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Design and synthesis of *N*-methylmaleimide indolocarbazole bearing modified 2-acetamino acid moieties as Topoisomerase I inhibitors

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ABSTRACT

A novel series of *N*-methylmaleimide indolocarbazole derivatives bearing modified 2-acetamino acid moieties are first reported. The cytotoxic effects of these compounds were tested in five human tumor cell lines. The potent compounds **9a, 9b, 9d, and 9e** have been further evaluated for their effect on Topoisomerase I (TOPO I) and cancer cell cycle. It is concluded that the indolocarbazoles with alkyl piperazine or morpholine substituent groups instead of esters or glycosyl residues would have better activities against tumors.

The indolocarbazole alkaloids are appealing molecules with broad spectrum of biological activities including anti-microbial,[1] anti-hypotension,[2] cytotoxic,[3] as well as the inhibition of platelet aggregation.[4] The most significant biological profile of these compounds is their potential anti-tumor effects. The indolocarbazole derivatives have shown complicated and distinct anti-tumor mechanisms including Topoisomerase I (TOPO I) poisoning, Protein Kinase C (PKC), Protein Kinase A (PKA), CDK1/cyclin B, and CDK5/p25 inhibition.[5] Their different mechanisms of action are owing to their structural diversity at some important substituted positions. For example, Rebeccamycin and NB-506 can form ternary complex with the DNA/TOPO I duplex via intercalation between the base pairs at the site of DNA cleavage, [6,7] while their analog Staurosporine exhibits high levels of PKC inhibition with no effect toward TOPO I (Fig. 1).[3,8]

Concerning for their multi-targeted anti-tumor properties, numerous attempts including adding other functional groups to the indolocarbazole framework as well as the modification of the carbohydrate moiety have been launched in the hope of obtaining analogs with improved pharmacological profiles.[9] Besides, it is well acknowledged that the introduction of amino acid and peptide-like groups are general strategies to improve the solubility and increase the capacity of molecules to the cellular targets,[10] which has already been applied in the modification of indolocarbazole derivatives.[11] In this letter, to improve the solubility of indolocarbazole scaffolds and to increase the binding affinity of the compounds with TOPO I, a series of *N*-methylmaleimide indolocarbazole derivatives bearing modified amino acids through 2-acetyl linker were synthesized and evaluated.

Indolocarbazole nucleus was synthesized from *N*-methyl pyrrole. Bromination and oxidation of *N*-methyl pyrrole gave *N*-methyl-dibromomaleimide **1**. Indolylmagnesium bromide was coupled with *N*-methyl-dibromomaleimide **1** to afford the bis-indolylmaleimides **2**. Oxidative cyclization of the bis-indolylmaleimides **2** in the presence of DDQ led to the indolocarbazole **3**. *N*-bromoacetyl amino acid methyl esters (**6a–e**) were prepared from the corresponding amino acids by esterification, neutralization and acylation of the amino group with bromoacetyl bromide. Alkylation of the indolocarbazole **3** with the esters **6a–e** in the presence of NaH gave the indolocarbazole amino acid ester derivatives **7a–e**. Hydrolysis of the methyl esters with NaH in DMF with small amount of water followed by the amidation of the indolocarbazole 8a–e with the piperazine or morpholine derivatives to give the amide derivatives **9a–e** (Scheme 1). The positive control JDC-108 (Fig. 1) was synthesized according to the literature procedures.[6b]

The cytotoxic evaluation of compounds were carried out on five human cancer cell lines by MTT assay: HT-29 (human colon carcinoma), HCT-8 (human ileocecal adenocarcinoma), Bel-7402 (human hepatoma cells), A549 (non-small cell lung carcinoma), and MCF-7 (human breast carcinoma). As shown in Table 1, most of the tested compounds were able to induce cell death in five human cancer cell lines. Among them, compound **7a, 9a, 9b, 9d**, and **9e** (one ester and four imide derivatives) showed comparable anti-tumor activity with JDC-108 which also bearing *N*-methyl indolocarbazole scaffold. Taking whole views of indolocarbazole analogs we

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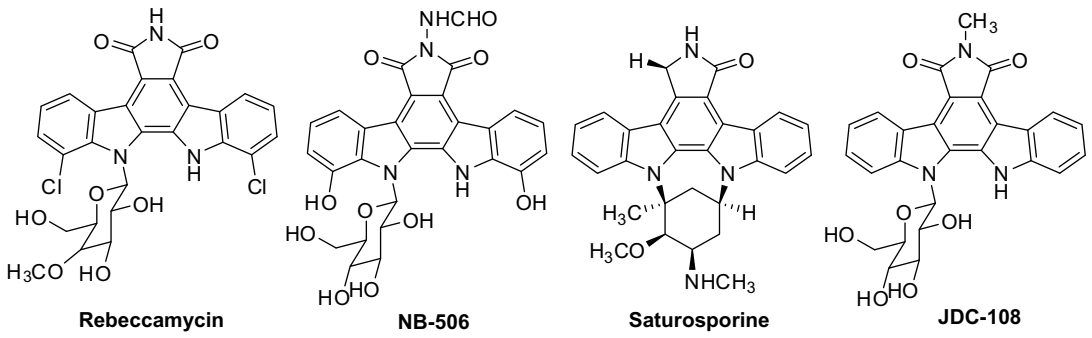
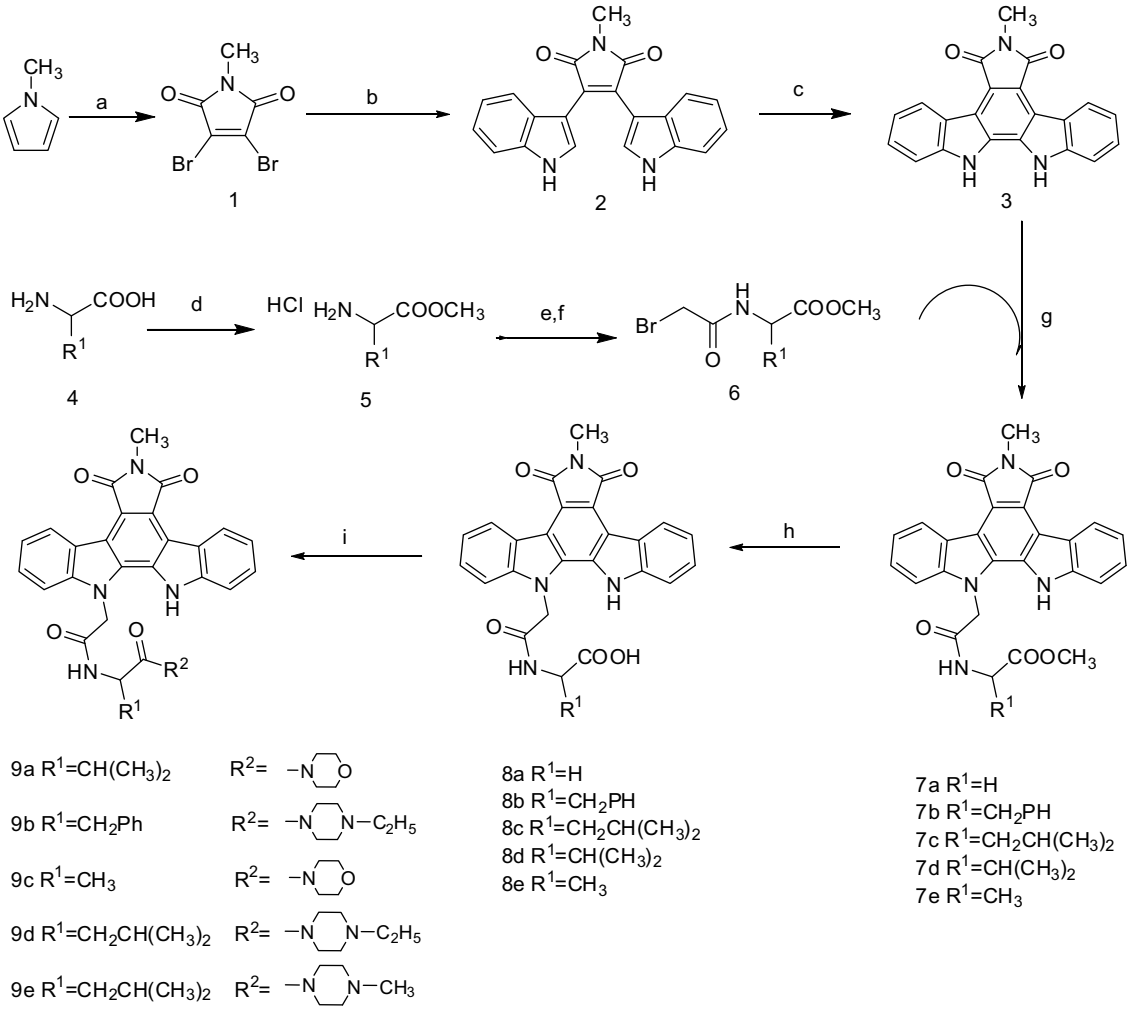


Figure 1. Structure of the mentioned indolocarbazole derivatives.

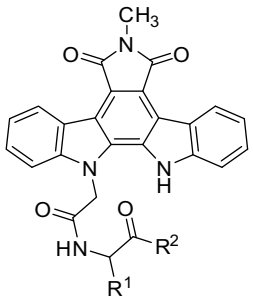


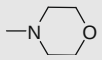
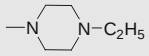
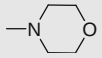
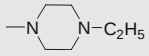
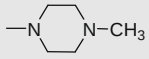
Scheme 1. The Synthesis of indolocarbazole analogs. Reagents and conditions: (a) Br_2, −78 °C, HNO_3; (b) CH_3CH_2Br, Mg, THF, indole, toluene; (c) DDQ, toluene; (d) Dry HCl, CH_3OH; (e) 10%NaOH, CH_2Cl_2; (f) $BrCH_2COBr$, TEA; (g) NaH, DMF; (h) NaH, DMF; (i) CDI, R_2H,THF.

synthesized, it is suggested that the amide analogs (**9a–e**) are more potential than those acid (**8a–e**) and ester (**7a–e**) analogs. Considering their better anti-proliferation activities, compounds **9a**, **9b**, **9d**, and **9e** were selected for further evaluation of their effect on DNA–TOPO I complex (Fig. 2) and their influence on TOPO I expression in HT-29 cells (Fig. 3).

The TOPO I inhibition assay was carried out on the TOPO I Drug Screening Kit (Cat. No. 1018, TopoGEN Corporation), following the protocol established by Trask et al.[12] According to the mechanism of TOPO I poison, compound like Camptothecin (CPT) is able to bind and stabilize the DNA/TOPO I binary complex which would result in an increase in the nicked DNA product (open-circular DNA). As shown in Figure 2, the conversions of supercoiled DNA (Form I) to open circular (OC DNA) is observed after treatment with CPT, **9a, 9b, 9d**, and **9e**, separately. At equivalent concentration, these indolocarbazole compounds showed comparable activities with CPT as TOPO I inhibitors. The changes of TOPO I expression in HT-29 cells induced by **9a, 9b, 9d**, and **9e** were also evaluated

Table 1
The in vitro MTT assay of 15 indolocarbazole analogs



Compound	R^1	R^2	IC_{50} (μM)				
			HT-29	HCT-8	Bel-7402	A549	MCF-7
7a	H	OCH_3	2.88	>10	>10	2.13	2.23
7b	CH_2Ph	OCH_3	>10	>10	>10	>10	>10
7c	$CH_2CH(CH_3)_2$	OCH_3	>10	>10	>10	>10	>10
7d	$CH(CH_3)_2$	OCH_3	>10	>10	>10	>10	>10
7e	CH_3	OCH_3	>10	>10	>10	>10	>10
8a	H	OH	>10	>10	>10	>10	>10
8b	CH_2Ph	OH	>10	>10	>10	>10	>10
8c	$CH_2CH(CH_3)_2$	OH	>10	>10	>10	>10	>10
8d	$CH(CH_3)_2$	OH	>10	>10	>10	>10	>10
8e	CH_3	OH	>10	>10	>10	>10	>10
9a	$CH(CH_3)_2$		2.66	>10	>10	5.11	>10
9b	CH_2Ph		2.88	1.09	1.91	1.86	1.91
9c	CH_3		>10	>10	>10	>10	>10
9d	$CH_2CH(CH_3)_2$		3.48	>10	>10	7.94	2.44
9e	$CH_2CH(CH_3)_2$		1.26	>10	2.23	1.84	1.94
JDC-108	–	–	7.44	>10	6.68	>10	>10

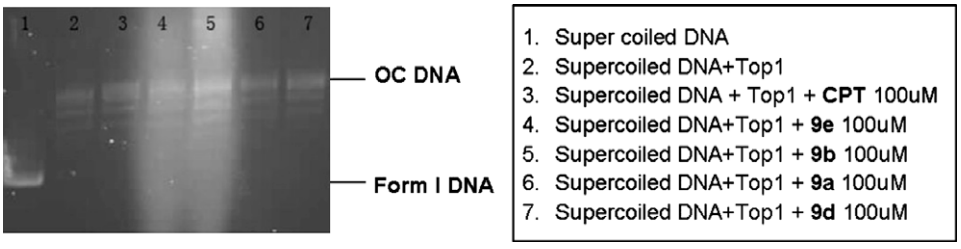


Figure 2. The effect of **9a**, **9b**, **9d**, and **9e** on DNA–TOPO I complex.

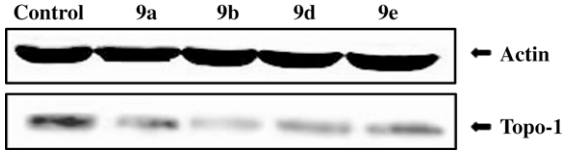


Figure 3. The influence of TOPO I expression in HT-29 cells.

following the common protocols with the commercial available TOPO I antibody.[13] As shown in Figure 3, after incubated with 20 μM of **9a, 9b, 9d**, and **9e** for 24 h, the expression levels of TOPO I on HT-29 cells were decreased compared to the control group, which were correlated with their cytotoxic IC_{50} values. Furthermore, the apoptosis-inducing activity of **9a, 9b, 9d**, and **9e** were evaluated using flow cytometry. As shown in Figure 4, incubation with 20 μM of **9a, 9b, 9d**, and **9e** for 24 h resulted in the increased percentage of apoptotic cells in Sub-G1 phase (2.49%, 10.3%, 8.86%, 14.33% compared to 0.65% in control group).

In conclusion, we have synthesized 15 novel *N*-methylmaleimide indolocarbazole analogs bearing modified 2-acetamino acid moieties and have tested their cell growth inhibitory activities. Compounds with the alkyl amide chains showed potent anti-proliferation activities against different tumor cell lines. Further studies

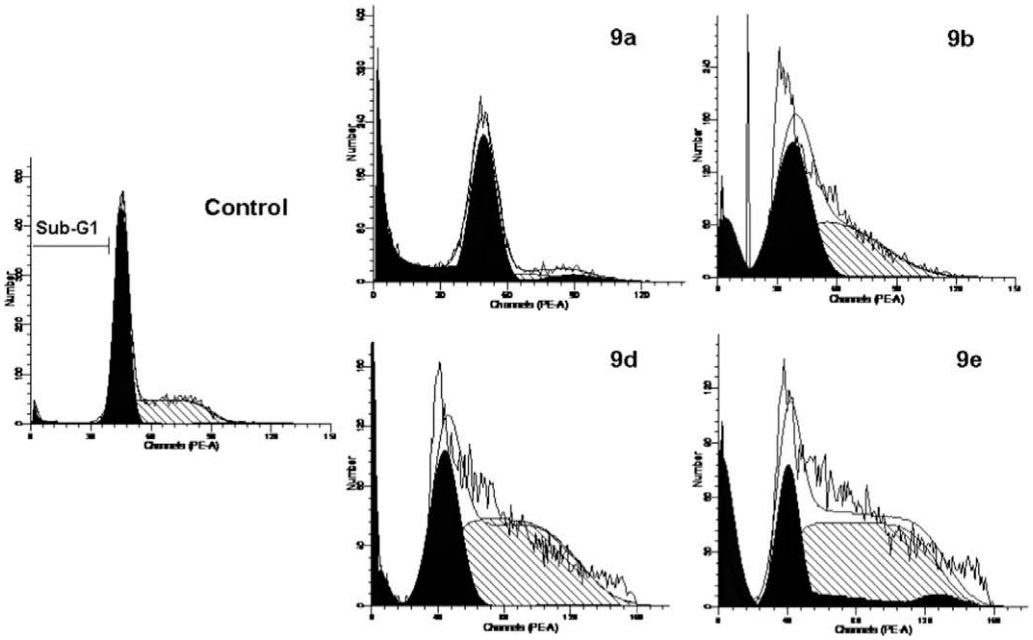


Figure 4. Compound **9a**, **9b**, **9d**, and **9e** could induce apoptosis in HT-29 cells.

on cellular level revealed that compound **9a**, **9b**, **9d**, and **9e** had comparable TOPO I inhibition activities with CPT. Moreover, the anti-tumor activities of **9a, 9b, 9d**, and **9e** were partly attributed to their abilities to modulate the expression levels of TOPO I proteins and to induce apoptosis in cancer cells. All the above results demonstrated that these compounds could be promising lead compounds of novel antitumor drugs. Further studies about the cellular mechanism of the potent compounds are in progress.

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Supplementary data

Supplementary data of synthesis and identification of the 15 compounds in this article can be found in the online version at www.sciencedirect.com and doi:10.1016/j.bmcl.2008.11.061.

References and notes

1. Omura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchiya, H.; Takahashi, Y.; Masuma, R. J. *Antibiotics* **1997**, *30*, 275.
2. (a) Omura, S.; Iwai, Y.; Hirano, A. Japan Kokai 78 73, 501, Chem. Abstr. 1978, 89, 178086b; (b) Moura, S.; Iwai, Y.; Hirano, A. Ger. Offen., 2,745,326, *Chem. Abstr.* **1978**, *89*, 58348.
3. Tamaoki, T.; Nomoto, H.; Takahishi, I.; Kato, Y.; Morimoto, M.; Tomita, F. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397.
4. Oka, S.; Kodama, M.; Takada, H.; Tomizuka, N.; Suzuki, H. *Agric. Biol. Chem.* **1986**, *50*, 2723.
5. For review, see: Prudhomme, M. *Curr. Med. Chem. Anti-Cancer Agents* **2004**, *4*, 509.
6. (a) Yamashita, Y.; Fujii, N.; Murakata, C.; Ashizawa, T.; Okabe, M.; Nakano, H. *Biochemistry* **1992**, *31*, 12069; (b) Anizon, F.; Belin, L.; Moreau, P.; Sancelme, M.; Voldoire, A.; Prudhomme, M.; Oltier, M.; Severe, D.; Riou, J. F.; Bailly, C.; Fabbro, D.; Meyer, T. *J. Med. Chem.* **1997**, *40*, 3456; (c) Moreau, P.; Anizon, F.; Sancelme, M.; Prudhomme, M.; Severe, D.; Riou, J.-F.; Goossens, J. F.; Henichart, J. P.; Bailly, C.; Labourier, E.; Tazzi, J.; Fabbro, D.; Mayer, T.; Auberting, A. M. *J. Med. Chem.* **1999**, *2*, 1816; (d) Bailly, C.; Qu, X.; Chaires, J. B.; Colson, P.; Houssier, C.; Ohkubo, M.; Nishimura, S.; Yoshinari, T. *J. Med. Chem.* **1999**, *42*, 2927.
7. Akinaga, S.; Sugiyama, K.; Akiyama, T. *Anti-Cancer Drug Des.* **2000**, *15*, 43.
8. (a) Nakano, H.; Kobayashi, E.; Takahashi, I.; Tamaoki, T.; Kuzuu, Y.; Iba, H. *J. Antibiot.* **1987**, *40*, 706; (b) Ruegg, U. T.; Burgess, G. M. *Trends Pharmacol. Sci.* **1989**, *10*, 218; (c) Davis, P. D.; Bit, R. A.; Hurst, S. A. *Tetrahedron Lett.* **1990**, *31*, 2353; (d) Harris, C. H.; Keech, E.; Malsher, P. *Tetrahedron Lett.* **1993**, *34*, 8361.
9. Reviews see (a) Prudhomme, M. *Curr. Med. Chem.* **2000**, *7*, 1189; (b) Prudhomme, M. *Curr. Med. Chem. Anti-Cancer Agents* **2004**, *4*, 509.
10. (a) Hudkins, R. L.; Iqbal, M.; Park, C.-H.; Goldstein, J.; Herman, J. L.; Shek, E.; Murakata, C.; Mallamo, J. P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1873; (b) Xie, G.; Gupta, R.; Atchison, K.; Lown, J. W. *J. Med. Chem.* **1996**, *39*, 1049; (c) Morier-Teissier, E.; Boitte, N.; Helbecque, N.; Bernier, J. L.; Pommery, N.; Duvalet, J. L.; Fournier, C.; Hecquet, B.; Catteau, J. P.; Hénichart, J. P. *J. Med. Chem.* **1993**, *36*, 2084; (d) Sassatellia, M.; Aboaba, B.; Debitonb, É.; Moreau, P.; Prudhommea, M. *Eur. J. Med. Chem.* **2006**, *41*, 709.
11. Moreau, P.; Sancelme, M.; Bailly, C.; Léonce, S.; Pierré, A.; Hickman, J.; Pfeiffer, B.; Prudhomme, M. *Eur. J. Med. Chem.* **2001**, *36*, 887.
12. Trask, D. K.; DiDonato, J. A.; Muller, M. T. *EMBO J.* **1984**, *3*, 671.
13. Yang, H.; Yong, Y.; Qidong, Y.; Wei, L.; Hongyan, G.; Li, Z.; Kun, Z.; Wei, W.; Xiaotang, W.; Qinglong, G. *Biochem. Biophy. Res. Commun.* **2006**, *351*, 521.