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WELL-DIVIDED AND pH-DEPENDENT BIMODAL CHEMILUMINESCENCE OF 2-METHYL-6-PHENYL-8-(4-SUBSTITUTED PHENYL)-IMIDAZO[1,2-*a*]PYRAZIN-3(7*H*)-ONES INDUCED BY SUPEROXIDE ANION

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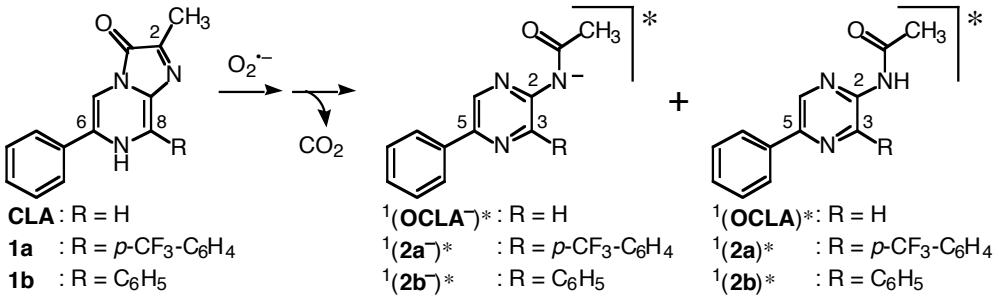
Abstract – An unprecedented pH-dependent bimodal chemiluminescence of 2-methyl-6-phenyl-8-(4-substituted phenyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-ones (**1a** and **1b**) initiated by superoxide anion ($O_2^{\bullet-}$) in phosphate buffer solutions is described. The intensity ratio of the bimodal luminescence due to two light-emitting species, the singlet-excited neutral 2-acetamido-5-phenyl-3-(4-substituted phenyl)pyrazine [^{1}(**2a**)* or ^{1}(**2b**)*] and its amide anion [^{1}(**2a**$^-$)* or ^{1}(**2b**$^-$)*], varied as the pH rose. The percentage of the anionic luminescence intensity [$I_{anion}/(I_{neutral}+I_{anion})$] for **1a** showed a good linear correlation with the pH value, demonstrating the usefulness of **1a** as a pH indicator as well as an $O_2^{\bullet-}$ probe.

Superoxide anion ($O_2^{\bullet-}$) is a toxic species involved in the pathology of many diseases, and recently has been found to be associated with apoptosis.[1] These disease states usually accompany homeostatic disorders such as alteration of intra-cellular pH.[2] Therefore, sensitive sensors that have ability to detect $O_2^{\bullet-}$ along with the local pH i*n vivo* can be helpful tools for mechanistic studies and diagnoses of related diseases. Recently, 2-methyl-6-phenylimidazo[1,2-*a*]pyrazin-3(7*H*)-one, *Cypridina* Luciferin Analogue (CLA), and its derevatives have been receiving much attention in the field of clinical pathology and medicine, since they have found to be good chemiluminescent $O_2^{\bullet-}$ probes in biological systems.[3-6] CLA emits bluish purple light (λ_{max} = 380 nm) in buffer solutions at pH 7.0, while blue light (λ_{max} = 450 nm) is produced at a higher pH (> 8.5).[7] The former arises from singlet-excited 2-acetamido-5-phenylpyrazine, oxy-luciferin analogue ^{1}(OCLA)*, while the latter from the singlet-excited amide anion of OCLA, ^{1}(OCLA$^-$)* as shown in Scheme 1.[7,8] This bimodal luminescence property can be applicable to monitoring medium pH values with spectral variation. However, no attention has been given to such

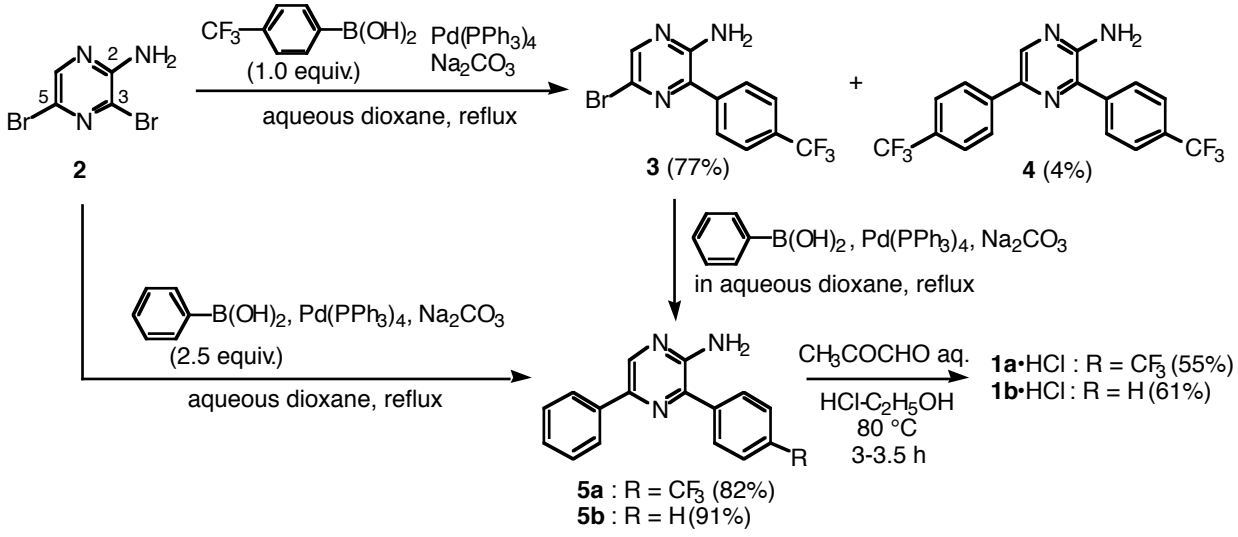
observation probably because the two spectra largely overlap each other and this makes it hard to estimate precisely medium pH from the luminescence intensities without any data processing.



Scheme 1 Chemiluminescent reaction of imidazopyrazinones with superoxide anion ($O_2^{\bullet-}$).

We have been studying substituent effects on imidazopyrazinone-related chemiluminescence system.[9,10] In the course of extending our study, it was found that 2-methyl-6-phenyl-8-(4-substituted phenyl)-imidazo[1,2-*a*]pyrazin-3(7*H*)-ones (**1a** and **1b**) showed distinctly divided and pH-dependent bimodal chemiluminescence in buffer solutions. Herein we present the chemiluminescent properties of **1a** and **1b** and their usefulness as pH-$O_2^{\bullet-}$ double sensors.

The imidazopyrazinones (**1a**) and (**1b**) were synthesized from 2amino-3,5-dibromopyrazine (**2**)[11] and obtained as hydrochloride salts (Scheme 2).[12,13] Regioselective introduction at C3 position of **2** was achieved by Suzuki coupling reaction with equimolar 4-trifluoromethylphenylboronic acid to afford monobrominated aminopyrazine (**3**) in 77% yield along with the small amount of 3,5-diarylated products (**4**). This C3 selectivity had previously demonstrated by Nakamura and co-workers in Stille and Sonogashira coupling reactions with **2**.[11,14] Subsequent cross-coupling of **3** with phenylboronic acid gave 3,5-diarylaminopyrazine (**5a**) in 82% yield.[15] 3,5-Diphenylaminopyrazine (**5b**) was directly synthesized from **2** with 2.5 equivalent of phenylboronic acid in 91% yield.[16]



Scheme 2 Synthesis of 6-phenyl-8-(4-substituted phenyl)imidazopyrazinones (**1a**) and (**1b**).

The reaction of imidazopyrazinone (**1a**) with $O_2^{\bullet-}$, which was generated by xanthine-xanthine oxidase system,[17] in phosphate buffer solutions at pH 7.0 gave the chemiluminescence at 400 nm (Figure 1). At pH > 7.0, dual luminescence with maxima at 400 nm and 530 nm was observed. As the pH rose, the emission at 530 nm intensified along with decrement of the neutral emission at 400 nm. Like CLA and other imidazopyrazinones, the emission at 400 nm was supposed to be produced from the singlet-excited state of acetylaminopyrazine ^{1}(**2a**)*, and the emission at 530 nm from its amide anion, ^{1}(**2a**$^-$)*. This was confirmed by that the chemiluminescence of **1a** in DMSO, which is commonly used for chemiluminescence studies as a solvent that produces the singlet-excited amide anions, was observed at 550 nm and the spent solution, in which the neutral acylaminopyrazine is usually included as the product of the reaction, showed fluorescence at 400 nm. Compound (**1b**) showed the similar pH-dependent bimodal emissions at 400 and 500 nm, except the neutral luminescence decreased as the pH rose to 8.5, above which the emission increased. This behavior is due to that the whole emission intensity of **1b** increased under such high pH conditions. These chemiluminescence for **1a** and **1b** was completely quenched by adding superoxide dismutase, confirming that these luminescent reactions were surely caused by $O_2^{\bullet-}$. Control experiments under the basic conditions demonstrate that the background luminescence caused by the spontaneous oxidation with dissolved triplet oxygen does not substantially influence the $O_2^{\bullet-}$-triggered luminescence.

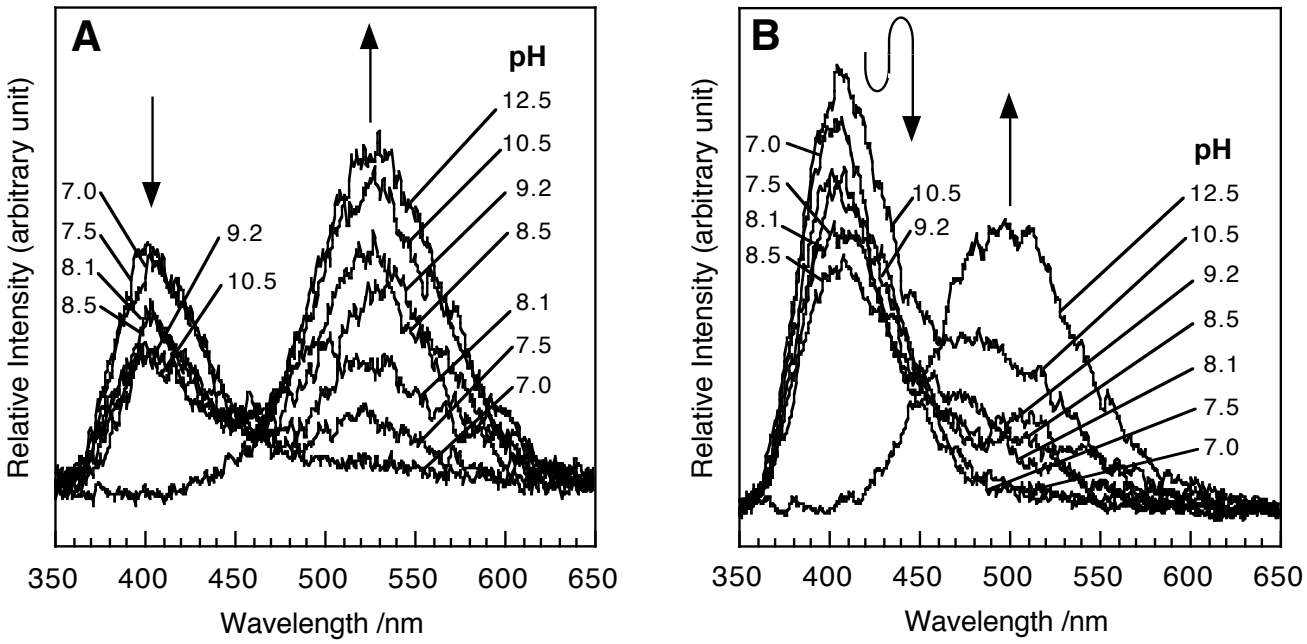


Figure 1 Chemiluminescence specra of (A) **1a** and (B) **1b** induced with superoxide anion in phosphate buffer solutions at 25 °C under various pH conditions.

The largely bathochromic-shifted anionic emissions for both **1a** and **1b** are supposed to be caused by the large energy stabilization in ^{1}(**2a**$^-$)* and ^{1}(**2b**$^-$)* with π-conjugation at the 8-position. The electron-

withdrawing trifluoromethyl group would be responsible for the larger bathochromic shift in the anionic luminescence of **1a**. Noteworthy is that the neutral and anion spectra for **1a** are well apart each other, whereas those for **1b** in part overlapped each other.

To evaluate their pH-sensing ability, the luminescence intensity ratio of the anion luminescence versus the sum of the bimodal luminescence, $I_{anion}/(I_{neutral}+I_{anion})$, for each of the imidazopyrazinones was plotted against pH (Figure 2). Compound (**1a**) showed a good linear correlation between the $I_{anion}/(I_{neutral}+I_{anion})$ value and pH, while **1b** gave a somewhat dispersed plot probably because the spectral overlap in **1b** makes the intensity values inaccurate.

Thus, the present study demonstrates the usefulness of **1a** as pH indicator as well as $O_2^{\bullet-}$ probe. Based on the luminescence ratio, $I_{anion}/(I_{neutral}+I_{anion})$, the direct measurement of pH is feasible in the range of pH 7-12. This bimodal luminescence as well as spectral (color) variation may allow us to apply **1a** for monitoring two concurrent events in biological systems. Application of **1a** to monitoring $O_2^{\bullet-}$ and the local pH in a biological system will be reported elsewhere.

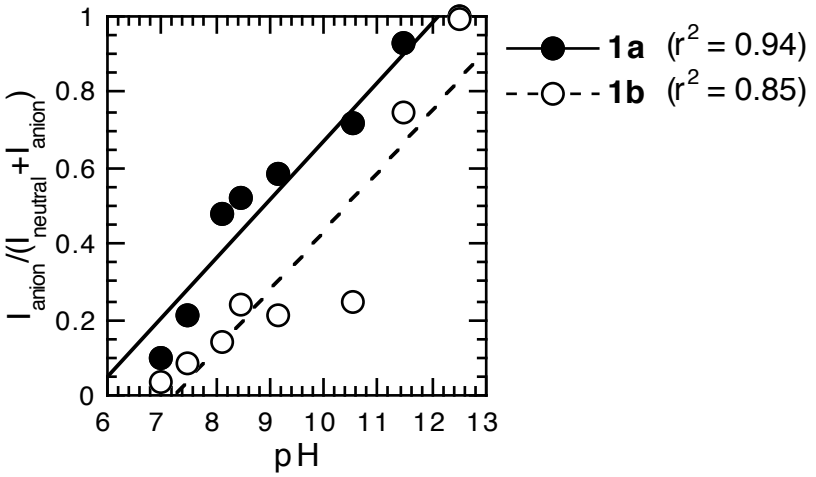


Figure 2 Relationship between pH and the percentage of anion luminescence, $I_{anion}/(I_{neutral}+I_{anion})$, for **1a** (closed circle) and **1b** (open circle) in phosphate buffer solutions in the presence of xanthine-xanthin oxidase system at 25 °C. I_{anion} donates the intensity of the anion luminescence, and $I_{neutral}$ the intensity of the neutral luminescence.

ACKNOWLEDGEMENTS

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12. Selected data for **1a**: pale yellow solid mp 153 °C (decomp); ^{1}H-NMR (400 MHz, CD_3OD) δ/ppm 2.55 (s, 3H), 7.49-7.59 (m, 3H), 8.00 (d, J = 8.1 Hz, 2H), 8.15-8.18 (m, 2H), 8.30 (d, J = 8.1 Hz, 2H), 8.79 (s, 1H); IR (KBr) ν_{max}/cm^{-1} 3403 (br, νOH), 3067, 2931 (νCH), 1658, 1572, 1532, 1493 (νC=C; ring stretching), 1323 (νCF), 848, 770, 695 (γCH). HRMS (positive-EI) Calcd for $C_{20}H_{14}N_3OF_3$: 369.1089. Found: 369.1107.
13. Selected data for **1b**: yellow needles mp 128 °C (decomp); ^{1}H-NMR (400 MHz, CD_3OD) δ/ppm 2.55 (s, 3H), 7.48-7.58 (m, 3H), 7.67-7.73 (m, 3H), 8.06-8.10 (m, 2H), 8.14-8.16 (m, 2H), 8.75 (s, 1H); IR (KBr) ν_{max}/cm^{-1} 3401 (br, νOH), 3061, 2924 (νCH), 1658, 1572, 1528, 1492 (νC=C; ring stretching), 773, 694 (γCH). HRMS (positive-EI) Calcd for $C_{19}H_{15}N_3O$: 301.1215. Found: 301.1263.
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15. Selected data for **5a**: mp 190-190 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.80 (s, 2H), 7.39 (t, J = 7.3 Hz, 1H), 7.47 (t, J = 7.3 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 7.97 (d, J = 8.0 Hz, 2H), 7.99 (d, J = 7.3 Hz, 2H), 8.52 (s, 1H); IR (KBr) ν_{max}/cm^{-1} 3405 (ν_{as}NH), 3305 (ν_sNH), 3172 (νCH), 1641 (δNH), 1614, 1530, 1465, 1406 (νC=C; ring stretching), 1322 (νCF), 849, 762, 695 (γCH). Anal. Calcd for $C_{17}H_{12}N_3F_3$: C, 64.76; H, 3.84; N, 13.33. Found: C, 64.49; H, 4.07; N, 13.51.
16. Selected data for **5b**: mp 134-135 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ/ppm 4.83 (s, 2H), 7.36 (t, J =

7.3 Hz, 1H), 7.43-7.48 (m, 3H), 7.53 (t, *J* = 7.3 Hz, 2H), 7.83 (d, *J* = 7.1 Hz, 2H), 7.97 (d, *J* = 7.3 Hz, 2H), 8.46 (s, 1H); IR (KBr) ν_{max}/cm^{-1} 3461 (ν_{as}NH), 3282 (ν_{s}NH), 3144 (νCH), 1625 (δNH), 1536, 1459, 1436 (νC=C; ring stretching), 746, 688 (γCH). HRMS (positive-EI) Calcd for $C_{16}H_{13}N_3$: 247.1108. Found: 247.1085.

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