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

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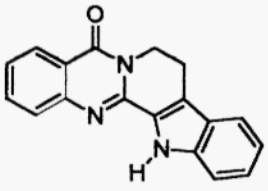
Abstract: A series of 2-aryl-4(3*H*)quinazolinones were prepared as parent systems of rutaecarpine and luoitonin A and their biological properties (cytotoxicity and COX-2 inhibitory activity) were evaluated.

Attractiveness of alkaloids rutaecarpine (**1**) (1) and luotonin A (**2**) (2) stemmed from their promising cytotoxicities (IC_{50}'s = 18.9 and 6.6 μM, 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(3*H*)-quinazolinone and C2 of either 1*H*-indole or quinoline in **1** and **2**, respectively (5).



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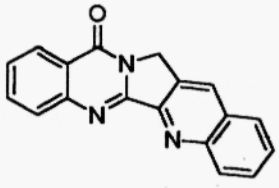

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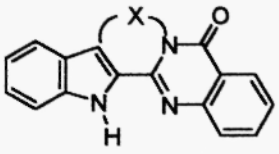
Previous studies on the conformations of annulated aza-biaryls such as 3,3'-polymethylene-2,2'-bipyridines (6) and 3,2'-polymethylene-2-phenylpyridines (7) revealed that the conformations of the unbridged parent compounds lay in between di- and tri-methylene bridged system. The relationship between the length of methylene-bridge and the dihedral angles of the 3,3'-polymethylene-2-(1*H*-indol-2-yl)-4(3*H*)-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(3*H*)-quinazolinones (**3**) whose conformations would be in between di- and trimethylene bridged systems.

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



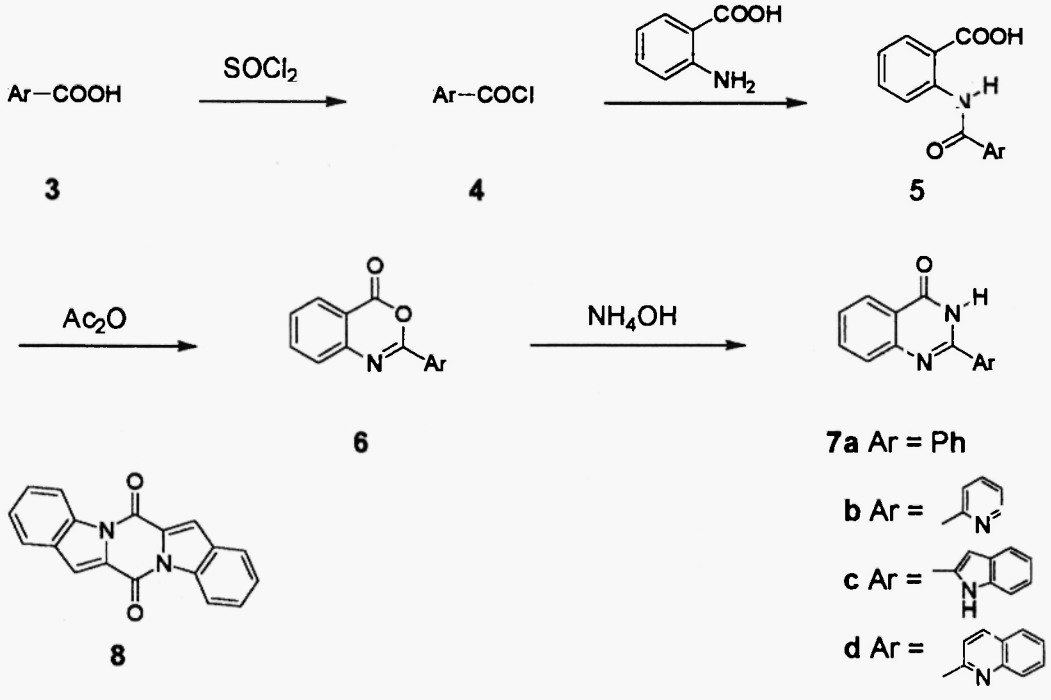
X	Dihedral Angle, α^{o} [a]
$-CH_2-$	0
$-(CH_2)_2-$	16(18, 6.3[b])
$-(CH_2)_3-$	52(35)
H, H	- (32)

[a]Values are obtained from Dreiding Model and values in parenthesis are calculated from CS Chem3D Program. (8)

[b]Data was taken from X-ray crystal structure. (9)

Synthesis

The designed compounds **3** were prepared by employing previously reported method such as ammonolysis of 4*H*-3,1-benzoxazolin-4-ones. (10) Although early studies claimed that the ammonolysis of 4*H*-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 $ZnCl_2$, (12) the reactions of **6** with NH_4OH 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 $SOCl_2$ in dry $CHCl_3$ 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 $CHCl_3$ 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 downfiled-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 D_2O-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 triemthylene-bridged homologue of rutaecarpine. (5)

Cytotoxicity: Cytotoxicites 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 μM.

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

Compounds	IC_{50} (μM)		Selectivity	IC_{50} (μM)
	COX-1	COX-2	(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 ^{1}H 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) υ 3050, 1750, 1610, 1475, 1260, 1245, 1220, 1180, 981 cm^{-1}. ^{1}H 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) υ 3260, 2965, 2890, 1640, 1488, 1362, 1090 cm^{-1}. ^{1}H 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-carboyl)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 1h. 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) υ 3305, 3049, 1745, 1741, 1630, 1605, 1474, 1264, 1236, 1220, 1180, 973, 764 cm^{-1}. ^{1}H NMR (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 $C_{16}H_{12}N_2O_3 \cdot 1/2H_2O$: 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) υ 3306, 3050, 1745, 1740, 1631, 1604, 1473, 1265, 1235, 1221, 972 cm^{-1}. ^{1}H NMR (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 $C_{17}H_{12}N_2O_3 \cdot H_2O$: 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) υ 3296, 1661, 1607, 1540, 1450, 1412, 1315, 1262 cm^{-1}. ^{1}H 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) υ 3297, 1662, 1610, 1540, 1450, 1412, 1315, 1261 cm^{-1}. ^{1}H 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 $C_{17}H_{12}N_2O_2$: 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]. ^{1}H 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) υ 3376, 3066, 1649, 1603, 1581, 1524, 1504, 1451, 1404, 1297, 1265, 770, 752 cm^{-1}. ^{1}H 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 $C_{17}H_{11}N_3O{\cdot}H_2O$: C, 70.09; H, 4.50; N, 14.43. Found. C, 70.16; H, 4.52; N, 14.39.

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