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Synthesis of boradiazaindacene–imidazopyrazinone conjugate as lipophilic and yellow-chemiluminescent chemosensor for superoxide radical anion

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ABSTRACT

As a chemiluminescent chemosensor that emits yellow light on reacting with a superoxide radical anion ($O_2^{\bullet-}$) and has a lipophilic character, a 6-phenylimidazo[1,2-*a*]pyrazin-3(7*H*)-one derivative possessing a boradiazaindacene (BODIPY) at the *para* position of 6-phenyl (**1**) was synthesized. The lipophilicity of **1** was investigated by reversed-phase liquid chromatography, and its log P_{ow} value was found to be 3.57. This value was much higher than that of 2-methyl-6-(4-methoxypheyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one (MCLA, log P_{ow}=1.19) and 6-[4-[2-{*N*′-(5-fluoresceinyl)thioureido}ethoxy]phenyl]-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (FCLA, log P_{ow}=−0.08), and it was comparable to that of benzenoid hydrocarbons. The $O_2^{\bullet-}$-induced chemiluminescence of **1** was investigated using the hypoxanthine/xanthine oxidase system as the source of $O_2^{\bullet-}$, and as a result, yellow emission was observed. The maximum wavelength was observed at 542 nm, and it was longer than that of FCLA.

1. Introduction

A superoxide radical anion ($O_2^{\bullet-}$) is a highly reactive free radical formed by the univalent reduction of oxygen during normal metabolism, and it plays important roles in biological systems. In mammalian cells, for example, $O_2^{\bullet-}$ acts as a signaling molecule that regulates important biological events such as protein phosphorylation,[1] cell growth,[2] and apoptosis.[3] Polymorphonuclear leukocytes and other phagocytes also produce $O_2^{\bullet-}$ as a chemical bomb to kill infections and ingested bacteria, respectively,[4] and to maintain healthy bodies. However, the increased production or insufficient extinction of $O_2^{\bullet-}$ causes cellular or tissue damage, inducing serious disease states such as ischemia reperfusion injury, Alzheimer's disease, and carcinogenesis.[4] Thus, the detection of production, distribution, and quantity of $O_2^{\bullet-}$ in cells and tissues is of particular clinical relevance.

2-Methyl-6-(4-methoxyphenyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one (MCLA) has been widely recognized as the most popular probe used for this purpose. MCLA can specifically react with $O_2^{\bullet-}$ immediately after its production to emit visible light (λ_{max}=465 nm in buffers at physiological pH conditions) without any light sources for excitation, accordingly enabling sensitive and real-time detection of $O_2^{\bullet-}$.[5] A fluorescein-linking MCLA derivative, 6-[4-[2-{*N*′-(5-fluoresceinyl) thioureido}ethoxy]phenyl]-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (FCLA), has been developed to detect $O_2^{\bullet-}$ at longer wavelength than MCLA.[6] On reacting with $O_2^{\bullet-}$ at physiological pH conditions, FCLA emits green light (λ_{max}=532 nm). This feature makes FCLA more useful than MCLA, because the blue light emitted by MCLA is absorbed by some biomaterials.[7] FCLA has the additional advantage of better water solubility over MCLA owing to the hydrophilicity of the fluorescein moiety, which is charged at the physiological pH conditions. However, because of the hydrophilic property, fluorescein-linking small molecules cannot easily permeate cell membranes. Therefore, the development of MCLA-based lipophilic chemiluminescent probes with emission at a longer wavelength than MCLA is still required for the successful detection of $O_2^{\bullet-}$ inside cells. For this purpose, we have previously synthesized imidazo[1,2-*a*]pyrazin-3(7*H*)-ones that have two lipophilic phenyl groups at the 6- and 8-positions of the imidazopyrazinone skeleton. Although these compounds exhibited $O_2^{\bullet-}$-induced chemiluminescence with an emission maxima around 500–530 nm in buffer solutions under basic conditions, their chemiluminescence spectra measured under neutral conditions showed peaks at around 400 nm.[8] In the present study, as part of our ongoing efforts to develop long-wavelength-light emitting imidazopyrazinones, we have designed a BODIPY-attached imidazopyrazinone, **1**. BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene) fluorophores are currently of special interest as substitutes for fluoresceins because of their superior photostability to fluorescein,[9] insensitivity toward solvent polarity and pH,[10] and sufficiently high lipophilicity to permeate cell membranes, as well as high extinction coefficients and fluorescence quantum yields approaching unity.[11–14]

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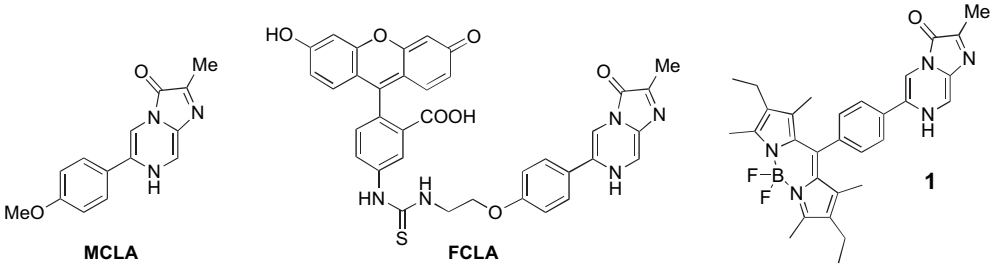
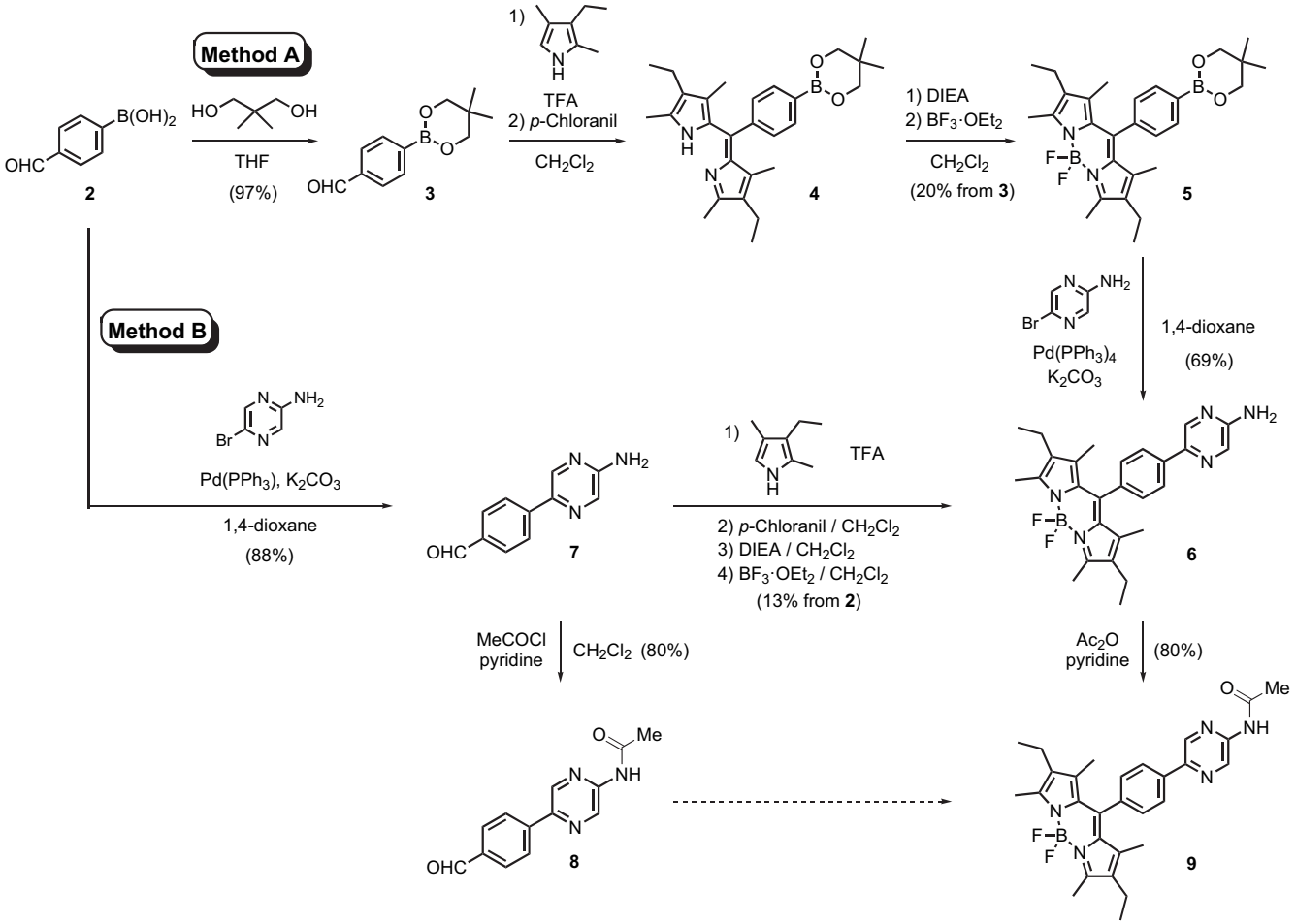
Herein, we describe the synthesis, lipophilicity, and chemiluminescence of the BODIPY-attached imidazopyrazinone that emits yellow light both in an aprotic organic solvent and in a buffer with an $O_2^{\cdot-}$-generating system at a physiological pH condition.



2. Results and discussion

2.1. Synthesis

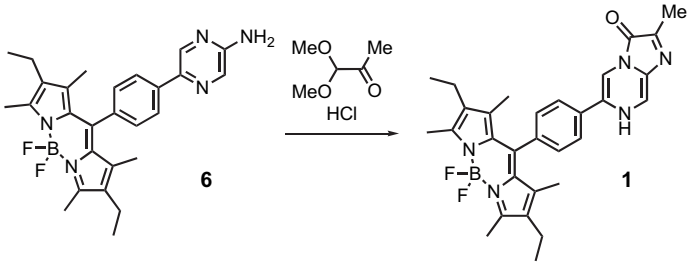
The synthesis of the BODIPY-attached imidazopyrazinone **1** was achieved as shown in Scheme 1. First, boronic acid **2** was protected with 2,2-dimethylpropan-1,3-diol to give boronate **3**.[15] Compound **3** was then reacted with 3-ethyl-2,4-dimethylpyrrole to give **4**, followed by successive treatment with chloranil and boron trifluoride etherate to afford borondipyrromethene **5**. The palladium-catalyzed cross-coupling reaction of **5** with 2-amino-5-bromopyrazine, prepared by the bromination of 2-aminopyrazine, gave BODIPY–aminopyrazine conjugate **6** (method A). We also followed an alternative reaction procedure (method B) that involves shorter steps than method A, beginning with the same starting material **2**. In this method, **2** was reacted with 2-amino-5-bromopyrazine to give 2-amino-5-(4-formylphenyl)pyrazine **7**, which was then successively reacted with pyrrole, chloranil, and boron trifluoride etherate to give **6**. The total yield of **6** from **2** via method A was 13%, while that via method B was 3%, apparently indicating that method A is superior to method B even though the number of steps in method A is more than method B. We considered that the existence of free amino group in **7** was unfavorable for the conversion of **7** to **6** and lead to the low yield via method B. To confirm this, we attempted the same reaction with *N*-acetyl derivative of **7** (**8**) to obtain **9** (in Scheme 1) but failed because **8** was hardly soluble in conventional organic solvents including dimethyl sulfoxide (DMSO).



Scheme 1.

Compound **6** was reacted with pyruvinaldehyde dimethyl acetal to form **1** (Table 1). For the first trial, we adopted the most popular reaction condition: the precursor aminopyrazines are reacted with α-ketoaldehydes or α-ketoalcetals in an alcohol in the presence of catalytic amount of concentrated hydrochloric acid.[8,16] The detailed reaction conditions are described as run 1 in Table 1. A thin-layer chromatographic analysis of the reaction mixture after 3 h at 80 °C indicated that multiple products were formed, none of them were fluorescent, and target compound **1** was not obtained. This result suggests that the borondipyrromethene moiety was unstable under the condition employed in this reaction, i.e., heating in ethanol in the presence of concentrated hydrochloric acid. To confirm this, **6** was treated with deuterium chloride in methanol-d_4 and monitored by ^{1}H NMR spectroscopy. The time course of the spectral change in the aromatic region is depicted in Figure 1. Obviously, the signals exhibited downfield shift within 330 min. This indicates that **6** was converted into another species. The direction of this spectral shift was opposite to that of borondipyrromethene formation from the corresponding dipyrromethene with boron trifluoride. Moreover, a fast-atom-bombardment mass spectrum of the converted product exhibited an m/z value at 426. This value coincided with the value calculated for [$C_{27}H_{31}N_5$+H$^+$] that corresponded to the protonated form of **6**. From these results, it could be safely concluded that the borondipyrromethene moiety was decomposed to give the corresponding deboronated dipyrromethene under the conditions employed for run 1 in Table 1. Then, we examined another experimental condition: 1,4-dioxane was employed as a solvent (run 2, Table 1). This condition has also been used to synthesize imidazopyrazinones.[17] Further, it has been reported that a BODIPY skeleton resisted a reaction involving treatment with 4-M HCl/1,4-dioxane mixture during the synthesis of a BODIPY-labeled sphingosine.[18] Consequently, **6** was successfully converted into target imidazopyrazinone **1**, as shown in Table 1. These results provided not only a successful method for preparing **1** but also useful information about handling BODIPY-containing compounds under strong acidic conditions.

Table 1
Reaction conditions for the synthesis of compound **1**



Run	Solvent	Temperature	Reaction period	Yield
1	EtOH	80 °C	3.5 h	(Decomp. of **6**)
2	1,4-Dioxane	80 °C	5.5 h	24%

2.2. Lipophilicity of BODIPY-attached imidazopyrazinone (1)

Lipophilicity is an important property that affects the transmembrane transport of chemical substances. Reversed-phase high performance liquid chromatography (RP-HPLC) using a C18 analytical column is one of the commonly adopted experimental methodologies for the measurement of lipophilicity of chemicals. Thus, the lipophilicity of newly synthesized imidazopyrazinone **1** was investigated by means of RP-HPLC. For comparison, MCLA and FCLA were also analyzed under the same conditions. As a result, the retention time (t_R) of **1**, MCLA, and FCLA was observed to be 11.04, 5.07, and 3.95 min, respectively (Table 2). This result suggests that **1** was the most lipophilic among the three compounds. In order to describe this result more quantitatively, the *n*-octanol/water partition coefficient (log P_{ow}), which is the common lipophilicity scale of chemicals, was estimated from the observed t_R. Generally, t_R obtained by RP-HPLC is related to the capacity factor (k') given by the following equation:[19]

$$k' = (t_R - t_0)/t_0 \quad (1)$$

where t_0 is the dead time. log P_{ow} of a test compound can be obtained by substituting the calculated k' value into the following equation:[19]

$$\log P_{ow} = a \log k' + b \quad (2)$$

where a and b are linear regression coefficients, which can be obtained by linearly regressing log P_{ow} of reference substances against their log k'. The k' and log P_{ow} values for **1**, MCLA, and FCLA were calculated using these equations and are listed after the t_R values in Table 2. As expected, FCLA was the most hydrophilic among them with a negative log P_{ow} value (−0.06), showing that FCLA was miscible with water up to the same extent as methylformamide (log P_{ow}=−0.06).[20] The lipophilicity of MCLA (log P_{ow}=1.18) was found to be almost the same as that of benzylalcohol (log P_{ow}=1.1).[20] log P_{ow} of **1** was estimated to be 3.57, and it was much larger than that of MCLA and FCLA. Judging from its log P_{ow} value, the lipophilicity of **1** was higher than that of benzenoid hydrocarbons such as *p*-xylene (log P_{ow}=3.15)[20] and naphtharene (log P_{ow}=3.37)[21] and similar to that of propranolol (log P_{ow}=3.56),[22] a naphtharene-containing beta-adrenergic receptor blocking agent. Thus, it was confirmed that introducing the BODIPY unit markedly increased the lipophilicity of the parent molecule, and the lipophilicity of **1** was much higher than that of the other two imidazopyrazinones.

2.3. Chemiluminescent property of BODIPY-linking imidazopyrazinone (1)

First, in order to verify that the emission from **1** originated from the BODIPY unit, we carried out the chemiluminescent reaction of **1** in aerated DMSO, which has been commonly used for performing chemiluminescence measurements of imidazopyrazinones and for affording brighter luminescence as compared to the reaction in aqueous solutions.[23] A chemiluminescence spectrum of **1** is shown in Figure 2, along with the chemiluminescence spectra of MCLA and FCLA measured under the same conditions. Compound **1** was confirmed to emit yellow light with a luminescence maximum at 557 nm, while FCLA gave luminescence with a maximum at 542 nm. These luminescence peaks apparently originated from the BODIPY moiety of **1** and the fluorescein moiety of FCLA, respectively. It is noteworthy that the emission band of **1** was red-shifted as compared to that of FCLA. These spectra revealed the emission of a small amount of blue light at about 460 nm, indicating that the luminescence energy produced at the MCLA moiety was not completely transferred to the energy acceptors, BODIPY, and fluorescein units during the chemiluminescent reaction in DMSO. The BODIPY fluorophore in **1** was directly connected to the imidazopyrazinone-phenyl group π conjugating system, and therefore, we did not expect **1** to exhibit bimodal luminescent behavior at first. Presumably, the phenyl ring and BODIPY unit were twisted owing to the two methyl groups at the C1 and C7 positions of the BODIPY skeleton, and they were conjugatively disconnected.[24]

The chemiluminescence of **1** with $O_2^{\cdot-}$ was measured in a phosphate buffer using the hypoxanthine/xanthine oxidase system as an $O_2^{\cdot-}$ generator.[8] Chemiluminescence spectra of MCLA and FCLA were

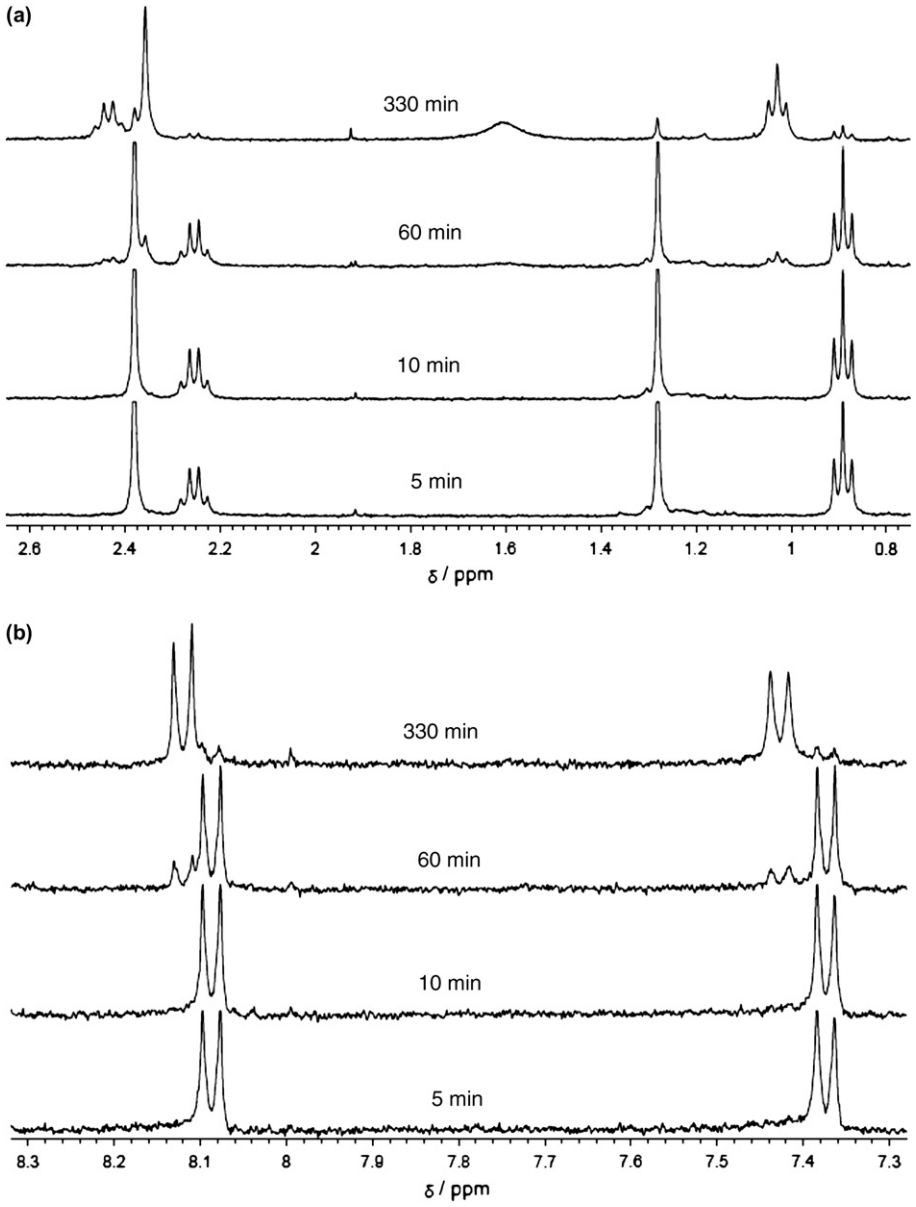


Figure 1. Time course of change in the ^{1}H NMR spectrum of **6** (a) in the alkyl region (δ 0.8–2.5 ppm) and (b) in the aromatic region (δ 7.3–8.7 ppm) measured in methanol-d_4 containing deuterium chloride.

Table 2
Retention time (t_R), capacity factor (k'), and log P_{ow} of the imidazopyrazinones (**1**, MCLA, and FCLA) obtained from the RP-HPLC analysis at 25 °C[a]

Compound	t_R/min	k'	log P_{ow}
1	11.04	0.46	3.57
MCLA	5.07	−0.12	1.18
FCLA	3.95	−0.42	−0.06

[a] Column: SHISEIDO Capcell Pak C18; id: 250 mm×4.6 mm; mobile phase: 80% MeOH; flow rate: 0.7 mL/min.

measured under the same conditions for comparison, and all of the observed spectra are shown in Figure 3. As shown in the figure, the chemiluminescence maximum of **1** was observed at 545 nm. This luminescence was consistent with the fluorescence of **9**, prepared separately from **6** as shown in Scheme 1, proving the light emitter involved in the chemiluminescent reaction of **1** was the corresponding oxidized product **9** (see Supplementary data). The luminescence maximum of **1** was observed at longer wavelength than that of FCLA (532 nm), similar to the observation in DMSO. Under this condition, either **1** or FCLA exhibited a single luminescence peak; the absence of emission originating from the MCLA moiety. This indicated that the luminescence energy generated at the MCLA moiety was efficiently transferred to the fluorescent chromophores under the $O_2^{\bullet-}$-induced chemiluminescent reaction condition. The reason for the difference in the emission intensities from the MCLA moiety in DMSO and in buffer solution was unclear. One possible reason was that the electronic structures of the light emitters involved in the reaction of **1** and FCLA in DMSO were different from those in the buffer solution. During the oxidative chemiluminescent reaction, an imidazopyrazinone reacts with a molecular oxygen or reactive oxygen species such as $O_2^{\bullet-}$ to afford a singlet-excited amidopyrazine, which decays to the ground state, accompanied by light emission, as shown in Scheme 2. The electronic structures of the singlet-exited amidopyrazines involved in the reactions in DMSO have been confirmed to be of amide anion

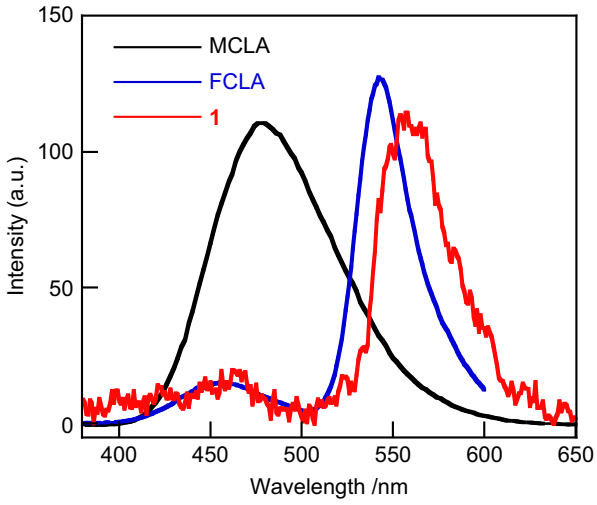


Figure 2. Chemiluminescence spectra of MCLA (black line), FCLA (blue line), and **1** (red line) in aerated DMSO at 25 °C. The final concentration of each probes, 50 μM.

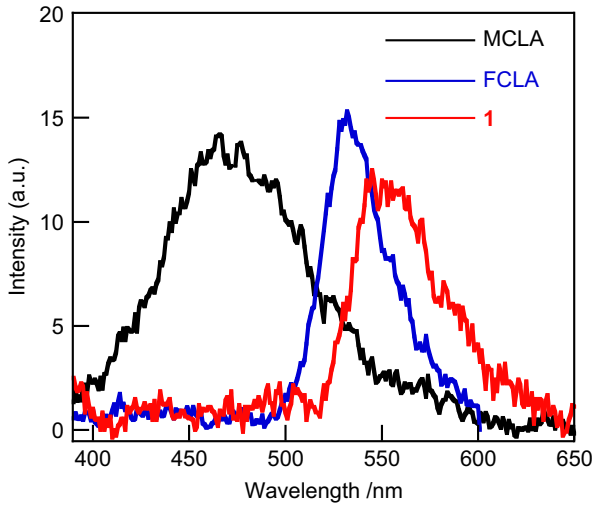


Figure 3. $O_2^{\bullet-}$-induced chemiluminescence spectra of MCLA (black line), FCLA (blue line), and **1** (red line) in 10 mM phosphate buffer (pH 8.6) containing 100 mM KCl, 0.05 mM EDTA, 0.15 mM hypoxanthine, and 0.02 unit/mL xanthine oxidase at 25 °C. The final concentration of each probes, 50 μM.

forms,[16b] while those in the reaction with an $O_2^{\bullet-}$-producing system at physiological pH conditions are known to be neutral.[8,25] Another possible reason for the difference in the emission intensities was that the emission was rather quenched in buffer solution. Researches on the luminescent properties of amidopyrazines have shown that their fluorescence intensity decreased in protic solvents.[26] This fluorescence quenching may be caused by the vibrational deactivation of the singlet-excited energy through hydrogen bonding. Therefore, the luminescence intensity for **1** as well as FCLA was likely to reduce to levels where the emission originating from the MCLA moiety could not be detected.

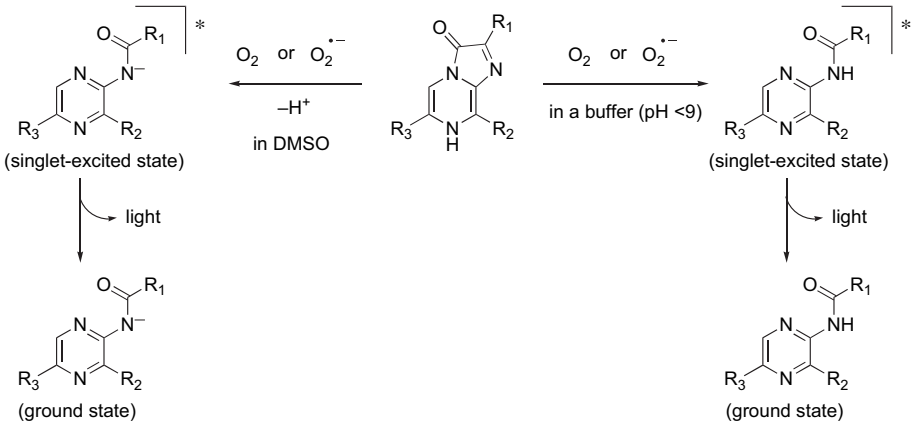
3. Conclusion

We have successfully synthesized BODIPY-attached imidazopyrazinone **1**. The synthetic investigation also revealed that the BODIPY unit is unstable when the reaction is carried out using hydrochloric acid in ethanol. The RP-HPLC analyses have clearly demonstrated that the BODIPY unit substantially enhances the lipophilicity of the imidazopyrazinones. Further, it may be worth noting that this work provides the first quantitative data, log P_{ow} values, of the imidazopyrazinones. The chemiluminescence studies have revealed that the luminescence energy produced at the imidazopyrazinone moiety in **1** is effectively transferred to the BODIPY unit to emit yellow light with a maximum wavelength longer than that of FCLA. Overall, we may say that we have succeeded in developing a yellow-light-chemiluminescent $O_2^{\bullet-}$ probe with a rather lipophilic character.

4. Experimental

4.1. General

All melting points were measured on MP-21 apparatus (Yamato Scientific Co., Ltd., Japan) in open capillary tubes; the values were uncorrected. ^{1}H NMR spectra were recorded on a JNM-ECP400 spectrometer (JEOL Ltd., Japan). Chemical shifts (δ) are reported in ppm and were measured using tetramethylsilane or an undeuteriated solvent as an internal standard in the deuterated solvent used. Coupling constants (J) are given in Hz. Chemical shift multiplicities are reported as s=singlet, d=doublet, t=triplet, q=quartet, and m=multiplet. Infrared (IR) spectra were obtained using FT/IR-4100, FT/IR-460plus, or FT/IR-660plus spectrophotometers (JASCO Co., Ltd., Japan). Fast-atom-bombardment (FAB) mass spectra were measured on a JMS-600-H mass spectrometer (JEOL Ltd., Japan). Xenon was used as a bombardment gas, and all the analyses were carried out in a positive mode with



Scheme 2.

the ionization energy and accelerating voltage set at 70 eV and 3 kV, respectively. A mixture of dithiothreitol and α-thioglycerol (1:1 or 1:2) was used as a liquid matrix. High and low resolution electron impact (EI) mass spectra were obtained with a JMS-AM II 50 mass spectrometer (JEOL Co., Ltd., Japan). The ionization energy was 70 eV, and the accelerating voltages were 0.3 kV for the low resolution and 0.5 kV for the high resolution analyses. Absorption spectra were measured on a V-650 spectrophotometer (JASCO Co., Ltd., Japan), and combustion analyses were performed on a MT-6 analyzer (Yanaco New Science Inc., Japan). Column chromatography was carried out on silica gel (particle size: 63–210 μm; Kanto Chemical Co.). Chemiluminescence spectra were collected with a JASCO F-777 spectrofluorometer (excitation light was shut off and only the light detector was used to measure the emitted light; bandpass of the detector: 10 nm; scan speed: 1000 nm/min for chemiluminescence in DMSO and 2000 nm/min for $O_2^{\cdot-}$-induced chemiluminescence).

MCLA, FCLA, and hypoxanthine were purchased from Tokyo Chemical Industry Co., LTD. (Japan). HPLC-grade methanol and water were obtained from Kanto Chemical Co. Inc. (Japan). Xanthine oxidase (from buttermilk) was purchased from EMB Bioscience Inc. (CA, USA). Other conventional chemicals used in the present study are commercially available and were used as received.

4.2. Synthesis

*4.2.1. 2,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-8-[4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl]-4-bora-3a,4a-diaza-s-indacene (**5**).* To a solution of **3** (401 mg, 1.84 mmol) and 2,4-dimethyl-3-ethylpyrrole (0.46 g, 3.7 mmol) in 200 mL of dichloromethane was added one drop of trifluoroacetic acid (TFA), and the mixture was stirred overnight at room temperature under argon. After confirming the disappearance of **3** by thin-layer chromatography (TLC), *p*-chloranil (453 mg, 1.84 mmol) in dichloromethane (50 mL) was added to the reaction mixture over a 35-min time period. *N*,*N*-Diisopropylethylamine (DIEA, 1.5 g, 11.5 mmol) was then added to the mixture, which was stirred at room temperature for another 30 min under argon. Boron trifluoride–diethyl ether complex (1.9 g, 12 mmol) was added dropwise to the reaction mixture, and stirring was continued for 2 h. The reaction mixture was washed with water and dried over $MgSO_4$. After evaporating the solvent, the obtained crude materials were purified by column chromatography on silica gel (46–50 μm, chloroform) to give **5** as an orange solid (185 mg, 20%). Mp 247 °C (decomp.); IR (KBr) ν_{max}/cm^{-1} 2962, 2928, 2870 (νC–H), 1540, 1475 (C=C, C=N, ring stretching), 1318 (νB–O), 1190, 977 (νB–N), 717 (γC–H); ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 7.89 (d, 2H, *J*=8.4 Hz), 7.27 (d, 2H, *J*=8.4 Hz), 3.82 (s, 4H), 2.53 (s, 6H), 2.29 (q, 4H, *J*=7.5 Hz), 1.26 (s, 6H), 1.07 (s, 6H), 0.97 (t, 6H, *J*=7.5 Hz); MS (FAB$^+$, *m*-NBA) 492 [M]$^+$; HRMS (positive EI) calcd for $C_{28}H_{36}B_2F_2N_2O_2$ 492.2931, found: 492.2938.

*4.2.2. 3-Ethyl-5-((1Z)-(4-ethyl-3,5-dimethyl-2H-pyrrol-2-ylidene)(4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)phenyl)methyl)-2,4-dimethyl-1H-pyrrole (**4**).* In order to confirm that **4** was formed during the above synthesis of **5** from **3**, the following experiment was achieved: To a solution of **3** (251 mg, 1.15 mmol) and 3-ethyl-2,4-dimethyl-1*H*-pyrrole (0.30 g, 2.5 mmol) in 100 mL of dichloromethane was added one drop of trifluoroacetic acid (TFA), and the mixture was stirred overnight at room temperature under argon. After confirming the disappearance of **3** by TLC, *p*-chloranil (285 mg, 1.16 mmol) in dichloromethane was added to the reaction mixture. The mixture was further stirred for 1.5 h, and then, the reaction was quenched with water. The organic layer was separated, and the water layer was further extracted with chloroform. The combined organic layer was evaporated and partially purified by column chromatography on alumina (Merck, 300 mesh) with hexane/ethyl acetate (7/3, v/v) as the eluent to give the target product as a reddish powder. ^{1}H NMR (DMSO-d_6, 400 MHz) δ/ppm 7.80 (d, 2H, *J*=7.7 Hz), 7.20 (d, 2H, *J*=7.7 Hz), 4.14 (s, 6H), 3.79 (s, 6H), 2.23 (q, 4H, *J*=7.4 Hz), 0.98 (s, 6H), 0.91 (t, 6H, *J*=7.4 Hz). This material was not further assigned.

*4.2.3. 8-[4-(5-Aminopyrazin-2-yl)phenyl]-2,6-diethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (**6**) via method A.* To a solution of 2-amino-5-bromopyrazine (17.3 mg, 99.4 μmol) and **5** (50.0 mg, 102 μmol) in 10 mL of 1,4-dioxane were successively added tetrakis(triphenylphosphine)palladium (8.9 mg, 7.7 μmol) and 0.25 mL of 2-M K_2CO_3, and the mixture was refluxed for 3 h under argon. After cooling to room temperature, the reaction mixture was washed with water and dried over $MgSO_4$. The evaporation of the solvent gave the crude product, which was purified by column chromatography on silica gel (46–50 μm) with hexane/ethyl acetate (7/3, v/v) as the eluent to afford **6** as a red solid (32.5 mg, 69%). Mp 258 °C (decomp.); IR (KBr) ν_{max}/cm^{-1} 3423, 3318 (νN–H), 2960, 2925, 2870 (νC–H), 1634 (δN–H), 1541, 1475 (C=C, C=N, ring stretching), 1194, 979 (νB–N), 721 (γC–H); ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 8.56 (d, 1H, *J*=1.4 Hz), 8.11 (d, 1H, *J*=1.4 Hz), 8.03 (d, 2H, *J*=8.1 Hz), 7.38 (d, 2H, *J*=8.1 Hz), 2.54 (s, 6H), 2.30 (q, 4H, *J*=7.5 Hz), 1.35 (s, 6H), 0.98 (t, 6H, *J*=7.5 Hz); MS (FAB$^+$, DTT/TG=1/2) 473 [M]$^+$; HRMS (positive EI) calcd for $C_{30}H_{32}BF_2N_5O$ 473.2562, found: 473.2539.

*4.2.4. 8-[4-(5-Aminopyrazin-2-yl)phenyl]-2,6-diethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (**6**) via method B.* To a solution of **7** (50.4 mg, 0.253 mmol) and 2,4-dimethyl-3-ethylpyrrole (73 mg, 0.59 mmol) in 150-mL dichloromethane was added one drop of trifluoroacetic acid (TFA), and the mixture was stirred overnight at room temperature under argon. After confirming the disappearance of **7** by TLC, *p*-chloranil (63.4 mg, 0.258 mmol) in dry CH_2Cl_2 was added to the stirred reaction mixture over a 45-min time period. *N*,*N*-Diisopropylethylamine (DIEA, 0.22 g, 1.7 mmol) was added to the reaction mixture, and the resulting mixture was stirred at room temperature for 20 min under argon. Boron trifluoride–diethyl ether complex (0.23 g, 1.6 mmol) was added dropwise to the mixture. After stirring for additional 1.5 h, the reaction mixture was washed with water and dried over $MgSO_4$. After evaporating the solvent, the obtained crude product was purified by column chromatography on silica gel (46–50 μm) with chloroform as the eluent to afford **6** as an orange solid (5.2 mg, 43%).

*4.2.5. 2-Amino-5-(4-formylphenyl)pyrazine (**7**).* To a solution of 2-amino-5-bromopyrazine (80.3 mg, 0.461 mmol) and 2-(4-formylphenyl)boronic acid (79.8 mg, 0.532 mmol) in 1,4-dioxane (2.0 mL) was successively added tetrakis(triphenylphosphine)palladium (22.1 mg, 19.1 μmol) and 2-M K_2CO_3 (0.6 mL), and the reaction mixture was refluxed for 3 h under argon. After cooling to room temperature, chloroform (20 mL) and water (20 mL) were added to the reaction mixture, and the organic phase was separated. The aqueous layer was extracted with one portion of chloroform (20 mL), and the combined organic phase was dried over $MgSO_4$. After evaporating the solvent, the resulting brownish yellow solid was purified by recrystallization from ethyl acetate/hexane to give **7** as a yellow powder (45.3 mg, 88%). The residual filtrate was concentrated and further purified by preparative TLC on silica gel to afford **7** as a yellow powder (21.7 mg). The total yield of **7** was 67.0 mg (73%). Mp>360 °C; IR (KBr) ν_{max}/cm^{-1} 3407, 3317 (νN–H), 2855, 2731 (νH–CO), 1687 (νC=O), 1605 (δN–H), 1545, 1395 (C=C, C=N, ring stretching), 829 (γC–H); ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm 10.05 (s, 1H), 8.54 (s, 1H), 8.09 (s, 1H), 8.06 (d, 2H, *J*=8.4 Hz), 7.96 (d, 2H, *J*=8.4 Hz); MS (FAB$^+$,

m-NBA) 200 $[M+H]^+$; HRMS (positive EI) calcd for $C_{11}H_9N_3O$ 199.0746, found: 199.0744.

*4.2.6. 2-Acetamido-5-(4-formylphenyl)pyrazine (**8**).* To a solution of 2-amino-5-(4-formyl)pyrazine (190.4 mg, 0.956 mmol) and pyridine (10 mL, 0.124 mol) in chloroform (20 mL) was added dropwise acetyl chloride (473 mg, 6.03 mmol), and the mixture was stirred under argon for 40 h. After evaporation of the solvent, water was added to the residue and the resulting precipitates were collected by suction filtration. The obtained materials were washed with water and methanol to afford the aimed compound as yellow solids. (184.3 mg, 80%). Mp>360 °C; IR (KBr) ν_{max}/cm^{-1} 3476, 3285 (νN–H), 3066 (νC–H), 2846, 2741 (νH–CO), 1693 (νC=O), 1603, 1544, 1508, 1468 (C=C, C=N, ring stretching), 837 (γCH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ/ppm 11.00 (1H, s), 10.08 (1H, s), 9.44 (1H, s), 9.13 (1H, s), 8.34 (1H, d, *J*=7.7 Hz), 8.04 (1H, d, *J*=7.7 Hz), 2.17 (3H, s); MS (FAB$^+$, DTT:TG=1:1) 242 $[M+H]^+$; HRMS (positive EI) calcd for $C_{13}H_{11}N_3O_2$ 241.0851, found: 241.0852.

*4.2.7. 6-[4-(2,6-Diethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacen-8-yl)phenyl]-2-methyl-3,7-dihydroimidazo[1,2-a]pyrazin-3(7H)-one (**1**).* To a solution of **6** (15.6 mg, 33.0 μmol) and pyruvicaldehyde dimethyl acetal (32 mg, 273 μmol) in 1,4-dioxane (1.5 mL), through which argon gas was passed for 30 min to drive out dissolving oxygen, was added two drops of 2-M HCl, and the mixture was stirred at 80 °C for 5.5 h. After cooling to room temperature, the solvent was evaporated and crude materials were purified by chromatography on silica gel (46–50 μm) with chloroform/methanol (6/1, v/v) as the eluent to give **1** as a red solid (4.0 mg, 24%). Mp 213 °C (decomp.); IR (KBr) ν_{max}/cm^{-1} 3422 (νN–H), 2962, 2926, 2869 (νC–H), 1629 (νC=O), 1542, 1476 (C=C, C=N, ring stretching), 1193, 981 (νB–N), 712 (γC–H); ^{1}H NMR (CD_3OD, 400 MHz) δ/ppm 8.03 (s, 1H, 6-pyrazine), 7.95 (d, 2H, *J*=8.0 Hz), 7.92 (s, 1H), 7.52 (d, 2H, *J*=8.0 Hz), 2.49 (s, 6H), 2.47 (s, 3H), 2.36 (q, 4H, *J*=7.5 Hz), 1.39 (s, 6H), 1.00 (t, 6H, *J*=7.5 Hz); MS (FAB$^+$, DTT/TG=1/2) 528 $[M+H]^+$; HRMS (positive EI) calcd for $C_{30}H_{32}BF_2N_5O$ 527.2668, found: 527.2666.

*4.2.8. 2-Acetamido-5-[4-(2,6-diethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacen-8-yl)phenyl]pyrazine (**9**).* To a solution of **6** (59.7 mg, 126 μmol) in pyridine (1.0 mL) was added acetic anhydride (389 mg, 3.81 mmol), and the mixture was stirred at 50 °C for 9 days. After cooling to room temperature, ethyl acetate (15 mL) was added to the reaction mixture and washed with 5% citric acid (15 mL, three times). The organic phase was dried over sodium sulfate, and the solvent was removed under reduced pressure. The resulting material was purified by column chromatography on silica gel (63–200 μm, 34 g) with chloroform as an eluent to give **9** as a solid (52.3 mg, 80%). Mp 260 °C (decomp.); IR (KBr) ν_{max}/cm^{-1} 2965, 2928, 2870 (νC–H), 1665 (νC=O), 1544, 1473 (C=C, C=N, ring stretching), 1192, 978 (νB–N), 758 (γC–H); ^{1}H NMR (CD_3OD, 400 MHz) δ/ppm 9.61 (s, 1H), 8.77 (d, 1H, *J*=1.5 Hz), 8.14 (d, 2H, *J*=8.4 Hz), 7.91 (s, 1H), 7.43 (d, 2H, *J*=8.4 Hz), 2.54 (s, 6H), 2.30 (q, 4H, *J*=7.3 Hz), 2.30 (s, 3H), 1.35 (s, 6H), 0.98 (t, 6H, *J*=7.3 Hz); MS (EI$^+$, DTT/TG=1/2) 516 $[M+H]^+$; HRMS (positive EI) calcd for $C_{29}H_{32}BF_2N_5O$ 515.2668, found: 515.2678.

4.3. Determination of lipophilic parameter log P_{ow} by reversed-phase HPLC

An HPLC analysis was performed on a Capcell Pak C18 SG120 column (particle size: 5 μm, id: 250 mm×4.6 mm, SHISEIDO Co., Ltd., Japan) with an SSC-3461 pump (SENSHU SCIENTIFIC Co. Ltd., Japan), a DG-1110 degasser (SENSHU SCIENTIFIC Co. Ltd., Japan), and an 875-UV Intelligent UV/VIS detector (JASCO Co., Ltd., Japan). HPLC-grade methanol and water were used as eluents. Analytes were eluted with 80% (v/v) methanol in water at a flow rate of 0.7 mL/min and were detected at 254 nm. Measurements were performed at 25 °C, controlled by a SSC-2300 column oven (SENSHU SCIENTIFIC Co. Ltd., Japan). Tartrazin (purchased from Tokyo Chemical Industry Co., LTD., Japan) was used as an unretained compound to determine the dead time (t_0).

A series of standard compounds including phenol (log P_{ow}=1.46), benzene (log P_{ow}=2.13), bromobenzene (log P_{ow}=2.99), naphtharene (log P_{ow}=3.37), biphenyl (log P_{ow}=3.98), and phenanthrene (log P_{ow}=4.57) were analyzed to generate a calibration curve. Each standard was injected three times, and the average log k' values obtained from the RP-HPLC analysis were plotted versus their known literature log P_{ow} values. The data points were subjected to least-squares fitting to give a straight line (Fig. 4). Parameters *a* and *b* in Eq. 2 were determined from the slope and the intercept of this straight line, respectively. The imidazopyrazinones (**1**, MCLA, and FCLA) were also analyzed three times each to obtain their average log k' values. Substituting the obtained values for log k' in Eq. 2 yielded the log P_{ow} values of the imidazopyrazinones. The variability in the determination of the k' value from the replicate injections of the analytes was between 2.3 and 5.3% (Table 3).

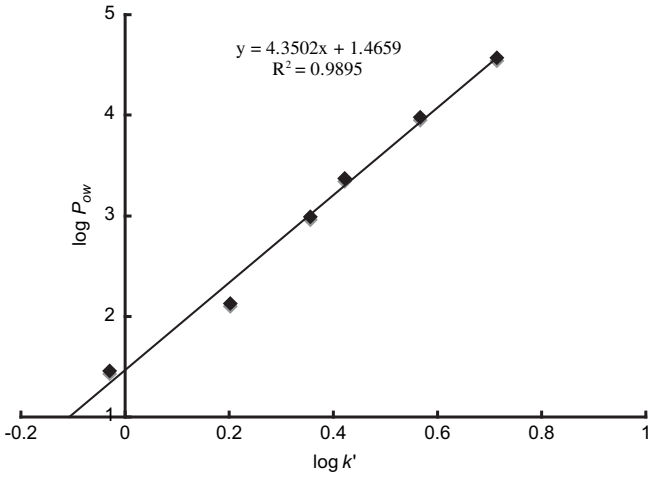


Figure 4. Calibration curve generating by plotting log P_{ow} against log k' obtained by the RP-HPLC for a series of standards (phenol, benzene, bromobenzene, naththarene, biphenyl, and phenanthrene).

Table 3
Precision of k' value determination by RP-HPLC for **1**, MCLA, FCLA, and standards

Compound	k'	%RSD
1	3.04	3.2
MCLA	0.86	3.3
FCLA	0.45	3.5
Phenol	0.90	5.3
Benzene	1.59	0.0
Bromobenzene	2.27	4.0
Naphtharene	2.64	0.0
Biphenyl	3.69	2.3
Phenanthrene	5.11	3.4

4.4. Chemiluminescence measurement

Chemiluminescent reactions in DMSO were achieved by mixing 1.0-mM solution of an imidazopyrazinone in methanol (50 μL) and DMSO containing 0.1 vol % of 10% NaOH (950 μL) in a quartz cuvette (path length: 1 cm) at 25 °C. The chemiluminescence induced by $O_2^{\cdot-}$ was measured in a mixture of 50-μL methanol solution of an imidazopyrazinone (1 mM) and 930 μL of 0.01 mol/L phosphate

buffer (pH 8.6) containing 100-mM KCl, 0.05-mM EDTA, and 0.15-mM hypoxanthine; light emission was started by adding xanthine oxidase (1.0 unit/mL, 20 μL) at 25 °C.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2009.11.086.

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