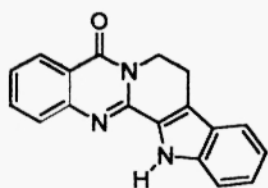


SYNTHESIS AND BIOLOGICAL PROPERTIES OF SELECTED 2-ARYL-4(3H)-QUINAZOLINONES

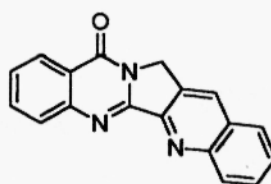
Eung Seok Lee, Jong Keun Son, Young Hwa Na, Yurngdong Jahng*
College of Pharmacy, Yeungnam University, Kyongsan 712-749, Korea

Abstract: A series of 2-aryl-4(3H)quinazolinones were prepared as parent systems of rutaecarpine and luotonin A and their biological properties (cytotoxicity and COX-2 inhibitory activity) were evaluated.

Attractiveness of alkaloids rutaecarpine (1) (1) and luotonin A (2) (2) stemmed from their promising cytotoxicities (IC_{50} 's = 18.9 and 6.6 μ M, respectively, for human leukemia cancer cell line) (3) as well as a variety of intriguing biological properties including anti-inflammatory activity (4). We have recently involve in the synthesis and selective inhibitory activities of in 1 and 2 as well as their homologues on COX-2, in which the inhibitory activities are highly dependent on the length of the methylene bridge connecting N3 of 4(3H)-quinazolinone and C2 of either 1H-indole or quinoline in 1 and 2, respectively (5).



1

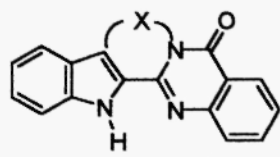


2

Previous studies on the conformations of annulated aza-biaryls such as 3,3'-polymethylene-2,2'-bipyridines (6) and 3,2'-polymethylene-2-phenylpyridines (7) revealed that the conformations of the unbridged parent compounds lay in between di- and tri-methylene bridged system. The relationship between the length of methylene-bridge and the dihedral angles of the 3,3'-polymethylene-2-(1H-indol-2-yl)-4(3H)-quinazolinones are shown in Figure 1. We, thus, reason that the non-bridged parent compounds may have readily adoptable conformations for the corresponding receptor sites for the maximal biological activity.

Anti-inflammatory activity of well known indomethacin and our findings (5) on the biological properties of the rutaecarpine and luotonin A as well as their homologues on COX-2 and against selected human cancer cell lines prompted us to synthesize and evaluate biological properties of 2-aryl-4(3H)-quinazolinones (3) whose conformations would be in between di- and trimethylene bridged systems.

Figure 1. Dihedral angle between 4(3H)-quinazolinone and 1H-indole ring



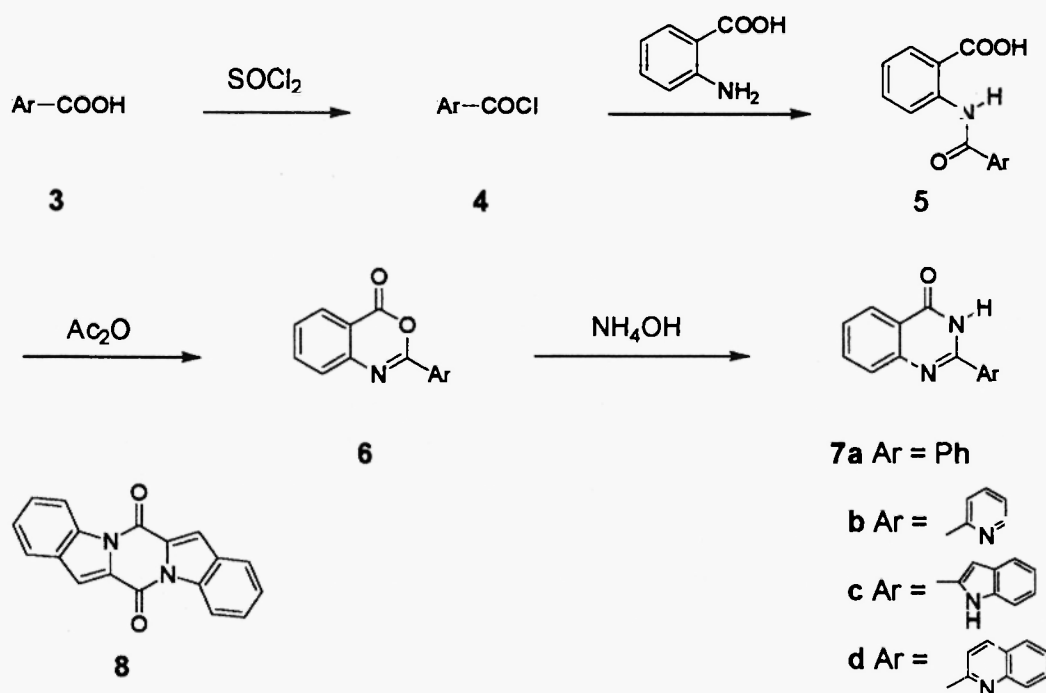
X	Dihedral Angle, $\alpha^{\circ a)}$
-CH ₂ -	0
-(CH ₂) ₂ -	16(18, 6.3 ^{b)})
-(CH ₂) ₃ -	52(35)
H, H	- (32)

^aValues are obtained from Dreiding Model and values in parenthesis are calculated from CS Chem3D Program. (8)

^bData was taken from X-ray crystal structure. (9)

Synthesis

The designed compounds **3** were prepared by employing previously reported method such as ammonolysis of 4H-3,1-benzoxazolin-4-ones. (10) Although early studies claimed that the ammonolysis of 4H-3,1-benzoxazolin-4-ones with an aromatic substituent at C2 position required drastic reaction condition such as heating 240-250 °C (11) or with anhydrous ZnCl₂, (12) the reactions of **6** with NH₄OH in EtOH under reflux afforded the desired product in good yield.



It is worthy to noting that the reaction of 1*H*-indole-2-carboxylic acid in equimolar SOCl₂ in dry CHCl₃ has afforded 1*H*-indole-2-carbonyl chloride in quantitative yield, although it has been claimed to afford bisindolodioxopiperazine (**8**). (13) Unlike dioxopiperazine **8**, acid chloride **4c** was very soluble in CHCl₃ and shown a well-resolved proton resonances required for the structure. A resonance for H3 appeared at δ 7.55 as an one-proton doublet ($J_{1,3} = 2.1$ Hz), which is downfield-shifted by 0.16 ppm due to the stronger electron withdrawing effect of COCl at C2 compared to the parent carboxylic acid, confirmed such a conversion. (14) In addition, a broad D₂O-exchangeable singlet at δ 8.94 also supported the presence of N-H. We were not able to either isolate or identify any trace of **8**. The bisindolodioxopiperazine (**8**), however, could be prepared by running the reaction in the presence of pyridine or triethylamine, which might uptake HCl formed, and thus catalyze formation of bisamide (**8**).

Biological Properties

COX Inhibitory Activity: Inhibitory activities of the compounds **7** on cyclooxygenase-1 and 2 (COX-1 and 2) were evaluated as compared to indomethacin and selective COX-2 inhibitor NS-398 by employing previously described method, (5) and summarized in Table 1. The inhibitory activities on COX-1 and COX-2 were significantly increased in **7c** and **7d** with COX-2 selectivity of 10.7 and 4.7, respectively. Selectivity of **7c** on COX-2 is somewhat surprising compared to previous result of a loss of selectivity on triethylene-bridged homologue of rutaecarpine. (5)

Cytotoxicity: Cytotoxicities of compounds **7** against human leukemia cancer cell line (CCRF-CEM) were evaluated by employing previously described method (15) and summarized in Table 1. Cytotoxicities of **7c,d** on CCRF-CEM are approximately 2- and 0.5-fold increase compared to the parent rutaecarpine and luotonin A, respectively, while **7a,b** did not show any significant cytotoxicities up to 50 μ M.

Table 1. Inhibitory Activities of Compound **7** on COX-1 and COX-2 and Human Cancer Cell Line

Compounds	IC ₅₀ (μ M)		Selectivity (COX-1/COX-2)	IC ₅₀ (μ M)
	COX-1	COX-2		CCRF-CEM
7a	>100	>100	-	>50
7b	>100	>100	-	>50
7c	78.3	7.3	10.7	9.3
7d	67.5	14.5	4.7	12.3
Indomethacin	0.016	0.009	1.9	
NS-398	1.67	< 0.002	> 8,300	

In conclusion, 2-(1*H*-indol-2-yl)-4(3*H*)-quinazolinone and 2-(quinol-2-yl)-4(3*H*)-quinazolinone were shown significant inhibitory activities on COX-2 and on cell growth of CCRF-CEM enough to be a possible lead for the future study as an anti-inflammatory and/or antitumor agent.

Experimental

Melting points were determined using a Fischer-Jones melting points apparatus and are not corrected. IR spectra were obtained using a Perkin-Elmer 1330 spectrophotometer. NMR spectra were obtained using a Bruker-250 spectrometer 250 MHz or 400 MHz for ^1H NMR and 62.5 MHz or 100 MHz for ^{13}C NMR and are reported as parts per million (ppm) from the internal standard tetramethylsilane (TMS). The compounds 7a (16) and 7b (17) were previously reported in the literature. Chemicals and solvents were commercial reagent grade and used without further purification. Elemental analyses were taken on a Hewlett-Packard Model 185B elemental analyzer.

1*H*-Indole-2-carbonyl Chloride (4c)

To a solution of indole-2-carboxylic acid (3.64 g, 0.023 mol) in 50 mL of CHCl_3 at room temperature was added SOCl_2 (5.51 mL, 0.23 mol). Resulting mixture was stirred for 8-12 h at room temperature and evaporated *in vacuo* to give 3.37 g (88%) of pale yellow solid. The obtained solid was used for the next step without any further purification. Unreported spectral data are as follows: IR (KBr) ν 3050, 1750, 1610, 1475, 1260, 1245, 1220, 1180, 981 cm^{-1} . ^1H NMR (400 MHz, CDCl_3) δ 8.94 (br s, NH), 7.74 (dd, J = 8.9, 0.8 Hz, H7), 7.55 (d, J = 2.1 Hz, H3), 7.45 (dd, J = 8.4, 1.0 Hz, H4), 7.43 (ddd, J = 8.4, 7.8, 0.8 Hz, H5), 7.23 (ddd, J = 8.4, 8.0, 1.0 Hz, H6).

Quinoline-2-carbonyl Chloride (4d)

The same procedure described for 4c was employed with quinoline-2-carboxylic acid (5.00 g, 0.029 mol) to give 4.42 g (85%) of pale yellow needles (ether): mp 96-97 °C [lit. (18) mp 96-97 °C]. Unreported spectral data are as follows: IR (KBr) ν 3260, 2965, 2890, 1640, 1488, 1362, 1090 cm^{-1} . ^1H NMR (400 MHz, CDCl_3) δ 8.35 (d, J = 8.8 Hz, H4), 8.34 (d, J = 8.4 Hz, H8), 8.13 (d, J = 8.8 Hz, H3), 7.92 (d, J = 8.0 Hz, H5), 7.85 (td, J = 7.6, 0.8 Hz, H7), 7.73 (ddd, J = 7.6, 1.0 Hz, H6).

N-(1*H*-Indole-2-carbonyl)anthranilic acid (5c)

To the acid chloride 4c (0.023 mol) in 30 mL of CHCl_3 was added anthranilic acid (3.09 g, 0.023 mol) in pyridine (8 mL) and CHCl_3 (30 mL). The reaction mixture was stirred for 8 h and refluxed for 1 h. The pale yellow precipitate formed was collected and washed with CHCl_3 to give 6.25 g (97%) as a pale green needles (EtOAc): mp > 260 °C. IR (KBr) ν 3305, 3049, 1745, 1741, 1630, 1605, 1474, 1264, 1236, 1220, 1180, 973, 764 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ 12.28 (s, 1H), 11.96 (s, 1H), 8.72 (d, J = 7.6 Hz, 1H), 8.07 (d, J = 8.0 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.25 (td, J = 8.0, 0.8 Hz, 1H), 7.19 (t, J = 7.6 Hz, 1H), 7.13 (s, 1H, H3'), 7.09 (td, J = 7.6, 0.8 Hz, 1H). *Anal.* Calcd for $\text{C}_{16}\text{H}_{12}\text{N}_2\text{O}_3 \cdot 1/2\text{H}_2\text{O}$: C, 66.43; H, 4.53; N, 9.68. Found: C, 66.36; H, 4.52; N, 9.70.

N-(Quinoline-2-carbonyl)anthranilic acid (5d)

The same procedure described for 5c was employed with 4d (0.023 mol) to give 6.18 g (92%) pale yellow needles (EtOAc): mp 241-242 °C. IR (KBr) ν 3306, 3050, 1745, 1740, 1631, 1604, 1473, 1265, 1235, 1221, 972 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ 13.43 (s, 1H), 8.92 (d, J = 8.4 Hz, 1H), 8.65 (d, J = 8.4 Hz, 1H), 8.29 (d, J = 8.4 Hz, 1H), 8.13 (d, J = 8.4 Hz, 2H), 8.09 (dd, J = 8.4, 1.4 Hz, 1H), 7.92 (td, J = 8.4, 1.6 Hz, 1H), 7.77 (td, J = 8.4, 1.2 Hz, 1H), 7.70 (td, J = 8.4, 1.5 Hz, 1H), 7.67 (td, J =

8.4, 1.6 Hz, 1H), 7.24 (td, $J = 8.4, 1.2$ Hz, 1H). ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 169.4, 163.1, 149.9, 146.1, 140.7, 138.7, 134.5, 131.8, 131.1, 129.5, 129.4, 128.8, 128.5, 123.4, 120.0, 118.9, 117.3. *Anal.* Calcd for $\text{C}_{17}\text{H}_{12}\text{N}_2\text{O}_3\cdot\text{H}_2\text{O}$: C, 66.43; H, 4.53; N, 9.68. Found: C, 66.36; H, 4.52; N, 9.70.

2-(1*H*-Indol-2-yl)-4*H*-3,1-benzoxazolin-4-one (6c)

A suspension of **5c** (1.40 g, 0.005 mol) in Ac_2O (10 mL) was refluxed for 2 h. Evaporation of the solvent *in vacuo* gave pale yellow solid, which was suspended in water and stirred for 30 min. The precipitate formed was collected and washed with water, followed by EtOH to give 1.26 g (96%) of pale yellow needles: mp 210–212 °C [lit. (19) mp 198–200 °C]. Unreported spectral data are as follows: IR (KBr) ν 3296, 1661, 1607, 1540, 1450, 1412, 1315, 1262 cm^{-1} . ^1H NMR (DMSO- d_6 , 400 MHz) δ 11.32 (s, 1H, N-H), 8.10 (d, $J = 7.9$ Hz, 1H), 7.76 (d, $J = 7.9$ Hz, 1H), 7.60–7.55 (m, 2H), 7.47 (d, $J = 8.4$ Hz, 1H), 7.43 (t, $J = 8.0$ Hz, 1H), 7.29 (s, 1H, H3), 7.19 (t, $J = 7.2$ Hz, 1H), 7.02 (td, $J = 7.6, 0.8$ Hz, 1H). ^{13}C NMR NMR (DMSO- d_6 , 100 MHz) δ 157.9, 151.5, 146.1, 137.5, 135.7, 127.6, 126.9, 126.8, 126.6, 125.6, 124.0, 120.9, 119.5, 115.8, 111.6, 106.9.

2-(Quinol-2-yl)-4*H*-3,1-benzoxazolin-4-one (6d)

The same procedure described above for **6c** was employed with **5d** (1.46 g, 0.005 mol) to give 1.26 g (92%) of pale pink needles (EtOAc): mp 182–183 °C. IR (KBr) ν 3297, 1662, 1610, 1540, 1450, 1412, 1315, 1261 cm^{-1} . ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.60 (d, $J = 8.9$ Hz, 1H), 8.46 (d, $J = 8.4$ Hz, 1H), 8.24 (d, $J = 8.0$ Hz, 1H), 8.23 (td, $J = 7.6, 0.8$ Hz, 1H), 8.11 (d, $J = 8.4$ Hz, 1H), 8.01 (td, $J = 8.0, 1.4$ Hz, 1H), 7.90 (ddd, $J = 8.0, 7.6, 0.8$ Hz, 1H), 7.84 (d, $J = 8.0$ Hz, 1H), 7.76 (td, $J = 8.0, 0.8$ Hz, 1H), 7.71 (td, $J = 7.6, 1.0$ Hz, 1H). ^{13}C NMR NMR (DMSO- d_6 , 100 MHz) δ 159.0, 155.3, 148.0, 147.0, 145.9, 137.5, 136.9, 130.7, 129.7, 129.5, 128.6, 128.6, 128.1, 128.1, 127.3, 120.3, 117.6. *Anal.* Calcd for $\text{C}_{17}\text{H}_{12}\text{N}_2\text{O}_2$: C, 74.45; H, 3.67; N, 10.21. Found: C, 74.25; H, 3.74; N, 10.31.

2-(1*H*-Indol-2-yl)-4(3*H*)-quinazolinone (7c)

A solution of **6c** (1.32 g, 0.005 mol) and 35% NH_4OH (35 mL) in EtOH (200 mL) was refluxed for 6 h with addition of additional 35% NH_4OH (35 mL) every 2 h. The creamy precipitate formed was collected and washed with water to give 2.09 g (80%) of solid which was purified by sublimation. mp 319–320 °C [lit. (19) mp 318–320 °C]. ^1H NMR (DMSO- d_6 , 400 MHz) δ 12.62 (s, N1'-H), 11.80 (s, N3-H), 8.17 (dd, $J = 7.9, 1.2$ Hz, H5), 7.84 (td, $J = 7.9, 0.8$ Hz, H7), 7.74 (dd, $J = 8.0, 1.0$ Hz, H8), 7.68 (s, H3'), 7.65 (dd, $J = 8.0, 0.8$ Hz, H4'), 7.54 (dd, $J = 8.0, 1.2$ Hz, H7'), 7.50 (t, $J = 8.0$ Hz, H6), 7.23 (t, $J = 7.6$ Hz, H6'), 7.06 (t, $J = 7.6$ Hz, H5'). ^{13}C NMR NMR (DMSO- d_6 , 100 MHz) δ 161.8, 148.8, 146.6, 137.7, 134.7, 130.0, 127.5, 126.9, 126.3, 126.1, 124.1, 121.5, 121.2, 120.0, 112.4, 105.0.

2-(Quinol-2-yl)-4(3*H*)-quinazolinone (7d)

The same procedure described above for **7c** was employed with **6d** (2.74 g, 0.01 mol) to give 2.05 g (75%) of fine white needles (CHCl_3): mp 267–268 °C. IR (KBr) ν 3376, 3066, 1649, 1603, 1581, 1524, 1504, 1451, 1404, 1297, 1265, 770, 752 cm^{-1} . ^1H NMR (DMSO- d_6 , 400 MHz) δ 8.80 (d, $J = 8.4$ Hz, 1H), 8.42 (d, $J = 8.4$ Hz, 1H), 8.25 (d, $J = 8.4$ Hz, 1H), 8.13 (d, $J = 8.4$ Hz, 1H), 7.94 (d, $J = 8.4$ Hz, 1H), 7.80 (dd, $J = 8.4, 1.4$ Hz, 1H), 7.78 (ddd, $J = 8.4, 8.0, 1.0$ Hz, 1H), 7.63 (ddd, $J = 8.4, 8.0, 1.0$ Hz,

1H), 7.48 (ddd, $J = 8.4, 8.0, 1.0$ Hz, 1H), 7.10 (ddd, $J = 8.4, 8.0, 1.0$ Hz, 1H). ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 170.5, 162.6, 149.7, 145.8, 138.7, 137.3, 131.5, 129.8, 129.3, 128.7, 128.3, 127.7, 127.4, 122.3, 120.9, 120.1, 118.3. Anal. Calcd for $\text{C}_{17}\text{H}_{11}\text{N}_3\text{O}_2$: C, 70.09; H, 4.50; N, 14.43. Found: C, 70.16; H, 4.52; N, 14.39.

Acknowledgement: Support by Korean Research Foundation Grant (KRF-2002-005-E00019) is gratefully acknowledged.

References

- (1) a) Y. Ashima and K. Kashiwaki, *J. Pharm. Soc. Jpn.* **35**, 1293 (1915). b) E. S. Lee, J. G. Park, and Y. Jahng, *Tetrahedron Lett.* **44**, 1883 (2003), and references therein.
- (2) a) Z.-Z. Ma, Y. Hano, T. Nomura, and Y.-J. Chen, *Heterocycles* **46**, 541 (1997). b) A. Cagir, S. H. Jones, R. Gao, B. M. Eisenhauer, and S. M. Hecht, *J. Am. Chem. Soc.* **125**, 13628 (2003), and references therein.
- (3) a) L.-M. Yang, C.-F. Chen, and K.-H. Lee, *Bioorg. Med. Chem. Lett.* **5**, 465 (1995). b) Z.-Z. Ma, Y. Hano, T. Nomura, and Y.-J. Chen, *Heterocycles* **55**, 1555 (2001).
- (4) a) T. C. Moon, M. Murakami, I. Kudo, K. H. Son, H. P. Kim, S. S. Kang, and H. W. Chang, *Inflamm. Res.* **48**, 621 (1999). b) P.-G. Xiao, G.-L. Quo, H.-L. Wang, L.-S. Lui, Y.-L. Zheng, Z.-J. Jia, and Z.-B. Deng, *Chinese J. Pharmacol. Toxicol.* **232** (1988). c) Y. Jahng, Unpublished results.
- (5) a) H. W. Chang, S. I. Kim, H. Jung, and Y. Jahng, *Heterocycles* **60**, 1359 (2003). b) Y. Jahng, Unpublished results.
- (6) R. P. Thummel, F. Lefoulon, and R. Mahadevan, *J. Org. Chem.* **50**, 3824 (1985).
- (7) R. P. Thummel and Y. Jahng, *J. Org. Chem.* **52**, 71 (1987).
- (8) Available from CambridgeSoft Corporataion, Cambridge, MA 02140-2317, USA.
- (9) I. Fujii, Y. Kobayashi, and N. Hirayama, *Z. Kristallogr.* **215**, 762 (2000).
- (10) C. A. Fetscher and M. T. Bogert, *J. Org. Chem.* **4**, 71 (1939).
- (11) a) W. L. F. Armarego, *Part I Quinazolines in Fused Pyrimidines* ed. by D. J. Brown, Interscience Publishers, New York, 1967, and references therein. b) M. Claesen and H. Vanderhaeghe, *Bull. Soc. Chim. Belges.* **68**, 220 (1959). c) S. S. Joshi and I. R. Gambhir, *J. Org. Chem.* **26**, 3714 (1961).
- (12) D. T. Zentmeyer and E. C. Wagner, *J. Org. Chem.* **14**, 967 (1949).
- (13) A.-R. Lee, W.-H. Huang, and J. -Y. Cheng, *Chin. Pharm. J.* **46**, 307 (1994).
- (14) G. Cignarella, D. Barlocco, P. Vianello, S. Villa, G. A. Pinna, P. Fadda, W. Fratta, L. Toma, and S. Gessi, *Il Farmaco* **53**, 667 (1998). They reported 1H-indole-2-carbonyl chloride as an intermediate without any physical and spectral data.
- (15) M. R. Boyd, *Princ. Pract. Oncol.* **3**, 1 (1989).
- (16) M. T. Bogert, R. A. Gortner, and C. G. Amend, *J. Am. Chem. Soc.* **33**, 949 (1911).
- (17) L. Berezowski and W. Dymek, *Acta Poloniae Pharm.* **27**, 11 (1970).
- (18) J. W. David, Jr., *J. Org. Chem.* **24**, 1691 (1959).
- (19) J. Bergman and N. Eklund, *Chemica Scripta* **19**, 193 (1982).

Received on February 4, 2004.