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Synthesis and *In Vitro* Cytotoxicity of 1-Azaanthraquinone-3-Carboxamides

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Five 1-azaanthraquinone-3-carboxamides were synthesized and evaluated in vitro cytotoxicity against four human cancer cell lines. The most active compound, **7b**, exhibited cytotoxic activity comparable to doxorubicin.

Key words : Drug resistance,Chemotherapy, Azaanthraquinone, Cytotoxic activity, Doxorubicin, Acridine-4-carboxamide

INTRODUCTION

The development of drug resistance is one of the causes of severe limitation to the chemotherapy in cancer patients. The use of inhibitors of topoisomerase I and II has shown synergistic effect against the resistant tumor cells (Cortes and Pinero, 1994; Bonner and Kozelsky, 1996). Recently, interest has focused on the mixed topoisomerase I/II inhibitors because these are more effective at overcoming "atypical" drug resistance. Examples of such mixed inhibitors which show broad-spectrum activity against solid tumors which are in clinical trial include acridine-4-carboxamide DACA (**1**) (Atwell *et al.*, 1987; Baguley et *al.*, 1995) and various tetracyclic chromophores (e.g., **2**) (Utsugi *et al.*, 1996; Ishida *et al.*, 1996) (Fig. 1).

Our previeous work on the azaanthraquinone derivatives revealed that diazaanthraquinone chromophore was more potent than monoazaanthraquinone derivative (Lee *et al.*, 1998). These facts prompted us to prepare the monoazaanthraquinone-carboxamides and evaluate their cytotoxicities. Incorporation of carboxamide side chain to the azaanthraquinone chromophore might replace the role of the additional nitrogen in the diazaanthraquinone system in terms of electronic nature. The substituents on the nitrogen of carboxamide were varied to probe the effect on their *in vitro* cytotoxicities.

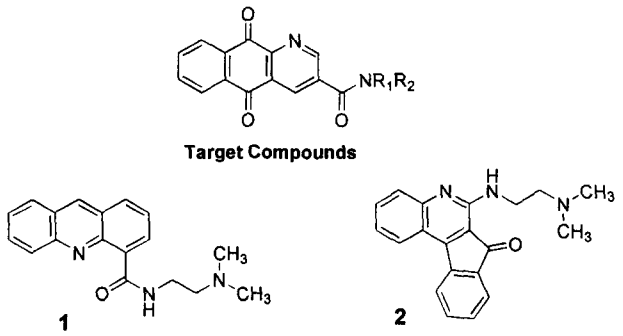

Fig. 1. The mixed topoisomerase I/II inhibitors

MATERIALS AND METHODS

Melting points were recorded on a Electrothermal IA9100 digital melting point apparatus and are uncorrected. IR spectra were determined with a Jasco FT/IR-300E spectrophotometer and reported in cm^{-1}. ^{1}H-NMR spectra were recorded on Bruker DPS300 NMR spectrometer using TMS as an internal standard and chemical shifts are reported as ppm δ units. Thin-layer chromatography was performed on E. Merck silica gel GF-254 precoated plates and the identification was done with UV light and colorization with spray of concentrated sulfuric acid followed by heating. Column chromatography was carried out on silica gel 60 (230-400 mesh ASTM). Commercially available reagents and solvents were used without additional purification unless otherwise stated. RPMI1640 media was obtained form Gibco BRL. Dimethyl sulfoxide (DMSO) and other chemicals were purchased from Sigma.

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5,10-Dioxo-5,10-dihydrobenzo[g]quinoline-3-carboxylic acid (6)

A solution of 3-methyl-5,10-dihydrobenzo[g]quinoline-5,10-dione (**5**) (1.639 g, 7.342 mmol) in 18% nitric acid (245 ml) was heated at 190-200°C in a titanium autoclave. After cooling, the precipitate was collected by suction and washed with water. After drying 5,10-dioxo-5,10-dihydrobenzo[g]-quinoline-3-carboxylic acid (**6**) (1.177 g, 4.648 mmol, 52%) was obtained as a yellow solid: mp 258-259°C; IR (KBr) 2926, 1718, 1684, 1384, 1293 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) 9.62 (s, 1H, Ar-H), δ 9.26 (s, 1H, Ar-H), 8.40 (m, 2H, Ar-H), 7.97 (m, 2H, Ar-H).

***N*3-Propyl-5,10-dioxo-5,10-dihydrobenzo[g]quinoline-3-carboxamide (7a)**

To a solution of 5,10-dioxo-5,10-dihydro-benzo[g]-quinoline-3-carboxylic acid (**6**) (100 mg,0.395 mmol) in dichloro-methane (10 ml) was added dicyclohexyl-carbodiimide (DCC, 98.6 mg, 0.474 mmol) and propyl-amine (28.02 mg, 0.474 mmol) in ice bath. The resulting mixture was stirred for 1 h. The precipitate was filtered by suction and washed with dichloromethane (3 × 5 ml). The filtrate was concentrated in vacuo and purified by flash column chromatography (5% MeOH in CH_2Cl_2) to give **7a** : mp 179°C (dec.); IR (KBr) 3307, 2932, 1683, 1451, 1372, 1294 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) 9.15 (d, *J*=2.0Hz, 1H, Ar-H), 8.69 (d, *J*=2.0Hz, 1H, Ar-H), 8.38 (m, 2H, Ar-H), 7.83 (m, 2H, Ar-H), 6.23 (brs, 1H, NH), 3.47 (m. 2H, CH_2), 1.92 (m, 2H, CH_2), 0.95 (t, *J*=7.3Hz, 3H, CH_3).

3-(Tetrahydro-1*H*-1-pyrrolylcarbonyl)-5,10-dihydrobenzo[g]quinoline-5,10-dione (7b)

The product was obtained in 15% yield: mp 175-176°C; IR 2925, 1733, 1683, 1373, 1258 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 9.26 (d, *J*=2.2Hz, 1H, Ar-H), 8.77 (d, *J*=2.2Hz, 1H, Ar-H), 8.38 (m, 2H, Ar-H), 7.88 (m, 2H, Ar-H), 3.73 (t, *J*=7.1Hz, 2H, CH_2), 3.49 (t, *J*=6.4Hz, 2H, CH_2), 2.06 (m, 4H, $2CH_2$).

3-(Morpholinocarbonyl)-5,10-dihydrobenzo[g]quinoline-5,10-dione (7c).

The product was obtained in 13% yield: mp 178-180°C; IR 2924, 1730, 1684, 1589, 1299 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 8.87 (s, 1H, Ar-H), 8.35 (m, 2H, Ar-H), 8.26 (m, 1H, Ar-H), 7.77 (m, 2H, Ar-H), 4.15 (m, 4H, $2CH_2$), 3.41 (m, 4H, $2CH_2$).

3-[(4-Methylpiperazino)carbonyl]-5,10-dihydrobenzo[g]quinoline-5,10-dione(7d).

The product was obtained in 9% yield: mp 183°C; IR 3412, 2925, 1733, 1683, 1377, 1300 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 8.87 (d, *J*=2.0Hz, 1H, Ar-H), 8.30 (m, 3H, Ar-H), 7.80 (m, 2H, Ar-H), 4.12 (m, 4H, $2CH_2$), 3.41 (m, 4H, $2CH_2$), 2.51 (s, 3H, CH_3).

***N*3-[2-(Diethylamino)ethyl]-5,10-dihydrobenzo[g]quinoline-3-carboxamide (7e).**

The product was obtained in 43.5% yield: mp 180-182°C (dec.); IR 2924, 1733, 1683, 1373, 1243 cm^{-1}; ^{1}H-NMR ($CDCl_3$-CD_3OD(1:1)/TMS) δ 9.49 (s, 1H, Ar-H), 9.08 (s, 1H, Ar-H), 7.83 (m, 2H, Ar-H), 4.83 (brs, 1H, NH), 3.26 (m, 2H, CH_2), 1.95 (m, 6H, $3CH_2$), 1.21 (t, *J*=7.0Hz, 6H, $2CH_3$).

Cells

Four human cancer cell lines, HOP62, SK-OV-3, HCT15, and SF295 were used in this study. SK-OV-3 and HCT15 were purchased from American Type Culture Collection. HOP62 and SF295 were obtained from national cancer institute, U.S.A. These cells were maintained in Dulbecco's modified eagle media supple-mented with 10% fetal calf serum at 37°C under a humidified atmosphere of 5% CO_2.

***In vitro* cytotoxicity assay**

Cell numbers were measured indirectly by sulforhodamine B (SRB) method according to the NCI (USA)'s protocol (Skehan *et al.*, 1990). Cells were plated into 96 well plate at a density of 2×10^3 cells per well. Next day (day 0), compounds of interest dissolved in DMSO/media were added in quadriplicate. The final concentrations of each compound were 1 nM-10 μM and the final concentration of DMSO was <0.1%. 72 hours later, cells were fixed with 10% trichloroacetic acid (TCA) for overnight at 4°C. The TCA-treated cells were extensively washed with distilled water and dried in the air. Then, SRB solution (0.4% in 1% acetic acid) was added to the well at room temperature for one hour. Bound dye was solubilized with 10 mM Tris after washing the wells with 1% acetic acid, and absorbances at 690 nm were measured using a micro-plate reader. The absorbance value of day 0 was subtracted from the absorbance values of day 3.

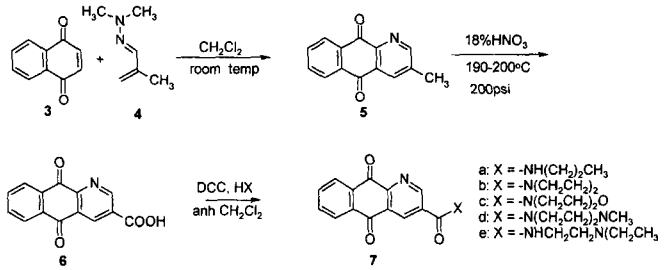
RESULTS AND DISCUSSION

Synthesis of **5** was carried out as described in our previous reports (Lee *et al.*, 1996) using hetero Diels-Alder reaction of 1,4-naphthaquinone with 1-dimethylamino-3-methyl-1-aza-1,3-butadiene.Oxidation of the compound **5** using conventional oxidizing agents ($KMnO_4$, $K_2Cr_2O_7$, or SeO_2) failed to give the desired intermediate **6**. Benzylic oxidation of the compound **5** was accomplished to afford the compound **6** using auto-clave (parr reactor, 190-200°C). The 1-azaanthra-quinone-3-carboxylic acid (**6**) was then converted to the target amides (**7a - e**) by

treating with the corresponding amines in the presence of dicyclohexylcarbodiimide(DCC) (Luzzio *et al.*, 1995) (Scheme 1).

The evaluations of the biological activity for the compounds were performed *in vitro* following the protocols developed by the National Cancer Institute (Skehan *et al.*, 1990). The *in vitro* cytotoxic activities of the azaanthraquinone derivatives (**5** and **7a** - **e**) against human cancer cell lines originated from lung (HOP62), ovarian (SK-OV-3), colon (HCT-15), CNS carcinoma (SF295) along with comparative data for doxorubicin are listed in Table I.

The novel 1-azaanthraquinone-3-carboxamides, with the exception of compound **7b**, were generally 10 to 100 folds less potent than doxorubicin in the four tumor cells. The most active compound, **7b**, exhibited cytotoxic activity comparable to that of doxorubicin in HOP62 cell and two-fold more potent activity than doxorubicin in SF295 cell. The existence of the additional hetero atom (O; **7c** or N; **7d**, **7e**) on the carbox-amide substituent decreased the activity which was the same as our previous observation (Lee *et al.*, 1998). The carboxamide substituent of 1-azaanthraquinone chromophore was designed to make a 1-azaanthraquinone system more π - deficient. It was expected that this would bring about a favorable π −π interaction with the base-pairs of electron-rich DNA. However, we could not obtain the significantly better activity with 1-azaanthraquinone-3-carboxamides than the compound without carboxamide except for the compound **7b**. Work is in progress to design, synthesize, and evaluate additional compounds in this and related systems.



Scheme 1. Synthesis of 1-azaanthraquinone-3-carboxamides

Table I. *In Vitro* cytotoxic activity of 1-azaanthraquinone-3-carboxamides

Compounds	IC_{50}[a](μM) of Cell Line[b]			
	HOP62	SK-OV-3	HCT15	SF295
5	2.6	6.1	2.5	1.5
7a	1.1	5.6	1.8	1.5
7b	0.2	1.8	0.23	0.048
7c	1.6	4.2	2.2	1.7
7d	1.8	4.2	1.7	0.49
7e	3.1	35	8.0	2.2
Doxorubicin	0.11	0.068	0.036	0.09

[a]IC_{50}= concentration of compound (mM) required to inhibit the cellular growth by 50% after 72 h of drug exposure, as determined by the SRB assay. Each experiment was run at least three times, and the results are presented as an average value. [b]Human cancer cell lines:HOP62(lung cancer cell), SK-OV-3(ovarian cancer cell), HCT-15(colon cancer cell), SF295 (CNS carcinoma cell).

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