Effect of Active Synthetic 2-Substituted Quinazolinones on Anti-Platelet Aggregation and the Inhibition of Superoxide Anion Generation by Neutrophils

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Quinazolinones, 2-substituted and 3-substituted, mainly synthesized by microwave irradiation, were subjected to anti-platelet aggregation and inhibition of superoxide anion generation assays. Interestingly, 2-phenyl-4-quinazolinone (4) exhibited significant inhibitory activities toward platelet aggregation and neutrophil activation, and it might therefore serve as a prototype lead compound.

Key words: Hydrangea chinensis, Quinazolinones, Anti-platelet aggregation, Superoxide anion generation, Microwave

INTRODUCTION

The roots of Hydrangea chinensis were used for the treatment of malaria and cardiovascular diseases (Chiang-Su, 1978). In a previous investigation on this species, the quinazolinones, febrifugine and isofebrifugine, exhibited significant anticancer activity against mouse mammalian tumor FM3A cell line (Kobayashi et al., 1999). Moreover, quinazolinones are one of the frequently encountered heterocycles in medical chemistry literature with such applications as anticonvulsant (Mannschreck et al., 1984), antibacterial agent (Ravikanth et al., 2000), anti-malarial agent (Murata et al., 1999), inhibitor of DNA repair enzyme poly(ADP-ribose) polymerase (PARP) (Griffin et al., 1998), and antagonist of angiotensin (De Laszlo et al., 1993).

Currently, there has been increasing interest in the use of microwave irradiation techniques in organic syntheses (Seijas et al., 1999). A number of synthetically useful organic reactions have been carried out in the microwave oven in open vessels (Seger et al., 1998; Sharma et al., 1989; Malamas et al., 1991). In each case, the reactions proceeded in a highly accelerated manner, with final product yields and purity comparable to those obtained with traditional protocols. Due to the excellent biological functions of quinazolinones, a series of 2-substituted and 3-substituted derivatives were synthesized in the current investigation by using a domestic microwave machine, and all products were subjected to anti-platelet aggregation and inhibition of superoxide anion generation assays.

MATERIALS AND METHODS

Instruments and reagents

Melting points were determined on a Laboratory Devices Mel-Temp II and were uncorrected. The UV spectra were obtained on a Hitachi 200-20 spectrophotometer, IR spectra on a Hitachi 260-30 spectrophotometer, and ¹H-NMR (400 and 200 MHz, using CDCl₃ as solvent) spectra on a Varian NMR spectrometer (Unity Plus). Low-resolution EIMS were collected on a JEOL JMS-SX/SX 102 A mass spectrometer or Quattro GC/MS spectrometer featuring a direct inlet system. The reactant mixtures in a test tube were irradiated in a domestic microwave (530 W, Sunhow) oven for 10 minutes. Silica gel 60 (Merck, 230-400 mesh) was used for column chromatography. The spots were detected by spraying with 50% H₂SO₄ and then heating on a hot plate.
General experimental procedure for the synthesis of 2-substituted quinazolinones

A mixture of 2-amino benzoic acid (1 mmol) and an amide (1.5 mmol) in a test tube was irradiated in a microwave oven for 10 minutes. After cooling, the crude reaction product was purified by column over silica (or alumina) gel to afford the 2-substituted quinazoline (Seger et al., 1998).

4-Quinazolinone (1)
White powder; mp 215-217 °C; IR (Neat) \( \nu_{\text{max}} \): 1697, 1657, 1607 cm\(^{-1} \); UV (MeOH) \( \lambda_{\text{max}} \): 223, 263, 270(sh); \(^1\)H-NMR (CD\(_2\)OD): \( \delta \) 8.23 (1H, dd, \( J = 8.8, 0.8 \) Hz), 8.10 (1H, s), 7.84 (1H, td, \( J = 8.8, 0.8 \) Hz), 7.70 (1H, dd, \( J = 8.8, 0.8 \) Hz), 7.60 (1H, td, \( J = 8.8, 0.8 \) Hz); EIMS \( m/z \): 146 [M]\(^+ \); yield 60%.

2-Methyl-4-quinazolinone (2)
White powder; mp 240-242 °C; IR (Neat) \( \nu_{\text{max}} \): 2956, 2928, 2868, 1726, 1123, 1069 cm\(^{-1} \); UV (MeOH) \( \lambda_{\text{max}} \): 223, 263, 270(sh); \(^1\)H-NMR (CD\(_2\)OD): \( \delta \) 8.18 (1H, dd, \( J = 8.0, 1.6 \) Hz), 7.80 (1H, td, \( J = 8.0, 1.6 \) Hz), 7.61 (1H, dd, \( J = 8.0, 1.6 \) Hz), 7.49 (1H, td, \( J = 8.0, 1.6 \) Hz), 2.46 (3H, s); EIMS \( m/z \): 160 [M]\(^-I \); yield 57%.

2-Phenyl-4-quinazolinone (4)
White powder; mp 220-222 oC; IR (Neat) \( \nu_{\text{max}} \): 1666, 1599, 1477, 765, 691 cm\(^{-1} \); UV (CHCl\(_3\)) \( \lambda_{\text{max}} \): 225, 243, 290, 321(sh) nm; \(^1\)H-NMR (CDCl\(_3\)): \( \delta \) 8.31 (1H, dd, \( J = 8.0, 0.6 \) Hz), 7.80 (1H, dd, \( J = 8.0, 0.6 \) Hz), 7.28 (2H, m), 7.25 (1H, m), 7.00-7.30 (5H, m), 5.12 (1H, dd, \( J = 9.2, 3.2 \) Hz), 4.45 (1H, dd, \( J = 14.0, 3.2 \) Hz), 4.54 (1H, dd, \( J = 14.0, 3.2 \) Hz), 3.93 (1H, dd, \( J = 13.2, 9.2 \) Hz); EIMS \( m/z \): 251 [M-1]\(^+ \); yield 55%.

Synthesis of 3-(2-hydroxy-2-phenylethyl)-3,4-dihydroquinazolin-4-one (9)
4-Quinazolinone (1) (110 mg, 0.75 mmol) was suspended in 10 mL isopropanol containing 0.05 mL pyridine. While the mixture was heated slowly to 120 °C (oil bath) 0.1 mL (8.8 mmol) of styrene oxide was added. After dissolution of the components the reaction mixture turned to brown from yellow. After 5 h at reflux, the reaction mixture was allowed to cool slowly in the oil bath. Column chromatography of the reaction mixture gave 86 mg (43 %) of 9. White powder; mp 160-162°C; IR (Neat) \( \nu_{\text{max}} \): 3404, 2922, 1671, 1608, 1472, 1373, 772, 753, 696 cm\(^{-1} \); UV (MeOH) \( \lambda_{\text{max}} \): 243, 270, 303 nm; \(^1\)H-NMR (CDCl\(_3\)): \( \delta \) 8.20 (1H, s), 8.25 (1H, dd, \( J = 7.8, 1.2 \) Hz), 7.80 (1H, m), 7.67 (1H, br, d, \( J = 8.0 \) Hz), 7.50-7.60 (3H, m), 7.40-7.30 (3H, m), 5.12 (1H, dd, \( J = 9.2, 3.2 \) Hz), 4.45 (1H, dd, \( J = 13.2, 3.2 \) Hz), 3.93 (1H, dd, \( J = 13.2, 9.2 \) Hz); EIMS \( m/z \): 265 [M-1]\(^+ \), 247, 160 (base peak).

Synthesis of 3-(2-chloro-2-phenylethyl)-3,4-dihydroquinazolin-4-one (10)
Hydroxyl compound 9 (60 mg, 0.23 mmol) was added in droplets to 0.2 mL (2.7 mmol) of thionyl chloride in 5 mL of absolute benzene (rapid HCl and SO\(_2\) evolution). After 1 h of reflux, the product was precipitated by addition of 5 mL ether. Column chromatography of the reaction mixture gave 61 mg (93 %) of 10. White powder; mp 160-162 °C; IR (Neat) \( \nu_{\text{max}} \): 3404, 2922, 1671, 1608, 1472, 1373, 772, 753, 696 cm\(^{-1} \); UV (MeOH) \( \lambda_{\text{max}} \): 243, 270, 303 nm; \(^1\)H-NMR (CDCl\(_3\)): \( \delta \) 8.20 (1H, s), 8.25 (1H, dd, \( J = 7.8, 1.2 \) Hz), 7.80 (1H, m), 7.67 (1H, br, d, \( J = 8.0 \) Hz), 7.50-7.60 (3H, m), 7.40-7.30 (3H, m), 5.12 (1H, dd, \( J = 9.2, 3.2 \) Hz), 4.45 (1H, dd, \( J = 13.2, 3.2 \) Hz), 3.93 (1H, dd, \( J = 13.2, 9.2 \) Hz); EIMS \( m/z \): 284 [M]\(^+ \), 160 (base peak).

Anti-platelet activity assay
Blood anticoagulated with ethylenediaminetetraacetic acid (EDTA) was collected from New Zealand rabbits.