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SYNTHESIS AND EVALUATION OF QUINDOLINE AND ITS ANALOGUE AS POTENTIAL ANTICANCER AGENTS

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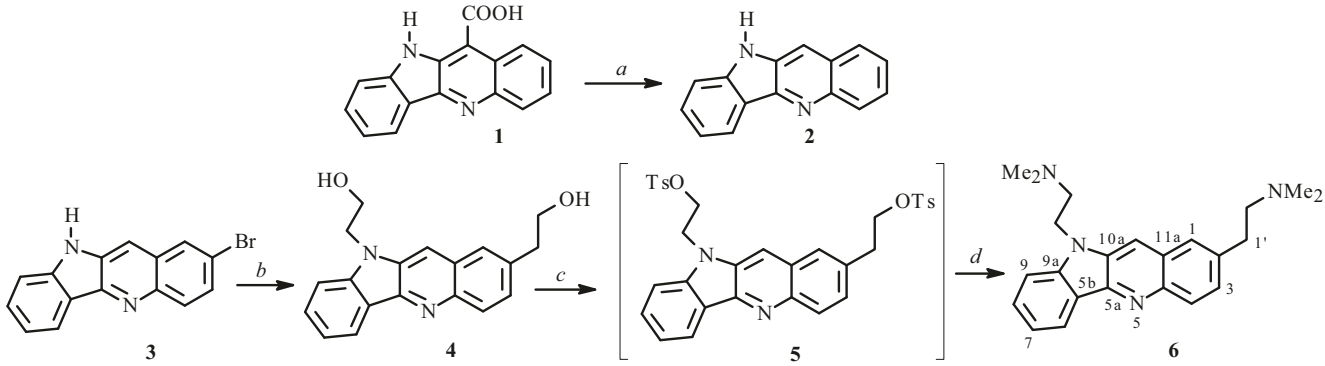
*Several derivatives of quindoline, 10*H*-(indolo[3,2-b]quinoline), alkaloids were prepared by the modification of the Pfitzinger quinoline reaction. The conversion of quindoline was 71% while that of another compound, 2,10-bis(dimethylaminoethyl)-indolo[3,2-b]quinoline, was 64%. In the evaluation of the cytotoxicities of the two compounds using five human ovarian cancer cell lines, namely SKOV-3, A2780, A2780R, CHI, and CHIR, quindoline gave minimum inhibitory concentration (IC_{50}) results of 66, 21.5, 24.5, 15.5, and 30 M, respectively whiles the more potent compound, 2,10-bis(dimethylaminoethyl)-indolo[3,2-b]quinoline, gave 6.3, 12.5, 10.5, 8.4, and 12.5 M, respectively. A third compound, 2-(3′-hydroxypropan-1′-yl)-10*H*-indolo[3,2-b]quinoline, was prepared by the Heck reaction in a yield of 70%.*

Keywords: quindoline, cytotoxicity, minimum inhibitory concentration, cancer.

The interesting pharmacology exhibited by quindoline compounds or indoloquinoline alkaloids, systematically called 10*H*-indolo[3,2-b]quinolines, ensures their popularity as targets for chemical synthesis and biological examination. These alkaloids and their analogues have been isolated from the plant *Cryptolepis sanguinolenta* (Lindl.) Schltr., a member of the Asclepiadaceae family and Periplocaceae subfamily [1]. In West Africa the root decoctions of the plant have been used in the treatment of a variety of illnesses including hepatitis, diabetes, malaria, and urinary and upper respiratory tract infections [2–4].

The method of synthesis of quindoline-11-carboxylic acid (**1**) is basically the Pfitzinger quinoline synthesis in which the *O*,*N*-indoxyl replaces the ketone [5]. The synthesis was that reported in the literature on the reaction between isatin and *O*,*N*-diacetylindoxyl to afford the quindoline-11-carboxylic acid (**1**), which was decarboxylated to yield quindoline (**2**) with overall yield of 71% [6]. No attempt was made to vary the synthetic procedure since the method was concise and the yield was very high.

It was noted that the synthetic procedure between *O*,*N*-diacetylindoxyl and 5-bromoisatin also gave rise to 2-bromoquindoline **3** (60% overall). Compound **3** was treated with BuLi in the cold, and ethylene oxide was added (Scheme 1).



a. Paraffin, heat, 200°C; *b*. BuLi, THF, –78°C, ethylene oxide; *c*. *p*-TsCl, 0°C, pyridine; *d*. Dimethylamine, 0°C

Scheme 1

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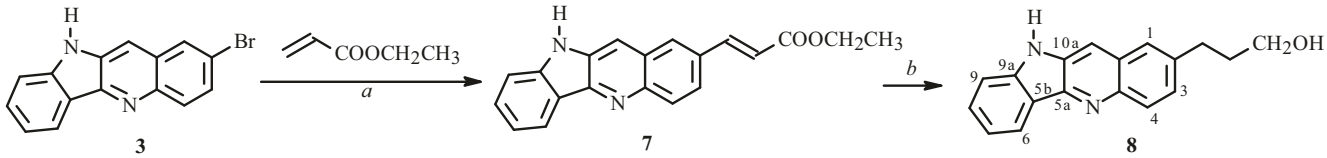
TABLE 1. Cytotoxicity (IC_{50}, μM) of **2** and **6** Compared to the Analogous 2,6-Dimethylamino Anthraquinone (2,6-AQ)

Compound	A2780	$A2780^R$	CHI	CHI^R	SKOV-3
2	21.5	24.5	15.5	30	66
8	12.5	10.5	8.4	12.5	6.3
2,6-AQ	2.6	N.d	1.8	N.d	2.9



a. $Pd(OAc)_2$, Ph_3P, Et_3N, DMF; *b*. $NaBH_4$, ethanol, 5 days

Scheme 2

The reaction afforded a solid, which was column chromatographed (silica gel, CH_2Cl_2, methanol) to yield dialcohol **4** after recrystallization from ethanol. Compound **4** melted at 217°C and gave a molecular ion peak at *m/z* 306.1366 in its mass spectrum. Elemental analysis confirmed the molecular formula of **4** as $C_{19}H_{18}N_2O_2$. The compound showed absorbance at 3200 cm^{-1} in the infrared spectrum and two singlet peaks.

Quindoline compounds are stable under Heck reaction conditions [7]. The reactions of 2-bromoquindoline (**3**) with ethyl acrylate catalyzed by palladium acetate yielded the desired 2-substituted quindoline, 2-(carboethoxyethenyl)-10*H*-indolo-[3,2- b]quinoline (**7**) in 56% (Scheme 2). Other unsaturated carbonyl compounds, such as acrylamide and *N*,*N*-dimethylacrylamide, all gave their desired Heck reaction products. Allyl alcohol, however, failed to give the desired product, which might be due to the more reactive hydroxyl functional group.

The reduction with sodium borohydride is usually associated with the conversion of aldehydes to alcohols. In this work the unsaturated ester functional group in compound **7** was successfully reduced with sodium borohydride in ethanol to yield the compound 2-(3′-hydroxypropan-1′-yl)-10*H*-indolo[3,2-b]quinoline (**8**) in 70% yield. The procedure is simple and can easily be replicated, but its long duration (5 days) makes it less attractive.

Elemental analysis confirmed the molecular formula of **8** as $C_{19}H_{16}N_2O$. An absorbance at 3200 cm^{-1} in the infrared spectrum and one proton singlet peak at 4.61 ppm in the ^1H NMR spectrum confirmed the presence of the hydroxyl functionality. The relative configuration of the quindoline analogue, 2-(3′-hydroxypropan-1′-yl)-10*H*-indolo[3,2-b]quinoline (**8**), was determined unambiguously by single crystal X-ray analysis.

Biological Activity. The cytotoxicities of quindoline **2** and 2,10-bis(dimethylaminoethyl)-indolo[3,2-b]quinoline (**6**) were evaluated using five human ovarian cancer cell lines: three parent lines SKOV-3, A2780, and CHI and two drug-resistant ones CHIR and A2780R. The minimum inhibitory concentrations (IC_{50}) values are shown in Table 1. The IC_{50} values were also compared to the analogous compound 2,6-dimethylaminoanthraquinone (2,6-AQ).

The results showed that quindoline **2** had moderate activity, although it had comparable activity against the resistant cell lines. The synthesized analogue, 2,10-bis(dimethylaminoethyl)-indolo[3,2-b]quinoline (**6**), showed more potent activity than quindoline **2** itself by a factor of 2. These results indicate that the analogues of quindoline are propitious as potential anticancer agents.

EXPERIMENTAL

General Experimental Procedure. Anhydrous/dry solvents were prepared as reported in the literature. Analytical TLC was performed on Merck silica gel 60 F254 precoated plates (250 mm thickness) and analyzed using UV light. Flash chromatography was performed on Merck silica gel 60 (230–400 mesh) under nitrogen pressure. IR spectra were recorded on a Shimadzu FTIR-8201 PC spectrophotometer. NMR was recorded on a Bruker AM 400 instrument for samples dissolved in DMSO-d_6 solution. ^1H and ^{13}C NMR were recorded at 400 and 100 MHz, respectively. NMR shifts are expressed in ppm (δ) downfield from TMS. Mass spectrometry was performed on a Kratos MS 50 spectrometer. Combustion microanalysis was performed at the Brunel University, Middlesex, England. Melting points are uncorrected. Compounds **1**, **2**, and **3** were prepared by the general literature methods [5].

Synthesis of 2,10-Bis(2-hydroxyethyl)-indolo-[3,2-b]quinoline (4). 2-Bromoquindoline (**3**, 3.3 g, 11.2 mmol) was dissolved in THF (100 mL) under nitrogen atmosphere. The solution was held at –78°C while 2.5 M BuLi solution in hexane (10 mL, 25 mmol) was added dropwise via a syringe to the solution over 10 min. The solution was stirred for a further 1 h. Ethylene oxide (1.3 g, 25 mmol) was bubbled through the solution, and stirring was continued for a further 1 h at –78°C and then at 0°C for another 2 h. The reaction mixture was poured carefully into a solution of saturated ammonium chloride (20 mL), and the upper organic layer was separated. The solvent was removed under reduced pressure to give a solid. Column chromatography (silica gel, DCM, methanol) of the solid followed by recrystallization from ethanol afforded (**4**, 2.1 g, 61%), mp 217–219°C, R_f 0.41 (EtOAc). IR (nujol mull, ν_{max}, cm^{-1}): 3200 (OH), 1600 (C=C), 1120, 1080, 1050, 880, 650. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.91 (2H, t, J = 6.6, H-2′), 3.73 (2H, t, J_{vic} = 5.1, 6.7, H-2″), 3.81 (2H, t, J_{vic} = 5.5, 6.2, H-1′), 4.5 (2H, t, J_{vic} = 5.5, 5.1, H-1″), 4.71 (1H, s, OH), 4.92 (1H, s, OH), 7.32 (1H, t, J = 8.3, H-8), 7.55 (1H, dd, J = 8.0, 2.0, H-3), 7.65 (1H, t, J = 8.3, H-7), 7.70 (1H, d, J = 8.3, H-6), 7.88 (1H, s, H-1), 8.12 (1H, d, J = 8.3, H-9), 8.33 (1H, s, H-11), 8.36 (1H, d, J = 8.0, H-4). ^{13}C NMR (100.4 MHz, DMSO-d_6, δ, ppm): 59.3 (C-2′), 62.1 (C-2″), 109.9 (C-1′), 111.5 (C-1″), 119.3 (C-9), 120.9 (C-11), 121.1 (C-7), 121.2 (C-5b), 126.4 (C-6), 126.6 (C-2), 126.7 (C-3), 128.2 (C-11a), 128.4 (C-1), 129 (C-4), 133 (C-8), 136.5 (C-10a), 142.3 (C-4a), 144.4(C-9a), 144.5 (a). EI-MS, *m/z*: 306 (50), 275 (100), 244 (35), 230 (25), 190 (5), 115 (5). [Found 306.1366 (M^+). Calcd for $C_{19}H_{18}N_2O_2$, 306.1364].

2,10-Bis(*p*-tosyloxyethyl)-indolo[3,2-b]quinoline (5). 2,10-Bis(hydroxyethyl)-indolo[3,2-b]quinoline (**4**, 750 mg, 2.5 mmol) and *p*-TsCl (1.9 g, 10 mmol) in dry pyridine (20 mL) were stirred at 0°C under nitrogen. The solution was kept in a refrigerator for 3 days. DCM (50 mL) and water (10 mL) were added and separated. The upper organic layer was extracted with water (3 × 10 mL) followed by 2 M hydrochloric acid (3 × 10 mL) and 5% sodium carbonate (3 × 10 mL) and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to give **5** (529 mg, 41%). EI-MS, *m/z*: 615 (15), 478 (75), 442 (10), 429 (15), 416 (5), 342 (25), 306 (5), 293 (100), 280 (5), 257 (55), 244 (30), 231 (20), 122 (10), 91 (15), 65 (5).

2,10-Bis(dimethylaminoethyl)-indolo[3,2-b]quinoline (6). 2,10-Bis(tosylethyl)-indolo[3,2b]-quinoline (**5**, 362 mg, 0.59 mmol) in dry DCM (20 mL) at –78°C was treated with an excess of dimethylamine, $(CH_3)_2NH$ (5 g, 5 mL, 0.1 mol). The reaction mixture was kept in the refrigerator for 3 days. The white crystals that separated were filtered off and the filtrate extracted with 5% NaOH (3 × 10 mL) followed by water (3 × 10 mL). The solution was dried over magnesium sulfate and evaporated under reduced pressure to give a solid, which was recrystallized from methanol to yield **6** (136 mg, 64%, mp 196–198°C, R_f 0.21 (EtOAc). IR (nujol mull, ν_{max}, cm^{-1}): 1600, 1120, 970, 900, 850, 750, 670. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 2.92 (12H, s, 2 × $N(CH_3)_2$), 3.11 (1H, m, H-2′), 3.31 (1H, m, H-2′), 3.42 (1H, m, H-1′), 4.69 (1H, m, H-1″), 7.31 (1H, m, H-7), 7.62–7.81 (3H, m, H-6, 8, 9), 8.01 (1H, d, $J_{4,3}$ = 6.7, H-4), 8.11–8.52 (3H, m, H-1, 11, 3). ^{13}C NMR (100.4 MHz, DMSO-d_6, δ, ppm): 27.2 (CH_3), 28.7 (CH_3), 59.2 (C-2′), 62.2 (C-2″), 109.1 (C-1′), 111.7 (C-1″), 119.4 (C-9), 120.9 (C-11), 121.2 (C-7), 121.3 (C5b), 126.4 (C-6), 126.6 (C-2), 126.7 (C-3), 128.3 (C-11a), 128.5 (C-1), 129.4 (C-4), 133.8 (C-8), 136.6 (C-10a), 142.3 (C-4a), 144.4 (C-9a), 144.5a). EI-MS, *m/z*: 360 (35), 290 (100), 232 (25), 58 (80). [Found 360.2372 (M^+). Calcd for $C_{23}H_{28}N_4$, 360.2308].

2-(Carboethoxyethenyl)-10*H*-indolo[3,2-b]quinoline (7). A mixture of 2-bromoquindoline (**3**, 0.5 g, 1.7 mmol), ethyl acrylate (341 mg, 3.4 mmol), palladium acetate (76.4 mg, 0.34 mmol), triphenylphosphine (178.5 mg, 0.68 mmol), triethylamine (5 mL), and DMF (10 mL) was heated at 100°C under nitrogen with stirring overnight. The mixture was cooled to room temperature. Water (10 mL) and hexane (20 mL) were added and the mixture filtered. The solid obtained was recrystallized from alcohol to give **7** (300 mg, 56%, mp 249–251°C, R_f 0.41 (EtOAc). IR (nujol mull, ν_{max}, cm^{-1}): 3350 (N-H), 1690 (C=O), 1620 (C=C), 1300, 1190, 980, 820, 750. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.31 (3H, t, J_o = 6.6, 7.3, H-5′), 4.32 (2H, q, J_o = 6.9, 6.9, 6.3, H-4′), 6.79 (1H, d, J_o = 16.1, H-2′), 7.31 (1H, t, $J_{7,6}$ = 7.3, 6.9, H-7), 7.59 (2H, m, H-8, 9), 7.82 (1H, d, J_o = 15.7, H-1′), 8.01 (1H, d, $J_{3,4}$ = 8.7, H-3), 8.12 (1H, d, $J_{4,3}$ = 8.7, H-4), 8.21 (1H, s, H-1), 8.29 (1H, d, $J_{6,7}$ = 7.7, H-6), 8.42 (1H, s, H-11), 11.51 (1H, s, NH). CI-MS *m/z*: 317 (100), 297 (5), 271 (15), 243 (10), 218 (5), 180 (5), 130 (15), 85 (30), 61 (10). [Found 317.1293 (M + 1)$^+$. Calcd for $C_{20}H_{16}N_2O_2$, 316.1208].

2-(3′-Hydroxypropan-1′-yl)-10*H*-indolo[3,2-b]quinoline (8). To a stirred solution of 2-(carboethoxyethenyl)-10*H*-indolo[3,2-b]quinoline (**7**, 0.8 g, 2.5 mmol) in absolute ethanol (100 mL) was added sodium borohydride $NaBH_4$ (1 g, 27 mmol) and the whole stirred at room temperature for 5 days. It was filtered and the solvent removed *in vacuo* to give a solid, which was purified by column chromatography (silica gel, ethyl acetate, methanol) to afford **8** (75 mg, 70%), mp 240–243°C, R_f 0.35 (EtOAc). IR (nujol mull, ν_{max}, cm^{-1}): 3210 (OH), 1600 (C=C), 1310, 1210, 1050, 890, 810, 750 (aromatic). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.81 (2H, t, J = 7.3, 7.1, H-1′), 2.83 (2H, t, J = 7.7, 7.3, H-2′), 3.52 (2H, d, J = 5.1, H-3′), 4.61 (1H, s, OH), 7.31 (1H, t, J = 7.3, 7.1, H-7), 7.49 (3H, m, H-6, 8, 9), 7.91 (1H, s, H-1), 8.09 (1H, t, J = 8.5, H-3), 8.21 (1H, s, H-11), 8.31 (1H, d, J = 7.7, H-4), 11.41 (1H, s, NH). ^{13}C NMR (100.4 MHz, DMSO-d_6, δ, ppm): 31.5 (C-2′), 33.8 (C-1′),

59.9 (C-3), 112.5 (C-9), 115 (C-11), 117 (C-7), 120.6 (C-5b), 120.9 (C-6), 121.7 (C-2), 122.9 (C-3), 126.1 (C-11a), 126.3 (C-1), 131.7 (C-4), 132.5 (C-8), 133.1 (C-10a), 133.3 (C-4a), 140.7 (C-9a), 144.9 (C-5a). CI-MS, *m/z*: 277 (100), 245 (20), 231 (65), 205 (15), 140 (5), 115 (10). [Found 277.1348 $(M + 1)^+$. Calcd for $C_{18}H_{16}N_2O$, 276.1259].

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