Synthesis and Antitumor Activity of Fused Quinoline Derivatives. II.[1] Novel 4- and 7-Hydroxyindolo-[3,2-*b*]quinolines

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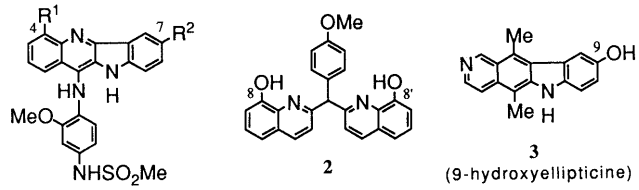
Novel indolo[3,2-*b*]quinoline derivatives (1c—f), which carried a methoxy or a hydroxy group at the 4- or 7-position of the lead compound 1a, were prepared and their antitumor activities against P388 in mice were examined. Except for the 4-hydroxy derivative (1d), these showed remarkably potent activity. Among these compounds, the 7-hydroxy derivative (1f) was the most potent one (optimal dose = 50 mg/kg, the median survival time of treated group/control group (T/C) > 330%, cure = 5/6).

Keywords indolo[3,2-*b*]quinoline; antitumor activity; P388 leukemia; synthesis; molecular modification; hydroxy group; methoxy group

We have previously reported the synthesis of novel indolo[3,2-*b*]quinoline derivatives[1,2] (**1a**, Fig. 1) having potent antitumor activity, and intercalative properties with deoxyribonucleic acid (DNA). We have also reported the antitumor activity of 7-glycosylaminoindolo[3,2-*b*]quinoline derivatives[3] (**1b**). This paper describes the syntheses of 4-methoxy- (**1c**), 4-hydroxy- (**1d**), 7-methoxy- (**1e**), and 7-hydroxy- (**1f**) indolo[3,2-*b*]quinoline derivatives.

The 4-hydroxy derivative (**1d**) was designed based on the information that the bis(8-hydroxyquinoline) derivative (**2**) exhibits potent antitumor activity due to its ability to form a chelate with the ferous ion necessary for ribonucleotide reductase (Fig. 1).[4] Compound **1d** contains a 8-hydroxyquinoline part and was expected to have an additional property similar to that of **2**. It seemed resonable to assume that this property of **1d** might influence its antitumor activity. 7-Hydroxy derivative (**1f**) was also designed based on the information that the ellipticine congener (**3**, Fig. 1) having a hydroxy group at 9-position shows more potent antitumor activity than ellipticine itself.[5] A mechanism of antitumor activity of ellipticine analogue was generally considered as follows. The hydroxyindole moiety of DNA-intercalative **3** is easily oxidized *in vivo* to afford the corresponding quinone imine type of metabolite, which attacks nucleophilic components of an enzyme.[5] We also synthesized the 7-hydroxy congener **1f** with the expectation of a similar additional effect as that of **3**.



1a: $R^1 = R^2 = H$
1b: $R^1 = H$, R^2 = NH-glycosyl
1c: R^1 = OMe, $R^2 = H$
1d: R^1 = OH, $R^2 = H$
1e: $R^1 = H$, R^2 = OMe
1f: $R^1 = H$, R^2 = OH

Fig. 1

The 4-methoxy- (**1c**) and 4-hydroxy- (**1d**) indolo[3,2-*b*]quinoline derivatives were prepared from 3-methoxy-2-[(*N*-phenylamino)acetamido]benzoic acid (**7**) *via* the key intermediate 11-chloro-4-methoxyindolo[3,2-*b*]quinoline (**9c**) (Chart 1). The intermediate (**7**) was afforded by the chloroacetylation of 2-amino-3-methoxybenzoic acid (**4**)[6] followed amination with aniline (**6a**). The intermediate (**8**) was prepared through the intramolecular condensation of **7** by heating with polyphosphoric acid (PPA). Treatment of **8** with phosphorus oxychloride ($POCl_3$) gave **9c**. The demethylation of **9c** by heating with 47% hydrobromic acid gave 11-chloro-4-hydroxyindolo[3,2-*b*]quinoline de-

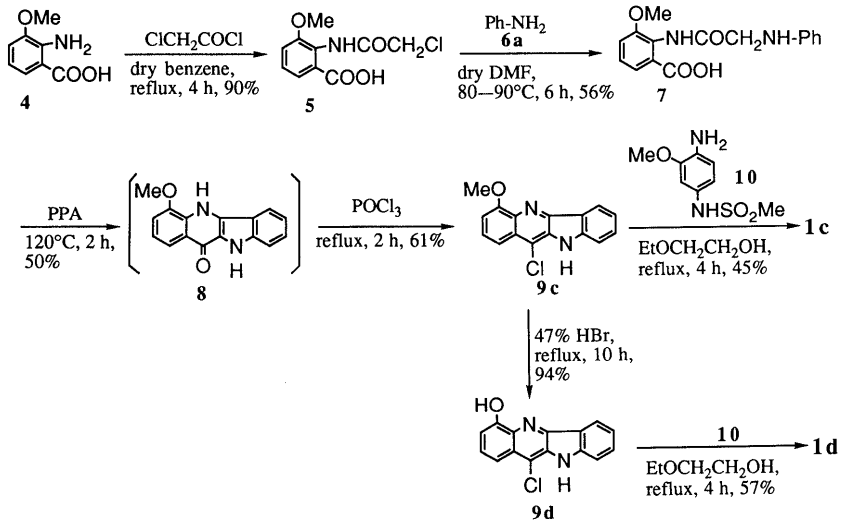


Chart 1

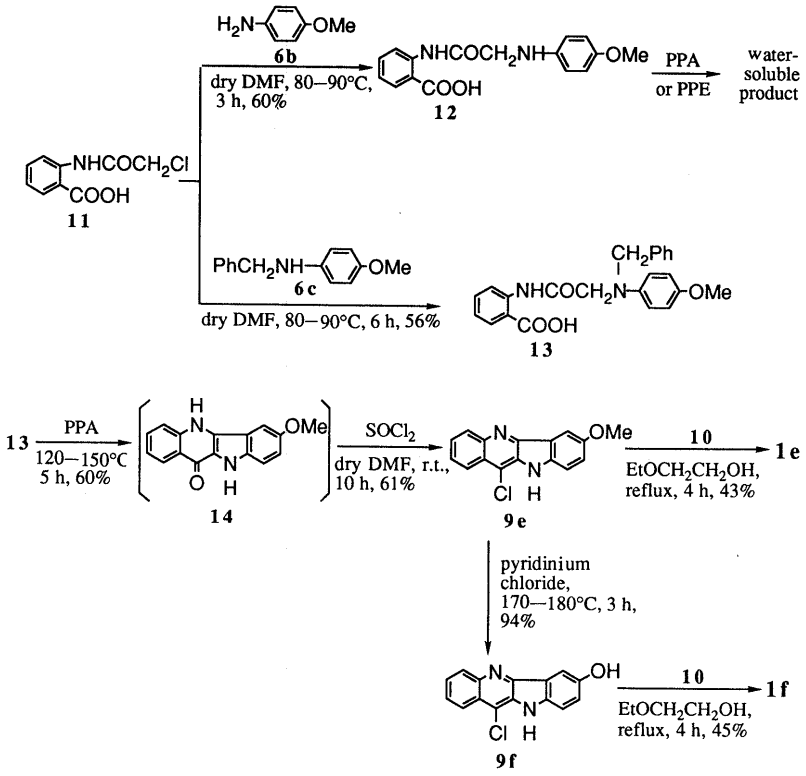
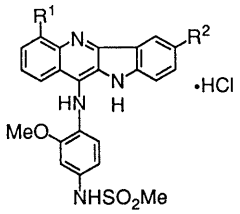


Chart 2

TABLE I. Antitumor Activity of Indolo[3,2-*b*]quinolines



1a—f

Compound			Antitumor act. P388 in mice		
No.	R^1	R^2	Dose (mg/kg)[a]	T/C (%)[b]	Cure[c]
1a	H	H	50	68	
			25	111	
			12.5	203	2/6
			6.25	300	3/6
			3.13	177	
1c	OMe	H	50	>319	3/6
			25	255	2/6
			12.5	213	
1d	OH	H	50	149	
			25	157	
			12.5	142	
1e	H	OMe	50	>330	3/6
			25	231	1/6
			12.5	187	
1f	H	OH	50	>330	5/6
			25	264	1/6
			12.5	198	
Amsacrine[7]			40	223	
			20	198	
			10	174	

a) The dose listed was given i.p. once a day on days 1 and 5. *b*) The median survival time of treated group/control group, T/C > 120%, active. *c*) The cure rates were observed at day 30.

rivative (**9d**). Amination of **9c** and **9d** with *N*-(4-amino-2-methoxyphenyl)methanesulfonamide hydrochloride (**10**)[7] successfully afforded **1c** and **1d**, respectively.

In the preparation of **1e** and **1f**, the key intermediate, 7-methoxy-5*H*,10*H*-indolo[3,2-*b*]quinolin-11-one (**14**) could not be obtained by the same procedure for the synthesis of **8**. Namely, treatment of 2-{[*N*-(4-methoxyphenyl)amino]acetamido}benzoic acid (**12**) with PPA or polyphosphoric acid ethyl ester (PPE) gave only water-soluble inseparable products. Therefore, *N*-protected congeners of **12** introduced by an acetyl, tosyl, benzyl, or 3,4-dimethoxybenzyl group to the nitrogen atom of aniline moiety (**13**) were synthesized and were attempted to give a corresponding derivative of **14** by the same procedure for the synthesis of **8**. Among these examinations, only the *N*-benzyl derivative **13** afforded the desired intermediate **14** with loss of the benzyl group. Other congeners gave but water-soluble inseparable products. Compounds **1e** and **1f** were prepared from **9e** according to the similar method for the preparation of **1c** and **1d**, respectively.

The antitumor activity of these compounds (**1c—1f**) were tested against leukemia P388 in mice (Table I). The 4-methoxy (**1c**) and 7-methoxy (**1e**) derivatives were found to have equally high potency and significant cure rate. On the other hand, in the case of hydroxy derivatives, the 7-hydroxy derivative (**1f**) showed excellent activity and the highest cure rate in this series of compounds. However, the 4-hydroxy derivative (**1d**) was extremely less active than the 7-hydroxy derivative (**1f**).

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra were taken on a Hitachi R-24 spectrometer at 60 MHz with Me_4Si as an internal standard. Fast atom bombardment

mass spectra (FAB-MS) were recorded on a VG-70SE spectrometer and infrared (IR) absorption spectra on a JASCO A-102 spectrometer.

2-Chloroacetamido-3-methoxybenzoic Acid (5) Chloroacetyl chloride (0.4 ml, 20 mmol) was added dropwise to a solution of 2-amino-3-methoxybenzoic acid (**4**; 3.50 g, 20 mmol)[6] in dry benzene (40 ml) at room temperature and the reaction mixture was heated at reflux for 4 h. The resulting precipitates were collected and recrystallized from a mixture of hexane and benzene to give 4.80 g (90%) of **5** as yellow crystals. mp 131—132 °C. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ: 3.83 (3H, s, OCH_3), 4.20 (2H, s, CH_2Cl), 6.97—7.60 (3H, m), 9.33 (1H, br, NH), 10.41 (1H, br, COOH). *Anal.* Calcd for $C_{10}H_{10}ClNO_4$: C, 49.30; H, 4.14; N, 5.75. Found: C, 49.19; H, 4.05; N, 5.48.

3-Methoxy-2-[(*N*-phenylamino)acetamido]benzoic Acid (7) A mixture of **5** (4.5 g, 19 mmol), distilled aniline (**6a**; 6 ml, 50 mmol), and dry dimethyl formamide (DMF) (6 ml) was heated at 80—90 °C for 4 h. The reaction mixture was poured into ice water and then basified with a 10% KOH solution and extracted with CH_2Cl_2. The aqueous layer was neutralized with a 10% HCl solution and the resulting precipitates were collected to give 3.1 g (56%) of **7** as yellow crystals. mp 125—127 °C. IR (Nujol): 3300, 1680 cm^{-1}. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ: 3.67 (3H, s, OCH_3), 3.76 (2H, s, CH_2), 6.43—6.74 (3H, m), 6.85—7.44 (5H, m), 9.10 (1H, br, NH). *Anal.* Calcd for $C_{16}H_{16}N_2O_4$: C, 63.99; H, 5.37; N, 9.33. Found: C, 63.93; H, 5.19; N, 9.09.

11-Chloro-4-methoxy-10*H*-indolo[3,2-*b*]quinoline (9c) A mixture of **7** (1.50 g, 5.00 mmol) and PPA (50 g) was heated with mechanical stirring at 120—130 °C for 2 h. The reaction mixture was poured into ice water and basified with a saturated $KHCO_3$ solution. The resulting precipitates were collected and then washed with water and dried to give 0.72 g (50%) of 4-methoxy-5*H*,10*H*-indolo[3,2-*b*]quinolin-11-one (**8**).

A mixture of **8** (0.72 g, 2.45 mmol) and $POCl_3$ (7 ml) was heated at reflux for 2 h. The excess $POCl_3$ was removed and then the residue was basified with a saturated $KHCO_3$ solution and extracted with $CHCl_3$. The organic layer was washed with a saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent gave 350 mg (61%) of **9c**. mp 220—224 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.03 (3H, s, OCH_3), 6.75—7.93 (6H, m), 8.45 (1H, d, J=8 Hz), 11.68 (1H, br, NH). FAB-MS (positive ion mode) m/z: 285 [(M+1)$^+$+2], 283 [(M+1)$^+$]. *Anal.* Calcd for $C_{16}H_{11}ClN_2O$: C, 67.97; H, 3.92; N, 9.91. Found: C, 67.99; H, 3.95; N, 9.95.

11-Chloro-4-hydroxy-10*H*-indolo[3,2-*b*]quinoline (9d) A mixture of **9c** (200 mg, 0.71 mmol) and a 47% hydrobromic acid aqueous solution (10 ml) was heated at reflux for 10 h and evaporated to dryness under reduced pressure. The residual solid was taken up in boiling water and basified with a saturated $KHCO_3$ solution. The precipitates were collected and recrystallized from a mixture of CH_2Cl_2 and MeOH to give 180 mg (94%) of **9d**. mp 181—183 °C (dec.). IR (Nujol): 3400, 3340 cm^{-1}. ^{1}H-NMR (DMSO-d_6 + $CDCl_3$) δ: 7.00—7.87 (6H, m), 8.42 (1H, d, J=8 Hz), 9.20 (1H, br, OH), 11.50 (1H, br, NH). FAB-MS (positive ion mode) m/z: 271 [(M+1)$^+$+2], 269 [(M+1)$^+$]. *Anal.* Calcd for $C_{15}H_9ClN_2O$: C, 67.05; H, 3.38; N, 10.43. Found: C, 67.01; H, 3.39; N, 10.64.

***N*-[4-*N*-(4-Methoxy-10*H*-indolo[3,2-*b*]quinolin-11-yl)amino-3-methoxyphenyl]methanesulfonamide (1c) Hydrochloride (General Procedure)** A mixture of **9c** (480 mg, 1.70 mmol) and 4-(methylsulfonyl)amino-2-methoxyaniline (**10**; 430 mg, 1.87 mmol) was heated at reflux in 2-ethoxyethanol (20 ml) for 4 h. The resulting precipitates were collected and recrystallized from MeOH to give 410 mg (45%) of the hydrochloride of **1c** as yellow crystals. Free base: mp 223—225 °C (dec.). ^{1}H-NMR (DMSO-d_6 + $CDCl_3$) δ: 2.94 (3H, s, SO_2CH_3), 3.89, 4.08 (6H, each s, OCH_3 × 2), 6.25—6.83 (2H, m), 6.96—7.84 (8H, m), 8.54 (1H, d, J=8 Hz). FAB-MS (positive ion mode) m/z: 463 [(M+1)$^+$]. *Anal.* Calcd for $C_{24}H_{22}N_4O_4S$: C, 62.32; H, 4.80; N, 12.11. Found: C, 62.68; H, 4.57; N, 12.32. Compounds **1d—f** were prepared similarly from **9d—f** and **10**.

***N*-[4-*N*-(4-Hydroxy-10*H*-indolo[3,2-*b*]quinolin-11-yl)amino-3-methoxyphenyl]methanesulfonamide (1d)** Free base: mp 178—180 °C (dec.). ^{1}H-NMR (DMSO-d_6 + D_2O) δ: 2.90 (3H, s, SO_2CH_3), 3.92 (3H, s, OCH_3), 6.35—6.79 (2H, m), 6.85—7.89 (8H, m), 8.37 (1H, d, J=8 Hz). FAB-MS (positive ion mode) m/z: 448 (M$^+$). *Anal.* Calcd for $C_{23}H_{20}N_4O_4S$: C, 61.59; H, 4.49; N, 12.49. Found: C, 61.46; H, 4.57; N, 12.37.

***N*-[4-*N*-(7-Methoxy-10*H*-indolo[3,2-*b*]quinolin-11-yl)amino-3-methoxyphenyl]methanesulfonamide (1e)** Free base: mp 223—225 °C. IR (Nujol): 3460, 3350 cm^{-1}. ^{1}H-NMR (DMSO-d_6 + $CDCl_3$) δ: 2.91 (3H, s, SO_2CH_3), 3.96, 3.99 (6H, s, OCH_3 × 2), 6.37 (1H, d, J=8 Hz), 6.76 (1H, dd, J=8, 2 Hz), 7.14 (1H, d, J=2 Hz), 7.22—7.70 (5H, m), 7.77—8.28 (2H, m), 8.45 (1H, br, NH), 9.27 (1H, br, NH). FAB-MS (positive ion mode) m/z: 463 [(M+1)$^+$]. *Anal.* Calcd for $C_{24}H_{22}N_4O_4S$: C, 62.32; H, 4.80; N, 12.11. Found: C, 62.32; H, 4.57; N, 11.87.

***N*-[4-*N*-(7-Hydroxy-10*H*-indolo[3,2-*b*]quinolin-11-yl)amino-3-methoxyphenyl]methanesulfonamide (1f)** Free base: mp 178—180 °C (dec.). ^{1}H-NMR (DMSO-d_6 + D_2O) δ: 2.58 (3H, s, SO_2CH_3), 3.89 (3H, s, OCH_3), 6.12 (1H, d, J=8 Hz), 6.49 (1H, dd, J=8, 2 Hz), 6.90 (1H, d, J=2 Hz), 7.62—7.91 (2H, m), 8.20 (1H, br, NH), 9.01 (1H, br, NH). FAB-MS (positive ion mode) m/z: 448 (M$^+$). *Anal.* Calcd for $C_{23}H_{20}N_4O_4S$: C, 61.59; H, 4.50; N, 12.49. Found: C, 63.12; H, 4.32; N, 12.46.

2-{[*N*-(4-Methoxyphenyl)amino]acetamido}benzoic Acid (12) A mixture of 2-chloroacetamidobenzoic acid (**11**, 2.13 g, 10 mmol), *p*-ansidine (**6b**; 2.50 g, 20 mmol), and dry DMF (6 ml) was heated at 90 °C for 4 h. The reaction mixture was poured into ice water and then basified with a 10% KOH solution and extracted with CH_2Cl_2. The aqueous layer was neutralized with a 10% HCl solution. The resulting precipitates were collected and recrystallized from a mixture of $CHCl_3$ and MeOH to give 2.89 g (60%) of **12** as yellow crystals. mp 176—178 °C ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ:3.67 (3H, s, OCH_3), 3.79 (2H, s, CH_2), 6.12 (2H, br, NH × 2), 6.68 (4H, s), 6.88—7.27 (1H, m), 7.36—7.73 (1H, m), 7.98 (1H, dd, J=8, 2 Hz), 8.80 (1H, dd, J=8, 2 Hz), 12.15 (1H, br, COOH).

2-{[*N*-Benzyl-*N*-(4-methoxyphenyl)amino]acetamido}benzic Acid (13) A mixture of 2-chloroacetamidobenzoic acid (**11**, 5.33 g, 25 mmol), *N*-benzyl-*p*-ansidine (**6c**; 6.50 g, 50 mmol), and dry DMF (6 ml) was heated at 100 °C for 12 h. The reaction mixture was poured into ice water and then basified with a 10% KOH solution and extracted with CH_2Cl_2. The aqueous layer was neutralized with a 10% HCl solution. The resulting precipitates were collected and recrystallized from a mixture of $CHCl_3$ and MeOH to give 4.8 g (56%) of **13** as yellow crystals. mp 153—155 °C. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6) δ: 3.67 (3H, s, OCH_3), 4.12 (2H, s, $COCH_2$), 4.76 (2H, s, Ar–CH_2), 6.76 (4H, s), 6.98—7.68 (7H, m), 7.87—8.26 (1H, m), 8.50—8.86 (1H, m). *Anal.* Calcd for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.68; N, 7.17. Found: C, 70.77; H, 5.44; N, 7.04.

11-Chloro-7-methoxy-10*H*-indolo[3,2-*b*]quinoline (9e) A mixture of **6b** (1.50 g, 2.50 mmol) and PPA (50 g) was heated with mechanical stirring at 120—150 °C for 5 h. The reaction mixture was poured into ice water and basified with a saturated $KHCO_3$ solution. The resulting precipitates were collected and then washed with water and dried to give 0.72 g (50%) of 7-methoxy-5*H*,10*H*-indolo[3,2-*b*]quinolin-11-one (**14**).

A mixture of **14** (0.72 g, 2.45 mmol), $SOCl_2$ (2 ml), and dry DMF (8 ml) was stirred at room temperature for 10 h. The excess $SOCl_2$ was removed and the reaction mixture was poured into ice water and then basified with a saturated $KHCO_3$ solution and extracted with $CHCl_3$. The organic layer was washed with a saturated NaCl solution and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on silica gel (AcOEt : hexane = 1 : 4) to give 200 mg (61%) of **9e**. mp 220—224 °C. ^{1}H-NMR (DMSO-d_6 + D_2O) δ: 3.88 (3H, s, OCH_3), 7.36—7.79 (5H, m), 7.94—8.42 (2H, m). FAB-MS (positive ion mode) m/z: 285 [(M+1)$^+$+2], 283 [(M+1)$^+$]. *Anal.* Calcd for $C_{16}H_{11}ClN_2O$: C, 69.99; H, 3.92; N, 9.91. Found: C, 69.97; H, 3.55; N, 9.65.

11-Chloro-7-hydroxy-10*H*-indolo[3,2-*b*]quinoline (9f) A mixture of **9e** (200 mg, 0.71 mmol) and dry pyridinium chloride (1.50 g) was heated at 170—180 °C for 3 h. The reaction mixture was poured into ice water. The resulting precipitates were collected and recrystallized from a mixture of CH_2Cl_2 and MeOH to give 180 mg (94%) of **9e** as yellow crystals. mp 190—192 °C (dec.). IR (Nujol): 3400 cm^{-1}. ^{1}H-NMR (DMSO-d_6 + $CDCl_3$) δ: 7.17—7.56 (5H, m), 7.74—8.23 (2H, m). FAB-MS (positive ion mode) m/z: 271 [(M+1)$^+$+2], 269 [(M+1)$^+$]. *Anal.* Calcd for $C_{15}H_9ClN_2O$: C, 67.05; H, 3.38; N, 10.43. Found: C, 67.24; H, 3.26; N, 10.43.

References and Notes

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