

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).

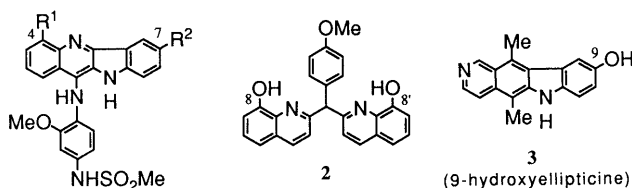
Keywords indolo[3,2-*b*]quinoline; antitumor activity; P388 leukemia; synthesis; molecular modification; hydroxy group; methoxy group

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 derivatives³⁾ (**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 ferrous 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 reasonable 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**.

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 (POCl₃) gave **9c**. The demethylation of **9c** by heating with 47% hydrobromic acid gave 11-chloro-4-hydroxyindolo[3,2-*b*]quinoline de-



- 1a:** R¹ = R² = H
1b: R¹ = H, R² = NH-glycosyl
1c: R¹ = OMe, R² = H
1d: R¹ = OH, R² = H
1e: R¹ = H, R² = OMe
1f: R¹ = H, R² = OH

Fig. 1

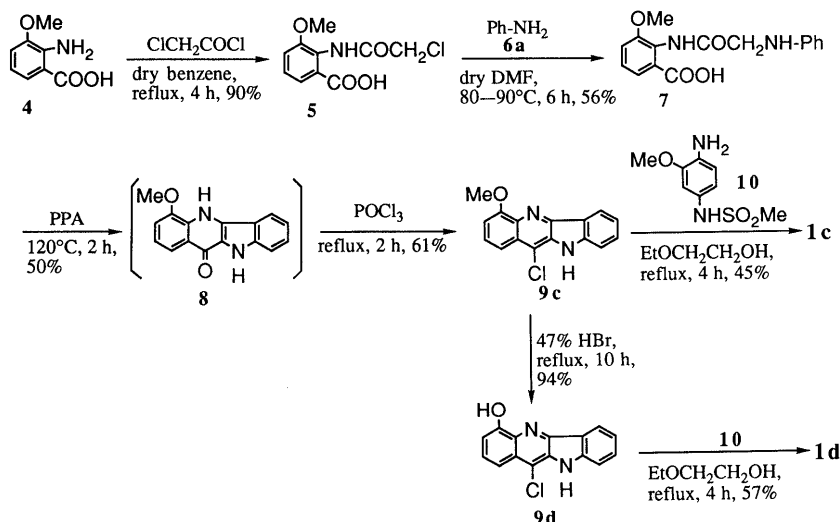


Chart 1

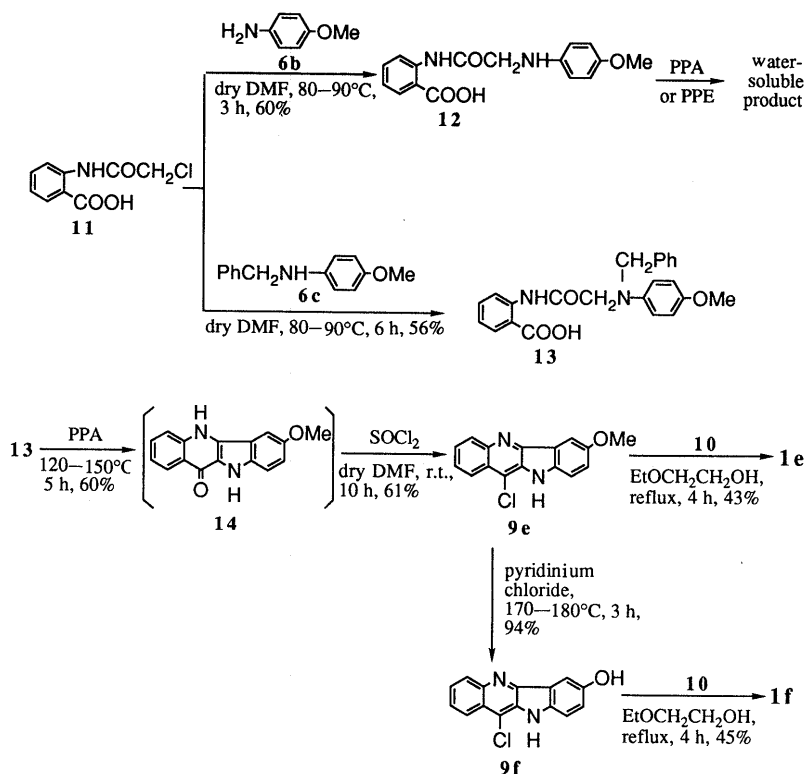
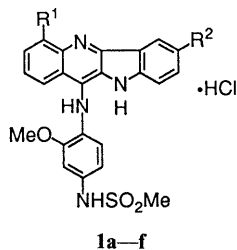


Chart 2

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

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. ¹H-nuclear magnetic resonance (¹H-NMR) spectra were taken on a Hitachi R-24 spectrometer at 60 MHz with Me₄Si 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. ¹H-NMR (CDCl₃+DMSO-*d*₆) δ: 3.83 (3H, s, OCH₃), 4.20 (2H, s, CH₂Cl), 6.97–7.60 (3H, m), 9.33 (1H, br, NH), 10.41 (1H, br, COOH). *Anal.* Calcd for C₁₀H₁₀ClNO₄: 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 CH₂Cl₂. 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. ¹H-NMR (CDCl₃+DMSO-*d*₆) δ: 3.67 (3H, s, OCH₃), 3.76 (2H, s, CH₂), 6.43–6.74 (3H, m), 6.85–7.44 (5H, m), 9.10 (1H, br, NH). *Anal.* Calcd for C₁₆H₁₆N₂O₄: C, 63.99; H, 5.37; N, 9.33. Found: C, 63.93; H, 5.19; N, 9.09.

11-Chloro-4-methoxy-10H-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 KHCO₃ 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 POCl₃ (7 ml) was heated at reflux for 2 h. The excess POCl₃ was removed and then the residue was basified with a saturated KHCO₃ solution and extracted with CHCl₃. The organic layer was washed with a saturated NaCl solution and dried over MgSO₄. Removal of the solvent gave 350 mg (61%) of **9c**. mp 220–224°C. ¹H-NMR (DMSO-*d*₆) δ: 4.03 (3H, s, OCH₃), 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 C₁₆H₁₁ClN₂O: C, 67.97; H, 3.92; N, 9.91. Found: C, 67.99; H, 3.95; N, 9.95.

11-Chloro-4-hydroxy-10H-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 KHCO₃ solution. The precipitates were collected and recrystallized from a mixture of CH₂Cl₂ and MeOH to give 180 mg (94%) of **9d**. mp 181–183°C (dec.). IR (Nujol): 3400, 3340 cm⁻¹. ¹H-NMR (DMSO-*d*₆+CDCl₃) 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 C₁₅H₉ClN₂O: 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.). ¹H-NMR (DMSO-*d*₆+CDCl₃) δ: 2.94 (3H, s, SO₂CH₃), 3.89, 4.08 (6H, each s, OCH₃ × 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 C₂₄H₂₂N₄O₄S: 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.). ¹H-NMR (DMSO-*d*₆+D₂O) δ: 2.90 (3H, s, SO₂CH₃), 3.92 (3H, s, OCH₃), 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 C₂₃H₂₀N₄O₄S: 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. ¹H-NMR (DMSO-*d*₆+CDCl₃) δ: 2.91 (3H, s, SO₂CH₃), 3.96, 3.99 (6H, s, OCH₃ × 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 C₂₄H₂₂N₄O₄S: 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.). ¹H-NMR (DMSO-*d*₆+D₂O) δ: 2.58 (3H, s, SO₂CH₃), 3.89 (3H, s, OCH₃), 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 C₂₃H₂₀N₄O₄S: 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 CH₂Cl₂. The aqueous layer was neutralized with a 10% HCl solution. The resulting precipitates were collected and recrystallized from a mixture of CHCl₃ and MeOH to give 2.89 g (60%) of **12** as yellow crystals. mp 176–178°C. ¹H-NMR (CDCl₃+DMSO-*d*₆) δ: 3.67 (3H, s, OCH₃), 3.79 (2H, s, CH₂), 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]benzoic 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 CH₂Cl₂. The aqueous layer was neutralized with a 10% HCl solution. The resulting precipitates were collected and recrystallized from a mixture of CHCl₃ and MeOH to give 4.8 g (56%) of **13** as yellow crystals. mp 153–155°C. ¹H-NMR (CDCl₃+DMSO-*d*₆) δ: 3.67 (3H, s, OCH₃), 4.12 (2H, s, COCH₂), 4.76 (2H, s, Ar-CH₂), 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 C₂₃H₂₂N₂O₄: C, 70.75; H, 5.68; N, 7.17. Found: C, 70.77; H, 5.44; N, 7.04.

11-Chloro-7-methoxy-10H-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 KHCO₃ 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), SOCl₂ (2 ml), and dry DMF (8 ml) was stirred at room temperature for 10 h. The excess SOCl₂ was removed and the reaction mixture was poured into ice water and then basified with a saturated KHCO₃ solution and extracted with CHCl₃. The organic layer was washed with a saturated NaCl solution and dried over MgSO₄. 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. ¹H-NMR (DMSO-*d*₆+D₂O) δ: 3.88 (3H, s, OCH₃), 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 C₁₆H₁₁ClN₂O: C, 69.99; H, 3.92; N, 9.91. Found: C, 69.97; H, 3.55; N, 9.65.

11-Chloro-7-hydroxy-10H-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 CH₂Cl₂ and MeOH to give 180 mg (94%) of **9f** as yellow crystals. mp 190–192°C (dec.). IR (Nujol): 3400 cm⁻¹. ¹H-NMR (DMSO-*d*₆+CDCl₃) 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 C₁₅H₉ClN₂O: C, 67.05; H, 3.38; N, 10.43. Found: C, 67.24; H, 3.26; N, 10.43.

References and Notes

- 1) M. Yamato, Y. Takeuchi, M.-r. Chang, K. Hashigaki, T. Tsuruo, and S. Tsukagoshi, *Chem. Pharm. Bull.*, **38**, 3048 (1990).
- 2) M. Yamato, Y. Takeuchi, K. Hashigaki, Y. Ikeda, M.-r. Chang, K. Takeuchi, M. Matsushima, T. Tsuruo, T. Tashiro, S. Tsukagoshi, and Y. Yamashita, *J. Med. Chem.*, **32**, 1295 (1989).
- 3) Y. Takeuchi, M.-r. Chang, K. Hashigaki, and M. Yamato, *Chem. Pharm. Bull.*, **39**, 1629 (1991).
- 4) M. Yamato, K. Hashigaki, Y. Yasumoto, J. Sakai, S. Tsukagoshi, T. Tashiro, and T. Tsuruo, *Chem. Pharm. Bull.*, **34**, 3466 (1986).
- 5) C. Auclair and C. Paoletti, *J. Med. Chem.*, **24**, 289 (1981).
- 6) J. Rieche, *J. Chem. Soc.*, **119**, 1429 (1921).
- 7) B. F. Cain, G. J. Atwell, and W. A. Denny, *J. Med. Chem.*, **18**, 1111 (1975).