Arch Pharm Res Vol 34, No 4, 519-526, 2011
DOI 10.1007/s12272-011-0401-z

Archives of Pharmacal Research
www.springer.com/12272

Synthesis and Cytotoxicity of Novel 3-Amino-4-indolylmaleimide Derivatives

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(Received April 29, 2010/Revised September 1, 2010/Accepted September 2, 2010)

In an attempt to develop potent and selective antitumor agents, a series of novel 3-amino-4-indolylmaleimides were designed and synthesized. The reaction showed high regioselectivity. The structure of compound **7a** was determined by an X-ray single crystal diffraction method. The cytotoxicities of the title compounds were evaluated against HeLa, SMMC 7721 and HL 60 cancer cell lines by a standard MTT assay *in vitro*. The pharmacological results showed that some of the title compounds displayed moderate or high cytotoxic activity against the tested cell lines. Compound **7d** was the most promising compound against the tested cancer cell lines. Structure-activity relationships are discussed based on the experimental data obtained. A hydroxyethylamino group at the 3-position in the side chain of indolylmaleimide is associated with an increase in cytotoxicity.

Key words: 3-Amino-4-indolylmaleimide, Synthesis, Crystal structure, Cytotoxicity

INTRODUCTION

Cancer is the second leading cause of death in the world. Angiogenesis is the process of generating new capillary blood vessels, and it plays an important role in the proliferation, invasion and metastasis of malignant tumors (Argyriou et al., 2009). Blocking tumor-induced angiogenesis continues to be an attractive strategy for cancer therapy (Fujita et al., 2008; Ali et al., 2009).

Protein kinase C (PKC), a family of serine/threonine specific kinases, composed of at least 12 isozymes, 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 organization of cytoskeletal and extracellular matrix proteins that regulate vascular function (Serova et al., 2006; Martiny -Baron and Fabbro, 2007). PKC overexpression has been linked to several types of cancer. A PKC isoform was suspected to be involved in vascular endothelial growth factor (VEGF)-induced tumor development and angiogenesis, and in the apoptosis-regulating phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Recently, protein kinase C was shown to be an important target in cancer chemotherapy (Serova et al., 2006). The development of PKC inhibitors as cancer chemotherapy agents is an active area of research (Jirousek et al., 1996; Pajak et al., 2008; Rucci et al., 2008; Zhao et al., 2009). The natural product, staurosporine, although a potent inhibitor of PKC, has limited selectivity *in vitro* for both ATP-dependent kinases and individual PKC isozymes (Tamaoki et al., 1986). A series of novel indolylmaleimides were developed for clinical trials (Zhao et al., 2008), such as Enzastaurin (Chen and LaCasce, 2008) and Sotrastaurin (Wagner et al., 2009). Enzastaurin is an acyclic bisindolylmaleimide derivative that potently and selectively inhibits PKC isoforms. Enzastaurin displayed anticancer efficacy in several preclinical cancer models and in clinical trials in patients with advanced cancers. It is currently undergoing phase III clinical trials for relapsed glioblastoma multiforme and diffuse B-cell lymphoma (Chen and LaCasce, 2008).

Indolylmaleimides (*e.g.*, JTT 010), were reported to

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exhibit remarkable PKC inhibition (Tanaka et al., 2006). We reasoned that by introducing nitrogen atoms into the indolylmaleimide ring, one might be able to enhance the binding of the compounds to PKC. On the other hand, SAR studies on indolylmaleimides indicated that attaching hydrophilic substituents on the appropriate position of the compounds could enhanced their inhibitory potency toward PKC (Tanaka et al., 2004; Dodo et al., 2005). This result prompted us to design a series of novel 3-amino-4-indolylmaleimide analogues in an attempt to improve potency against PKC and to find more potent anticancer compounds.

Recently, our research group has been interested in the synthesis and development of indolylmaleimide derivatives as anticancer agents (Zhao et al., 2008, 2009; Jiang et al., 2010). In our continuing research program directed toward the synthesis of novel indolylmaleimide derivatives in this area, we describe herein the synthesis and preliminary biological evaluation of novel 4-amino-3-indolylmaleimide derivatives against various human cancer cell lines *in vitro*.

MATERIALS AND METHODS

Chemicals

Melting points were determined with an RY-1 apparatus, and are 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, $CDCl_3$ and acetone-d_6 with tetramethylsilane as an internal standard. EI mass spectra were recorded on a Shimadzu QP-2010 GC-MS system and a Waters Micromass GCT system. ESI mass spectra were obtained on a Shimadzu 2010A LC-MS instrument. Elemental analyses were performed using a Vario EL III elemental analyser. Crystal structures were determined using a Bruker SMART CCD area detector diffractometer. All chemicals and reagents were purchased from known commercial suppliers and were used without further purification. 3,4-Dibromomaleimide, 3,4-dibromo-N-methylmaleimide, 2-bromo-3-(1*H*-indol-3-yl)-N-methylmaleimide and bisindolylmaleimide were synthesized according to published procedures with minor changes (Scharf et al., 1965; Brenner et al., 1988; Zhao et al., 2009). Target compounds were prepared according to the synthetic routes shown in Scheme 1 and Scheme 2.

General procedure for preparation of compound 5

4 (5 mmol) and triethylamine (10 mmol) were mixed thoroughly in DMF (10 mL). To this solution, an amine (6 mmol) was added. The reaction mixture was stirred at 100ºC for 16 h. Water (20 mL) was added. The mixture was extracted with ethyl acetate (40 mL × 2), and washed with saturated $NaHCO_3$ (40 mL × 2) and water (40 mL × 2). The solvent was removed and purified by flash column chromatography (dichloromethane/methanol = 10:1, v/v) to give red yellow solids **5**. Compound **7** was synthesized from **6** following the procedure given above. Compound **8** was synthesized from **4** or **6** using ethanolamine as solvent. The physical and spectral data of compounds **5a-5h**, **7a-7d** and **8** are as follows.

3-n-propylamino-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (5a)

Red crystalline solid; Yield 73%, m.p. 199~202ºC; IR ν_{max} (KBr r, cm^{-1}): 3332, 1757, 1698, 1534, 1457, 1384, 745; ^{1}H-NMR (DMSO-d_6): δ 0.84 (t, 3H, J = 8.4 Hz, CH_3), 1.15-1.24 (m, 2H, CH_2), 2.90 (s, 3H, CH_3), 2.95-2.99 (m, 2H, CH_2), 4.02 (s, 1H, NH), 6.99 (t, 1H, J = 8.0 Hz, Ar-H), 7.09 (t, 1H, J = 8.0 Hz, Ar-H), 7.27-7.34 (m, 2H, Ar-H), 7.39 (t, 1H, J = 8.0 Hz, Ar-H), 11.22 (s, 1H, NH); MS (ESI) *m/e* $[M+H]^+$ 284.2, $[M+Na]^+$ 306.3; Anal. Calcd. for $C_{16}H_{17}N_3O_2$: C, 67.83; H, 6.05; N, 14.83. Found: C, 67.65; H, 6.08; N, 14.40%.

3-isopropylamino-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (5b)

Red crystalline solid; Yield 67%, m.p. 203~206ºC; IR ν_{max} (KBr, cm^{-1}): 3448, 3319, 1700, 1647, 1615, 1549, 1385, 745; ^{1}H-NMR ($CDCl_3$): δ 0.97 (d, 6H, J = 8.4 Hz, $2CH_3$), 3.07 (s, 3H, CH_3), 3.68-3.77 (m, 1H, CH), 5.05 (d, 1H, J = 8.4 Hz, NH), 7.14 (t, 1H, J = 7.4 Hz, Ar-H), 7.21-7.26 (m, 2H, Ar-H), 7.39 (d, 1H, J = 8.0 Hz, Ar-H), 7.48 (d, 1H, J = 8.0 Hz, Ar-H), 8.34 (s, 1H, NH); MS (ESI) *m/e* $[M+H]^+$ 284.2, $[M+Na]^+$ 306.1; Anal. Calcd. for $C_{16}H_{17}N_3O_2$: C, 67.83; H, 6.05; N, 14.83. Found: C, 67.50; H, 6.08; N, 14.51%.

3-n-butylamino-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (5c)

Red crystalline solid; Yield 77%, m.p. 183~186ºC; IR ν_{max} (KBr, cm^{-1}): 3401, 2861, 1700, 1653, 1541, 1456, 1350; ^{1}H-NMR (acetone-d_6): δ 0.58 (t, 3H, J = 8.2 Hz, CH_3), 0.97-1.01 (m, 2H, CH_2), 1.29-1.32 (m, 2H, CH_2), 2.96 (s, 3H, CH_3), 3.17 (t, 2H, J = 8.4 Hz, CH_2), 7.02-7.06 (m, 1H, Ar-H), 7.10-7.14 (m, 1H, Ar-H), 7.33 (s, 1H, Ar-H), 7.42-7.47 (m, 2H, Ar-H), 10.55 (brs, 1H, NH); MS (ESI) *m/e*: $[M+H]^+$ 298.2, $[M+Na]^+$ 320.2; Anal. Calcd. for $C_{17}H_{19}N_3O_2$: C, 68.67; H, 6.44; N, 14.13. Found: C, 68.44; H, 6.50; N, 13.90%.

3-benzylamino-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (5d)
Red crystalline solid; Yield 78%, m.p. 193~195ºC; IR ν_{max} (KBr, cm^{-1}): 3409, 1692, 1653, 1537, 1456, 1339; ^{1}H-NMR (DMSO-d_6): δ 2.90 (s, 3H, CH_3), 2.99 (s, 2H, CH_2), 4.23 (s, 1H, NH), 6.83 (d, 1H, *J* = 8.4 Hz, Ar-H), 6.87 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.10-7.15 (m, 4H, Ar-H), 7.28 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.38 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.72 (d, 1H, *J* = 8.0 Hz, Ar-H), 11.22 (d, 1H, NH); MS (ESI, *m/z*) *m/e*: $[M+H]^+$ 332.3, $[M+Na]^+$ 354.3, $[M-H]^-$ 330.1. This compound was reported in the literature (Schultz et al., 1991).

3-(di-n-butylamino)-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (5e)
Red crystalline solid; Yield 84%, m.p. 197~199ºC; IR ν_{max} (KBr, cm^{-1}): 3365, 3278, 1695, 1646, 1542, 1350, 1238; ^{1}H-NMR (acetone-d_6): δ 0.70-0.74 (t, *J* = 8.4 Hz, 6H, $2CH_3$), 1.00 -1.05 (m, 4H, $2CH_2$), 1.44-1.48 (m, 4H, $2CH_2$), 2.94 (s, 3H, CH_3), 3.50 (t, 4H, *J* = 8.4 Hz, $2CH_2$), 7.03-7.05 (m, 1H, Ar-H), 7.09-7.14 (m, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 7.40-7.44 (m, 2H, Ar-H), 10.55 (s, 1H, NH); MS (ESI, *m/z*): 354.3 $[M+H]^+$, 376.4 $[M+Na]^+$. Anal. Calcd. for $C_{21}H_{27}N_3O_2$: C, 71.36; H, 7.70; N, 11.89. Found: C, 71.02; H, 7.41; N, 11.59%.

3-(pyrrolidin-1-yl)-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (5f)
Red crystalline solid; Yield 88%, m.p. 209~212ºC; IR ν_{max} (KBr, cm^{-1}): 3384, 3269, 1683, 1630, 1542, 1358, 1243; ^{1}H-NMR ($CDCl_3$): δ 1.73-1.76 (m, 4H, $2CH_2$), 3.06 (s, 3H, CH_3), 3.53-3.58 (m, 4H, $2CH_2$), 7.09-7.13 (m, 2H, Ar-H), 7.16-7.20 (m, 1H, Ar-H), 7.35 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.46 (d, 1H, *J* = 7.8 Hz, Ar-H), 8.28 (s, 1H, NH); MS (ESI, *m/e*): $[M+H]^+$ 296.3, $[M+Na]^+$ 318.1. Anal. Calcd. for $C_{17}H_{17}N_3O_2$: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.04; H, 5.57; N, 14.12%.

3-(morpholin-4-yl)-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (5g)
Red crystalline solid; Yield 87%, m.p. 209~213ºC; IR ν_{max} (KBr, cm^{-1}): 3391, 3282, 1701, 1651, 1539, 1452, 1386; ^{1}H-NMR ($CDCl_3$): δ 3.09 (s, 3H, CH_3), 3.67 (m, 8H, $4CH_2$), 7.18 (t, 1H, *J* = 8.4 Hz, Ar-H), 7.24 (t, 1H, *J* = 8.4 Hz, Ar-H), 7.37-7.40 (m, 1H, Ar-H), 7.46 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.55 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.38 (s, 1H, NH); MS (ESI) *m/e*: $[M^+ + H]$ 312.3, $[M^+ + Na]$ 334.2; Anal. Calcd. for $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.58; H, 5.45; N, 13.32%.

3-(2-hydroxyethylamino)-4-(1H-indol-3-yl)-1-methyl-1H-pyrrole-2,5-dione (5h)
Red crystalline solid; Yield 78%, m.p. 219~222ºC; IR ν_{max} (KBr, cm^{-1}): 3461, 3316, 3218, 3027, 2930, 1699, 1644, 1550, 1358, 1056; ^{1}H-NMR (DMSO-d_6): δ 2.91 (s, 3H, CH_3), 3.11 (t, *J* = 5.8 Hz, 2H, CH_2), 3.24 (t, *J* = 5.8 Hz, 2H, CH_2), 4.58 (s, 1H, OH), 6.90 (s, 1H, NH), 7.00-7.03 (m, 1H, Ar-H), 7.09-9.12 (m, 1H, Ar-H), 7.29-7.31 (m, 1H, Ar-H), 7.37-7.40 (m, 2H, Ar-H), 11.17 (s, 1H, NH); MS (ESI, *m/z*): 286.2 $[M+H]^+$, 308.3 $[M+Na]^+$. Anal. Calcd. for $C_{15}H_{15}N_3O_3$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.08; H, 5.01; N, 14.42%.

3-(dimethylamino)-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione (7a)
Red crystalline solid; Yield 62%, m.p. 161~163ºC; IR ν_{max} (KBr, cm^{-1}): 3370, 3290, 2945, 1650, 1544, 1422, 1367; ^{1}H-NMR (DMSO-d_6): δ 2.96 (s, 6H, CH_3), 7.00-7.04 (m, 1H, Ar-H), 7.09-7.12 (m, 1H, Ar-H), 7.26-7.29 (m, 1H, Ar-H), 7.36-7.39 (m, 2H, Ar-H), 10.32 (s, 1H, NH), 11.22 (s, 1H, NH); MS (EI, *m/z*): 255 (M^+), 240, 207, 169, 128, 113, 92, 77. HRMS (EI): Calcd for $C_{14}H_{13}N_3O_2$ 255.1008 (M^+). Found 255.1011.

3-(pyrrolidin-1-yl)-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione (7b)
Red crystalline solid; Yield 79%, m.p. 147~149ºC; IR ν_{max} (KBr, cm^{-1}): 3434, 3287, 2972, 1743, 1687, 1622, 1534, 1430, 1236; ^{1}H-NMR (DMSO-d_6): δ 1.62-1.66 (m, 4H, $2CH_2$), 3.37 (t, 4H, *J* = 6.4 Hz, $2CH_2$), 6.92-6.96 (m, 1H, Ar-H), 7.02-7.05 (m, 1H, Ar-H), 7.18 (d, 1H, *J* = 2.4 Hz, Ar-H), 7.28-7.33 (m, 2H, Ar-H), 10.24 (s, 1H), 11.09 (s, 1H); MS (EI, *m/e*): $[M]^+$ 281, 252, 238, 168, 127. HRMS (EI): Calcd. for $C_{16}H_{15}N_3O_2$ 281.1164 (M^+). Found 281.1166.

3-(morpholin-4-yl)-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione (7c)
Red crystalline solid; Yield 79%, m.p. 147~149ºC; IR (KBr, cm^{-1}): 3386, 2981, 1701, 1624, 1531, 1431, 1110; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.44 (d, 4H, *J* = 3.6 Hz, $2CH_2$), 3.50 (d, *J* = 3.6 Hz, 4H, $2CH_2$), 7.04 (t, 1H, *J* = 7.4 Hz, Ar-H), 7.12 (t, 1H, *J* = 7.4 Hz, Ar-H), 7.375-7.40 (m, 2H, Ar-H), 7.46 (d, *J* = 7.8 Hz, 1H, Ar-H), 10.46 (s, 1H, NH), 11.33 (s, 1H, NH); MS (EI, *m/e*): $[M]^+$ 297, 252, 238, 226, 168, 140, 127, 84. Anal. Calcd. for $C_{16}H_{15}N_3O_3$: C, 64.64; H, 5.09; N, 14.13. Found: C, 64.38; H, 5.31; N, 14.40%.

3-(2-hydroxyethylamino)-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione (7d)
Red crystalline solid; Yield 65%, m.p. 179~182ºC; IR ν_{max} (KBr, cm^{-1}): 3488, 3294, 3157, 1697, 1648, 1554, 1421, 1350, 1059; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 3.10 (t, 2H, *J* = 5.8 Hz, CH_2), 3.22 (q, 2H, *J* = 5.7 Hz, CH_2), 4.62 (t, 1H, *J* = 5.3 Hz, OH), 6.72 (s, 1H, NH),

7.00 (d, 1H, J = 7.2 Hz, Ar-H), 7.08 (t, 1H, J = 7.7 Hz, Ar-H), 7.29 (t, J = 2.4 Hz, 1H, Ar-H), 7.37 (t, 2H, J = 7.2 Hz, Ar-H), 10.35 (s, 1H, NH), 11.22 (s, 1H, NH); MS (EI, *m/e*): $[M]^+$ 271, 240, 238, 197, 129. HRMS (EI): Calcd. for $C_{14}H_{14}N_3O_3$ 271.0957 (M^+). Found 271.0955.

3-(2-hydroxyethylamino)-1-(2-hydroxyethyl)-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione (8)

Red crystalline solid; Yield 78%, m.p. 232~235ºC; IR ν_{max} (KBr, cm^{-1}): 3488, 3294, 1759, 1697, 1647, 1554, 1421, 1350; ^{1}H-NMR (DMSO-d_6): δ 3.12 (t, 2H, J = 5.8 Hz, CH_2), 3.22 (t, 2H, J = 5.8 Hz, CH_2), 3.47-3.50 (m, 4H, $2CH_2$), 4.60 (brs, 1H, OH), 4.84 (brs, 1H, OH), 6.92-6.95 (m, 1H, Ar-H), 7.01-7.03 (m, 1H, Ar-H), 7.07-7.10 (m, 1H, Ar-H), 7.30 (s, 1H, NH), 7.39-7.42 (m, 2H, Ar-H), 11.23 (s, 1H, NH); MS (EI, *m/z*): 315 $[M]^+$, 296, 284, 238, 197, 169, 155, 128. Anal. Calcd. for $C_{16}H_{17}N_3O_4$: C, 60.94; H, 5.43; N, 13.33. Found: C, 60.58; H, 5.76; N, 12.99%.

X-ray crystallography

Crystals for *3-(dimethylamino)-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione* **7a** were obtained by dissolving compound **7a** (0.3 g, 1.7 mmol) in ethyl acetate (5 mL) and ethanol (5 mL) and evaporating the solvent slowly at room temperature for about 2 days.

The following were the procedures and the programs we used to do these procedures: Data collection: *SMART*; cell refinement: *SAINT*; data reduction: *SAINT*; programs used to solve structure: *SHELXS97*; programs used to refine structure: *SHELXL97*; molecular graphics: *SHELXTL*; software used to prepare material for publication: *SHELXTL*.

Crystallographic data (excluding structural factors) for the structure of compound **7a** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 759817. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Cntre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033.

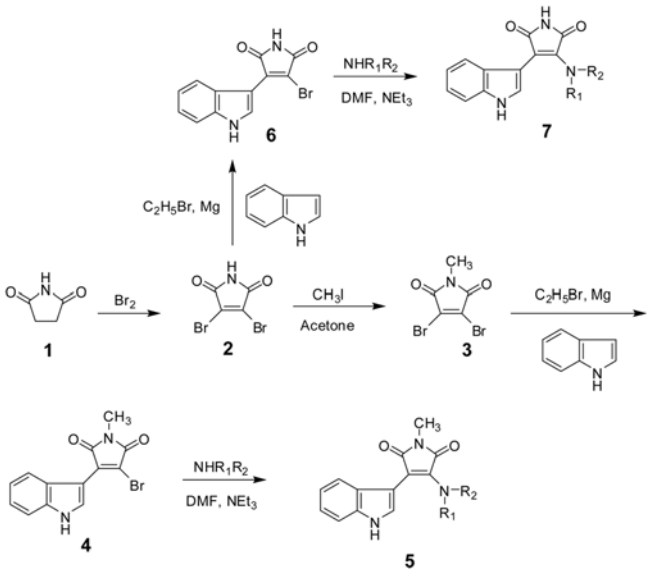
Cytotoxicity assay

In vitro cytotoxicity of novel 3-amino-4-indolylmaleimide derivatives against HeLa, SMMC 7721 and HL 60 cells were evaluated by a standard MTT assay using bisindolylmaleimide as a positive control (Zhao et al.,2009). Cells were seeded in 96-well plates at a concentration of 40,000 cells per well in 100 mL RPMI 1640 medium. After culture for 24 h at 37ºC and 5% CO_2, cells were incubated with various concentrations of test drugs for 48 h. MTT was added at a final concentration of 0.5 mg/mL and incubated with cells for 4 h. The formazan crystals that formed in each well were dissolved in 100 μL DMSO, and the optical density was measured at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength). Antitumor potency of the target compounds were expressed as IC_{50} values that were calculated by linear regression analysis of the concentration-response curves obtained for the target compounds. Assays were performed in triplicate in each of three independent experiments.

RESULTS AND DISCUSSION

Chemistry

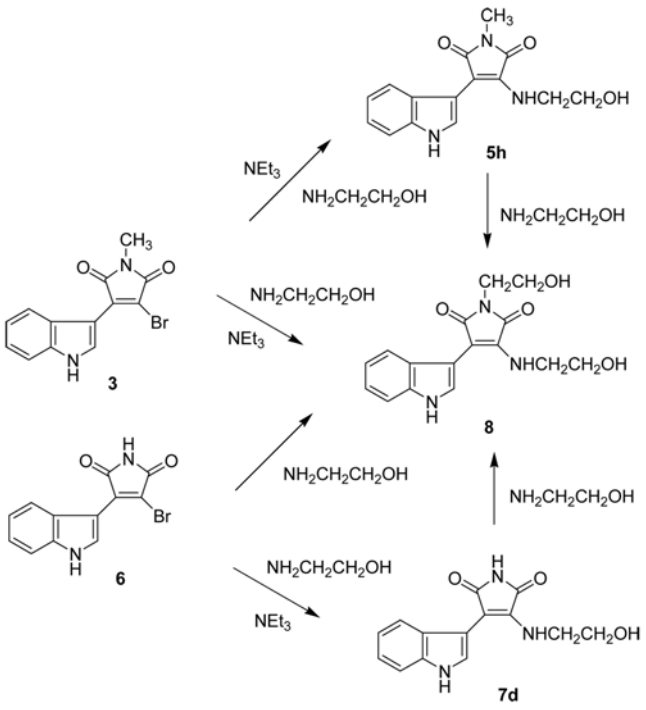
The target compounds were synthesized according to the route shown in Scheme 1, and the yields were not optimized. 2-Bromo-3-(1*H*-indol-3-yl)-N-methylmaleimide **4** as the key intermediate was easily synthesized from succinimide by bromination with bromine, methylation with methyl iodide and by the reaction of indole with 2,3-dibromo-N-methylmaleimide in the presence of magnesium and ethyl bromide in 35% yield for the three steps (Zhao et al., 2009). Bisindolylmaleimide, a side-product, was formed if toluene was used as the solvent (Brenner et al., 1988). 2-Bromo-3-(1*H*-indol-3-yl)maleimide **6** was obtained from compound **2** by the reaction of indole with 2,3-dibromomaleimide in the presence of magnesium and



Scheme 1. Synthetic route for compounds **5a**-**5h** and **7a**-**7d**.

ethyl bromide in 49% yield for the two steps. Compounds **4** and **6** were then treated with different amines in DMF to give 3-amino-4-indolyl-N-methylmaleimides **5** and 3-amino-4-indolylmaleimides **7** as red crystals.

We found that when compounds **4** and **6** were treated with primary amines, besides the main products **5** and **7**, a side-product also was isolated from the reaction mixture. For example, first, we treated compound **4** (1.0 equiv.) with ethanolamine (1.2 equiv.) in the presence of triethylamine (1.5 equiv.) as a base, in DMF at 100°C to give 3-(2-hydroxyethylamino)-4-indolyl-N-methylmaleimide **5h** as the main product with a 78% yield. When compound **4** (1.0 equiv.) was treated with ethanolamine (2.0 equiv.), a new compound was detected by TLC analysis besides compound **5h**. With increasing amounts of ethanolamine, the yield of the new compound increased. It turned out to be a main product when ethanolamine was used as a solvent. After purification by flash column chromatography, a red crystal was isolated using spectral detection. The ^{1}H-NMR spectrum showed that the proton signal of the methyl group at δ 3.0 ppm had disappeared, and one new signal was exhibited in the range of δ 2 ~ 4 ppm. Further, the EI mass spectrum of this compound showed it to be a molecular ion at 315 (M^+). On the basis of spectral data, we assumed that ring opening and closing reactions happened in this reaction and assigned to the obtained compound the structure of 1-(2-hydroxylethyl)-3-(2-hydroxyethylamino)-4-indolylmaleimide **8**. Using the same procedure, compound **6** was treated with ethanolamine (1.2 equiv. and 2.0 equiv.), and compounds **7d** and **8** were obtained in 65% and 80% yield, respectively. When treating compound **5h** or **7d** with ethanolamine, compound **8** was obtained in 72% and 78% yield, respectively (Scheme 2).



Scheme 2. Synthetic route for compound **8**.

However, when compounds **4** and **6** were treated with secondary amines, such as piperidine or di-n-butylamine, a main product of 3-aminoindolylmaleimides were obtained in 70~85% yield.

Crystals of compound **7a** suitable for X-ray analysis were obtained by slow evaporation of a solution of this compound in a mixture of ethyl acetate and ethanol (1:1). A single red crystal with dimensions 0.470× 0.392×0.351 mm was chosen for X-ray diffraction study. The data were collected on a CAD-4 diffractometer equipped with graphite-monochromatic MoKα radiation (λ = 0.71037 Å) using an ω scan mode at 293

Table I. Crystal data and structure refinement for compound **7a**

CCDDC deposit no.	CCDC759817
Molecular formula	$C_{16}H_{17}N_3O_3$
Molecular weight	299.33
Temperature (K)	293 (2)
Radiation λ	0.71073 A
Crystal system	Monoclinic
Space group	P2 (1)/n
a / Å	13.5807 (13)
b / Å	8.5960 (8)
c / Å	26.395 (3)
V / Å^3	3010.2 (5)
Z, calculated density (g cm^{-3})	8
Dcalc (g cm^{-3})	1.321
Crystal size (mm)	0.470 × 0.392 × 0.351
Crystal colour	Red crystal
Absorption coefficient (cm^{-1})	0.093
Absorption correction	Empirical
Tmin and Tmax	1.0000 and 0.0441
F(000)	1264
Range for data collection	1.87-25.50°
Limiting indices	$-12\le h\le 16$, $-10\le k\le 10$, $-29\le l\le 31$
Reflections collected/unique	15441/5622 [R(int)=0.0519]
Completeness to θ = 25.50	99.9%
Refinement method	Full-matrix least-squares on F^2
Goodness-of fit on F^2	1.031
Final R indices [I > 2σ (I)]	R_1 = 0.0683, wR_2 = 0.2035
R indices (all data)	R_1 = 0.0921, wR_2 = 0.2235
Extinction coefficient	0.0012 (11)
Largest diff. Peak and hole (eÅ^{-3})	0.466 and –0.34

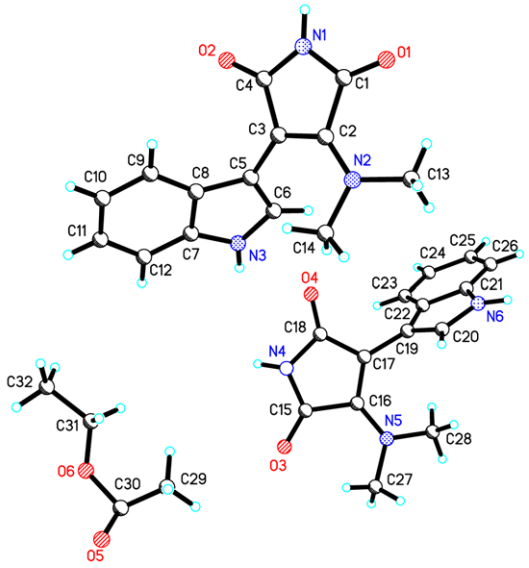


Fig. 1. ORTEP structure of the compound **7a**, showing 50% probability ellipsoids.

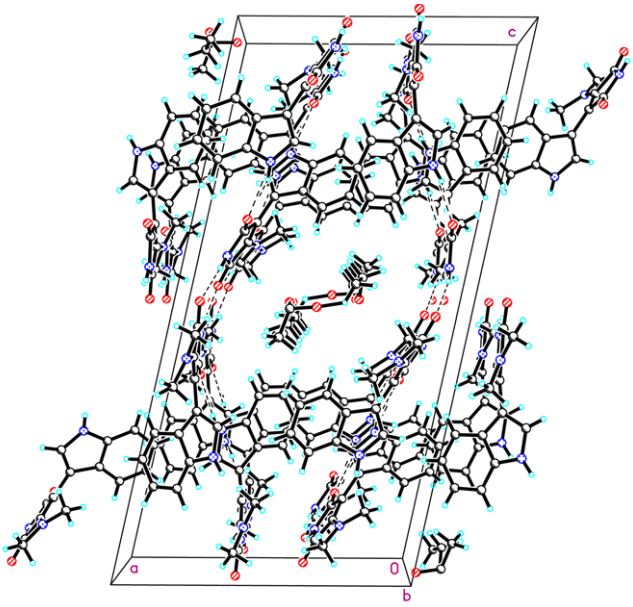


Fig. 2. Packing view of compound **7a** in the unit cell, showing N-H ··· O hydrogen-bonding interaction.

(2) K. In the range of $1.87^{o} < \theta < 25.50^{o}$, a total of 15441 reflections were collected, of which 5622 were independent (R_{int} = 0.0519) and 3856 were observed with $I > 2\sigma$ (I).

The crystal data and refinement details are listed in Table I. A view of the title structure is illustrated in Fig. 1. A perspective view of the crystal packing in the unit cell is shown in Fig. 2.

The asymmetric unit contains two 3-dimethylamine-4-indolylmaleimide molecules and one ethyl acetate molecule. The average molecular formula is $C_{16}H_{17}N_3O_3$. The average molecular weight is 299.33. Two 3-dimethylamine-4-indolylmaleimide molecules show different conformations. In one of them, the indole ring and maleimide are nearly coplanar, whereas in the other one, they are highly twisted with respect to each other. In the crystal packing, indolylmaleimide molecules are linked by intermolecular N-H···O hydrogen bonds. The layers are further connected into a three-dimensional network by N-H···O hydrogen bonds involving the maleimide molecules as H-donors and by weak π–π stacking interactions involving neighbouring indole and maleimide rings. Atoms N1, N3, N4 and N6 act as hydrogen bond donors, respectively, via O3, O2, O1 and O4 atoms to generate the supramolecular structures. The ethyl acetate molecule was located in the middle of the cave of the crystal cell.

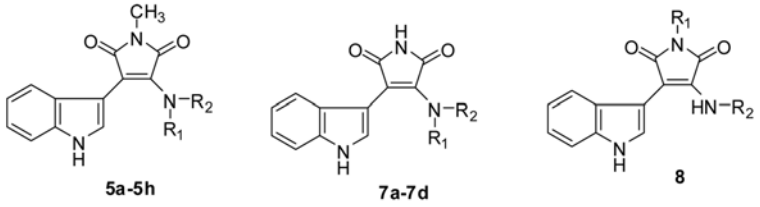
Cytotoxicity assay

The cytotoxicity of the novel 3-aminoindolylmaleimide derivatives **5a-5h, 7a-7d** and **8** was evaluated with HeLa, SMMC 7721 and HL 60 *in vitro* by a standard MTT assay using bisindolylmaleimide as a positive control. Antitumor potencies of the title compounds were expressed as IC_{50} values that were calculated by linear regression analysis of the concentration-response curves obtained for each compound. The results are summarized in Table II.

As shown in Table II, while most of the prepared compounds showed potent inhibitory effects on SMMC 7721 cell lines, and the potencies of some compounds were better or comparative to the lead compound bisindolylmaleimide, some compounds showed moderate cytotoxicity against tested cancer cell lines, although they were less potent than bisindolylmaleimide. It can be concluded that the cytotoxicity of the tested compounds against SMMC 7721 is higher than against HeLa and HL 60 cell lines, reflecting the excellent selectivity for a particular human hepatocellular cancer cell type.

The cytotoxicities of the resulting derivatives appeared to be related to the nature of the leaving group at 1-position in the maleimide ring, which was in agreement with the cytotoxic mechanism reported. It has been observed from Table II that most of the derivatives with hydroxyethyl moieties have higher cytotoxicity than the derivatives with an alkyl group. For example, cytotoxicities of compound **7d** were 22.7, 7.5 and 19.2 μM against HeLa, SMMC 7721 and HL 60, respectively. A hydroxyethylamino group at the 3-

Table II. 3-aminoindolylmaleimide derivatives and their cytotoxicity



Compound	R_1 R_2	IC_{50} (μM)		
		HeLa	SMMC 7721	HL 60
5a	-H -$CH(CH_3)_2$	30.5	21.6	33.6
5b	-H -$(CH_2)_2CH_3$	39.1	27.2	46.2
5c	-H -$(CH_2)_3CH_3$	30.5	21.6	33.6
5d	-H -$PhCH_2$	>100	45.6	>100
5e	-$(CH_2)_3CH_3$ -$CH_2)_3CH_3$	62.7	18.1	43.6
5f	-$CH_2CH_2CH_2CH_2$-	>100	56.5	>100
5g	-$CH_2CH_2OCH_2CH_2$-	59.7	39.8	78.7
5h	-H -CH_2CH_2OH	31.5	18.4	18.9
7a	-CH_3 -CH_3	29.6	28.9	25.3
7b	-$CH_2CH_2CH_2CH_2$-	87.6	30.1	46.5
7c	-$CH_2CH_2OCH_2CH_2$-	41.8	25.5	34.2
7d	-H -CH_2CH_2OH	22.7	7.5	19.2
8	-CH_2CH_2OH -CH_2CH_2OH	>100	33.5	>100
bisindolylmaleimide		56.8	20.2	33.4

position in the side chain of indolylmaleimide is associated with an increase in cytotoxicity. We suppose specific hydrogen bonds interactions with PKC may play an important role in cytotoxicity (He et al., 1994; Levy et al., 2008).

On the other hand, the cytotoxicity of compounds **7a**-**7d** is superior to compound **5a**-**5h** against tested cancer cell lines. The results indicate that the series bearing N-methylmaleimide displays lower activity than the series **7a**-**7d**. This study may provide valuable information for further designing more potent anticancer agents. Structural optimizing of indolylmaleimide derivatives are currently underway in our laboratories.

In summary, we have synthesized a series of novel 3-amino-4-indolylmaleimides from succinimide. The structure of compound **7a** was determined by X-ray single crystal diffraction method. Cytotoxicity was evaluated for the synthesized compounds against cervical cancer HeLa cells, human hepatocellular cancer SMMC 7721 cells, and HL 60 cell lines *in vitro* by a standard MTT assay. Some of the target compounds exhibited more potent cytotoxic effects against HeLa and SMMC 7721 cell lines. Structure-activity relationships were discussed based on the experimental data obtained. A hydroxyethylamino group at the 3-position in the side chain of indolylmaleimide is associated with an increase in cytotoxicity. This study may provide valuable information for further designing more potent anticancer drugs. Further biological evaluation and structural optimization of indolylmaleimide derivatives are currently underway in our laboratories.

ACKNOWLEDGEMENTS

We thank the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry of China (No.[2008]890) and Donghua Univesity for financial support for funding the outstanding young faculty (S.Y. Zhao) and Foshan University for initial funding for the investigator (Y. Yun).

REFERENCES

Ali, A. S., Ali, S., Ei-Rayes, B. F., Philip, P. A., and Sarkar, F. H., Exploitation of protein kinase C: a useful target for cancer therapy. *Cancer Treat. Rev.*, 35, 1-8 (2009).

Argyriou, A. A., Giannopoulou, E., and Kalofonos, H. P.,

Angiogenesis and anti-angiogenic molecularly targeted therapies in malignant gliomas. *Oncology*, 77, 1-11 (2009).

Brenner, M., Rexhausen, H., Steffan, B., and Steglich, W., Synthesis of arcyriarubin b and related bisindolylmaleimides. *Tetrahedron*, 44, 2887-2892 (1988).

Chen, Y. B. and LaCasce, A. S., Enzastaurin. *Expert Opin. Investig. Drugs*, 17, 939-944 (2008).

Dodo, K., Katoh, M., Shimizu, T., Takahashi, M., and Sodeoka, M., Inhibition of hydrogen peroxide-induced necrotic cell death with 3-amino-2-indolylmaleimide derivatives. *Bioorg. Med. Chem. Lett.,* 15, 3114-3118 (2005).

Fujita, Y., Abe, R., and Shimizu, H., Clinical approaches toward tumor angiogenesis: past, present and future. *Curr. Pharm. Des.,* 14, 3820-3834 (2008).

He, M., Buisine, E., Tartar, A., and Sergheraert, C., Design and synthesis of new leads for PKC bisubstrate inhibitors. *Bioorg. Med. Chem. Lett.*, 4, 2845-2850 (1994).

Jiang, D.-F., Yang, Y.-W., Shao, Z.-Y., and Zhao, S.-Y., Regioselective amination of 3-bromoindolylmaleimide with amines. *Lett. Org. Chem.*, 7, 144-148 (2010).

Jirousek, M. R., Gillig, J. R., Gonzalez, C. M., Heath, W. F., McDonald, J. H., 3rd., Neel, D. A., Rito, C. J., Singh, U., Stramm, L. E., Melikian-Badalian, A., Baevsky, M., Ballas, L. M., Hall, S. E., Winneroski, L. L., and Faul, M. M., (S)-13-[(Dimethylamino)methyl]-10,11,14,15-tetrahydro-4,9: 16,21-dimetheno-1H,13H-dibenzo[e,k]pyrrolo[3,4-h][1,4,13] oxadiazacyclohexadecene-1,3(2H)-dione (LY333531) and related analogues: isozyme selective inhibitors of protein kinase Cβ. *J. Med. Chem.,* 39, 2664-2671 (1996).

Levy, D. E., Wang, D. X., Lu, Q., Chen, Z., Perumattam, J., Xu, Y. J., Liclican, A., Higaki, J., Dong, H., Laney, M., Mavunkel, B., and Dugar, S., Aryl-indolylmaleimides as inhibitors of CaMKII δ. Part 1: SAR of the aryl region. *Bioorg. Med. Chem. Lett.*, 18, 2390-2394 (2008).

Martiny-Baron, G. and Fabbro, D., Classical PKC isoforms in cancer. *Pharmacol. Res.*, 55, 477-486 (2007).

Pajak, B., Orzechowska, S., Gajkowska, B., and Orzechowski, A., Bisindolylmaleimides in anti-cancer therapy-more than PKC inhibitors. *Adv. Med. Sci.,* 53, 21-31 (2008).

Rucci, N., Susa, M., and Teti, A., Inhibition of protein kinase c-Src as a therapeutic approach for cancer and bone metastases. *Anticancer Agents Med. Chem.*, 8, 342-349 (2008).

Scharf, H. D., Korte, F., Seidler, H., and Dittmar, R., Preparative photochemical C4-ring synthesis. I. *Chem. Ber.*, 98, 764-780 (1965).

Schultz, M., Tsaklakidis, C., Haag, R., Scheuer, W., and Russmann, E., Preparation of 4-(3-indolyl)maleimides as antiallergics and immunotherapeutic agents. DE 4005970, (1991).

Serova, M., Ghoul, A., Benhadji, K. A., Cvitkovic, E., Faivre, S., Calvo, F., Lokiec, F., and Raymond, E., Preclinical and clinical development of novel agents that target the protein kinase C family. *Semin. Oncol.*, 33, 466-478 (2006).

Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M., and Tomita, F., Staurosporine, a potent inhibitor of phospholipid/Ca^{2+} dependent protein kinase. *Biochem. Biophys. Res. Commun.,* 135, 397-402 (1986).

Tanaka, M., Sagawa, S., Hoshi, J., Shimoma, F., Matsuda, I., Sakoda, K., Sasase, T., Shindo, M., and Inaba, T., Synthesis of anilino-monoindolylmaleimides as potent and selective PKCβ inhibitors. *Bioorg. Med. Chem. Lett.,* 14, 5171-5174 (2004).

Tanaka, M., Sagawa, S., Hoshi, J., Shimoma, F., Yasue, K., Ubukata, M., Ikemoto, T., Hase, Y., Takahashi, M., Sasase, T., Ueda, N., Matsushita, M., and Inaba, T., Synthesis, SAR studies, and pharmacological evaluation of 3-anilino-4-(3-indolyl) maleimides with conformationally restricted structure as orally bioavailable PKCβ-selective inhibitors. *Bioorg. Med. Chem.,* 14, 5781-5794 (2006).

Wagner, J., Von Matt, P., Sedrani, R., Albert, R., Cooke, N., Ehrhardt, C., Geiser, M., Rummel, G., Stark, W., Strauss, A., Cowan-Jacob, S. W., Beerli, C., Weckbecker, G., Evenou, J. P., Zenke, G., and Cottens, S., Discovery of 3-(1H-indol-3-yl)-4-[2-(4-methylpiperazin-1-yl)quinazolin-4-yl]pyrrole -2,5-dione (AEB071), a potent and selective inhibitor of protein kinase C isotypes. *J. Med. Chem.,* 52, 6193-6196 (2009).

Zhao, S.-Y., Shao, Z.-Y., Qin, W.-M., and Zhang, D.-Q., Recent progress in indolylmaleimide derivatives as protein kinase C inhibitors. *Chin. J. Org. Chem.,* 28, 1676-1684 (2008).

Zhao, S. Y., Mo, S. W., Chen, Z. L., Yue, Y., and Sun, Y., Synthesis and cytotoxicity of novel 3-amido-4-indolylmaleimide derivatives. *J. Chem. Res.*, 3, 198-200 (2009).