Synthesis and Antiulcer Activity of the Metabolites of 2-(4-Ethyl-1-piperazinyl)-4-phenylquinoline Dimaleate (AS-2646), a Novel Gastric Antisecretory and Antiulcer Agent

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The metabolites 3 and 4 of 2-(4-ethyl-1-piperazinyl)-4-phenylquinoline dimaleate (AS-2646, 1), a candidate as a gastric antisecretory and antiulcer drug, were synthesized to confirm the proposed structures. The effects of the metabolites 2—4 on ulcer induced by stress were determined.

Keywords antiulcer agent; gastric antisecretion; metabolite; 2-(4-ethyl-1-piperazinyl)-4-phenylquinoline

In the past decade, many successful treatments of peptic ulcer diseases have been developed based on the use of a number of antisecretory agents which act on peripheral sites.[1] Recently, some agents acting on the central nervous system have attracted interest because of their antiulcer effects.[2] We have previously reported the synthesis and antiulcer activity of 4-phenyl-2-(1-piperazinyl)quinoline derivatives.[3] Among them, 2-(4-ethyl-1-piperazinyl)-4-phenylquinoline dimaleate (AS-2646, **1**) was found to possess potent inhibitory effects on gastric secretion and on ulcer induced by exposure to restraint and water-immersion stress in rats. It was supposed that **1** exerts its effects by acting mainly *via* a central mechanism.[4] Thus, compound **1** was selected for evaluation in humans as a promising novel agent for the treatment of peptic ulcers. In studies on the metabolism of **1**, three metabolites were detected in the plasma and/or urine in rats, dogs and/or monkeys, and these metabolites were provisionally assigned as the deethyl (**2**), N-oxido (**3**), and 6-hydroxy (**4**) derivatives on the basis of their mass spectra (MS) and proton nuclear magnetic resonance (^{1}H-NMR) spectra.[5] The metabolites **2** and **3** were also found in human plasma.[6] As for **2**, its synthesis and the effect on ulcer induced by stress in rats, examined at a single dose of 5 mg/kg, *p.o.* as a preliminary screening test, have been reported in the previous paper.[3]

The present study was undertaken to obtain conclusive proof for the structures of **3** and **4** through synthesis and to determine their effects on ulcer induced by stress in rats. This paper also includes the results of a more detailed examination of the effect of **2** in the above test.

The oxidation of the free base of **1** with *m*-chloroperbenzoic acid in chloroform ($CHCl_3$) afforded the N-oxide (**3**) as slightly hygroscopic crystals. The MS of **3** showed the molecular ion peak (M^+) at *m/z* 333. The ^{1}H-NMR spectrum of **3** showed a signal attributable to methylene protons of the ethyl group at lower field (δ 3.32) as compared with that for **1** (δ 2.48). The spectral data indicate that the oxidation occurred at the piperazine distal nitrogen of **1**. Besides the α-methylenes (4H, δ 3.22—3.31) adjacent to the N-ethyl group in **3**, the signals of four protons ascribable to the β-methylenes were observed as two separate signals at δ 4.10 and 4.43 (each 2H). The unusually low field shift of the latter signal (probably due to two axial protons), compared with the case of **1** (4H, δ 3.81), may be explained in terms of the deshielding anisotropic effect of the N-oxido moiety on these protons, though no ^{1}H-NMR spectral data for piperazine N-oxide derivatives are available in the literature.

The synthesis of **4** was then carried out as follows. 4′-Methoxybenzoylacetanilide (**5**), obtained by condensation of 4-methoxyaniline with ethyl benzoylacetate, was subjected to cyclization with polyphosphoric acid (PPA) to give 6-methoxy-4-phenylcarbostyril (**6**) in 56% yield. The chlorination of **6** with thionyl chloride ($SOCl_2$) and *N*,*N*-dimethylformamide (DMF) in $CHCl_3$, afforded the 2-chloride **7** (88%), which was subjected to the displacement reaction with *N*-ethylpiperazine to give **8** in 75% yield. The demethylation of **8** with pyridine hydrochloride afforded

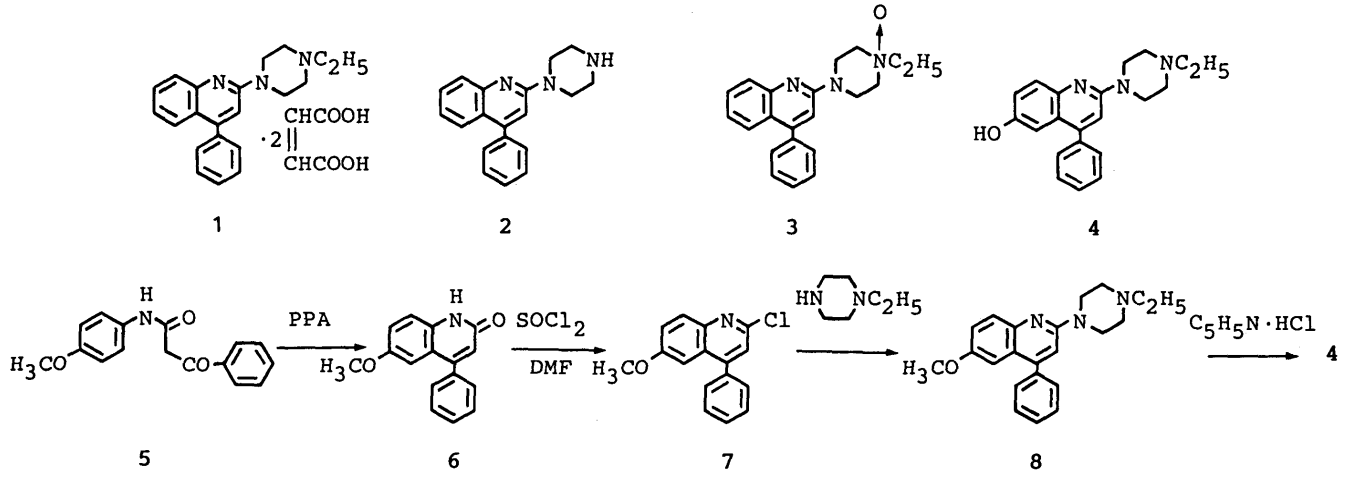

Chart 1

TABLE I. Inhibitory Effects of the Metabolites **2**—**4** and the Reference Compounds **1** and **8** on Stress-Induced Ulcer in Rats

Compd. No.	Dose (mg/kg, *p.o.*)	Inhibition (%)	ED_{50} (mg/kg)
2	0.2	46	0.3 (0.03—3.3)[a]
	0.5	56[b]	
	1	55[c]	
	2	74[c]	
	5	72[b]	
3	1	28	1.9 (0.76—4.8)
	2	57[c]	
	5	74[b]	
4	20	35	
	100	74[c]	
1	1	45	1.2 (0.4—3.2)
	2	67[c]	
	5	90[b]	
8	10	53[b]	
	50	61[b]	

a) 95% confidence limits. *b*) $p<0.01$, significant difference between means. *c*) $0.01<p<0.05$.

the target 6-hydroxy derivative **4** (64% yield), whose MS showed M^+ at *m/z* 333. The ^{1}H-NMR spectrum and the analytical results for **4** were consistent with the proposed structure. The synthetic compounds **2**—**4** were confirmed to be identical with the corresponding metabolites of **1** on the basis of thin layer chromatography, gas chromatography (GC)-MS, and ^{1}H-NMR comparisons.

The metabolites **2**—**4** were evaluated for their effects on ulcer induced by exposure to restraint and water-immersion stress in rats. The results are shown in Table I together with those for **1** and the intermediate **8**. As is clear from the lower ED_{50} value for **2** (0.3 mg/kg) than that for **1** (1.2 mg/kg), **2** was more potent than **1**. The metabolite **3** was fairly active. Drugs having a tertiary amino group are often subject to oxidative dealkylation or N-oxidation *in vivo*, giving metabolites with a similar potency to that of the parent drug, as is well known for imipramine, for example.[7] In contrast with **2** and **3**, the metabolite **4** exhibited a greatly decreased potency. The intermediate **8** showed a moderate effect.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a Hitachi 260-10 spectrometer in KBr disks and MS were taken on a JEOL JMS-D300 spectrometer. ^{1}H-NMR spectra were teken on a Varian A-60 or XL-300 spectrometer, using tetramethylsilane as an internal standard. Chemical shifts are given as δ (ppm). Abbreviations are as follows: s, singlet; d, doublet; q, quartet; m, multiplet. Organic extracts were dried over anhydrous magnesium sulfate.

2-(4-Ethyl-1-piperazinyl)-4-phenylquinoline (The Free Base of 1) The preparation of **1** was described previously.[3] This salt was treated in a usual manner to be converted into the free base, which was recrystallized from ether, mp 100 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.15 (3H, t, J=7.2 Hz, $NCH_2C\underline{H}_3$), 2.48 (2H, q, J=7.2 Hz, $NC\underline{H}_2CH_3$), 2.59 [4H, m, $(CH_2C\underline{H}_2)_2NEt$], 3.81 [4H, m, $(C\underline{H}_2CH_2)_2NEt$], 6.91 (1H, s, 3-H), 7.16 (1H, ddd, J=8.3, 6.9, 1.3 Hz, 6-H), 7.40—7.55 (5H, m, C_6H_5), 7.53 (1H, ddd, J=8.4, 6.9, 1.5 Hz, 7-H), 7.62 (1H, ddd, J=8.3, 1.5, 0.6 Hz, 5-H), 7.77 (1H, ddd, J=8.4, 1.3, 0.6 Hz, 8-H). *Anal.* Calcd for $C_{21}H_{23}N_3$: C, 79.46; H, 7.30; N, 13.24. Found: C, 79.48; H, 7.37; N, 13.13.

1-Ethyl-4-(4-phenylquinoline-2-yl)piperazine 1-Oxide (3) *m*-Chloroperbenzoic acid (80%, 0.86 g, 0.004 mol) was added portionwise to a solution of **1** (1.27 g as the free base, 0.004 mol) in $CHCl_3$ (8 ml) under cooling with ice-water. The reaction mixture was stirred for 1 h under the same conditions, washed with dilute aqueous sodium hydroxide solution, dried, and evaporated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (20:1) as an eluent to give **3** (1.1 g, 80%) after recrystallization from EtOAc, mp 167—168 °C. MS *m/z*: 333 (M^+). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.47 (3H, t, J=7.1 Hz, $NCH_2C\underline{H}_3$), 3.22—3.31 [4H, m, $(CH_2C\underline{H}_2)_2NEt$], 3.32 (2H, q, J=7.1 Hz, $NC\underline{H}_2CH_3$), 4.10 and 4.43 [each 2H, m, $(C\underline{H}_2CH_2)_2NEt$], 6.93 (1H, s, 3-H), 7.22 (1H, ddd, J=8.3, 6.8, 1.2 Hz, 6-H), 7.45—7.55 (5H, m, C_6H_5), 7.57 (1H, ddd, J=8.4, 6.8, 1.4 Hz, 7-H), 7.66 (1H, ddd, J=8.3, 1.4, 0.6 Hz, 5-H), 7.78 (1H, ddd, J=8.4, 1.2, 0.6 Hz, 8-H). *Anal.* Calcd for $C_{21}H_{23}N_3O\cdot 1/2H_2O$: C, 74.64; H, 7.01; N, 12.43. Found: C, 74.61; H, 6.93; N, 12.29.

4′-Methoxybenzoylacetanilide (5) A solution of ethyl benzoylacetate (78 g, 0.4 mol) in toluene (120 ml) was added dropwise to a solution of 4-methoxyaniline (50 g, 0.4 mol) in toluene (120 ml) during 3 h at 120 °C and the mixture was stirred at this temperature for 3 h, while volatile materials were allowed to distill off. After the reaction mixture had cooled, the resulting crystals were collected, dissolved in 10% aqueous sodium hydroxide solution, and treated with charcoal. The solution was acidified with dilute hydrochloric acid, and the resulting crystals were collected and recrystallized from EtOH–DMF–H_2O to give **5** (51 g, 47%), mp 123 °C (lit.[8] mp 121 °C). IR: 1650 and 1680 (C=O) cm^{-1}. MS *m/z*: 233 (M^+).

4-Phenyl-6-methoxycarbostyril (6) A mixture of **5** (37 g, 0.137 mol) and PPA (370 g) was stirred at 100 °C for 5 h. The cooled mixture was poured onto ice and water, and the resulting solid was collected and recrystallized from $CHCl_3$–ether to give **6** (19.3 g, 56%), mp 255 °C. IR: 1650 (C=O) cm^{-1}. MS *m/z*: 251 (M^+). *Anal.* Calcd for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.34; H, 5.07; N, 5.52.

2-Chloro-6-methoxy-4-phenylquinoline (7) The treatment of **6** with $SOCl_2$ and DMF in $CHCl_3$ in the same manner as described previously[3] afforded **7** (88%), mp 94 °C ($CHCl_3$–hexane). MS *m/z*: 269 (M^+). *Anal.* Calcd for $C_{16}H_{12}ClNO$: C, 71.25; H, 4.48; Cl, 13.14; N, 5.19. Found: C, 71.30; H, 4.52; Cl, 13.20; N, 5.18.

2-(4-Ethyl-1-piperazinyl)-6-methoxy-4-phenylquinoline (8) The treatment of **7** with *N*-ethylpiperazine in the same manner as described previously[3] afforded **8** as an oil. MS *m/z*: 347 (M^+). ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.16 (3H, t, J=7 Hz, $NCH_2C\underline{H}_3$), 2.4—2.8 [4H, m, $(CH_2C\underline{H}_2)_2NEt$], 2.50 (2H, q, J=7 Hz, $NC\underline{H}_2CH_3$), 3.5—3.9 [4H, m, $(C\underline{H}_2CH_2)_2NEt$], 3.73 (3H, s, OCH_3), 6.90 (1H, s, 3-H), 7.02 (1H, d, J=3 Hz, 5-H), 7.21 (1H, dd, J=8, 3 Hz, 7-H), 7.50 (5H, s, C_6H_5), 7.75 (1H, d, J=8 Hz, 8-H). This was converted into the maleate in a usual manner in 75% yield from **7**. mp 115—116 °C (MeOH–EtOAc). *Anal.* Calcd for $C_{22}H_{25}N_3O\cdot 2C_4H_4O_4\cdot 1/2H_2O$: C, 61.22; H, 5.82; N, 7.14. Found: C, 61.33; H, 5.71; N, 7.12.

2-(4-Ethyl-1-piperazinyl)-6-hydroxy-4-phenylquinoline (4) A mixture of **8** (10.4 g as the free base, 0.03 mol) and pyridine hydrochloride (17.3 g, 0.15 mol) was stirred at 170 °C for 7 h. The cooled mixture was dissolved in a mixture of EtOAc and dilute aqueous sodium bicarbonate solution. The organic layer was separated, washed with water, dried, and evaporated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (100:1) as an eluent to give **4** (6.5 g, 64%) after recrystallization from $CHCl_3$–ether, mp 193—194 °C. MS *m/z*: 333 (M^+). ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.30 (3H, t, J=7 Hz, $NCH_2C\underline{H}_3$), 2.3—2.8 [4H, m, $(CH_2C\underline{H}_2)_2NEt$], 2.47 (2H, q, J=7 Hz, $NC\underline{H}_2CH_3$), 3.4—3.9 [4H, m, $(C\underline{H}_2CH_2)_2NEt$], 5.3—6.1 (1H, br, OH), 6.77 (1H, s, 3-H), 6.88 (1H, d, J=3 Hz, 5-H), 7.10 (1H, dd, J=8, 3 Hz, 7-H), 7.40 (5H, s, C_6H_5), 7.66 (1H, d, J=8 Hz, 8-H). *Anal.* Calcd for $C_{21}H_{23}N_3O\cdot 2/5H_2O$: C, 74.05; H, 7.04; N, 12.34. Found: C, 74.00; H, 7.12; N, 12.13. This was converted into the maleate in a usual manner, mp 192—193 °C (MeOH–EtOAc). *Anal.* Calcd for $C_{21}H_{23}N_3O\cdot 2C_4H_4O_4$: C, 61.59; H, 5.52; N, 7.43. Found: C, 61.72; H, 5.22; H, 7.31.

Effect on the Ulceration Induced by Exposure to Restraint and Water-Immersion Stress Male Std-Wistar rats (180—270 g) were used in the experiment. Drugs were dissolved or suspended in 0.5% aqueous tragacanth and administered orally to groups of five animals. Compounds **1**, **2**, **4**, and **8** were used as the maleate salts. Rats were fed until just before the experiment. The rats were individually immobilized in a compartment of the stress cage. The cage was immersed vertically in a water bath at 23 °C to the height of the xiphoid process of the rats accroding to the method of Takagi and Okabe.[9] After 20 h, the rats were sacrificed. The stomachs were removed and cut open along the greater curvature. The maximum diameter of each lesion was measured with a dissection microscope (×12). The sum of the length (mm) of each lesion was taken as the ulcer index. Test compounds were administered orally 30 min before the immersion. The inhibitory rate was determined by comparing the ulcer index (mean value) in drug-treated groups with that in the control group. The ED_{50} values, the dose required for 50% reduction of the ulcer index, and the 95%

confidence limits were calculated according to the method of Litchfield and Wilcoxon.[10]

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