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SYNTHESIS AND CHEMILUMINESCENT ACTIVITY OF PYRIDAZINO[4,5-*b*]INDOLE-1,4(2*H*,3*H*)-DIONES

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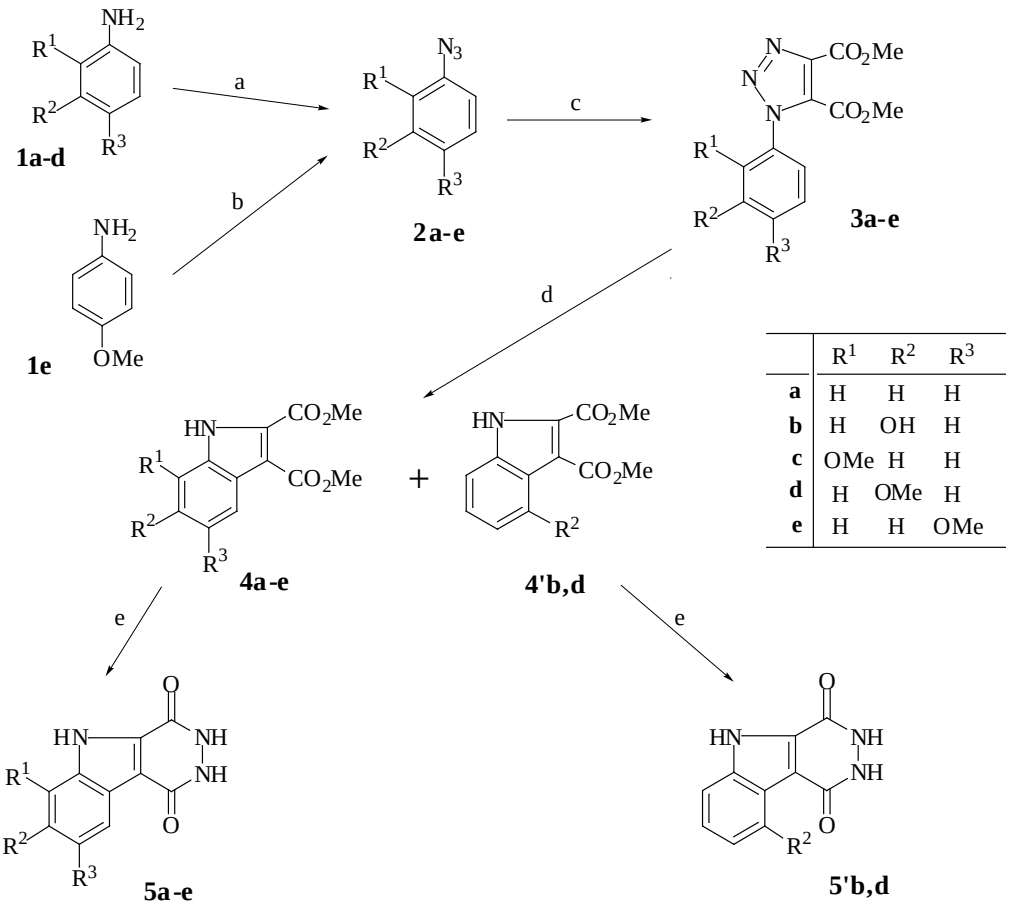
Abstract - Substituted and non-substituted anilines were transformed to the corresponding triazole derivatives, which were converted to dimethyl indole-2,3-dicarboxylates by photo-cyclization. The reaction of diester with hydrazine hydrate gave 1,2,3,4-tetrahydro-5*H*-pyridazino[4,5-*b*]indole-1,4(2*H*,3*H*)-diones (**5**). Some of compounds (**5**) were found to have chemiluminescent activity in a similarly to luminol in the presence of hydrogen peroxide and peroxidase in a basic solution.

Since the discovery of the chemiluminescence of luminol by Albrecht,[1] its analytical usefulness has been extensively studied.[2] The chemiluminescent assay is an attractive analytical method because of its high sensitivity, rapid reaction and wide dynamic range.[3] Polycyclic heterocyclic compounds containing the pyridazinedione moiety are well known to have chemiluminescent activity as luminol analogue.[4] And the development of luminol analogs has been performed actively to meet the requests of clinical analyses for something more useful than luminol. We describe here the synthesis of pyridazino[4,5-*b*]indole-1,4(2*H*,3*H*)-diones (**5**) as luminol analogue and evaluation of their chemiluminescent activity.

The synthetic route of pyridazinoindoles (**5**) from aniline derivatives (**1**) is shown in Scheme 1. Among final compounds (**5**), **5a**,[5] **5c**,[6] **5d**[6] and **5'd**[6] have been already known. All these compounds have been prepared by the reaction of the corresponding dimethyl (or diethyl) indole-2,3-dicarboxylates (**4**) with hydrazine hydrate. This synthetic method for preparing the pyridazine-1,4-dione moiety from diester is well known, so that we also employed this procedure in this study. As for preparing **4**, Diels *et al.*[7] have reported the synthesis of **4a** by the reaction of 1,2-diphenylhydrazine with DMAD. All other groups described above also employed this strategy. Especially Bare *et al.*[6] used this procedure for preparing



	R^1	R^2	R^3
a	H	H	H
b	H	OH	H
c	OMe	H	H
d	H	OMe	H
e	H	H	OMe

a: i) $NaNO_2$ /AcOH, conc.H_2SO_4. ii) NaN_3. **b**: i) $NaNO_2$ /10% HCl. ii) NaN_3.
c: DMAD/dioxane. **d**: *hv*/MeOH. **e**: NH_2NH_2•H_2O/EtOH.

Scheme 1

diethyl analogue of **4c**, **4d** and **4'd**, however, the yields of them were very low. Huntress *et al.*[8] also reported the synthesis of **4a** by the reaction of phenylhydroxylamine with DMAD followed by heating in xylene. But their method is also not so efficient. On the other hand, Nagawa *et al.*[9] has reported the good yield synthesis of diethyl 1*H*-benz[*g*]indole-2,3-dicarboxylate from naphthylamine in a few steps *via* photocyclization of diethyl 1-(1-naphthyl)-1*H*-1,2,3-triazole-4,5-dicarboxylate. We applied this procedure for preparing **4** in this work.

As shown in Scheme 1, at first, aniline derivatives (**1**) was converted to azidobenzenes (**2**) by the method of Forster *et al.*[10] In the case of **1e**, using hydrochloric acid instead of H_2SO_4 improved the yield of **2e**. These azides (**2**) were used as a starting material for the next step without further purification because of their sufficient purity. Compounds (**2**) were transformed to **4** *via* photocyclization reaction of triazole (**3**) accompanied by denitrogenation by the method of Nagawa *et al.*[9] In the case of the

photocyclization of **3b**, two isomers (**4b** and **4'b**) were obtained. Similarly, two isomers (**4d** and **4'd**) were obtained from **3d**. All structures of **4** were well consisted with their instrumental and analytical data. As described above compound (**4a**)[7,8] has been already reported, however, its instrumental data (including the ^{1}H-NMR assignment) have not been described in detail. So, we report its detail data in EXPERIMENTAL in this paper. Finally compounds (**4**) were derived to the corresponding desired pyridazinoindoles (**5**) in 92-40% yields by the reaction of hydrazine hydrate. This reaction promptly proceeded by using large excess of hydrazine hydrate and employing external heating at high temperature (120 °C), which is higher than the refluxing temperature of EtOH. Compounds (**5a**,[5] **5c**,[6] **5d**[6] and **5'd**[6]) are already reported. But their spectral data (especially assignments of their ^{1}H-NMR spectra) have not been reported as in the case of **4**. We now describe their detail data in EXPERIMENTAL in this paper. All these instrumental data consisted their structures well.

The evaluation of the chemiluminescent activity of compounds (**5**) was performed in the presence of Triton X-100, hydrogen peroxide, and *Arthromyces ramosus* peroxidase (ARP) in a phosphate buffer solution at pH 8.0 and 10. The chemiluminescent intensity of these pyridazinoindoles is shown in Table 1. All of methoxy derivatives had chemiluminescent activity, however, these activities were 1/20-1/40 of that of luminol at pH 10. These compounds showed increasing light intensity with rise in pH and this phenomenon is the same as that of luminol. It seems that the presence of methoxy group is important for chemiluminescent activity of this series.

Table 1 Chemiluminescent Production of Pyridazino[4,5-*b*]indole-1,4(2*H*, 3*H*)dione Drivatives at pH 8 and 10

Compound	pH 8	pH 10
5a	NP[a]	NP[a]
5b	NP[a]	NP[a]
5'b	NP[a]	NP[a]
5c	2.3×10^{-3}	2.5×10^{-2}
5d	2.7×10^{-3}	5.7×10^{-2}
5'd	2.9×10^{-3}	2.0×10^{-2}
5e	2.5×10^{-3}	3.1×10^{-2}
Luminol	3.7×10^{-1}	1.0[b]

Arthromyces ramosus peroxidase (ARP) was used as peroxydase. Amount of ARP was 55 Unit and hydrogen peroxide was 0.22 mmol per 3 mL of test solution containing 0.3 mg of each compound.
[a] NP means no production of chmiluminescence. [b] Value of the total chmiluminescence intensity of luminol at pH 10 is presented as 1.0 for standard.

EXPERIMENTAL

All melting points were determined on a Yanagimoto micro-melting point apparatus, and are uncorrected. Elemental analyses were performed on a Yanagimoto MT-5 CHN Corder elemental analyzer. The FAB-MS spectra were measured on a VG 70 mass spectrometer and glycerol or *m*-nitrobenzyl alcohol was used as a matrix agent. The IR spectra were recorded on a Japan Spectroscopic IRA-102 diffraction grating infrared spectrophotometer and frequencies are expressed in cm^{-1}. ^{1}H-NMR spectra were recorded on a Varian VXR-200 instrument working at 200 MHz in $CDCl_3$ with TMS as an internal standard. Chemical shifts are given in ppm (δ) and *J* values in Hz. Photocyclization reaction was performed using an Eikohsha High-Pressure Mercury Arcs EHB-W1 (500 watt). The chemiluminescence intensity was measured with a Shimadzu Multiconvertible Spectrophotometer Double-40.

General Procedure of Preparation of Azide (2)

To a mixture of aniline derivative (107 mmol) in AcOH (92 mL) and conc. H_2SO_4 (43 mL) was added sodium nitrite (7.70 g, 111 mmol) in water (50 mL) dropwise under vigorous stirring at 0-5 °C. After 10 min aqueous urea was added to the reaction mixture to remove excess sodium nitrite. Then sodium azide (7.70 g, 118 mmol) in water (50 mL) was added to the reaction mixture at 0-5 °C for 3 h. The reaction mixture was poured into ice water and the resulting mixture was basified with sodium hydroxide and extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated to give corresponding azide (**2**), which was employed as a starting material for the next step without purification, because it gave almost single spot on TLC using several solvent systems.

Azidobenzene (2a)

Aniline (**1a**) (10.0 g, 107 mmol) was used as a starting material and **2a** (12.4 g, 104 mmol) was obtained as pale yellow viscous oil (97%), IR (neat) cm^{-1}: 2100 (N_3).

3-Azidophenol (2b)

3-Aminophenol (**1b**) (6.00 g, 55.0 mmol) was used as a starting material and **2b** (6.80 g, 50.0 mmol) was obtained as brown viscous oil (91%), IR (neat) cm^{-1}: 2110 (N_3).

2-Azidoanisole (2c)

2-Aminoanisole (**1c**) (10.0 g, 81.3 mmol) was used as a starting material and **2c** (12.0 g, 80.5 mmol) was obtained as brown viscous oil (99%), IR (neat) cm^{-1}: 2110 (N_3).

3-Azidoanisole (2d)

3-Aminoanisole (**1d**) (1.00 g, 8.10 mmol) was used as a starting material and **2d** (1.14 g, 7.65 mmol) was obtained as brown viscous oil (94%), IR (neat) cm^{-1}: 2145 (N_3).

4-Azidoanisole (2e)

4-aminoanisole (**1e**) (233 mg, 1.90 mmol) was used as a starting material and 10% hydrochloric acid (10 mL) was used instead of AcOH and conc. H_2SO_4. Compound (**2e**) (167 mg, 1.12 mmol) was obtained as pale brown crystals (59%), IR (neat) cm^{-1}: 2105 (N_3).

General Procedure of Preparation of Triazole (3)

To a solution of **2** (100 mmol) in dioxane (200 mL) was added dimethyl acetylenedicarboxylate (DMAD, 56.8 g, 400 mmol) and the resulting mixture was stirred at rt for 10 d in a dark. After evaporation of the solvent the viscous oily residue was triturated with *n*-hexane. Precipitated solid was collected, washed with ether and recrystallized from appropriate solvent to give corresponding **3**.

Dimethyl 1-Phenyl-1*H*-1,2,3-triazole-4,5-dicarboxylate (3a)

Compound (**2a**) was employed as a starting material and **3a** was obtained as colorless needles (from MeOH), yield 60%, mp 124-125 °C. FAB-MS *m/z*: 262 (MH^+). IR (KBr) cm^{-1}: 1725 (CO). NMR ($CDCl_3$) δ: 3.92 (3H, s, 5-ester Me), 4.01 (3H, s, 4-ester Me), 7.56 (5H, s, Ph-H). *Anal*. Calcd for $C_{12}H_{11}N_3O_4$: C, 55.17; H, 4.24; N, 16.09. Found: C, 54.77; H, 4.25; N, 16.05.

Dimethyl 1-(3-Hydroxyphenyl)-1*H*-1,2,3-triazole-4,5-dicarboxylate (3b)

Compound (**2b**) was employed as a starting material and **3b** was obtained as colorless needles (from benzene), yield 58%, mp 158-160 °C. FAB-MS *m/z*: 555 ($2MH^+$), 278 (MH^+). IR (KBr) cm^{-1}: 3240 (OH), 1732 (CO). NMR (DMSO-d_6) δ: 3.85 (3H, s, 5-ester Me), 3.90 (3H, s, 4-ester Me), 6.95-7.06 (3H, m, 2'-, 4'- and 6'-H), 7.43 (1H, t, *J* = 7.9, 5'-H), 10.26 (1H, br s, OH). *Anal*. Calcd for $C_{12}H_{11}N_3O_5$: C, 51.99; H, 4.00; N, 15.16. Found: C, 51.92; H, 4.21; N, 15.34.

Dimethyl 1-(2-Methoxyphenyl)-1*H*-1,2,3-triazole-4,5-dicarboxylate (3c)

Compound (**2c**) was employed as a starting material. In this case solid did not precipitate. So the residue was column-chromatographed on silica gel and eluate by *n*-hexane-ethyl acetate (3:2, v/v) gave **3c** as yellow granules (from benzene-*n*-hexane), yield quantitatively, mp 68-69 °C. FAB-MS *m/z*: 292 (MH^+). IR (KBr) cm^{-1}: 1740 (CO). NMR ($CDCl_3$) δ: 3.78, 3.86, 4.01 (each 3H, each s, Me x 3), 7.05 (1H, dd, *J* = 8.0, *J* = 1.1, 3'-H), 7.14 (1H, td, *J* = 8.0, *J* = 1.1, 5'-H), 7.52 (1H, td, *J* = 7.9, *J* = 1.5, 4'-H), 7.57 (1H, dd, *J* = 8.0, *J* = 1.5, 6'-H). *Anal*. Calcd for $C_{13}H_{13}N_3O_5$: C, 53.61; H, 4.50; N, 14.43. Found: C, 53.37; H, 4.85; N, 14.58.

Dimethyl 1-(3-Methoxyphenyl)-1*H*-1,2,3-triazole-4,5-dicarboxylate (3d)

Compound (**2d**) was employed as a starting material. In this case also solid did not precipitate. So the residue was column-chromatographed on silica gel and eluate by *n*-hexane-ethyl acetate (7:3, v/v) gave **3d** as colorless needles (from benzene-*n*-hexane), yield 85%, mp 94-95 °C. FAB-MS *m/z*: 292 (MH^+). IR (KBr) cm^{-1}: 1725 (CO). NMR ($CDCl_3$) δ: 3.87, 3.93, 4.01 (each 3H, each s, Me x 3), 7.05-7.14 (3H, m, 2'-, 4'- and 6'-H), 7.44 (1H, td, *J* = 8, *J* = 1, 5'-H). *Anal*. Calcd for $C_{13}H_{13}N_3O_5$: C, 53.61; H, 4.50; N, 14.43. Found: C, 53.45; H, 4.50; N, 14.56.

Dimethyl 1-(4-Methoxyphenyl)-1*H*-1,2,3-triazole-4,5-dicarboxylate (3e)

Compound (**2e**) was employed as a starting material. In this case also solid did not precipitate. So the residue was column-chromatographed on silica gel and eluate by *n*-hexane-ethyl acetate (13:7, v/v) gave **3e** as colorless needles (from benzene-*n*-hexane), yield 94%, mp 96-97 °C. FAB-MS *m/z*: 292 (MH^+). IR (KBr) cm^{-1}: 1745, 1720 (CO). NMR ($CDCl_3$) δ: 3.88, 3.91, 4.00 (each 3H, each s, Me x 3), 7.02 (2H, br d, *J* = 6.8, 3'- and 5'-H), 7.46 (2H, br d, *J* = 6.8, 2'- and 6'-H). *Anal*. Calcd for $C_{13}H_{13}N_3O_5$: C, 53.61; H, 4.50; N, 14.43. Found: C, 53.38; H, 4.48; N, 14.49.

General Procedure of Preparation of 1*H*-Indole-2,3-dicarboxylates (4)

A solution of **3** in MeOH was irradiated with a 500 watt high pressure mercury lamp under nitrogen atmosphere at rt. After evaporation of the solvent the residue was column-chromatographed on silica gel to isolate the desired **4**.

Dimethyl 1*H*-Indole-2,3-dicarboxylate (4a)

Compound (**3a**) (300 mg, 1.15 mmol) in MeOH (500 mL) was employed as a starting material. The reaction time was 2 h. The eluate by benzene-ether (9:1, v/v) gave **4a** as colorless needles (from benzene-*n*-hexane), yield 77%, mp 112-113 °C (lit., 114 °C[7], 112-112.5 °C[8]). FAB-MS *m/z*: 202 (MH^+ - 32), 234 (MH^+). IR (KBr) cm^{-1}: 3305 (NH), 1725, 1690 (CO). NMR ($CDCl_3$) δ: 3.99 (6H, s, Me x 2), 7.22-7.42 (2H, m, 5- and 6-H), 7.45 (1H, br d, *J* = 7, 7-H), 8.06 (1H, br d, *J* = 8.2, 4-H), 9.36 (1H, br s, NH). *Anal*. Calcd for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.00. Found: C, 61.63; H, 4.75; N, 5.83.

Dimethyl 6-Hydroxy-1*H*-indole-2,3-dicarboxylate (4b) and Dimethyl 4-Hydroxy-1*H*-indole-2,3-dicarboxylate (4'b)

Compound (**3b**) (300 mg, 1.08 mmol) in MeOH (600 mL) was employed as a starting material. The reaction time was 3 h. The former eluate by *n*-hexane-ethyl acetate (3:1, v/v) gave **4b** as yellow granules (from benzene) and the latter eluate by the same eluent afforded a mixture of unreacted **3b** and **4'b**.

This mixture was re-chromatographed on silica gel and the eluate by chloroform-ethyl acetate (8:2, v/v) gave **4'b** as colorless needles (from benzene-ether).

4b: yield 28%, mp 222-223 ℃. FAB-MS *m/z*: 250 (MH^+). IR (KBr) cm^{-1}: 3320, 3260 (NH and OH), 1691, 1672 (CO). NMR ($CDCl_3$) δ: 3.82 and 3.86 (each 3H, each s, ester Me x 2), 6.75 (1H, dd, *J* = 8.8, *J* = 2.1, 5-H), 6.82 (1H, d, *J* = 2.1, 7-H), 7.66 (1H, d, *J* = 8.8, 4-H), 9.55 (1H, s, NH or OH), 12.14 (1H, br s, NH or OH). *Anal*. Calcd for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45; N, 5.62. Found: C, 58.01; H, 4.61; N, 5.62.

4'b: yield 18%, mp 143-144 °C (decomp). FAB-MS *m/z*: 250 (MH^+). IR (KBr) cm^{-1}: 3440, 3205 (NH and OH), 1740 (CO). NMR ($CDCl_3$) δ: 3.99 and 4.00 (each 3H, each s, ester Me x 2), 6.73 (1H, d, *J* = 8, 5-H), 6.92 (1H, d, *J* = 8, 7-H), 7.27 (1H, t, *J* = 8, 6-H), 9.25 (1H, br s, NH or OH), 10.73 (1H, s, NH or OH). *Anal*. Calcd for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.66; H, 4.44; N, 5.37.

Dimethyl 7-Methoxy-1*H*-indole-2,3-dicarboxylate (4c)

Compound (**3c**) (100 mg, 0.344 mmol) in MeOH (500 mL) was employed as a starting material. The reaction time was 1.5 h. The eluate by *n*-hexane-ethyl acetate (7:3, v/v) gave **4c** as colorless prisms (from benzene-*n*-hexane), yield 43%, mp 108-109 °C. FAB-MS *m/z*: 264 (MH^+). IR (KBr) cm^{-1}: 3300 (NH), 1700 (CO). NMR ($CDCl_3$) δ: 3.978, 3.982 and 3.99 (each 3H, each s, Me x 3), 6.76 (1H, d, *J* = 8, 6-H), 7.18 (1H, t, *J* = 8, 5-H), 7.62 (1H, d, *J* = 8, 4-H), 9.42 (1H, br s, NH). *Anal*. Calcd for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32. Found: C, 59.24; H, 4.87; N, 5.07.

Dimethyl 6-Methoxy-1*H*-indole-2,3-dicarboxylate (4d) and Dimethyl 4-Methoxy-1*H*-indole-2,3-dicarboxylate (4'd)

Compound (**3d**) (500 mg, 1.72 mmol) in MeOH (600 mL) was employed as a starting material. The reaction time was 3.5 h. Unreacted **3d** (75.0 mg) was recovered from the former eluate by *n*-hexane-ethyl acetate (3:1, v/v). The latter eluate by the same eluent afforded **4d** as colorless prisms (from benzene) and then **4'd** as colorless needles (from benzene) respectively.

4d: yield 48%, mp 138-140 ℃ (decomp). FAB-MS *m/z*: 232 (MH^+ - 32), 264 (MH^+). IR (KBr) cm^{-1}: 3325 (NH), 1730, 1685 (CO). NMR ($CDCl_3$) δ: 3.86, 3.976 and 3.982 (each 3H, each s, Me x 3), 6.83 (1H, d, *J* = 2.2, 7-H), 6.93 (1H, dd, *J* = 9.0, *J* = 2.2, 5-H), 7.91 (1H, d, *J* = 9.0, 4-H), 9.18 (1H, br s, NH). *Anal*. Calcd for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32. Found: C, 59.03; H, 4.91; N, 5.06.

4'd: yield 33%, mp 150-152 °C. FAB-MS *m/z*: 232 (MH^+ - 32), 264 (MH^+). IR (KBr) cm^{-1}: 3360 (NH), 1715 (CO). NMR ($CDCl_3$) δ: 3.92, 3.93 and 3.99 (each 3H, each s, Me x 3), 6.54 (1H, br d, *J* = 8, 5-H),

7.00 (1H, dd, *J* = 8, *J* = 1, 7-H), 7.27 (1H, t, *J* = 8, 6-H), 8.95 (1H, br s, NH). Anal. Calcd for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32. Found: C, 59.45; H, 4.86; N, 5.11.

Dimethyl 5-Methoxy-1-*H*-indole-2,3-dicarboxylate (4e)

Compound (**3e**) (200 mg, 0.687 mmol) in MeOH (500 mL) was employed as a starting material. The reaction time was 15 min. After evaporation of the solvent the crystalline residue was recrystallized from benzene-*n*-hexane to give **4e** as pale yellow granules and unchanged **3e** (20.3 mg) was recovered from its mother liquor.

4e: yield 69%, mp 152-154 ℃ (decomp). FAB-MS *m/z*: 232 (MH^+ - 32), 264 (MH^+). IR (KBr) cm^{-1}: 3285 (NH), 1720, 1690 (CO). NMR ($CDCl_3$) δ: 3.89 (3H, s, methoxy Me), 3.99 (6H, s, ester Me), 7.04 (1H, dd, *J* = 9.2, *J* = 2.5, 6-H), 7.32 (1H, d, *J* = 9.2, 7-H), 7.48 (1H, d, *J* = 2.5, 4-H), 9.16 (1H, br s, NH). *Anal*. Calcd for $C_{13}H_{13}NO_5$: C, 59.31; H, 4.98; N, 5.32. Found: C, 59.43; H, 4.90; N, 4.95.

General Procedure of Preparation of 1,2,3,4-Tetrahydro-5*H*-pyridazino [4,5-*b*]-indole-1,4 (2*H*,3*H*)- diones (5)

A mixture of **4** and 100% hydrazine hydrate in EtOH (280 mL) was refluxed for appropriate period. After evaporation of the reaction mixture the resulting crystalline residue was dissolved in DMSO. The solution was acidified with diluted hydrochloric acid and precipitated crystalline solid was washed with water and recrystallized from an appropriate solvent to afford **5**.

1,2,3,4-Tetrahydro-5*H*-pyridazino[4,5-*b*]indole-1,4(2*H*,3*H*)-dione (5a)

A mixture of **4a** (1.30 g, 5.58 mmol) and 100% hydrazine hydrate (55.0 g, 1.10 mol) in EtOH (280 mL) was employed. The reflux time was 5 h. Recrystallization from MeOH gave **5a** as pale yellow powder, yield 92%, mp >300 °C (lit., >300 °C[5a], >330 °C[5b], >330 °C[5c]). FAB-MS *m/z*: 202 (MH^+), 403 ($2MH^+$). IR (KBr) cm^{-1}: 3420, 3135, 2980 (NH), 1638 (CO). NMR (DMSO-d_6) δ: 7.30 and 7.47 (each 1H, each br t, *J* = 7.3, 7- and 8-H), 7.61 (1H, br d, *J* = 7.3, 6-H), 8.11 (1H, br d, *J* = 7.3, 9-H), 11.55 (2H, s, NH x 2), 12.54 (1H, s, NH). *Anal*. Calcd for $C_{10}H_7N_3O_2 \cdot 1/5H_2O$: C, 58.65; H, 3.64; N; 20.52. Found: C, 58.98; H, 4.03; N, 20.54.

7-Hydroxy-1,2,3,4-tetrahydro-5*H*-pyridazino[4,5-*b*]indole-1,4(2*H*,3*H*)-dione (5b)

A mixture of **4b** (670 mg, 2.70 mmol) and 100% hydrazine hydrate (13.5 g, 270 mmol) in MeOH (68 mL) was employed. The reflux time was 5 h. Recrystallization from acetone gave **5b** as pale yellow powder, yield 74%, mp >300 °C. FAB-MS *m/z*: 218 (MH^+), 435 ($2MH^+$). IR (KBr) cm^{-1}: 3308, 3248, 3148 (NH and OH), 1650 (CO). NMR (DMSO-d_6) δ: 6.77 (1H, dd, *J* = 8.5, *J* = 2.0, 8-H), 6.91 (1H, d, *J*

= 2.0, 6-H), 7.85 (1H, d, *J* = 8.5, 9-H), 9.65 (1H, br s, OH or NH), 11.40 (2H, br, OH or NH), 12.13 (1H, s, OH or NH). *Anal*. Calcd for $C_{10}H_7N_3O_3$: C, 55.30; H, 3.25; N; 19.35. Found: C, 55.31; H, 3.56; N, 19.17.

9-Hydroxy-1,2,3,4-tetrahydro-5*H*-pyridazino[4,5-*b*]indole-1,4(2*H*,3*H*)-dione (5'b)

A mixture of **4'b** (150 mg, 0.602 mmol) and 100% hydrazine hydrate (3.00 g, 60.0 mmol) in MeOH (15 mL) was employed. The reflux time was 5 h. Recrystallization from MeOH gave **5'b** as white powder, yield 40%, mp >300 °C. FAB-MS *m/z*: 218 (MH^+). IR (KBr) cm^{-1}: 3437, 3167, 3032 (NH and OH), 1620 (CO). NMR (DMSO-d_6) δ: 6.55 and 6.99 (each 1H, each d, *J* = 8.0, 6- and 8-H), 7.30 (1H, t, *J* = 8.0, 7-H), 11.95 (1H, br s, OH or NH), 12.48 (1H, s, OH or NH). *Anal*. Calcd for $C_{10}H_7N_3O_3 \cdot 1/3H_2O$: C, 53.82; H, 3.46; N; 18.83. Found: C, 53.95; H, 3.66; N, 18.65.

6-Methoxy-1,2,3,4-tetrahydro-5*H*-pyridazino[4,5-*b*]indole-1,4(2*H*,3*H*)-dione (5c)

A mixture of **4c** (1.40 g, 5.32 mmol) and 100% hydrazine hydrate (42.0 g, 840 mmol) in EtOH (280 mL) was employed. The reflux time was 2 h. Recrystallization from EtOH gave **5c** as white powder, yield 73%, mp >300 °C (lit.,[6] >260 °C). FAB-MS *m/z*: 232 (MH^+). IR (KBr) cm^{-1}: 3310, 3065, 2970, 2890 (NH), 1655 (CO). NMR (DMSO-d_6) δ: 3.96 (3H, s, OMe), 6.99 and 7.68 (each 1H, each d, *J* = 7.3, 7- and 9-H), 7.21 (1H, t, *J* = 7.3, 8-H). *Anal*. Calcd for $C_{11}H_9N_3O_3 \cdot 1/6H_2O$: C, 56.41; H, 4.02; N; 17.94. Found: C, 56.69; H, 4.32; N, 17.94.

7-Methoxy-1,2,3,4-tetrahydro-5*H*-pyridazino[4,5-*b*]indole-1,4(2*H*,3*H*)-dione (5d)

A mixture of **4d** (170 mg, 0.650 mmol) and 100% hydrazine hydrate (14.0 g, 280 mmol) in MeOH (30 mL) was employed. The reflux time was 8.5 h. Recrystallization from acetone-*n*-hexane gave **5d** as pale yellow powder, yield 82%, mp >300 °C (lit.,[6] >260 °C). FAB-MS *m/z*: 232 (MH^+), 463 ($2MH^+$). IR (KBr) cm^{-1}: 3150, 3040, 2960 (NH), 1630 (CO). NMR (DMSO-d_6) δ: 3.84 (3H, s, OMe), 6.93 (1H, dd, *J* = 8.7, *J* = 2.1, 8-H), 7.01 (1H, d, *J* = 2.1, 6-H), 7.94 (1H, d, *J* = 8.7, 9-H), 11.45 (2H, br s, NH x 2), 12.40 (1H, br s, NH). *Anal*. Calcd for $C_{11}H_9N_3O_3 \cdot 1/4H_2O$: C, 56.05; H, 4.06; N; 17.83. Found: C, 56.23; H, 4.15; N, 17.91.

9-Methoxy-1,2,3,4-tetrahydro-5*H*-pyridazino[4,5-*b*]indole-1,4(2*H*,3*H*)-dione (5'd)

A mixture of **4'd** (430 mg, 1.63 mmol) and 100% hydrazine hydrate (13.0 g, 260 mmol) in EtOH (86 mL) was employed. The reflux time was 2 h. Recrystallization from acetone-*n*-hexane gave **5'd** as colorless granules, yield 69%, mp >300 °C (lit.,[6] >260 °C). FAB-MS *m/z*: 232 (MH^+). IR (KBr) cm^{-1}: 3285, 3035, 2910, 2750 (NH), 1650 (CO). NMR (DMSO-d_6) δ: 4.00 (3H, s, OMe), 6.84 and 7.21 (each

1H, each d, *J* = 8.0, 6- and 8-H), 7.42 (1H, t, *J* = 8.0, 7-H), 10.30, 11.70 and 12.80 (each 1H, each br, NH x 3). *Anal*. Calcd for $C_{11}H_9N_3O_3 \cdot 1/5H_2O$: C, 56.27; H, 4.04; N; 17.90. Found: C, 56.32; H, 4.27; N, 17.78.

8-Methoxy-1,2,3,4-tetrahydro-5*H*-pyridazino[4,5-*b*]indole-1,4(2*H*,3*H*)-dione (5e)

A mixture of **4e** (400 mg, 1.50 mmol) and 100% hydrazine hydrate (12.0 g, 240 mmol) in MeOH (50 mL) was employed. The reflux time was 3 h. Recrystallization from acetone-*n*-hexane gave **5e** as pale brown powder, yield 63%, mp >300 °C. FAB-MS *m/z*: 232 (MH^+). IR (KBr) cm^{-1}: 3125, 3040, 2960 (NH), 1650 (CO). NMR (DMSO-d_6) δ: 3.83 (3H, s, OMe), 7.10 (1H, dd, *J* = 9.0, *J* = 2.6, 7-H), 7.49 (1H, d, *J* = 9.0, 6-H), 7.53 (1H, d, *J* = 2.6, 9-H), 11.49 (2H, br s, NH x 2), 12.40 (1H, br s, NH). *Anal*. Calcd for $C_{11}H_9N_3O_3 \cdot 1/5H_2O$: C, 56.27; H, 4.04; N; 17.90. Found: C, 56.56; H, 4.26; N, 17.61.

Evaluation of Chemiluminescent Activity

The reaction solution contains 10 mM phosphate buffer (for pH 8) or 10 mM carbonate buffer (for pH 10), Triton X-100 (0.05%) and test compound (0.1 mg/mL). The concentration of hydrogen peroxide solution was 3 mM. *Arthromyces ramosus* peroxidase (ARP) solution was prepared as 730 U/mL. The reaction solution (3 mL) was transferred to a sample tube 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 after addition of 75 μL of hydrogen peroxide solution (final concentration is 0.22 mmol of hydrogen peroxide/3 mL of the reaction solution) and 75 μL of ARP solution (final concentration is 55 Unit of ARP/3 mL of the reaction solution).

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