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Chemiluminescent 2,6-diphenylimidazo[1,2-*a*]pyrazin-3(7*H*)-ones: a new entry to Cypridina luciferin analogues†

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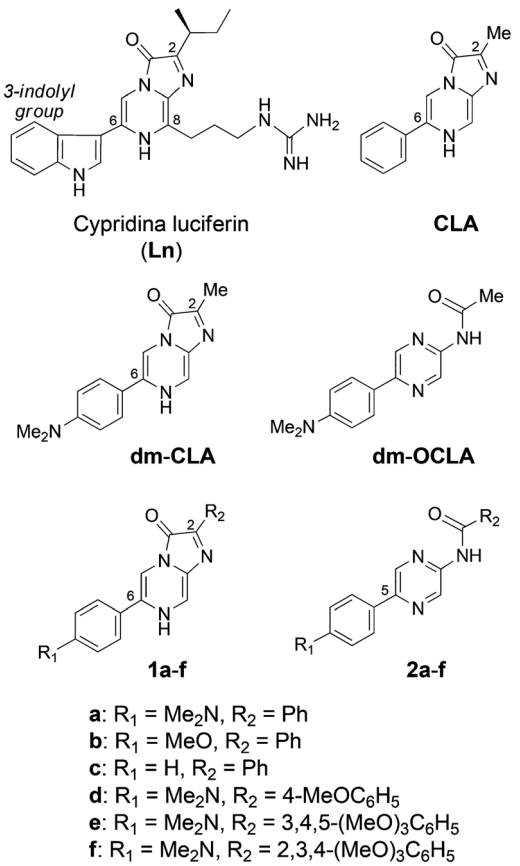
An investigation of the chemiluminescent properties of 2,6-diphenylimidazo[1,2-*a*]pyrazin-3(7*H*)-one derivatives (**1**), having substituted phenyl groups, is described. Among the derivatives **1**, the 6-[4-(dimethylamino)phenyl] derivatives (**1a,d–f**) gave a high quantum yield ($\Phi_{CL} \geq 0.0025$) in diglyme/acetate buffer, which is a model reaction condition for the *Cypridina* bioluminescence. Their efficient chemiluminescence is mainly caused by the electronic effect of the substituent at C6. In particular, the electron-donating 4-(dimethylamino)phenyl group at C6 of **1a,d–f** plays an essential role in increasing the chemi-excitation efficiency (Φ_S) by the charge transfer-induced luminescence (CTIL) mechanism. The results provide useful information for designing new Cypridina luciferin analogues showing efficient chemiluminescence.

Introduction

Cypridina luciferin (**Ln**) is the substrate for the bioluminescence of the luminous ostracod *Cypridina* (Scheme 1), and produces light by a reaction with molecular oxygen ($^3O_2$).[1,2] One of the characteristics of the bioluminescence is its high efficiency at light production ($\Phi_{BL} \approx 0.3$).[3] The imidazo[1,2-*a*]pyrazin-3(7*H*)-one (imidazopyrazinone) ring is the core for the luminescence reactivity of **Ln**. Imidazopyrazinone derivatives, including **Ln**, exhibit chemiluminescence reactivity with $^3O_2$ in aprotic solvents.[4,5]

To establish the mechanism of the bioluminescence, the chemiluminescence of imidazopyrazinone derivatives has been investigated as a model of the *Cypridina* bioluminescence reaction.[5–9] For this purpose, 6-aryl-2-methylimidazopyrazinones such as a Cypridina luciferin analogue (**CLA**) and its dimethylamino derivative (**dm-CLA**), whose π-electronic properties were modulated by a substituent at C6, have been employed (Scheme 1).[5c,d,6] The reason to choose 6-aryl imidazopyrazinone derivatives is that the 3-indolyl group at C6 on **Ln** is a π-electronically conjugated appendage. DMSO containing a base (DMSO/base)[4] and diglyme containing an acetate buffer (diglyme/acetate buffer)[5] have been used as the standard reaction conditions for imidazopyrazinone chemiluminescence. The diglyme/acetate buffer condition is predicted to be



Scheme 1 Cypridina luciferin (**Ln**), its analogues **CLA**, **dm-CLA** and **1a–f**, and *Cypridina* oxyluciferin analogues **dm-OCLA** and **2a–f**.

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† In memory of the late Professor Nicholas J. Turro.

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a favorable condition for the chemiluminescence reaction of **Ln** because it allows elementary processes similar to those of the bioluminescence reaction.[2a,5] Among the 6-aryl-2-methylimidazopyrazinones investigated, **dm-CLA** having an electron-donating 4-(dimethylamino)phenyl group gives the highest chemiluminescence quantum yield (Φ_{CL} = 0.015) in diglyme/acetate buffer.[6c,d] A mechanistic analysis of these substituent effects led to the conclusion that the 3-indolyl group of **Ln** plays an essential role as an electron-donating substituent that accelerates the oxygenation process and increases the chemiexcitation efficiency.

Despite the success described above, the chemiluminescence of an imidazopyrazinone still gives an efficiency lower than the Φ_{BL} value of the *Cypridina* bioluminescence. In an attempt to solve this problem, new imidazopyrazinone derivatives **1**, with phenyl groups added at C2 and C6 to electronically modulate the chemiluminescence, were designed as new Cypridina luciferin analogues (Scheme 1). It has been found that benzamidopyrazine **2a**, which is the product of the chemiluminescence reaction of **1a**, functions as a fluorophore.[10] This encouraged us to study the chemiluminescence of 2,6-diphenylimidazopyrazinones. We investigated the chemiluminescent properties of a series of substituted 2,6-diphenyl derivatives **1** in DMSO/base and in diglyme/acetate buffer, and found that the derivatives having an electron-donating 4-(dimethylamino)phenyl group at C6 show efficient chemiluminescence in diglyme/acetate buffer. To understand the origin of the efficient chemiluminescence of the 6-[4-(dimethylamino)-phenyl] derivatives, the factors that determine the Φ_{CL} values, including the reaction efficiency, the chemiexcitation efficiency and the fluorescence quantum yield, were evaluated mechanistically. We report herein the chemiluminescent properties of **1**, which can provide a guideline for designing new Cypridina luciferin analogues with efficient chemiluminescence.

Experimental

General

Melting points were obtained using a Yamato MP-21 apparatus. IR spectra were measured using a Horiba FT-720 spectrometer. High-resolution electro-spray ionization (ESI) mass spectra were recorded using a JEOL JMS-T100LC mass spectrometer. ^{1}H NMR spectra were recorded using a JEOL ECA-500 instrument (500 MHz). UV/visible absorption spectra were measured using a Varian Cary 50 spectrophotometer (scan speed, 600 nm min^{-1}; data interval, 1 nm). Fluorescence spectra and fluorescence quantum yields were obtained using a Hamamatsu Photonics Quantaurus-QY absolute PL quantum yield measurement system. Chemiluminescence spectra and chemiluminescence quantum yields were measured using an ATTO AB-1850 luminescence detecting spectrophotometer. The intensity of the total light (400–700 nm) emitted from a chemiluminescence reaction was monitored using a luminometer with a Hamamatsu R5929 photomultiplier tube powered by a Hamamatsu C4900 power supply. The signal from the luminometer was collected on a PC and the data were analyzed using the graphics program Igor Pro, Version 4.0 (Wave Metrics, Inc.). Spectroscopic measurements were made in a quartz cuvette (1 cm path length) at 25 ± 1 °C. Spectral-grade solvents were used for the measurement of UV/visible absorption, fluorescence and chemiluminescence. HPLC analyses were carried out using a JASCO 980 HPLC system with a Kanto Chemical Mightysil RP-18 GP-II column (250 mm × 4.6 mm, 5 μm). Density functional theory (DFT) calculations were performed using the Gaussian 09 program.[11] DFT includes Beck's three-parameter function combined with Lee, Yang and Parr's correlation function (B3LYP) along with the 6-31G(d) basis set.[12] Transition state structures were located using the Berny algorithm until the Hessian matrix had only one imaginary eigenvalue. Molecular graphics were generated using GaussView, Version 5.[13]

Preparation of imidazopyrazinones 1 and amidopyrazines 2

Imidazopyrazinone **1c** and amidopyrazine **2a** were prepared by previously reported procedures.[10,14] Other derivatives (**1a,b,d–f**) were synthesized from the corresponding 5-arylaminopyrazines and arylglyoxals as follows. Amidopyrazines **2b–f** were also synthesized by acylation of the corresponding 5-arylaminopyrazines.

Synthesis of 1a. To a solution of 5-[4-(dimethylamino)-phenyl]aminopyrazine (40 mg, 0.20 mmol) and phenylglyoxal monohydrate (92 mg, 0.60 mmol) in 1,4-dioxane (2.0 mL) was added conc. HCl (0.2 mL) under Ar. The reaction mixture was heated at 100 °C for 1 h. After cooling, the mixture was concentrated under reduced pressure. The red residue was purified by silica gel column chromatography four times [$CHCl_3$–methanol (20 : 1 and 5 : 1)], yielding **1a** (38 mg, 57%) as a brown powder: mp 256–257 °C. δ_H (CD_3OD) 3.00 (6 H, s), 6.83 (2 H, d, *J* 9.2 Hz), 7.37 (1 H, br t), 7.44 (2 H, br t), 7.52 (2H, d, *J* 8.6 Hz), 7.72 (1 H, br s), 8.00 (1 H, br s) and 8.41 (2 H, d, *J* 5.2 Hz). ν (KBr)/cm^{-1} 3056, 2927, 1608 and 1516. *m*/*z* (ESI) Found: 331.1597 ([M + H]$^+$). $C_{20}H_{19}N_4O$ requires 331.1559.

1b: orange powder, mp 89–90 °C. δ_H (CD_3OD) 3.85 (3 H, s), 7.08 (2 H, d, *J* 8.6 Hz), 7.32 (2 H, t, *J* 7.2 Hz), 7.38 (1 H, t, *J* 7.5 Hz), 7.66 (2 H, d, *J* 8.6 Hz), 7.78 (1 H, s), 8.03 (1 H, br s) and 8.41 (2 H, d, *J* 7.5 Hz). ν (KBr)/cm^{-1} 3411, 3060, 1610 and 1508. *m*/*z* (ESI) Found: 318.1263 ([M + H]$^+$). $C_{19}H_{16}N_3O_2$ requires 318.1243.

1d: dark red powder, mp >300 °C. δ_H (CD_3OD) 3.28 (6 H, s), 3.87 (3 H, s), 7.09 (2 H, d, *J* 9.2 Hz), 7.53 (2 H, d, *J* 8.6 Hz), 7.98 (2 H, d, *J* 8.6 Hz), 8.22 (2 H, d, *J* 8.6 Hz), 8.28 (1 H, s) and 8.49 (1 H, s). ν (KBr)/cm^{-1} 3401, 1608 and 1508. *m*/*z* (ESI) Found: 361.1698 ([M + H]$^+$). $C_{21}H_{21}N_4O_2$ requires 361.1665.

1e: dark red powder, mp >300 °C. δ_H (CD_3OD) 3.32 (6 H, s), 3.82 (3 H, s), 3.94 (6 H, s), 7.64 (2 H, s), 7.66 (2 H, d, *J* 9.2 Hz), 8.04 (2 H, d, *J* 8.6 Hz), 8.33 (1 H, s) and 8.54 (1 H, s). ν (KBr)/cm^{-1} 3401, 1646, 1616 and 1508. *m*/*z* (ESI) Found: 421.1907 ([M + H]$^+$). $C_{23}H_{25}N_4O_4$ requires 421.1876.

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1f: dark red powder, mp >300 °C. δ_H (CD_3OD) 3.26 (6 H, s), 3.92 (3 H, s), 3.95 (3 H, s), 3.97 (3 H, s), 7.02 (1 H, d, *J* 9.2 Hz), 7.52 (2 H, d, *J* 8.1 Hz), 7.98 (1 H, d, *J* 9.2 Hz), 8.12 (2 H, d, *J* 8.6 Hz) 8.69 (1 H, s) and 8.98 (1 H, s). ν (KBr)/cm^{-1} 2937, 1585 and 1499. *m*/*z* (ESI) Found: 421.1875 ($[M + H]^+$). $C_{23}H_{25}N_4O_4$ requires 421.1876.

Synthesis of 2c. A solution of 5-phenylaminopyrazine (69 mg, 0.41 mmol) and benzoyl chloride (70 μL, 0.60 mmol) in pyridine (1 mL) was stirred at room temperature under Ar for 2.5 h. The reaction was quenched by the addition of brine (20 mL) and the product was extracted with chloroform (20 mL × 3). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by recrystallization from a mixture of $CHCl_3$ and ethyl acetate (10 : 1), yielding **2a** (50 mg, 45%) as colorless cubes, mp 210–211 °C. δ_H ($CDCl_3$) 7.46 (1 H, t, *J* 7.5 Hz), 7.52 (2 H, t, *J* 8.0 Hz), 7.55 (2 H, t, *J* 8.0 Hz), 7.62 (1 H, t, *J* 7.5 Hz), 7.97 (2 H, d, *J* 7.5 Hz), 8.01 (2 H, d, *J* 7.5 Hz), 8.53 (1 H, br s), 8.72 (1 H, br s) and 9.77 (1 H, d, *J* 0.9 Hz). ν (KBr)/cm^{-1} 3366, 3053, 1668, 1540 and 1501. *m*/*z* (ESI) Found: 276.1149 ($[M + H]^+$). $C_{17}H_{14}N_3O$ requires 276.1137.

2b: yellow cubes, mp 189–190 °C. δ_H ($CDCl_3$) 3.88 (3 H, s), 7.02 (2 H, t, *J* 8.6 Hz), 7.53 (2 H, t, *J* 8.0 Hz), 7.61 (1 H, t, *J* 6.9 Hz), 7.95–7.97 (4 H, m), 8.49 (1 H, br s), 8.66 (1 H, d, *J* 1.8 Hz) and 9.71 (1 H, d, *J* 1.8 Hz). ν (KBr)/cm^{-1} 3369, 1668, 1610 and 1513. *m*/*z* (ESI) Found: 306.1266 ($[M + H]^+$). $C_{18}H_{16}N_3O_2$ requires 306.1243.

Synthesis of 2d. A solution of 5-[4-(dimethylamino)phenyl]-aminopyrazine (41 mg, 0.19 mmol), 4-methoxybenzoic acid (29 mg, 0.19 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (109 mg, 0.57 mmol) and 4-dimethylaminopyridine (23 mg, 0.19 mmol) in dichloromethane (2 mL) was heated under reflux under Ar for 3.5 h. The reaction was quenched by the addition of water (60 mL) and the product was extracted with chloroform (70 mL × 3). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel TLC [$CHCl_3$–ethyl acetate (5 : 1)], yielding **2d** (18 mg, 28%) as a yellow powder: mp 219–220 °C. δ_H ($CDCl_3$) 3.04 (6 H, s), 3.89 (3 H, s), 6.81 (2 H, d, *J* 9.2 Hz), 7.00 (2 H, d, *J* 8.6 Hz), 7.92 (4 H, m), 8.35 (1 H, br s), 8.62 (1 H, d, *J* 1.7 Hz) and 9.65 (1 H, d, *J* 1.8 Hz). ν (KBr)/cm^{-1} 3392, 1670, 1608 and 1540. *m*/*z* (ESI) Found: 371.1516 ($[M + Na]^+$). $C_{20}H_{20}N_4NaO_2$ requires 371.1484.

2e: yellow cubes, mp 177–178 °C. δ_H ($CDCl_3$) 3.04 (6 H, s), 3.92 (3 H, s), 3.95 (6 H, s), 6.81 (2 H, d, *J* 8.6 Hz), 7.16 (2 H, s), 7.92 (2 H, d, *J* 8.6 Hz), 8.36 (1 H, br s), 8.64 (1 H, d, *J* 1.2 Hz) and 9.65 (1 H, br s). ν (KBr)/cm^{-1} 1646, 1610 and 1533. *m*/*z* (ESI) Found: 431.1732 ($[M + Na]^+$). $C_{22}H_{24}N_4NaO_4$ requires 431.1695.

2f: yellow cubes, mp 212–213 °C. δ_H ($CDCl_3$) 3.04 (6 H, s), 3.92 (3 H, s), 3.95 (3 H, s), 4.13 (3 H, s), 6.81 (2 H, d, *J* 9.2 Hz), 6.85 (1 H, d, *J* 9.2 Hz), 7.91 (2 H, d, *J* 9.2 Hz), 8.03 (1 H, d, *J* 9.2 Hz), 8.65 (1 H, d, *J* 1.7 Hz), 9.68 (1 H, d, *J* 1.2 Hz) and 10.52 (1 H, br s). ν (KBr)/cm^{-1} 3332, 2974, 2939 and 1657. *m*/*z* (ESI) Found: 431.1684 ($[M + Na]^+$). $C_{22}H_{24}N_4NaO_4$ requires 431.1695.

Analysis of the chemiluminescence reactions of imidazopyrazinones

A small portion (20 μL) of a stock solution of imidazopyrazinone **1** (1.0×10^{-3} M) in methanol was added into a quartz cuvette placed in a luminometer and was mixed with aerated DMSO (2.0 mL) containing 0.10 M NaOH aqueous solution (1.0% v/v) or aerated diglyme (2.0 mL) containing 0.10 M of acetate buffer (acetic acid–sodium acetate buffer, pH 5.6, 0.66% v/v) at 25 ± 1 °C. The chemiluminescence reactions of **1** were traced by monitoring the intensity (*I*) of the emitted light. The pseudo-first-order rate constant (k_{CL}) was obtained by analysis using the equation: $\ln I_t = -k_{CL}t + \ln I_0$, where I_0 and I_t are the intensities at $t = 0$ and t, respectively. The Φ_{CL} values were determined as values relative to the Φ_{CL} (0.013) of luminol in DMSO containing *t*-BuOK/*t*-BuOH under air.[15]

The yields of amidopyrazines **2** obtained after chemiluminescence reactions of **1** in diglyme/acetate buffer were determined by HPLC. A spent solution (10 mL) obtained by a chemiluminescence reaction for 24 h was mixed with 2-ethylnaphthalene (1.0 μL) as an internal standard, and was analyzed three times by reverse phase HPLC with a H_2O–CH_3CN (1 : 4) eluent (1.0 mL min^{-1}). Yields of **2** were determined by relative peak integrations (monitoring the absorbance at 370 nm) and individual response factors against 2-ethylnaphthalene.

Results and discussion

Chemiluminescence of 1a–c in DMSO/NaOH (aq.) and in diglyme/acetate buffer

To investigate the chemiluminescent activity of a 2,6-diphenylimidazopyrazinone derivative, the substituent effect of the 6-phenyl group in **1a–c** was studied first. Chemiluminescence reactions of **1a–c** in aerated DMSO containing 0.10 M NaOH aqueous solutions were examined at 25 °C. The reactions of **1a–c** showed only a weak luminescence, and the Φ_{CL} values were below 10^{-4}. A chemiluminescence reaction of an imidazopyrazinone in basic DMSO gives light emission from the excited singlet (S_1) state of the amide anion of an amidopyrazine product.[5–7] Fluorescence measurements of the amide anions of **2a–c** in DMSO containing 0.10 M NaOH aqueous solutions showed quantum yields (Φ_F) below 0.005. Therefore, the low luminescence of **1a–c** was due to the fact that the amide anions of **2a–c** were not fluorescent.

Next, chemiluminescence reactions of **1a–c** in aerated diglyme containing acetate buffer (pH 5.6, 0.66% v/v) were examined at 25 °C. While **1b,c** showed only a weak chemiluminescence ($\Phi_{CL} < 10^{-4}$), **1a** gave an estimated Φ_{CL} value of 0.0025. The chemiluminescence emission spectrum of **1a** showed the maximum (λ_{CL}) at 521 nm. This coincides with the fluorescence spectrum of the neutral form of amidopyrazine **2a**, indicating that light emission occurred from the S_1 state of **2a**. The difference in chemiluminescence behavior between **1a–c** in diglyme/acetate buffer can be explained by the substituent effect of the 6-phenyl group with the reaction

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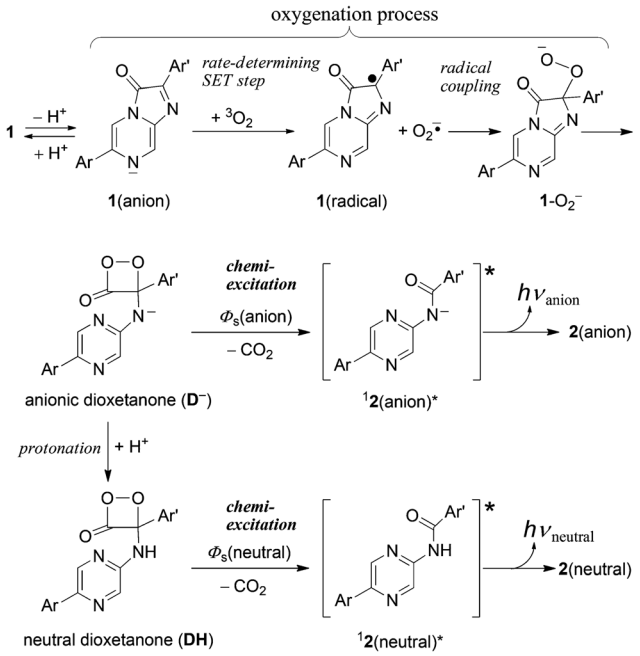

Scheme 2 Reaction mechanism of the chemiluminescence of **1**.

mechanism reported previously (Scheme 2).[6] In DMSO/NaOH (aq.), the chemiluminescence reactions of **1a–c** give the S_1 states of the amide anions of **2a–c** *via* anionic dioxetanones ($\mathbf{D}^-$). On the other hand, the chemiluminescence reaction of **1a** in diglyme/acetate buffer gives the S_1 states of the neutral form of **2a** *via* neutral dioxetanone (**DH**), while the chemiluminescence reactions of **1b,c** still give the excited amide anions of **2b,c** *via* $\mathbf{D}^-$. The difference between **1a–c** is because of competition between the decomposition of $\mathbf{D}^-$ to give the excited amide anion and the protonation of $\mathbf{D}^-$ to give **DH**. Because the 4-(dimethylamino)phenyl group is a good electron-donating substituent, the basicity of the anionic N atom of $\mathbf{D}^-$ is increased, which leads to the preferential protonation of $\mathbf{D}^-$. In the case of **1b,c**, the electron-donating properties of the 4-methyoxyphenyl and phenyl groups are not sufficient for the preferential protonation of $\mathbf{D}^-$. Therefore, **1b,c** gave negligible chemiluminescence in diglyme/acetate buffer in a manner similar to that in DMSO/NaOH (aq.). Only **1a**, with the electron-donating 4-(dimethylamino)phenyl group, is chemiluminescent in diglyme/acetate buffer.

Chemiluminescence of 1a,d–f in diglyme/acetate buffer

Based on the chemiluminescent structure of **1a**, with the 4-(dimethylamino)phenyl group at C6, we investigated the effect on chemiluminescence of substituents on the phenyl group at C2 with **1d–f**, all of which showed chemiluminescence in diglyme/acetate buffer. The observed properties are summarized in Table 1, together with those of **1a** and **dm-CLA**, for comparison.

Chemiluminescence emission spectra for **1a,d–f** are shown in Fig. 1. The λ_{CL} values for **1d–f** are also coincident with the fluorescence emission maxima of the neutral forms of **2d–f**, as

Table 1 Chemiluminescent properties of **1a,d–f** and **dm-CLA** in aerated diglyme/acetate buffer[a] at 25 °C

Substrate	λ_{CL}[b]/nm	k_{CL}[c]/10^{-4} s^{-1}	Φ_{CL}[d]/10^{-2}	Φ_R[e]	Φ_F[f]	Φ_S[g]
1a	521	1.3	0.25	0.89	0.16	0.018
1d	524	3.1	1.2	0.45	0.34	0.078
1e	523	1.2	0.62	0.32	0.29	0.067
1f	518	4.5	1.4	0.37	0.44	0.086
dm-CLA[h]	491	23	1.5	0.62	0.33	0.073

[a] Diglyme containing acetate buffer (0.10 M, pH 5.6, 0.66% v/v). [b] Emission maxima of the chemiluminescence spectra. [c] Pseudo-first-order rate constants. [d] Chemiluminescence quantum yields. [e] Chemical efficiencies to give the corresponding amidopyrazine products by the chemiluminescence reactions. [f] Fluorescence quantum yields of the corresponding amidopyrazines. [g] Chemiexcitation efficiencies. [h] Ref. 6*d*.

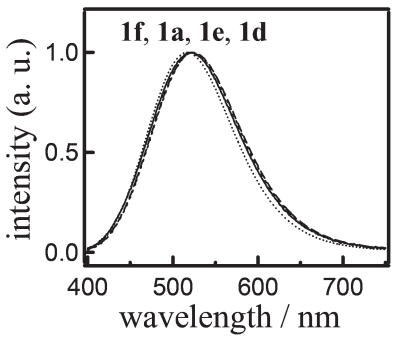

Fig. 1 Chemiluminescence emission spectra of **1a,d–f** (1.0×10^{-5} M) in aerated diglyme/acetate buffer at 25 °C.

observed for **1a**, indicating that light emitters are the S_1 states of **2d–f**. The λ_{CL} values for **1a,d–f** are similar to one another, indicating that the S_1 states of **2a,d–f** have a similar π-electronic properties which are not affected by the structure of the acyl groups. These π-electronic properties of the S_1 states of **2a, d–f** are consistent with our previous report.[10]

Decay curves of chemiluminescence intensities (I) over time for **1a,d–f** gave pseudo-first-order rate constants (k_{CL}) (Table 1). The chemiluminescence reaction rate of an imidazopyrazinone is regulated by the oxygenation process of an imidazopyrazinone anion as the rate-determining step (Scheme 2).[5a,6b] In particular, thermal electron transfer from an imidazopyrazinone anion to $^3O_2$ determines the value of k_{CL}.[6b] Because the k_{CL} values of **1a,d–f** are smaller than that of **dm-CLA**, the π-electronic conjugation of the phenyl group at C2 of the anions of **1a,d–f** reduces the reaction rate. The difference in the k_{CL} value between **1a,d–f** is explained by the introduction of the methoxy group. The Hammett σ_p and σ_m constants of the methoxy group are −0.27 and 0.12, respectively.[16] Thus, methoxy groups at the *ortho* and *para* positions of a phenyl group are electron donating and a methoxy group at the *meta* position is electron withdrawing. The order of the strength of electron donation, 2,3,4-trimethoxyphenyl (**1f**) > 4-methoxyphenyl (**1d**) > 3,4,5-trimethoxyphenyl (**1e**) ≈ phenyl (**1a**), corresponds to the order of the k_{CL} values, indicating that the

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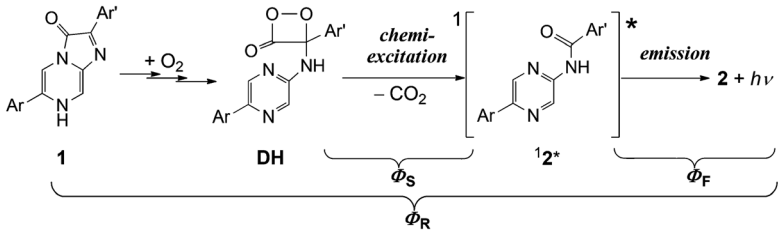

Scheme 3 Three factors to make chemiluminescence quantum yield (Φ_{CL}).

electron-donating properties of the phenyl group at C2 of an imidazopyrazinone anion effectively modulate the oxygenation reactivity.

The Φ_{CL} values for **1d–f** are in the range of 0.006–0.014 and are similar to that (0.015) for **dm-CLA** and higher than that (0.0025) for **1a** (Table 1). The value of Φ_{CL} tends to increase with increasing electron donation from the phenyl group at C2 of **1a,d–f**. To confirm the origin of the high Φ_{CL} values for **1a, d–f**, the elements of the Φ_{CL} value were evaluated. A Φ_{CL} value is a product of three efficiencies, $\Phi_{CL} = \Phi_R \times \Phi_S \times \Phi_F$, where Φ_R is the chemical efficiency to produce **2** by an appropriate chemiluminescence reaction pathway, Φ_S is the chemiexcitation efficiency for generating the S_1 state of **2** from a neutral dioxetanone **DH** and Φ_F is the fluorescence quantum yield of **2** (Scheme 3). The Φ_R values were determined by product analyses of the chemiluminescence reactions of **1a,d–f** in diglyme/acetate buffer, which indicated that **2a,d–f** were obtained in 32–89% yields. The Φ_F values of **2a,d–f** measured in diglyme/acetate buffer exceeded 0.16. Consequently, the Φ_S values were estimated to be greater than 0.018. It is noteworthy that the chemiluminescence of **1d,f** in diglyme/acetate buffer gives higher Φ_S values than that of **dm-CLA**.

Analysis of the chemiexcitation mechanism in the chemiluminescence of 1a,d–f

To understand the cause of efficient chemiexcitation in the chemiluminescence of **1a,d–f**, we analyzed the chemiexcitation processes mechanistically. The thermal decomposition of a 1,2-dioxetanone for efficient chemiexcitation can be explained by the charge transfer-induced luminescence (CTIL) mechanism.[6d,17] In the CTIL mechanism, a high Φ_S value requires that both the transition state (**TS**) of decomposition of **DH** for chemiexcitation and the S_1 state of **2** have a strong intramolecular charge transfer (ICT) character. Electronic properties of **DH** and **TS** for the chemiluminescence of **1a,d–f** and **dm-CLA** were predicted by DFT calculations with the restricted (R) and unrestricted (U) B3LYP/6-31G(d) methods, respectively. Fig. 2 shows selected optimized geometries of **DH** and **TS**, and calculation data are summarized in Table 2. The value of ΔE is the relative heat of formation of **TS** compared to that of the corresponding **DH**. The r_{CC} and r_{OO} values are the C–C and O–O bond lengths, respectively, and the q_{Do}, q_{NH}, q_{Py}, q_{Ar} and $q_{Ar'}$ values are the total Mulliken charge densities of the atoms constituting the dioxetanone (C_2O_3), NH, pyrazine ($C_4H_2N_2$) and two aryl parts, respectively (Scheme 4).

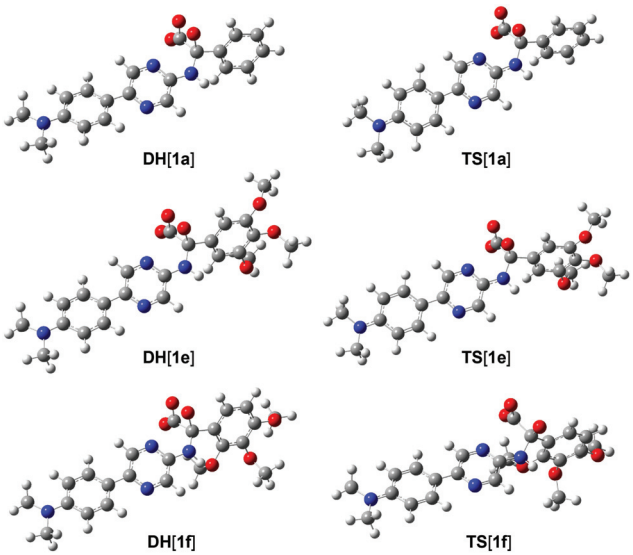

Fig. 2 Optimized geometries of **DH[1a,e,f]** and **TS[1a,e,f]** calculated by the R- and UB3LYP/6-31G(d) methods, respectively.

The ΔE values for **TS[1a,d–f]** are similar to that of **TS[dm-CLA]**. Because the value of ΔE corresponds to the activation energy of the decomposition of **DH**, the reaction rates of the chemiexcitation processes from **DH[1a,d,e]** and **DH[dm-CLA]** will be similar to one another. The dipole moments, molecular geometries and charge distributions of **DH[1a,d,e]** and **TS[1a,d,e]** are also similar to those of **DH[dm-CLA]** and **TS[dm-CLA]**, respectively. On the other hand, **TS[1f]** has a characteristically long O–O bond (2.25 Å) and the negative and positive charges in the structure of **TS[1f]** are localized at the dioxetanone and 5-[4-(dimethylamino)phenyl]pyrazinylamino moieties, respectively. In fact, the q_{Do} value (−0.344) of **TS[1f]** is much less than that (−0.156) of **TS[1a]** and the summation of the q_{NH}, q_{Py} and q_{Ar} values (+0.335) for **TS[1f]** is much greater than that (+0.249) for **TS[1a]**. **TS[1f]** has a larger dipole moment (14.7 D) than the other species (8.4–10.0 D). These characteristic properties of **TS[1f]** originate from the strong electron-donating properties of the 2,3,4-trimethoxyphenyl group and the steric hindrance of its *ortho* substituent, yet the properties of **TS[1f]** do not cause a drastic change in the Φ_S value. The differences in the $q_{Ar'}$ values between **TS[1a,d–f]** and **DH[1a,d–f]** are small (around −0.05). Thus, the phenyl (Ar′) groups at C2 of **1a,d–f** have only a small electronic effect on the properties of the corresponding **TS**. That is, **TS[1a,d–f]**

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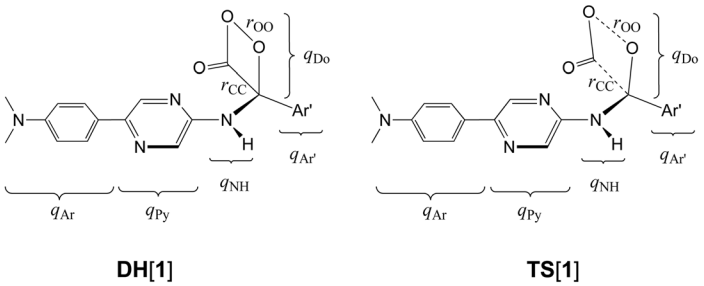
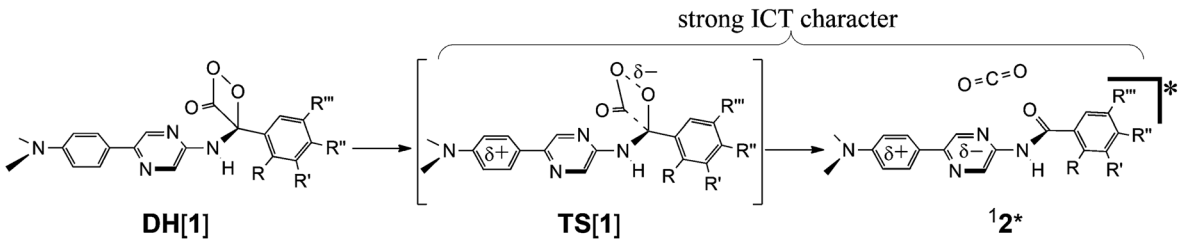
Table 2 Relative energies (ΔE), dipole moments (μ), C–C and O–O bond distances (r_{CC} and r_{OO}) and Mulliken charge densities (q) for dioxetanones (**DH**) and transition states (**TS**) of the dioxetanone decompositions for chemiluminescence of **1a,d–f** and **dm-CLA** calculated by the R- and UB3LYP/6-31G(d) methods

Dioxetanone or transition state[a]	ΔE^b/ kcal mol^{-1}	μ/D ($\Delta\mu$)[c]	r_{CC}/Å (Δr_{CC})[c]	r_{OO}/Å (Δr_{OO})[c]	q_{Do}[d] (Δq)[c]	q_{NH}[d] (Δq)[c]	q_{Py}[d] (Δq)[c]	q_{Ar}[d] (Δq)[c]	$q_{Ar'}$[d] (Δq)[c]
DH[1a]	0.0	4.1	1.546	1.492	0.014	−0.321	0.119	0.099	0.088
TS[1a]	17.5	10.0 (+5.9)	1.565 (+0.019)	1.963 (+0.471)	−0.156 (−0.170)	−0.291 (+0.030)	0.196 (+0.077)	0.209 (+0.110)	0.042 (−0.046)
DH[1d]	0.0	3.4	1.545	1.492	0.009	−0.323	0.117	0.097	0.100
TS[1d]	17.6	8.4 (+5.0)	1.566 (+0.021)	1.962 (+0.470)	−0.156 (−0.165)	−0.293 (+0.030)	0.190 (+0.073)	0.197 (+0.100)	0.062 (−0.038)
DH[1e]	0.0	3.2	1.546	1.492	0.022	−0.322	0.120	0.097	0.082
TS[1e]	17.4	8.7 (+5.5)	1.565 (+0.019)	1.961 (+0.469)	−0.155 (−0.177)	−0.289 (+0.033)	0.199 (+0.079)	0.209 (+0.112)	0.036 (−0.046)
DH[1f]	0.0	3.8	1.551	1.493	0.041	−0.297	0.102	0.091	0.064
TS[1f]	18.7	14.7 (+11.0)	1.557 (+0.006)	2.250 (+0.757)	−0.344 (−0.385)	−0.194 (+0.103)	0.257 (+0.155)	0.272 (+0.182)	0.008 (−0.056)
DH[dm-CLA]	0.0	4.3	1.538	1.492	0.044	−0.313	0.115	0.099	0.056[e]
TS[dm-CLA]	18.3	9.2 (+4.9)	1.558 (+0.020)	1.983 (+0.491)	−0.098 (−0.142)	−0.293 (+0.020)	0.179 (+0.064)	0.192 (+0.093)	0.019[e] (−0.036)[e]

[a] **DH** and **TS** denote dioxetanone and the transition state of the dioxetanone decomposition, respectively. **DH** and **TS** were calculated by the R- and UB3LYP/6-31G(d) methods, respectively. [b] Energy of a transition state relative to that of the corresponding dioxetanone. [c] Differences between the values for **TS** and the corresponding **DH**. [d] Skeletal parts for the Mulliken charge density are shown in Scheme 4. [e] Mulliken charge densities of the methyl group.



Scheme 4 Definitions of the C–C and O–O bond lengths (r_{CC} and r_{OO}) and the total Mulliken charge densities (q) of the skeletal parts of **DH[1]** and **TS[1]**.



Scheme 5 The CTIL mechanism for the chemiexcitation process from **DH[1]** to ^{1}**2*** *via* **TS[1]** in the chemiluminescence of **1a,d–f**.

maintain a favorable ICT character, like **TS[dm-CLA]**, for efficient chemiexcitation, because they have the common 4-(dimethylamino)phenyl group at the pyrazinylamino moiety. It has already been shown that **2a** has a large dipole moment (μ, 16 D) in the S_1 state.[10] Similarly, the S_1 states of **2d–f** will have large μ values. Thus, the S_1 states of **2a,d–f**, like the S_1 state of **dm-OCLA**, have a strong ICT character. Therefore, the decomposition of **DH[1a,d–f]** proceeds to give the S_1 states of **2a,d–f** *via* **TS[1a,d–f]** efficiently by following the CTIL mechanism in a manner similar to the decomposition of **DH[dm-CLA]** (Scheme 5).

Conclusion

We have demonstrated that **1a** having the electron-donating 4-(dimethylamino)phenyl group at C6 shows moderate chemiluminescent activity in diglyme/acetate buffer, while **1a** gives

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negligible chemiluminescence in DMSO/NaOH (aq.). The chemiluminescence reaction of **1a** proceeds to give the S_1 state of the neutral form of **2a**. Because **1b,c** having the 4-methoxyphenyl and phenyl groups at C6 are non-chemiluminescent substrates in DMSO/NaOH (aq.) and in diglyme/acetate buffer, the introduction of the dimethylamino group to the phenyl group at C6 is essential for the chemiluminescent activity of 2,6-diphenyl derivatives **1** *via* the generation of the S_1 state of a neutral amidopyrazine from a neutral dioxetanone **DH**. The derivatives **1d–f** having methoxy substituent(s) on the phenyl group at C2 together with the 4-(dimethylamino)phenyl group at C6 also show chemiluminescence in diglyme/acetate buffer, and their Φ_{CL} values are similar to the high Φ_{CL} value of **dm-CLA**. In the elements to determine the Φ_{CL} values of **1a,d–f**, the Φ_S values were greater than 0.018. The chemiexcitation processes for **1a,d–f** are explained by the efficient decompositions of **DH** to yield the S_1 states of **2a,d–f** with a strong ICT character *via* ICT **TS** through the CTIL mechanism, as in the case of **dm-CLA**. While the Φ_{CL} values of **1a,d–f** are still smaller than the Φ_{BL} value (*ca.* 0.3),[3] **1a,d–f** are in the group of derivatives with a high Φ_{CL} value greater than 0.001 in diglyme/acetate buffer like **dm-CLA**.[6c,d] Interestingly, the methoxy substituted phenyl groups at C2 of **1d–f** serves to maintain or slightly increase the Φ_S value, therefore contributing to a high Φ_{CL} value. This finding provides a guideline for designing a new Cypridina luciferin analogue with a Φ_{CL} value close to the Φ_{BL} value. To the 6-[4-(dimethylamino)phenyl]-2-phenylimidazopyazinone structure, for instance, an alkoxy-arm group can be introduced to the phenyl group at C2, which will have a supramolecular photochemical function of intramolecularly regulating the reactivity of the imidazopyrazinone core.[18] Based on this idea, we are studying the reaction mechanism of an efficient *Cypridina* bioluminescence, in which Cypridina luciferase has supramolecular functions that regulate the luminescence reaction and increase the Φ_{BL} value.

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