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Synthesis and *In Vitro* Cytotoxicity of 4-Alkyl- or 4-Arylaminosubstituted Cyclopenta[c]quinoline Derivatives

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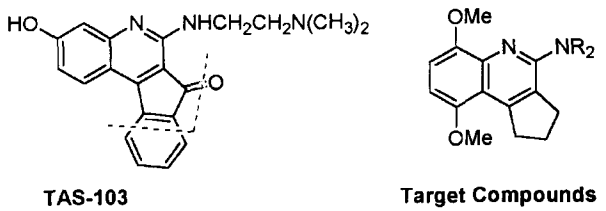
Twelve 4-substituted cyclopenta[c]quinoline derivatives were synthesized and evaluated *in vitro* cytotoxicity against four human cancer cell lines (HOP62, SK-OV-3, MD-MB-468 and T-47D). The compounds **6c** and **6e** bearing p-anisidine and pyrrolidine side chain were more active than the others.

Key words: Antitumor agents, Cyclopentaquinoline derivatives, Cytotoxic activity, Doxorubicin, and TAS-103

INTRODUCTION

6-[[2-(Dimethylamino)ethyl]amino]-3-hydroxy-7*H*-indeno [2,1-c]quinolin-7- one dihydrochloride (TAS-103) (Fig. 1) was a novel lead structure which has potential for the treatment of resistant tumors. TAS-103 was developed to target both topoisomerase (Topo I and Topo II) (Minderman *et al.*, 2000; Aoyagi *et al.*, 1999).

We previously reported the synthesis and *in vitro* cytotoxicities of 2-substituted 4-methylquinoline derivatives (Lee *et al.*, 2000). These compounds were designed as truncate analogues of TAS-103. Our previous result revealed that the bicyclic system would be too small to give a good activity. Therefore, we then turned our attention to introduce an additional cyclopentane ring system that is a structural unit of TAS-103. These analogues would further delineate the structure-activity relationship of the truncated analogs of TAS-103. In this report, we describe the synthesis and *in vitro* cytotoxic activities of 4-alkyl or arylaminosubstituted cyclopenta [c]quinoline derivatives (Fig. 1). Alkyl or aryl substituents on nitrogen were expected to have a favorable hydrophobic interaction with DNA backbone.



TAS-103 **Target Compounds**

Fig. 1. Structures of TAS-103 and target

MATERIALS AND METHODS

Melting points were recorded on a Electrothermal IA9 100 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 δ ppm 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 (230-400mesh ASTM). Commercially available regents 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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6,9-Dimethoxy-2,3,4,5-tetrahydro-1*H*-cyclopentaquinoline-4-one (3)

To a solution of dimethoxyaniline **1** (679 mg, 4.3 mmol) in toluene (15 ml) was added ethyl-2-oxo-1-cyclopentacarboxylate (1 g, 6.4 mmol). The reaction mixture was stirred at reflux for 2 h. The reaction mixture was concentrated *in vacuo* to give a crude 2-oxo-1-cyclopentane carboxamide (1.4 g). The crude product was then added portionwise to H_2SO_4 (10 ml) at 80°C. The reaction mixture was stirred at 80°C for 1 h and at 90°C for additional 15 min. The reaction mixture was cooled to room temperature and poured into ice-cold water (80 ml). The resulting mixture was neutralized with 5N NaOH solution and the precipitate was filtered to give **3** (820 mg, 63%) as white powder; mp 192~194°C; IR (KBr) 3358, 2953, 1625, 1494 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) 2.12 (q, *J*=7.7 Hz, 2H), 2.82 (t, *J*=7.7Hz, 2H), 3.43 (t, *J*=7.7Hz, 2H), 3.85 (s, 3H), 3.95 (s, 3H), 6.67(d, *J*=8.8Hz, 1H), 7.02 (d, *J*=8.8Hz, 1H), 7.28 (brs, 1H).

4-Chloro-6,9-dimethoxy-2,3-dihydro-1*H*-cyclopentaquinoline (4)

6,9-Dimethoxy-2,3,4,5-tetrahydro-1*H*-cyclopentaquinoline-4-one (**3**) (2 g, 8.16 mmol) was suspended in phosphorous oxychloride (25 ml). The mixture was stirred at reflux for 1 h. The reaction mixture was then cooled to room temperature and poured slowly into ice (100 g). The mixure was bascified with 5N NaOH solution and the precipitate was filtered to give **4** (1.75 g, 81%) as brown solid; mp 157~159°C; IR (KBr) 2929, 2834, 1612, 1571, 1504, 1470 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 2.20 (q, *J*=7.7Hz, 2H), 3.08 (t, *J*=7.7Hz, 2H), 3.62 (t, *J*=7.7Hz, 2H), 3.88 (s, 3H), 4.00 (s, 3H), 6.73 (d, *J*= 8.6Hz, 1H), 6.89 (d, *J*=8.6Hz, 1H)

4-Chloro-2,3,6,9-tetrahydro-1*H*-cyclopenta[c]quinoline-6,9-dione (5)

A solution of 4-chloro-6,9-dimethoxy-2,3-dihydro-1*H*-cyclopenta[c]quinoline (**4**) (200 mg, 0.76 mmol) in acetonitrile (5 ml) was treated with a solution of ceric ammonium nitrate (1250 mg, 2.28 mmol) in acetonitrile-water (4:1, 1 ml). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo and diluted with water (20 ml). The resulting mixture was extracted with CH_2Cl_2 (3 × 30 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash-column chromatography (EtOAc:CH_2Cl_2=1:99) to give **5** as a brown powder (141 mg, 72 %); mp 172~174°C; IR (KBr) 3046, 2654, 1665, 1605, 1550 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 2.28 (q, *J*=7.8Hz, 2H), 3,09 (t, *J*=7.8Hz, 2H), 3.54 (t, *J*=7.8Hz, 2H), 7.01 (d, *J*=8.5Hz, 1H), 8.30 (d, *J*=8.3Hz, 1H).

General procedure for 4-substituted 6,9-dimethoxy-2,3-dihydro-1*H*- cyclopenta[c]quinolines

4-Chloro-2,3,6,9-tetrahydro-1*H*-cyclopenta[c]quinoline-6,9-dione (**4**) (0.76 mmol) was treated with corresponding amine (5.32 mmol) in pyridine (5 mL). The reaction mixture was stirred at reflux for 24 h under nitrogen atmosphere. The solvent was removed *in vacuo*. The resulting residue was diluted with saturated $NaHCO_3$ solution (60 ml) and extracted with CH_2Cl_2 (3 × 30 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc:CH_2Cl_2=1:99).

***N*4-Phenyl-6,9-dimethoxy-2,3-dihydro-1*H*-cyclopenta[c] quinolin-4-amine (6a)**

The product was obtained in 88% yield as a brown powder; mp 187~189°C; IR(KBr) 3326, 2928, 1600, 1540 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 2.21 (q, *J*=7.6Hz, 2H), 2.84 (t, *J*=7.6Hz, 2H), 3.54 (t, *J*=7.6Hz, 2H), 3.86 (s, 3H), 4.02 (s, 3H), 6.46 (brs, 1H), 6.55 (d, *J*=8.5Hz, 1H), 6.86 (d, *J*=8.5Hz, 1H), 7.01 (m, 1H), 7.33 (m, 2H), 7.88 (d, *J*=7.7Hz, 2H).

***N*4-(3-Methoxyphenyl)-6,9-dimethoxy-2,3-dihydro-1*H*-cyclopenta [c]quinolin-4-amine (6b)**

The product was obtained in 60% yield as a brown powder; mp 153~155°C; IR (KBr) 3329, 2946, 1600, 1548 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 2.23 (q, *J*=7.6Hz, 2H), 2.85 (t, *J*=7.6Hz, 2H), 3.55 (t, *J*=7.6, 2H), 3.86 (s, 3H), 3.95 (s, 3H), 3.98 (s, 3H), 6.47 (brs, 1H), 6.56 (d, *J*=8.5Hz, 1H), 6.59 (s, 1H), 6.85 (d, *J*=8.5Hz, 1H), 6.97 (d, *J*=8.0Hz, 1H), 7.19 (m, 1H), 8.39 (m, 1H).

***N*4-(4-Methoxyphenyl)-6,9-dimethoxy-2,3-dihydro-1*H*-cyclopenta [c]quinolin-4-amine (6c)**

The product was obtained in 95% yield as a brown powder; mp 148~150°C; IR (KBr) 3322, 2924, 1603, 1509 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 2.18 (q, *J*=7.6, 2H), 2.77 (t, *J*=7.6, 2H), 3.51 (t, *J*=7.6, 2H), 3.81 (s, 3H), 3.85 (s, 3H), 4.00 (s, 3H), 6.36 (brs, 1H), 6.53 (d, *J*= 8.5Hz, 1H), 6,84 (d, *J*=8.5Hz, 1H), 6.90 (d, *J*=8.9Hz, 2H), 7.74 (d, *J*=8.9Hz, 2H).

***N*4-(4-Chlorophenyl)-6,9-dimethoxy-2,3-dihydro-1*H*-cyclopenta[c]quinolin-4-amine (6d)**

The product was obtained in 59% yield as a brown powder; mp 181~183°C; IR (KBr) 3339, 2925, 1538 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 2.23 (q, *J*=7.6Hz, 2H), 2.85 (t, *J*=7.6Hz, 2H), 3.54 (t, *J*=7.6Hz, 2H), 3.85 (s, 3H), 4.01 (s, 3H), 5.30 (brs, 1H), 6.56 (d, *J*=8.5, 1H), 6.86 (d, *J*=8.5Hz, 1H), 7.30 (d, *J*=8.8Hz, 2H), 7.88 (d, *J*=8.8Hz, 2H).

6,9-Dimethoxy-4-tetrahydro-1*H*-1-pyrrolyl-2,3-dihydro-1*H*-cyclopenta[c]quinoline (6e)

The product was obtained in 72 % yield as a yellow-brown powder; mp 149~151°C; IR (KBr) 2938, 1646, 1579 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 1.94 (m, 4H), 2.07 (q, *J*=7.6Hz, 2H), 3.16 (t, *J*=7.6Hz, 2H), 3.43 (t, *J*=7.6Hz, 2H), 3.78 (m, 4H), 3.83 (s, 3H), 3.98 (s, 3H), 6.43 (d, *J*=8.5Hz, 1H), 6.69 (d, *J*=8.5Hz, 1H).

4-(6,9-Dimethoxy-2,3-dihydro-1*H*-cyclopenta[c]quinolin-4-yl)morpholine (6f)

The product was obtained in 56 % yield as a cream colored powder; mp 115~117°C; IR (KBr) 2924, 2857, 1580 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 2.12 (q, *J*=7.5Hz, 2H), 2.94 (t, *J*=7.5Hz, 2H), 3.50 (t, *J*=7.5Hz, 2H), 3.54 (m, 4H), 3.86 (s, 3H), 3.88 (m, 4H), 3.99 (s, 3H), 6.55 (d, *J*=8.5Hz, 1H), 6.84 (d, *J*=8.5Hz, 1H).

6,9-Dimethoxy-4-piperidino-2,3-dihydro-1*H*-cyclopenta[c]quinoline (6g)

The product was obtained in 45% yield as a yellow-brown powder; mp 118~120°C; IR (KBr) 2921, 2822, 1579 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 1.69 (m, 6H), 2.09 (q, *J*=7.4Hz, 2H), 2.93 (t, *J*=7.4Hz, 2H), 3.46 (m, 4H), 3.50 (t, *J*=7.4Hz, 2H), 3.85 (s, 3H), 3.98 (s, 3H), 6.52 (d, *J*=8.5Hz, 1H), 6.81 (d, *J*=8.5Hz, 1H).

6,9-Dimethoxy-4-(4-methylpiperazino)-2,3-dihydro-1*H*-cyclopenta[c]quinoline (6h)

The product was obtained in 62 % yield as a cream colored powder; mp 141~143°C; IR (KBr) 2925, 2841, 1577 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 2.10 (q, *J*=7.4Hz, 2H), 2.36 (s, 3H), 2.59 (m, 4H), 2.94 (t, *J*=7.4Hz, 2H), 3.46 (t, *J*=7.4Hz, 2H), 3.59 (m, 4H), 3.85 (s, 3H), 3.99 (s, 3H), 6.53 (d, *J*=8.5Hz, 1H), 6.83 (d, *J*=8.5Hz, 1H).

***N*1-(6,9-dimethoxy-2,3-dihydro-1*H*-cyclopenta[c]quinolin-4-yl)-*N*2,*N*2-dimethyl-1,2-ethanediamine (6i)**

The product was obtained in 56 % yield as a cream colored powder; mp 117~119°C; IR(KBr) 3381, 2936, 2825, 1605, 1547 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 2.17 (q, *J*=7.6Hz, 2H), 2.28 (s, 6H), 2.58 (m, 2H), 2.76 (t, *J*=7.6Hz, 2H), 3.47 (t, *J*=7.6Hz, 2H), 3.73 (m, 2H), 3.84 (s, 3H), 3.97 (s, 3H), 5.10 (brs, 1H), 6.46 (d, *J*=8.5Hz, 1H), 6.80 (d, *J*=8.5Hz, 1H).

***N*1-(6,9-dimethoxy-2,3-dihydro-1*H*-cyclopenta[c]quinolin-4-yl)- *N*3,*N*3-diethyl-1,3-propanediamine (6j)**

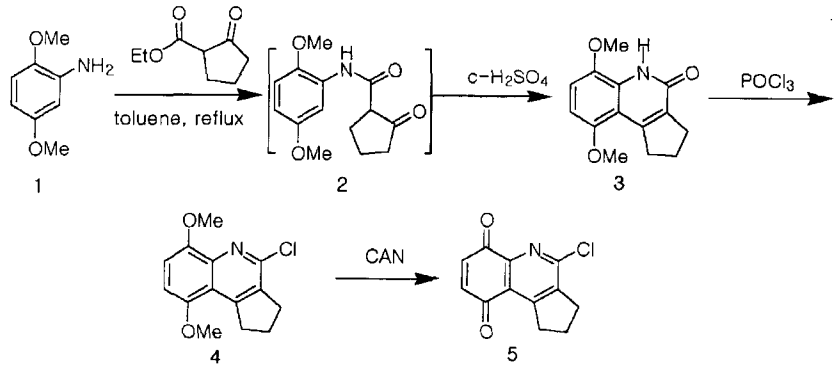
The product was obtained in 37 % yield as a cream colored powder; mp 121~123°C; IR (KBr) 3357, 2940, 2834, 1578 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 1.27~1.68 (m, 8H), 2.09 (q, *J*=7.4Hz, 2H), 2.58 (m, 6H), 2.94 (t, *J*=7.4Hz, 2H), 3.45 (m, 4H), 3.85 (s, 3H), 3.98 (s, 3H), 6.52 (d, *J*=8.5Hz, 1H), 6.81 (d, *J*=8.5Hz, 1H).

Cells

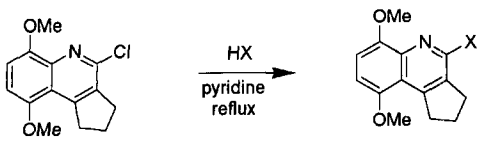
Four human cancer cell lines, HCT15, SK-OV-3, MD-MB-468 and T-47D were used in this study. SK-OV-3 and HCT15 were obtained from national cancer institute, U.S.A. MD-MB-468 and T-47D were purchased from American Type Culture Collection. These cells were maintained in Dulbecco's modified eagle media supplemented 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). Briefly, 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 h later, cells were fixed with 10% trichloroacetic acid (TCA) for overnight at 4°C. The TCA-treated cells were exten-



Scheme 1. Synthesis of 4-chloro-6,9-dimethoxy-2,3-dihydro-1*H*-cyclopenta[c]quinoline



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Compound No	HX	Yield(%)
6a	aniline	88
6b	*m* -anisidine	95
6c	*p* -anisidine	60
6d	*p* -chloroaniline	59
6e	$HN(CH_2CH_2)_2$	72
6f	$HN(CH_2CH_2)_2O$	56
6g	$HN(CH_2CH_2)_2CH_2$	45
6h	$HN(CH_2CH_2)_2NCH_3$	62
6i	$H_2NCH_2CH_2N(CH_3)_2$	56
6j	$H_2NCH_2CH_2CH_2N(CH_2CH_3)_2$	37

Scheme 2. Synthesis of 4-substituted cyclopenta[c]quinoline derivatives

sively 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 microplate reader. The absorbance value of day 0 was subtracted from the absorbance values of day 3.

RESULTS AND DISCUSSION

In order to synthesize cyclopenta[c]quinoline derivatives bearing 4-alkyl or arylaminosubstituent, 4-chloro-6, 9-dimethoxy-2,3-dihydro-1*H*-cyclopenta[c]-quinoline (**4**) was required. The synthesis of this intermediate was outlined in scheme 1. 6,9-Dimethoxy-2,3,4,5-tetrahydro-1*H*-cyclopenta[c]quinoline-4-one (**3**) was prepared using Knorr cyclization starting from dimethoxyaniline (**1**) and ethyl 2-oxocyclopentanecarboxylate (Kaslow *et al.*, 1946). Treatment of the compound **3** with $POCl_3$ at reflux afforded the compound **4**. Oxidative demethylation of **4** gave the compound **5** (Potts *et al.*, 1986).

The compound **4** was treated with various amines (aniline, *p*-anisidine, *m*-anisidine, *p*-chloroaniline, pyrrolidine, morpholine, piperidine, *N*-methylpiperazine, dimethylaminoethylamine, and diethylaminoethylamine) at reflux to give the compounds **6a**-j in 37 to 95% yield. (Scheme 2). The sutructure of the target compounds was assigned by spectroscopoic data.

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 cyclopenta[c]quinoline derivatives (**4**, **5**, **6a-j**) against human cancer cell lines originated from colon (HCT-15), ovarian (SK-OV-3), breast adenocarcinoma (MD-MB-468) and breast ductal carcinoma (T-47D) along with comparative data for doxorubicin are listed in Table I.

The compounds were generally less potent than doxorubicin. The tricyclic quinoline derivatives **6a-j** were more potent than the bicyclic quinoline derivatives (Lee

Table I. *In vitro* cytotoxic activity of 4-substituted cyclopenta[c]quinoline derivatives

Compound No	X	IC50 (μM)			
		HCT15	SK-OV-3	MD-MB-468	T-47D
4	-Cl	50	100	7	80
5	-Cl	21	30	30	30
6a	-aniline	50	>100	~100	>100
6b	-m-anisidine	21	40	13	12
6c	-p-anisidine	2.8	23	4	6
6d	-p-chloroaniline	12	90	20	40
6e	$-N(CH_2CH_2)_2$	3.1	20	6	21
6f	$-N(CH_2CH_2)_2O$	20	40	27	31
6g	$-N(CH_2CH_2)_2CH_2$	21	31	23	23
6h	$-N(CH_2CH_2)_2NCH_3$	12	40	22	32
6i	$-NH(CH_2)_2N(CH_3)_2$	4.4	24	5	11
6j	$-NH(CH_2)_3N(CH_2CH_3)_2$	21	50	30	20
Doxorubicin		0.08	0.05	0.005	0.02

[a]IC_{50}=concentration of compound (μM) 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: HCT15 (colon cancer cell), SK-OV-3 (ovarian cancer cell), MD-MB-468 (human breast adenocarcinoma), T-47D (human breast ductal carcinoma)

et al., 2000). There is no significant activity difference between quinoline **4** and quinolindione **5** as our previous results (Lee *et al.*, 2000). The compounds **6c** and **6e** bearing *p*-anisidine and pyrrolidine side chain were more potent than the others. This result suggests that additional ring system may be necessary for good activity. Work is in progress to design, synthesize, and evaluate additional compounds in this and related systems.

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