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Bisindolylmaleimides with Large Stokes Shift and Long-Lasting Chemiluminescence Properties

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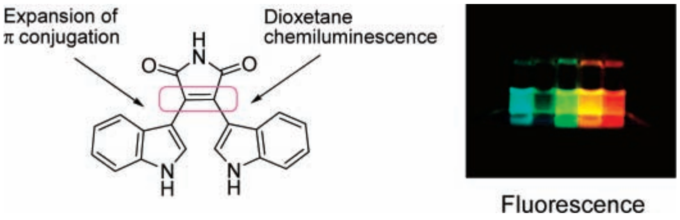
ABSTRACT



Various bisindolylmaleimides have fluorescence emission maxima wavelengths longer than 500 nm, large Stokes shifts longer than 200 nm, different fluorescence emission wavelengths at an excitation wavelength of 365 nm, and a long-lasting chemiluminescence. The expansion of the π-conjugation, the π-bond electronic structure, and oxidation of the C═C bond at the 2,3-position of the maleimide moiety are crucial for producing these fluorescence and chemiluminescence properties.

Fluorescence (FL), chemiluminescence (CL), and bioluminescence (BL) compounds have been extensively developed for luminescence assays.[1−3] Indole derivatives are known as FL and CL compounds.[4,5] Most indole derivatives previously investigated as FL and CL probes had disadvantageous properties of short FL maxima emission wavelength, small Stokes shifts, and weak CL intensity. These FL and CL properties caused limitations in highly selective and sensitive FL and CL assays. The widespread FL and CL assays with indole derivatives might be developed by removing the disadvantageous FL and CL properties. This led us to develop indole derivatives having FL emission maxima wavelengths longer than 500 nm, large Stokes shifts longer than 200 nm, and a strong CL intensity in the reaction with H_2O_2. We designed the indole derivatives based on two concepts as described below. First, the expansion of the indole π-conjugation will produce longer FL emission maxima wavelengths and large Stokes shifts.

Second, a C═C bond attached to the indole will react with H_2O_2 to give the desired dioxetane resulting in CL. Highly sensitive CL probes of dioxetane derivatives have been utilized for measuring enzyme activities and singlet oxygen.[6] When both strong maxima light emission intensity and the long-lasting CL are attained, an increase in total photon

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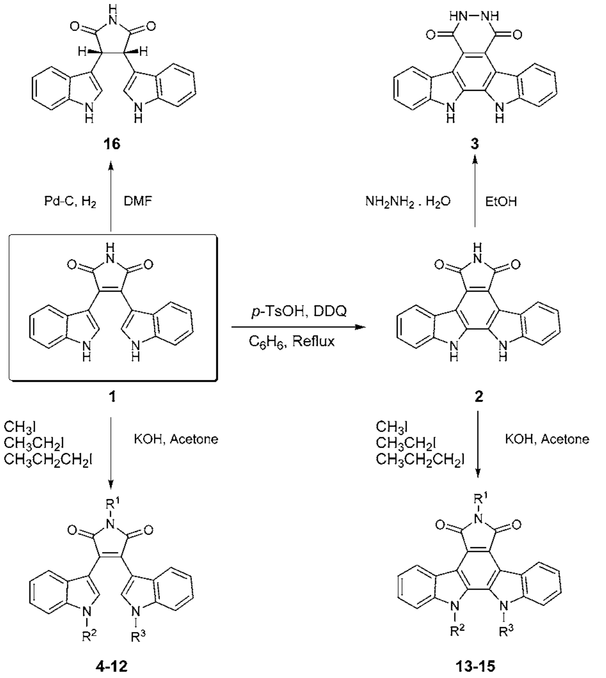
counts is obtained and an apparent increase in CL intensities is expected. On the basis of the above concepts, we selected bisindolylmaleimides (BIM) as candidates of a new probe. BIM have been developed as protein kinase inhibitors.[7] A little was studied about the FL and CL properties of BIM. BIM as red electroluminescence materials have been developed.[8] However, the CL properties of BIM were not evaluated. Recently, we found that 3,4-bis(3-indolyl)-1*H*-pyrrole-2,5-dione (**1**, Scheme 1) had a large Stokes shift and strong CL intensity when compared to those of indole.[9] Thus we synthesized various BIM and evaluated their FL and CL properties.

Scheme 1. Synthesis of **1**−**16**



compd	R^1	R^2	R^3	compd	R^1	R^2	R^3
4	CH_3	H	H	10	$CH_3CH_2CH_2$	H	H
5	CH_3	CH_3	H	11	$CH_3CH_2CH_2$	$CH_3CH_2CH_2$	H
6	CH_3	CH_3	CH_3	12	$CH_3CH_2CH_2$	$CH_3CH_2CH_2$	$CH_3CH_2CH_2$
7	CH_3CH_2	H	H	13	CH_3	CH_3	CH_3
8	CH_3CH_2	CH_3CH_2	H	14	CH_3CH_2	CH_3CH_2	CH_3CH_2
9	CH_3CH_2	CH_3CH_2	CH_3CH_2	15	$CH_3CH_2CH_2$	$CH_3CH_2CH_2$	$CH_3CH_2CH_2$

BIM **1**, which was synthesized from methyl 2-(3-indolyl)-glyoxylate and 2-(3-indolyl)acetamide in the presence of potassium *tert*-butoxide,[10] can be converted to the indolocarbazole, phtahlhydrazide, and *N*-alkyl substituted structures. Indolocarbazole **2** was synthesized via electrocyclization followed by aromaization with DDQ, and was converted into phthalhydrazide **3**. The *N*-alkylation of **1** and **2** with alkyl iodides in acetone rapidly proceeded to yield **4**−**12** and **13**−**15**, respectively. **16** was synthesized by the hydrogenation of **1** with 10% Pd−C catalyst (Scheme 1).[11]

The FL spectra of **1**−**3**, **6**, **13**, and luminol (3-aminophthalhydrazide) are shown in Figure 1. The Stokes shifts of **1**−**3** were 148, 192, and 201 nm, respectively. The FL quantum yields of **2** and **13** were 0.42 and 0.58, respectively.[12] The FL intensity of **2** was 7-fold higher than that of **1** in DMF (Table 1).

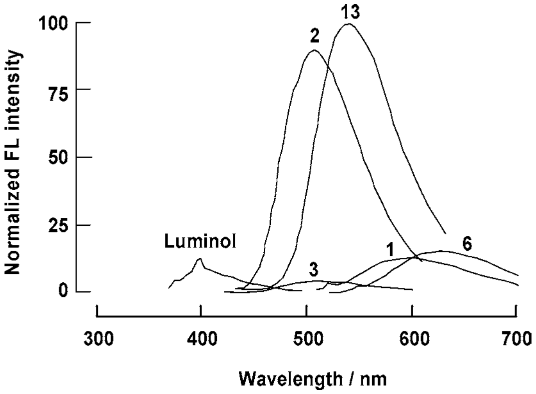


Figure 1. FL spectra of **1**−**3**, **6**, **13**, and luminol. 0.1 μM **1**−**3**, **6**, **13**, and luminol in DMF. The excitation wavelengths of **1**−**3**, **6**, **13**, and luminol were 452, 315, 316, 471, 323, and 357 nm, respectively.

The FL emission maximum wavelength of **3** was 517 nm. This wavelength is approximately 120 nm longer than that for luminol. The FL intensities of **4**−**12** were 0.25−1.4-fold compared to that of **1**. In contrast, the FL intensities of **13**−**15** apparently did not decrease compared to that of **2**. The FL emission maxima wavelengths of **4**−**12** were 615−630 nm and were longer than that (600 nm) of **1**. In the FL properties of **13**−**15**, similar results were observed. These results indicated that the FL emission maxima wavelengths shifted to longer wavelengths due to the *N*-alkylation of the maleimide and indole moieties. The FL lifetime of **13** was the longest among **1**−**3**, **6**, **13**, **16**, and luminol. According to Table 1, the FL lifetimes of **2** and **3** are similar but the FL quantum yield is very different. This means the difference of the quantum yield is not due to the contribution of the nonradiative path, but due to the difference of the real fluorescence rate constant.

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Table 1. Relative FL Intensities of **1**−**3**, **6**, **13**, **16**, and Luminol: Maximum Absorption Wavelength (λ_{abs}), Molar Absorption Coefficients (ϵ), Maximum Emission Wavelength (λ_{em}), FL Quantum Yield (Φ_f), and FL Lifetime (τ_f)

compd[a]	λ_{abs} (nm)	ϵ ($M^{-1}cm^{-1}$)	λ_{em} (nm)	Stokes shift (nm)	Φ_f	τ_f (ns)	RFL[b]
1	366	5300	595	229	0.31	11.5	52
	452	8150	600	148			100
2	315	42200	507	192	0.42	11.5	700
	400	4400	505	105			61
3	316	42700	517	201	0.02	12.8	34
6	373	5610	626	253	0.36	8.6	67
	471	8270	627	156			121
13	323	35700	538	215	0.58	19.8	776
16	282	9600					
luminol	295	7970	399	104	0.11	1.5	57
	357	8090	399	42	0.09		99

[a] 0.1 μM in DMF. [b] FL intensity (λ_{abs} 452 nm) of **1** in DMF was taken as 100.

The FL of 0.1 μM **16** in DMF was not observed. The FL emission wavelengths of 10 μM **16** in DMF were 326, 368, 387, and 503 nm at an excitation wavelength of 282 nm. The FL intensity and emission wavelengths of **16** were apparently low and short compared to that of **1**. **16** has no C═C bond in the linked moiety. Thus the presence of a C═C bond at the 2,3-position of the maleimide moiety affected the FL properties of BIM. The FL emission colors of **2**, **3**, **13**, **1**, and **9** were blue-green, green, green-yellow, yellow, and orange with UV irradiation at 365 nm, respectively (Figure 2). Different FL emission wavelengths were obtained at an excitation wavelength of 365 nm. BIM having full color FL emissions can be developed. These FL compounds should be useful for molecular imaging as FL probes at an excitation wavelength of 365 nm.

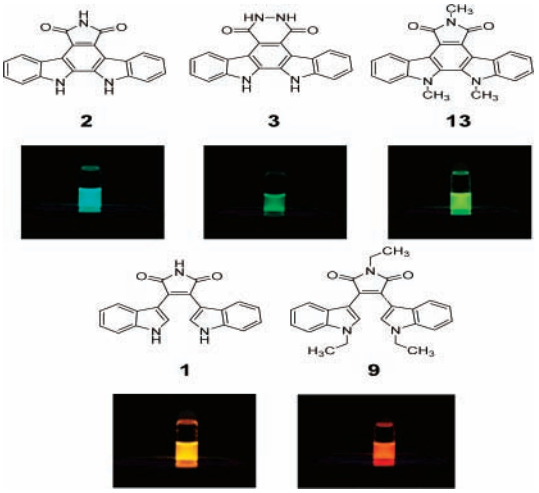

Figure 2. Photograph of **1**−**3**, **9**, and **13** with UV irradiation at 365 nm. The concentration of **1**−**3**, **9**, and **13** was 0.1 mM in DMF.

Table 2. Comparison of Experiments and Calculations at Absorption Peaks of Compounds **1** and **2**

compd	electronic state	exptl (nm)	calcd (nm)	E (cm^{-1})
$(\mathbf{1})^{1-}$	S_1	366	385	25959
$(\mathbf{1})^{2-}$	S_1	452	441	22697
$(\mathbf{2})^{1-}$	S_1	400	387	25870
$(\mathbf{2})^{1-}$	S_2	315	307	32545

To assign the absorption spectra listed in Table 2, ab initio molecular orbital (MO) configuration interaction (CI) calculations were carried out for **1** and **2**.[13] Table 2 lists the vertical excitation energies inferred from the present calculations by assuming the possible molecules involved in DMF; i.e., the monovalent and divalent anions ($(\mathbf{1})^{1-}$, $(\mathbf{1})^{2-}$, $(\mathbf{2})^{1-}$, and $(\mathbf{2})^{2-}$) and, moreover, the DMF complexes would exist. Our theoretical result suggested that monovalent ($(\mathbf{1})^{1-}$) and/or divalent ($(\mathbf{1})^{2-}$) anions would exist in DMF, because the two peaks (366 and 452 nm) measured in the experimental absorption spectrum of **1** would correspond to the vertical excitation energies, 385 nm of $(\mathbf{1})^{1-}$ and 441 nm of $(\mathbf{1})^{2-}$. The absorption spectra and FL intensities of **1** in 1−10% (v/v) triethylamine in DMF were similar to that of **1** in DMF. Thus the monovalent or divalent anions of **1** exist in the excited state in DMF.

The corresponding electronic structures to S_1 and S_2 states are described by the single electron excitation between the HOMO(85a) and LUMO(86a) and that between the HOMO-1(84a) and LUMO(86a), respectively. (See the Supporting Information for the numbering of MO.) Note that the HOMO-1, HOMO, and LUMO of all of the present possible molecules are quite similar to each of them. HOMO-1(84a), HOMO(85a), and LUMO(86a) feature the π bond of the indolyl group, the π and anti-π bonds around the 2,3-position of the maleimide moiety. In the case of **2**, we have a feature similar to **1**. The π-bond electronic structure around the maleimide moiety would be crucial for the FL properties of **1** and **2**.

The CL emission maxima of **1** were 420−440 nm. From the investigation of the products formed after the CL reaction of **1** using LC-ESI-MS, we speculated that the CL reaction of **1** proceeded via the decomposition of dioxetane formed after oxidation of the maleimide and indole moieties.[14] Futhermore, the molecular ion (*m/z* 419.37) of **17** was observed by using FAB MS (Figure 3).

The CL intensity data were recorded with very different conditions for **1**, **4**, **7**, **10**, and **16**. Apparently, the maxima light emission intensities and the lasting CL times of **1**, **4**, **7**, and **10** were stronger and longer compared to those of skatole (3-methylindole). **1** has a maximum CL intensity at about 2 min after the addition of H_2O_2, and the CL emission

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(13) The potential energies for the electronic excited states were computed with multireference configuration interaction (MRCI) methods. All these calculations were performed with the electronic structure program MOLPRO.

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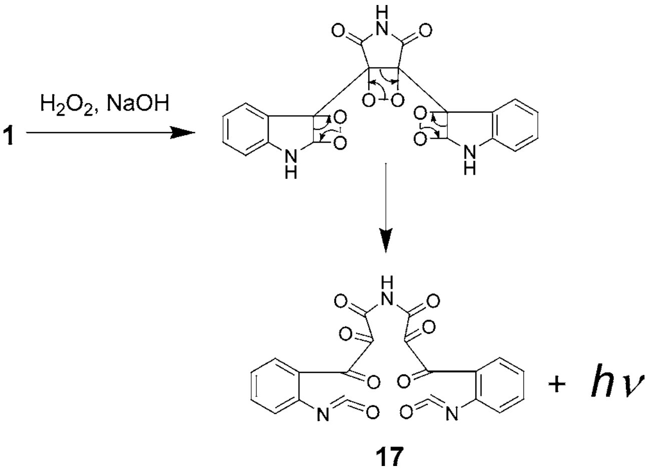

Figure 3. The possible CL mechanism of **1**.

decreases rapidly. In contrast, **4**, **7**, and **10** have maximum CL intensity within 10 min after the addition of H_2O_2, and the CL emissions decrease gradually (Figure 4).[15] The CL of **4**, **7**, and **10** showed a long-lasting CL property; thus, the lasting CL time was extended by the *N*-alkylation of the maleimide moiety in **1**. The CL intensities of **5**, **6**, **8**, **9**, **11**, and **12** in CH_3CN were in the range of 3−40% compared to those of **4**, **7**, and **10** (Table 3). This indicated that the CL intensity decreased apparently due to the *N*-alkylation of the indole ring.

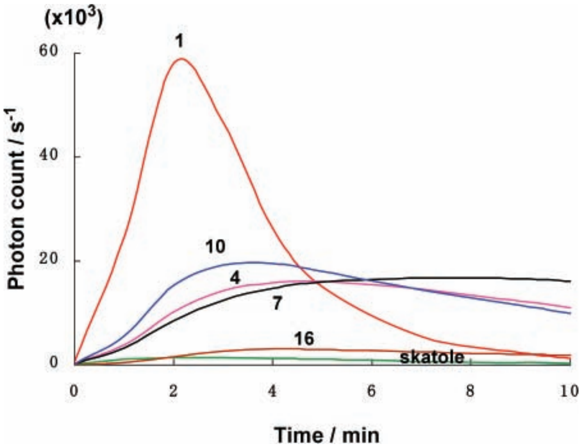

Figure 4. Time course of CL of **1**, **4**, **7**, **10**, **16**, and skatole. Red, purple, black, blue, brown, and green lines show the CL curves of **1**, **4**, **7**, **10**, **16**, and skatole, respectively. The concentrations of **1**, **4**, **7**, **10**, **16**, and skatole were 10 μM in CH_3CN. **1**: [NaOH] = 2.5 mM, [H_2O_2] = 63 mM. **4**: [NaOH] = 25 mM, [H_2O_2] = 6.3 mM. **7**: [NaOH] = 13 mM, [H_2O_2] = 6.3 mM. **10**: [NaOH] = 13 mM, [H_2O_2] = 13 mM. **16**: [NaOH] = 6.3 mM, [H_2O_2] = 63 mM. Skatole: [NaOH] = 100 mM, [H_2O_2] = 6.3 mM.

Table 3. Relative CL Intensities of **1**, **4**−**12**, **16**, and Skatole

compd[a]	NaOH (nM)	H_2O_2 (mM)	RCL[b]				
skatole	400	25	100				
1	10	250	2394	100			
4	100	25		65	100		
5	25	50			17		
6	5	250			4		
7	50	25		69		100	
8	25	10				26	
9	5	250				3	
10	50	50		75			100
11	25	25					40
12	25	250					3
16	25	250	270	11			

[a] 10 μM in CH_3CN. [b] CL intensity of skatole in CH_3CN was taken as 100.

1 may be a CL compound having this on−off CL property. This on−off CL property is utilized for probes measuring enzyme activities by synthesizing a CL substrate with functional groups which react with an enzyme. The CL intensity of **16** was 0.11-fold compared to that of **1**. This indicated that the dioxetane CL reaction proceeds in the C═C bond at the 2,3-position of the maleimide moiety of **1** and increased the CL intensity.

In summary, we developed BIM having long FL emission maxima wavelengths, large Stokes shifts, different FL emission wavelenghs at the exitation wavelength of 365 nm, and a long-lasting CL. The expansion of the π-conjugation, the π-bond electronic structure, and oxidation of the C═C bond at the 2,3-position of the maleimide moiety are crucial for producing these FL and CL properties based on our experiments and calculations. This study should provide significant facts for developing highly selective and sensitive chemical sensors with use of indole derivatives.

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Supporting Information Available: Experimental details and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The CL measurements of skatole, **1**, **4**−**12**, and **16** are as follows: To 200 μL of 10 μM skatole, **1**, **4**−**12**, and **16** in CH_3CN was added 100 μL of 0.5−600 mM NaOH. After allowing the solution to stand for 20 s, the CL reaction was initiated by the addition of 100 μL of 5−500 mM H_2O_2. The CL emission was measured for 10 min, and the integral photon counts were used for estimation of the CL intensities.