

SYNTHESIS AND CHEMILUMINESCENCE OF 10-HYDROXY- AND 10-AMINOPYRIDAZINO[4,5-*b*]QUINOLINE-1,4(2*H*, 3*H*)-DIONES

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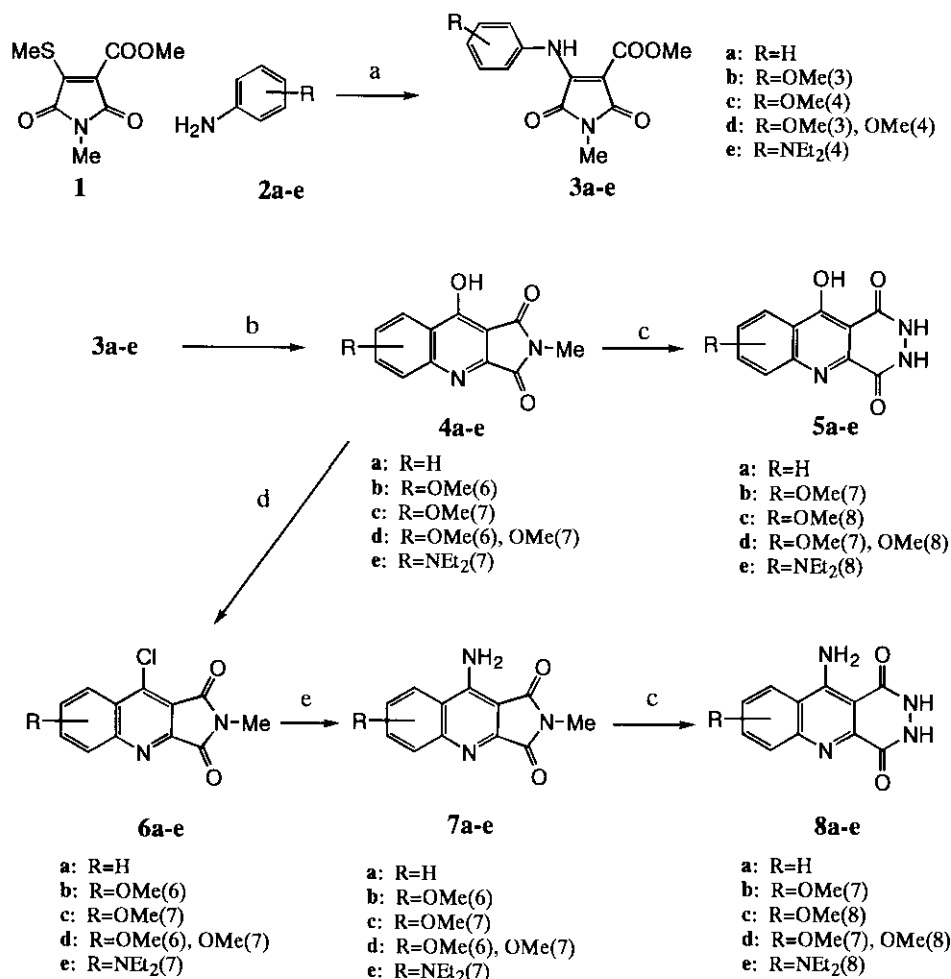
Abstract --- Substituted anilines reacted with methyl 1-methyl-4-methylthio-2,5-dioxo-3-pyrroline-3-carboxylate (**1**) in refluxing methanol to give the corresponding methyl 1-methyl-2,5-dioxo-4-phenylamino-3-pyrroline-3-carboxylates (**3a-e**) which were converted in good yields to 2-methylpyrrolo[3,4-*b*]quinoline derivatives (**4a-e**) by heating in diphenyl ether. Reaction of **4a-e** with hydrazine hydrate gave 10-hydroxypyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-diones (**5a-e**) in good yields. The desired 10-aminopyridazino[4,5-*b*]quinoline-1,4(2*H*, 3*H*)-diones (**8a-e**) were obtained by the chlorination of **4a-e** with phosphorus oxychloride followed by ammonolysis with 28% ammonium hydroxide in good yields.

Compounds (**5**) and (**8**) were found to be efficiently chemiluminescent in a similarly to luminol in the presence of H₂O₂ and horseradish peroxidase in a solution of a phosphate buffer pH 8.0.

Several derivatives of polycyclic heterocycles containing the pyridazinedione nucleus are known to possess chemiluminescent activity.¹ This suggested that other similarly heterocyclic fused pyridazine derivatives might also have useful chemiluminescent properties. We recently reported that the reaction of 4-aminopyrazole-3-carbonitriles with dimethyl acetylenedicarboxylate gave the 1,3-disubstituted 4-amino-1*H*-pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyridazine-5,8(6*H*,7*H*)-diones which showed higher chemiluminescence than luminol in a system of horseradish peroxidase (HRD) and hydrogen peroxide in a phosphate buffer solution at pH 8.0.² In the case of the synthesis of fused pyridazine derivatives, the key intermediates are fused 2-alkylpyrrole-1,3-dione derivatives. It has been reported that 4-cyano-3-

methylthiomaleimides smoothly reacted with various amines to give the corresponding aminopolycyclic pyrimidines containing a pyrroline ring, which are readily converted to the desired polycyclic pyridazine derivatives in good yields.³ A combination of the above process and Gould Jacobs reaction⁴ was considered well applicable to synthesis of the key compounds, 2-methylpyrrolo[3,4-*b*]quinoline derivatives. This paper presents a synthesis of 10-hydroxypyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-diones and 10-aminopyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-diones as chemiluminescent compounds through use of methyl 1-methyl-4-methylthio-2,5-dioxo-3-pyrroline-3-carboxylate (**1**).⁵

Reaction of **1** with various aniline derivatives (**2a-e**) in refluxing methanol gave readily the corresponding 3-phenylaminomaleimide derivatives (**3a-e**)⁶, in 85-98% yields, which were smoothly converted in 73-97% yields to pyrrolo[3,4-*b*]quinolines (**4a-e**)⁷ under refluxing in diphenyl ether for 1 h. The reaction of



a: reflux in methanol for 30 min -1 h; **b:** reflux in diphenyl ether for 1 h; **c:** reflux in a large excess of NH₂-NH₂ · H₂O; **d:** POCl₃ + *N,N*-diethylaniline; **e:** 28% NH₄OH, 180 °C in mini autoclave

Scheme 1

4a-e with a large excess of 80% hydrazine hydrate afforded the corresponding polycyclic hydroxy-pyridazinediones, 10-hydroxypyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-diones (**5a-e**)⁸ in 72-97% yields. The chlorination of **4a-e** with phosphorus oxychloride in the presence of diethylaniline was carried out to give the corresponding 9-chloro-2-methylpyrrolo[2,3-*d*]quinolines (**6a-e**)⁹, in 76-98% yields, which were induced to the expected 9-amino-2-methylpyrrolo[2,3-*d*]quinolines (**7a-e**)¹⁰ in 54-82% yields by ammonolysis with 28% ammonium hydroxide at 180°C in a mini autoclave. The desired 10-aminopyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-dion derivatives (**8a-e**)¹¹ were readily obtained in 70-93% yields, respectively, by the general reaction of compounds **7** with a large excess of 80% hydrazine hydrate under refluxing.¹²

The chemiluminescence(CL) experiments reported here were performed in the presence of Triton X-100, hydrogen peroxide, and HRD in a phosphate buffer solution at pH 8.0.¹³ The CL intensity in these pyridazinoquinoline series is shown in Table 1. Both 10-hydroxy- and 10-aminopyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-dione derivatives (**5a-d**, **8a-d**) showed nearly the same or somewhat stronger light intensity than luminol at pH 8.0. These compounds also showed increasing light intensity with rise in pH, as noted also for luminol. The methoxy and diethylamino groups on the quinoline ring are very important groups for CL production.

Table 1. Chemiluminescence Intensity of Hydroxy- and Aminopolycyclic Pyridazinedione Derivatives

Compound	CL(CPS) ^a pH 8	Compound	CL(CPS) ^a pH 8
5a	2.3x10 ⁴	8a	1.2x10 ⁵
5b	4.5x10 ⁶	8b	1.8x10 ⁷
5c	2.6x10 ⁴	8c	3.8x10 ⁵
5d	3.3x10 ⁵	8d	2.5x10 ⁶
Luminol	1.1x10 ⁵	8e	5.1x10 ³

a) Counts per 1.0 sec. (Their values were subtracted from each background.)

A reaction solution contains 10 mmol/L phosphate buffer pH 8.0, 0.5 ml/L Triton X-100, 2.5x10⁻⁷ mol/L test compound, and 2500 U/L HRP (Each test compound was prepared to obtain concentration of 1.5x10⁻⁵ mol/L in DMSO). The solution (3 mL of vol) was transferred to a Borosilicate glass tube (12x75 mm) and immediately placed in a water bath (37 °C) for 10 min. At the end of the incubation period, the sample tube to be counted was incorporated into a luminometer. Photons were counted for 1.0 sec. after addition of 0.3 mL of 1.1x10⁻⁹ mol/L H₂O₂ (5.0x10⁻¹⁰ mol/L as final concentration) and 0.3 mL of 0.2 mol/L glycine buffer pH 8.0.

In conclusion, 10-hydroxy- and 10-aminopyridazino[4,5-*b*]quinoline-1,4(2*H*,3*H*)-diones were found to be more efficient than luminol in light production. These pyridazinoquinoline-1,4-dione derivatives are the first example of chemiluminescence compounds in the pyridazinoquinoline derivatives.

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6. **3c**: Yield 92%, mp 151-152 °C, orange needles(MeOH). IR(KBr) ν max cm^{-1} : 3450(NH), 1715, 1662(CO). $^1\text{H-NMR}(\text{CDCl}_3)$ δ : 2.97(3H, s, NMe), 3.83(3H, s, OMe), 3.91(3H, s, OMe), 6.91(2H, d, $J=8.5$ Hz, phenyl-H), 7.22(2H, d, $J=8.5$ Hz, phenyl-H), 10.04(1H, br s, NH).
7. **4c**: Yield 94%, mp>360 °C, yellow needles(DMF). IR(KBr) ν max cm^{-1} : 3480(OH, br), 3060(OH), 1715, 1640(CO). $^1\text{H-NMR}(\text{DMSO-d}_6)$ δ : 2.97(3H, s, NMe), 3.87(3H, s, OMe), 7.40(1H, dd, $J=2.9, 9.0$ Hz, 7-H), 7.57(1H, d, $J=2.9$ Hz, 9-H), 7.77(1H, d, $J=9.0$ Hz, 6-H), 13.60(1H, br s, OH). MS m/z : 258(M^+ , 100), 215(7), 201(15), 175(11).
8. **5c**: Yield 96%, mp>360 °C, yellow needles(DMSO). IR(KBr) ν max cm^{-1} : 3450(OH, br), 3120(NH or OH), 3120-2600(OH, br), 1620(CO). $^1\text{H-NMR}(\text{DMSO-d}_6)$ δ : 3.91(3H, s, OMe), 7.55(1H, dd, $J=3.0, 9.1$ Hz, 7-H), 7.61(1H, d, $J=3.0$ Hz, 9-H), 8.10(1H, d, $J=9.1$ Hz, 6-H), 8.27(1H, s, NH or OH), 12.30(1H, br s, NH or OH). MS m/z : 259(M^+ , 78), 138(14), 91(38), 58(14), 45(100).
9. **6c**: Yield 93%, mp 287-289 °C, colorless needles(toluene). IR(KBr) ν max cm^{-1} : 1770, 1720(CO). $^1\text{H-NMR}(\text{CDCl}_3)$ δ : 3.33(3H, s, NMe), 4.08(3H, s, OMe), 7.48(1H, dd, $J=2.5, 9.1$ Hz, 6-H), 7.71(1H, d, $J=2.5$ Hz, 8-H), 8.30(1H, d, $J=9.1$ Hz, 5-H). MS m/z : 278(M^+ , 24), 276(M^+ , 100), 221(10), 219(23), 193(35), 184(24), 148(14).
10. **7c**: Yield 78%, mp >300 °C, yellow needles(toluene). IR(KBr) ν max cm^{-1} : 3440, 3330(NH), 1750, 1700(CO). $^1\text{H-NMR}(\text{CDCl}_3 + \text{one drop of CF}_3\text{COOH})$ δ : 3.25(3H, s, NMe), 3.99(3H, s, OMe), 7.61(1H, d, $J=2.5$ Hz, 8-H), 7.71(1H, dd, $J=2.5, 9.5$ Hz, 6-H), 7.98(1H, d, $J=9.5$ Hz, 5-H), 8.15(1H, br s, NH), 8.37(1H, br s, NH). MS m/z : 257(M^+ , 100), 242(20), 219(19), 200(13), 174(18).
11. **8c**: Yield 93%, mp >360°C, yellow needles(DMSO). IR(KBr) ν max cm^{-1} : 3200-2600(NH, OH, br), 1650, 1620(CO). $^1\text{H-NMR}(\text{DMSO-d}_6)$ δ : 3.94(3H, s, OMe), 7.64(1H, dd, $J=2.7, 9.3$ Hz, 7-H), 7.97(1H, d, $J=2.7$ Hz, 9-H), 8.15(1H, d, $J=9.3$ Hz, 6-H), 10.29(1H, br s, NH), 11.11(1H, br s, NH). MS m/z : 258(M^+ , 100), 173(15), 121(34), 44(96).
12. Satisfactory spectral (IR, UV, $^1\text{H-NMR}$, MS) data were obtained for all new compounds in this work.
13. CL intensity was measured with a Magic Lite Analyzer of CIBA-CONING.