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Synthesis of bicyclic dioxetanes bearing a 3-hydroxy-4-isoxazolylphenyl moiety: new CIEEL-active dioxetanes emitting light with remarkable high-efficiency in aqueous medium

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Abstract—Bicyclic dioxetanes bearing a 3-hydroxy-4-isoxazolylphenyl moiety (**3a**–**c**) were synthesized. All these dioxetanes (**3a**–**c**) underwent base-induced CIEEL-decay to afford light with high efficiency in an NaOH/H_2O system as well as in a TBAF (tetrabutylammonium fluoride)/acetonitrile system. Among them, a dioxetane bearing a 3-hydroxy-4-[5-(trifluoromethyl)isoxazol-3-yl]phenyl moiety (**3a**) emitted light in the aqueous system with the highest efficiency which parallels that attained in an aprotic solvent system. Fluorescence study and the AM1 calculations for **3a** and the parent CIEEL-active dioxetanes (**1**) suggested that one important factor affecting chemiluminescence efficiency in an aqueous system should be the hydrogen-bonding at the carbonyl oxygen site of an oxyanion of hydroxyarenecarboxylate as the emitter produced by the CIEEL-decay.

The intramolecular CIEEL (chemically initiated electron exchange luminescence)[1,2] of hydroxyarene-substituted dioxetanes has received a great deal of attention from the viewpoint of mechanistic interests related to bioluminescence and application to modern biological analysis using chemiluminescence.[3–5] An adamantylidene–dioxetane (**1**) is a typical example for such CIEEL-active dioxetanes and its phosphate-protected form is now used in chemiluminescence bioassays.[3,6,7] The base-induced CIEEL from **1** occurs to emit light effectively in an aprotic solvent, such as DMSO and acetonitrile, while its chemiluminescence efficiency (Φ^{CIEEL}) decreases markedly in aqueous medium (Φ^{CIEEL} in H_2O/Φ^{CIEEL} in DMSO = 1/39000).[6–9] This significant defect has been improved to some extent (ca. 400 times enhancement) by adding a surfactant or by tethering a fluorescer to a dioxetane.[5,10] However, further development of high-sensitive biological analysis using chemiluminescence would require realizing new CIEEL-active dioxetanes emitting light with high efficiency even in aqueous medium (Fig. 1).

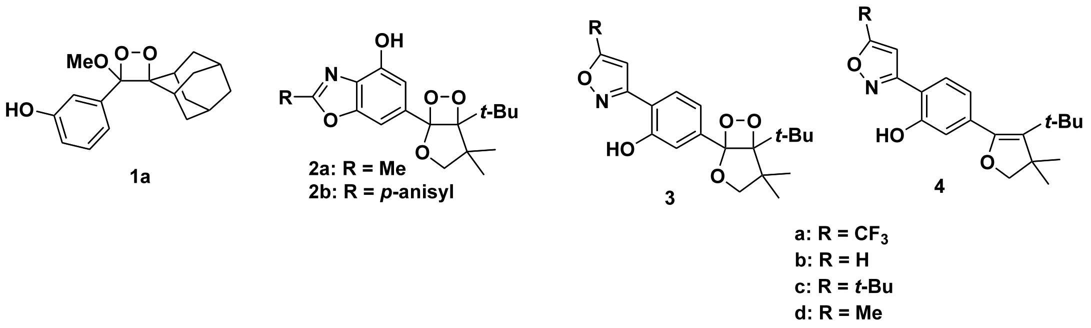

Figure 1.

Keywords: singlet oxygenation; 1,2-dioxetane; CIEEL; aqueous medium.

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A very recent study on the CIEEL of dioxetanes bearing a 4-hydroxybenzoxazole moiety (**2a** and **2b**) has revealed that (1) a marked drop of chemiluminescence efficiency (Φ^{CIEEL}) for the CIEEL-decay in an aqueous system is attributed to synergistic effect of the decreased chemiexcitation yield (Φ_S) and the decreased fluorescence yield (Φ^{fl}) of the emitter; and (2) the expansion of the π-system by introducing an aryl group at the 2-positon of the benzoxazole moiety leads to appreciable improvement of chemiexcitation yield as well fluorescence yield of the emitter, though the chemiluminescence efficiency is yet considerably lower in an aqueous system than in the DMSO system [$\Phi^{CIEEL}(H_2O)/\Phi^{CIEEL}(DMSO) = 1/35–1/63$].[9] These facts prompted us to design new CIEEL-active dioxetanes bearing a 4-aryl-substituted 3-hydroxyphenyl moiety, and it was found that 3-hydroxy-4-isoxazolylphenyl-substituted dioxetanes (**3**), especially a dioxetane bearing a 3-hydroxy-4-[5-(trifluoromethyl)isoxazol-3-yl]-phenyl moiety (**3a**), exhibit CIEEL-decay with remarkable high chemiluminescence efficiency even in an aqueous system.

Dioxetanes bearing a 3-hydroxy-4-isoxazolylphenyl moiety (**3**) were synthesized by singlet oxygenation of the corresponding dihydrofurans (**4**), which were prepared from 4-acetyl-3-methoxyphenyl-substituted dihydrofuran (**5**)[11] or its 3-hydroxy analog by several steps. The synthetic process of **3a** (Fig. 2) as a representative was as follows: an oxime (**6**) of **5** was lithiated[12] with BuLi (2 equiv.) at −78°C in THF and was successively quenched with *S*-ethyl trifluoroethanethioate to give an intermediary hydroxyisoxazoline, of which dehydration with TsOH/toluene afforded a dihydrofuran substituted with a 3-methoxy-4-[5-(trifluoromethyl)isoxazol-3-yl]-phenyl (**7a**) (yield based on **6** = 55%). Demethylation of **7a** with EtSNa in hot DMF gave a precursor (**4a**) in 84% yield. Dihydrofurans (**4b**–**d**) were similarly synthesized in 33–63% yields based on the oxime. When a solution of **4a** (100 mg) and tetraphenylporphin (TPP) (1 mg) in CH_2Cl_2 (5 mL) was irradiated with a 940-W Na lamp under an oxygen atmosphere at 0°C for 1 h, the desired dioxetane (**3a**) was produced in 87% isolated yield as pale yellow granules after chromatographic purification with SiO_2/Et_2O–hexane (1:20).[13] The other dioxetanes (**3b**: R = H) and (**3c**: R = *t*-butyl) were obtained by similar singlet oxygenation of the corresponding dihydrofurans (**4b** and **4c**) in isolated yield of 32 and 88%, respectively. It should be noted here that the singlet oxygenation of a methyl analog (**4d**) gave little of the desired dioxetane (**3d**) but complex mixture derived presumably from the oxygenation of the isoxazole ring. All dioxetanes (**3a**–**c**) synthesized here were quite stable enough to permit handling at room temperature.

When a dioxetane (**3a**) was treated with a large excess of tetrabutylammonium fluoride (TBAF) in acetonitrile at 25°C,[14] decomposition of **3a** occurred smoothly to follow pseudo-first-order kinetics independent of the TBAF concentration and to emit blue light (maximum wavelength: $\lambda_{max}^{CIEEL} = 481$ nm, chemiluminescence efficiency: $\Phi^{CIEEL} = 0.44$,[15] half-life of CIEEL-decay: $t_{1/2} =$ 11000 s). Similar treatment of the other dioxetanes (**3b** and **3c**) with TBAF/acetonitrile caused also the CIEEL-decay to afford light whose chemiluminescent properties are summarized together with those for **3a** and the parent dioxetane (**1**) in Table 1. Comparing the chemiluminescent properties for 3-hydroxy-4-isoxa-

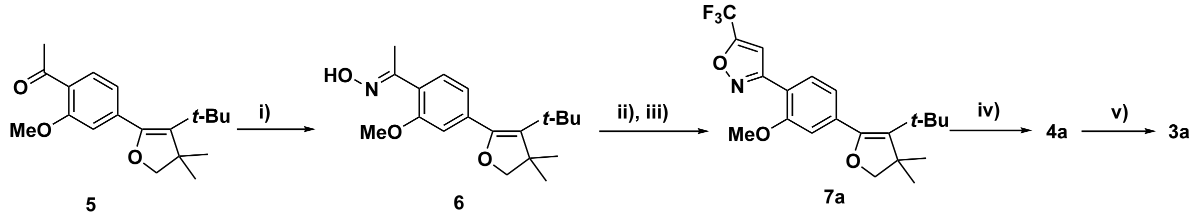


Figure 2. *Reagents and conditions*: (i) $NH_2OH/EtOH$; (ii) $BuLi/CF_3COSEt/THF/–78°C$; (iii) TsOH/toluene; (iv) EtSNa/DMF; (v) $h\nu/TPP/O_2$.

Table 1. Base-induced chemiluminescent decomposition of dioxetanes bearing a 3-hydroxy-4-(isoxazol-3-yl)phenyl group (**3**)

Dioxetane	TBAF/acetonitrile[a]			NaOH/H_2O[b]			
	λ_{max}^{CIEEL} (nm)	Φ^{CIEEL}	$t_{1/2}$ (s)	λ_{max}^{CIEEL} (nm)	Φ^{CIEEL}	$t_{1/2}$ (s)	$\Phi_{water}^{CIEEL}/\Phi_{acetonitrile}^{CIEEL}$
3a	481	0.44	11000 (1400)[c]	473	0.24	13000 (1200)[c]	1/1.8
3b	477	0.39	650	477	0.064	2800	1/6.1
3c	474	0.36	380	477	0.015	1500	1/24
1	466[d]	0.12[e]	13[e]	466[d]	7.5×10^{-6}[f]	180	1/16000

[a] A solution of a dioxetane in acetonitrile (1.0×10^{-5} M, 1 mL) was added to a TBAF solution in acetonitrile (1.0×10^{-2} M, 2 mL) at 25°C.

[b] A solution of a dioxetane in acetonitrile (1.0×10^{-3} M, 0.1 mL) was added to a NaOH solution in water (0.1 M, 2.9 mL) at 25°C.

[c] A figure in parenthesis means half-life of the CIEEL-decay at 45°C.

[d] Ref. 17.

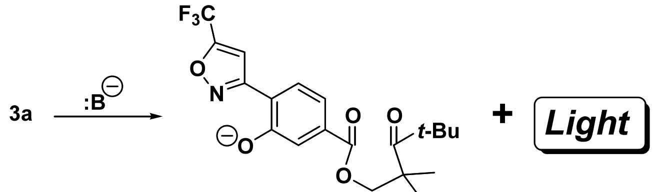
[e] Ref. 7.

[f] Ref. 6.

zolylphenyl-substituted dioxetanes (**3a–c**) with those for **1**, which bears an unsubstituted 3-hydroxyphenyl, one realizes that chemiluminescence efficiency (Φ^{CIEEL}) is 3–4 times higher for **3** than for **1**, while half-life of the CIEEL-decay is prolonged markedly for **3**. It is noteworthy that heating at 45°C accelerated the CIEEL-decay rate considerably for **3a** with little change of Φ^{CIEEL} and $\lambda_{\mathrm{max}}^{\mathrm{CIEEL}}$ ($t_{1/2}$ at 45°C = 1400 s).

Next, we carried out NaOH-induced decomposition of the present dioxetanes (**3a–c**). Treatment of **3a** (1×10^{-3} M in acetonitrile, 0.1 mL) with NaOH (0.1 M in H_2O, 2.9 mL) gave blue light ($\lambda_{\mathrm{max}}^{\mathrm{CIEEL}} = 473$ nm, $t_{1/2} = 13000$ s at 25°C) with remarkable high efficiency ($\Phi^{\mathrm{CIEEL}} = 0.24$), which parallels that attained in TBAF/acetonitrile system, and exceeds Φ^{CIEEL} of the parent dioxetane (**1**) in aprotic medium (acetonitrile). The CIEEL-decay rate of **3a** in an aqueous system was also accelerated on heating ($t_{1/2}$ at 45°C = 1200s). The other analogs (**3b** and **3c**) gave also light effectively in NaOH/H_2O though their Φ^{CIEEL} was inferior to that of **3a** (see Table 1).

The maximum wavelength ($\lambda_{\mathrm{max}}^{\mathrm{CIEEL}}$) of the CIEEL emission for **3a** in TBAF/acetonitrile coincided with fluorescence maximum wavelength ($\lambda_{\mathrm{max}}^{\mathrm{fl}}$) of the corresponding spent reaction mixture, from which a free form of ketoester (**8a**) was isolated exclusively (Fig. 3). The fluorescence of the authentic ketoester (**8a**), prepared from **3a** by thermolysis, was very effective ($\Phi^{\mathrm{fl}} = 0.70$) and its maximum wavelength ($\lambda_{\mathrm{max}}^{\mathrm{fl}}$) coincided also with $\lambda_{\mathrm{max}}^{\mathrm{CIEEL}}$ of the CIEEL emission for **3a** in TBAF/acetonitrile.[16] Using this fluorescence yield (Φ^{fl}) and chemiluminescence yield (Φ^{CIEEL}) shown in Table 1, singlet-chemiexcitation yield ($\Phi_s = \Phi^{\mathrm{CIEEL}}/\Phi^{\mathrm{fl}}$) is estimated to be 0.63 in TBAF/acetonitrile. The fluorescence of **8a** was also very effective even in NaOH/H_2O ($\Phi^{\mathrm{fl}} = 0.50$). Using this value, singlet-chemiexcitation yield (Φ_s) for **3a** is estimated roughly to be 0.48 in an aqueous system, though the fluorescence maximum wavelength of **3a** ($\lambda_{\mathrm{max}}^{\mathrm{fl}} = 466$ nm) was observed in a little shorter region than the case of the CIEEL emission ($\lambda_{\mathrm{max}}^{\mathrm{CIEEL}} = 473$ nm). These results show that both the singlet-chemiexcitation efficiency for the CIEEL of **3a** and the fluorescence efficiency of the emitter (**8a**) are unprecedentedly high for the CIEEL of hydroxyarene-substituted dioxetanes in an aqueous system.

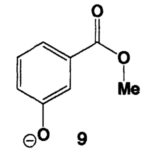
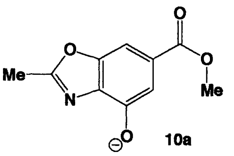
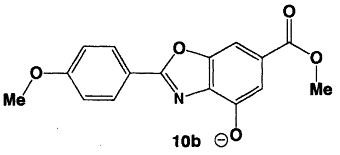
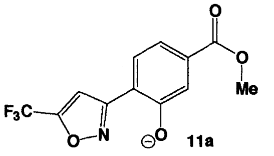
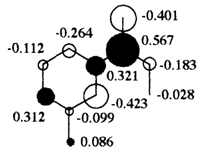
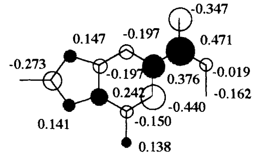
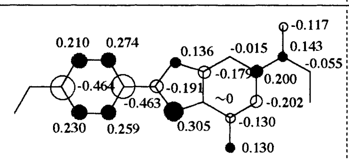
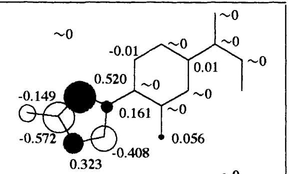
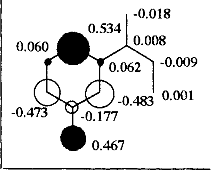
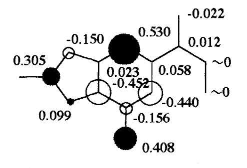
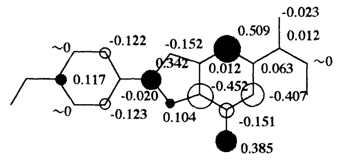
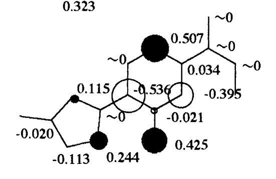


8a

Figure 3.

Hydrogen-bonding effects are presumably responsible for the significant decrease of chemiluminescent efficiency (Φ^{CIEEL}) of phenolate-substituted dioxetanes in an aqueous system.[4,9,10] Of the two factors (Φ_s and Φ^{fl}) relating to Φ^{CIEEL} directly, Φ^{fl} of the produced emitter would be influenced by hydrogen-bonding, though it is not quite clear how hydrogen-bonding affects singlet-chemiexcitation process. Qualitative comparison of the degree of hydrogen-bonding to an oxyanion of hydroxyarenecarboxylates as the emitter should lead to rationalizing the significant difference in chemiluminecence efficiency between **3** and **1** in an aqueous system. The semiempirical AM1 calculations are relevant to attaining this purpose, as has been used by Adam to rationalize the solvatochromism related to the CIEEL of **1** in an aqueous system.[17] Thus, we selected four typical CIEEL-active dioxetanes **1**, **2a**, **2b**, and the present dioxetane (**3a**), for which the order of Φ^{CIEEL} is **1**<**2a**≪**2b**≪**3a**, and carried out the AM1 calculations for their emitters simplified as a methyl ester, namely, oxyanions of an ester (**9**) for **1**, an ester (**10a**) for **2a**, an ester (**10b**) for **2b**, and an ester (**11a**) for **8a**. As shown in Table 2, the large HOMO coefficients on the phenolate oxygen atom guarantee appreciable hydrogen bonding at this site in the ground state for all

Table 2. HOMO and LUMO coefficients for oxyanions of methyl hydroxyarenecarboxylates (**9**, **10a**, **10b** and **11a**)

	 9	 10a	 10b	 11a
LUMO				
HOMO				

four oxyanions, while small LUMO coefficients there suggest far weaker hydrogen-bonding at the same site in the excited state. On the other hand, significant difference is shown in the HOMO and the LUMO coefficients on the ester carbonyl oxygen atom among the four oxyanions. For all oxyanions, the hydrogen bonding should be markedly weak at the carbonyl oxygen atom in the ground state due to the very small HOMO coefficients on this site. In contrast, the LUMO coefficients on the carbonyl oxygen atom are significantly different among **9**, **10a**, **10b**, and **11a**, and they decrease in the order **9**>**10a**>**10b**≫**11a** so that the hydrogen-bonding at this site should be appreciable for **9** whereas minimal for **11a** in the excited state. This order coincides with the order of the chemiluminescence efficiency, **1**<**2a**≪**2b**≪**3a**, in an aqueous medium. Conclusively, the AM1 calculations suggest that the hydrogen-bonding at the carbonyl oxygen site of the ester rather than the phenolate oxygen site in an oxyanion of the phenolic ester as the emitter plays an important role to decrease its fluorescence efficiency which affects the chemiluminescence efficiency for the CIEEL-active dioxetanes in an aqueous system.

Dioxetanes bearing a 3-hydroxy-4-isoxazolylphenyl moiety (**3a**–**c**) synthesized here, especially **3a**, were shown to exhibit remarkably high chemiluminescence efficiency even in an aqueous system. The AM1 calculations of the emitters for **1**, **2** and **3a** suggested that the hydrogen-bonding at the carbonyl oxygen site should affect Φ^{fl}. Further study on the CIEEL of **3** is expected to provide a clue to examining the relation of the chemiexcitation process to the hydrogen bonding, which remains unsolved.

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13. Selected data for **3a**: pale yellow granules melted at 126.0–128.0°C (from hexane, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ_H 1.02 (s, 9H), 1.17 (s, 3H), 1.39 (s, 3H), 3.85 (d, $J=8.3$ Hz, 1H), 4.60 (d, $J=8.3$ Hz, 1H), 7.28 (dd, $J=8.3$ and 1.5 Hz, 1H), 7.39 (d, $J=1.5$ Hz, 1H), 7.59 (d, $J=1.5$ Hz, 1H), 7.91 (d, $J=8.3$ Hz, 1H), 10.67 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 18.5, 25.1, 27.0, 36.8, 45.7, 80.5, 105.4, 110.3, 115.9, 117.9, 118.7, 119.7, 126.0, 127.5, 138.7, 141.8, 157.3, 162.7; IR (KBr) 3144, 2975, 2898, 1613, 1550; MS (*m*/*z*, %) 413 (M^+, 1), 381 (13), 366 (20), 357 (28), 273 (33), 256 (100), 228 (13). Anal. calcd for $C_{20}H_{22}F_3NO_5$: C, 58.11; H, 5.36; N, 3.39. Found: C, 58.16; H, 5.38; N, 3.74%.
14. A solution of **3a** in acetonitrile (1.0×10^{-5} M, 1 mL) was added to a TBAF solution in acetonitrile (1.0×10^{-2} M, 2 mL) at 25°C.
15. Chemiluminescence efficiency (Φ^{CIEEL}) was based on the reported value for *tert*-butyldimethylsilyl ether of **1**: $\Phi^{CIEEL}=0.29$ in DMSO (Ref. 7).
16. Quinine bisulfate was used as the fluorescence standard.
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