoxicity of novel 3-amido-4-indolylmaleimide derivatives

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A series of novel 3-amido-4-indolylmaleimides have been synthesised from succinimide in five steps sequence consisting of bromination, indole addition, azide substitution, reduction and selective acylation. Cytotoxicity was evaluated for the products against cervical cancer Hela cell lines and human hepatocellular cancer SMMC 7721 cell line by standard MTT assay *in vitro*. Some of these compounds showed moderate cytotoxic potencies.

Keywords: 3-amido-4-indolylmaleimide, synthesis, cytotoxicity

Angiogenesis is the process of generating new capillary blood vessels, which plays an important role in the proliferation, invasion and metastasis of malignant tumours. Blocking tumour-induced angiogenesis continues to be an attractive strategy for cancer therapy.[1]

Protein kinase C(PKC) is a family of serine/threonine specific kinases, composed of at least 12 isozymes, which are involved in signal transduction pathways that govern a wide range of physiological processes including differentiation, proliferation, gene expression, brain function, membrane transport and the organisation of cytoskeletal and extracellular matrix proteins that regulate vascular function.[2-4] PKC overexpression has been linked to several types of cancer, with the PKC isoform suspected to be involved in vascular endothelial growth factor(VEGF)-induced tumor development and angiogenesis and in the apoptosis-regulating phosphatidyl inositol 3-kinase(PI3K)/Akt pathway. Targeting PKC therefore represents a potentially effective strategy for the treatment of cancer.[5-7] The natural product staurosporine, although a potent inhibitor of PKC, has limited selectivity *in vitro* for both ATP-dependent kinases and individual PKC isozymes. A series of novel bisindolylmaleimides have been developed for clinical trail,[8] including Enzastaurin[9] and Sotrastaurin.[10] Enzastaurin is an acyclic bisindolylmaleimide that potently and selectively inhibits the PKC isoform. Enzastaurin displayed anticancer efficacy in several preclinical cancer models and in clinical trials in patients with advanced cancers. It is currently undergoing phase III development for relapsed glioblastoma multiforme and diffuse B-cell lymphoma.[9]

Recently, our research group has been interested in the synthesis and development of indolylmaleimide derivatives as anticancer agents.[8] In continuation of this research, we describe the synthesis and preliminary biological evaluation of some novel 3-amido-4-indolylmaleimide derivatives against cervical cancer Hela and human hepatocellular cancer SMMC 7721 cell line *in vitro*.

The title compounds were synthesised according to the route shown in Fig. 1, and the yields were not optimised.

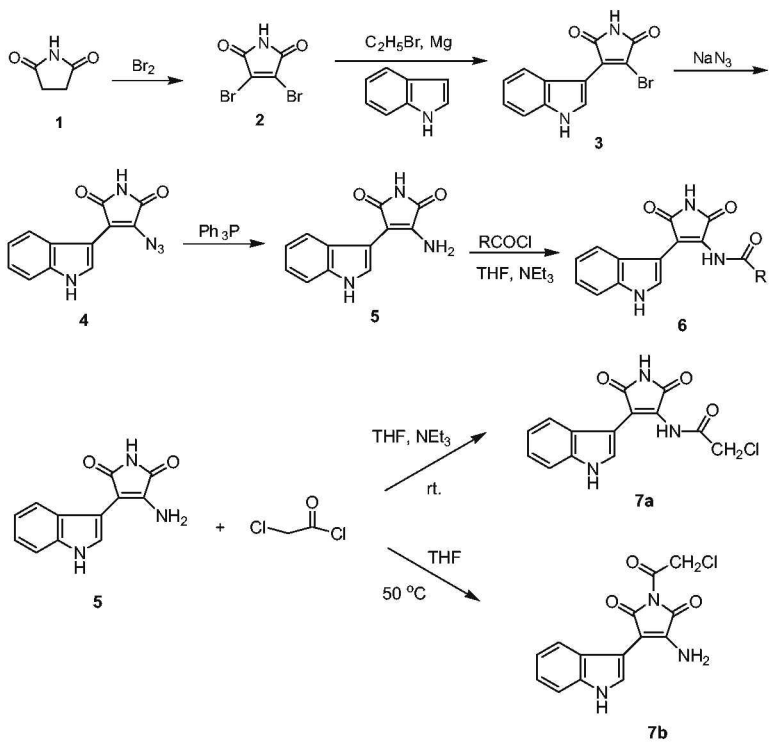

Fig.1 Synthetic route for compounds **6a–f** and **7a–b**.

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The key intermediate 2-bromo-3-(1*H*-indol-3-yl)maleimide **3** was easily synthesised from succinimide by bromination with bromine.[11] Indole was added to 2,3-dibromomaleimide in the presence of magnesium and ethyl bromide in 45% yield for the two steps. Bisindolylmaleimide, a side-product, was formed if toluene were used as solvent.[12] Compound **3** was treated with sodium azide in DMF to give 3-azideindolylmaleimide **4** which was reduced by PPh_3 in the presence of TsOH to give 3-aminoindolylmaleimides **5**.[13] There are three nitrogen atoms in the compound **5**, NH_2 group, N atom in the indole and N atom in the maleimide. Schultz and co-workers[14] reported that 3-amido-4-indolyl-N-methylmaleimide derivatives were synthesised from N-Boc-protected indolylmaleimides as starting material, and removed the Boc-protected group at the final step. Here, we developed a selective acylation procedure. Without purification compound **5** was treated with an acyl chloride in THF at the room temperature to provide the target compound **6** with 60% yield. The enamine reacted more readily with the acyl choride compared to the other groups.

When the compound **5** reacted with chloroacetyl chloride, the compounds **7a** and **7b** were obtained in THF at room temperature in the presence of NEt_3 and in THF at 50 °C in the absence of NEt_3, respectively. A reasonable explanation is that the formation of amine hydrochloride salt on NH_2 group makes it more difficult to obtain the acylation product **7a** in high temperature. The product **7b**, with chloroacetylation on the N atom of maleimide, was shown to be the main product by the existence of the signal of indole NH proton at 11.48 ppm.

The cytotoxicity of the novel compounds (**6a–f**) was evaluated with Hela and SMMC 7721 cells *in vitro* by MTT assay. The results expressed as IC_{50} were summarised in Table **1**. It was be found that these compounds (**6a–f**) show moderate cytotoxicity on Hela and SMMC 7721 cell line. For example, cytotoxicity of the compound **6f** against Hela and SMMC 7721 was 21.2 and 12.5 μM, respectively.

In conclusion, a series of novel 3-amido-4-indolylmaleimides have been synthesised from succinimide. Cytotoxicity was evaluated for the synthesised compounds against cervical cancer Hela cell lines and human hepatocellular cancer SMMC 7721 cell line by standard MTT assay. Some of the compounds exhibited potent cytotoxicity against Hela cell line and SMMC 7721 cell line. Further biological evaluation and structure optimisation of indolylmaleimide derivatives are currently underway in our laboratories.

Experimental

Melting points were determined with RY-1 apparatus, and were uncorrected. IR spectra were determined as KBr pellets on a Shimadzu model 470 spectrophotometer. ^{1}H NMR spectra were recorded using a Bruker AV 400 MHz spectrometer in DMSO-d_6 with tetramethylsilane as internal standard. Electrospray impact mass spectra were recorded on Simadzu LCMS-2010 system. Elemental analyses were performed on a Vario EL III elemental analyser.

Table 1 The cytotoxicity data of indolylmaleimide derivatives **6a–f** *in vitro*

Compound	IC_{50} (μM)	
	Hela	SMMC 7721
6a	35.5	9.2
6b	42.5	21.2
6c	29.5	26.2
6d	38.9	16.3
6e	31.2	20.4
6f	21.2	12.5
Ro 31-6233	23.8	10.3

General procedure for preparation of **6a–f** *and* **7a–b**

Compound **5** (5 mmol) and triethylamine (10 mmol) were mixed thoroughly in THF (10 ml). The acyl chloride in THF (5 ml) was added dropwise at room temperature to this solution. The reaction mixture was stirred for 6 h. Water (5 ml) was added, extracted with ethyl acetate and washed with saturated $NaHCO_3$ and water. The solvent was removed and purified by flash column chromatography to give a red-yellow solid. The physical and spectra data of the compounds **6a–f** and **7a–b** are as follows.

N-(4-(1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)acetamide (**6a**): Yellow crystals 70% yield; m.p. 248–250 °C; IR (cm^{-1}): 1704(C=O), 1642(C=O). ^{1}H NMR: δ 1.98(s 3H), 7.07(t, 1H, *J* = 7.2 Hz), 7.15(t, 1H, *J* = 7.2 Hz), 7.44–7.50 (m, 2H), 7.85(s, 1H), 9.99(s, 1H), 10.86(s, 1H), 11.81(s, 1H). MS(ESI, *m/z*): 270.1[(M + H)]$^+$, 292.2 [(M + Na)]$^+$. Anal. Calcd for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61. Found: C, 62.84; H, 4.31; N, 15.29%.

N-(4-(1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)butyramide (**6b**):Yellow crystals 65% yield; m.p. 240–243 °C. IR (cm^{-1}): 1701(C=O), 1638(C=O). ^{1}H NMR: δ 0.81(t, 3H, *J* = 7.6 Hz), 1.44(q, 2H, *J* = 7.2 Hz), 2.28(t, 2H, *J* = 7.6 Hz), 7.04(t, 1H, *J* = 7.2 Hz), 7.14(t, 1H, *J* = 7.2 Hz), 7.43–7.48(m, 2H), 7.84(s, 1H), 9.90(s, 1H), 10.87(s, 1H), 11.79(s, 1H). MS(ESI, *m/z*): 298.2[(M + H)]$^+$, 320.2 [(M + Na)]$^+$. Anal. Calcd for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13. Found: C, 64.39; H, 5.01; N, 13.92%.

N-(4-(1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl) benzamide (**6c**): Red crystals 59% yield; m.p. 232–235 °C. IR (cm^{-1}): 1697(C=O), 1648(C=O). ^{1}H NMR: δ 6.87(t, 1H, *J* = 7.2 Hz), 7.08(t, 1H, *J* = 7.2 Hz), 7.42–7.58(m, 5H), 7.88–7.94(m, 3H), 10.30(s, 1H), 10.96(s, 1H), 11.83(s, 1H). MS(ESI, *m/z*):332.2[(M + H)]$^+$, 354.1 [(M + Na)]$^+$. Anal. Calcd for $C_{19}H_{13}N_3O_3$: C, 68.88; H, 3.95; N, 12.68. Found: C, 68.98; H, 3.76; N, 12.95%.

N-(4-(1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-4-chlorobenzamide (**6d**): Red crystals 56% yield; m.p. 258–261 °C. IR (cm^{-1}): 1703(C=O), 1639(C=O). ^{1}H NMR: δ 6.88(t, 1H, *J* = 7.2 Hz), 7.08(t, 1H, *J* = 7.2 Hz), 7.43(d, 1H, J = 8.0 Hz), 7.51(d, 1H, *J* = 8.0 Hz), 7.58(d, 2H, *J* = 8.4 Hz), 7.93–7.95(m, 3H), 10.45(s, 1H), 10.99(s, 1H), 11.86(s, 1H). MS(ESI, *m/z*): 364.0 [(M-H)]$^-$. Anal. Calcd for $C_{19}H_{12}ClN_3O_3$: C, 62.39; H, 3.31; N, 11.49. Found: C, 62.67; H, 3.12; N, 11.18%.

N-(4-(1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-4-nitrobenzamide (**6e**): Red crystals 62% yield; m.p. 265–268°C. IR (cm^{-1}): 1712(C=O), 1657(C=O). ^{1}H NMR: δ 6.89(t, 1H, *J* = 7.2 Hz), 7.09(t, 1H, *J* = 7.2 Hz), 7.43-7.52(m, 2H), 7.96(d, 1H, *J* = 7.2 Hz), 8.13(d, 2H, *J* = 8.8 Hz), 8.33(d, 2H, *J* = 8.8 Hz), 10.76(s, 1H), 11.03(s, 1H), 11.89(s, 1H). MS(ESI, *m/z*): 375.0 [(M-H)]$^-$. Anal. Calcd for $C_{19}H_{12}N_4O_5$: C, 60.64; H, 3.21; N, 14.89. Found: C, 60.42; H, 3.41; N, 14.69%.

N-(4-(1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-4-hydroxy-3-nitrobenzamide (**6f**): Red crystals 42% yield; m.p. 260–262°C. IR (cm^{-1}): 1710(C=O), 1669(C=O). ^{1}H NMR: δ 6.89 (m, 1H, *J* = 7.2 Hz), 7.07 (t, 1H, *J* = 7.2 Hz), 7.20(d, 1H, *J* = 8.8 Hz), 7.43(d, 1H, *J* = 8.0 Hz), 7.49(d, 1H, *J* = 8.0 Hz), 7.93(s, 1H), 8.06(d, 1H, *J* = 8.8 Hz), 8.53(s, 1H), 10.47(s, 1H), 10.98(s, 1H), 11.80(s, 1H), 11.85(s,1H). MS(ESI, *m/z*):391.0 [(M-H)]$^-$. Anal. Calcd for $C_{19}H_{12}N_4O_6$: C, 58.17; H, 3.08; N, 14.28. Found: C, 58.42; H, 3.11; N, 13.96%.

N-(4-(1H-indol-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-2-chloroacetamide (**7a**): Yellow crystals 82% yield; m.p. 170–172°C. IR (cm^{-1}): 1706(C=O), 1653(C=O). ^{1}H NMR: δ 4.28(s, 2H), 7.08(t, 1H, *J* = 7.2 Hz), 7.16(t, 1H, *J* = 7.2 Hz), 7.45–7.49(m, 2H), 7.88(s, 1H), 10.37(s, 1H), 10.97(s, 1H), 11.86(s, 1H). MS(ESI, *m/z*): 303.9 [(M + H)]$^+$. Anal. Calcd for $C_{14}H_{10}ClN_3O_3$: C, 55.37; H, 3.32; N, 13.84. Found: C, 55.13; H, 3.61; N, 13.50%.

3-amino-1-(2-chloroacetyl)-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione (**7b**)**:** Yellow crystals 55% yield; m.p. 160–162°C. IR (cm^{-1}): 1695(C=O), 1646(C=O). ^{1}H NMR: δ 4.88(s, 2H), 7.03(t, 1H, *J*=7.2Hz), 7.13(t, 1H, *J* = 7.2 Hz), 7.26(s, 2H), 7.42(d, 1H, *J* = 8.0 Hz), 7.53(s, 1H), 7.59(d, 1H, *J* = 8.0 Hz), 11.48(s, 1H). MS(ESI, *m/z*): 304.0 [(M + H)]$^+$. Anal. Calcd for $C_{14}H_{10}ClN_3O_3$: C, 55.37; H, 3.32; N, 13.84. Found: C, 55.02; H, 3.41; N, 13.59%.

Bioassay of cytotoxicity testing

The cytotoxicity of the novel compounds (**6a–f**) were evaluated against Hela(cervical cancer cell lines) and SMMC-7721(human hepatocellular cancer cell lines) *in vitro* by MTT assay[15], Ro 31-6233 as the positive control.[16] The results expressed as IC_{50} were summarised in Table 1. The cells were seeded in 96-well plate at the concentration of 4000 cells per well in 100 mL RPM I 1640 medium. After being cultured for 12 h at 37 °C and 5% CO_2, the cells were

incubated with various concentrations of samples for 24 h. MTT was added at a terminal concentration of 5 mg/mL and incubated with cells for 4 h. The formazan crystals were dissolved in 100 mL DMSO each well, and the optical density was measured at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength).

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