Chemi- and Bio-luminescent Properties of Coelenterazine Analogues Possessing an Adamantyl Group

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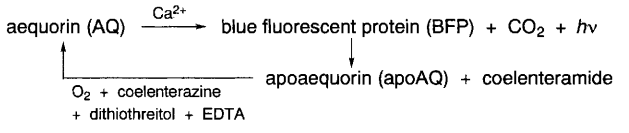
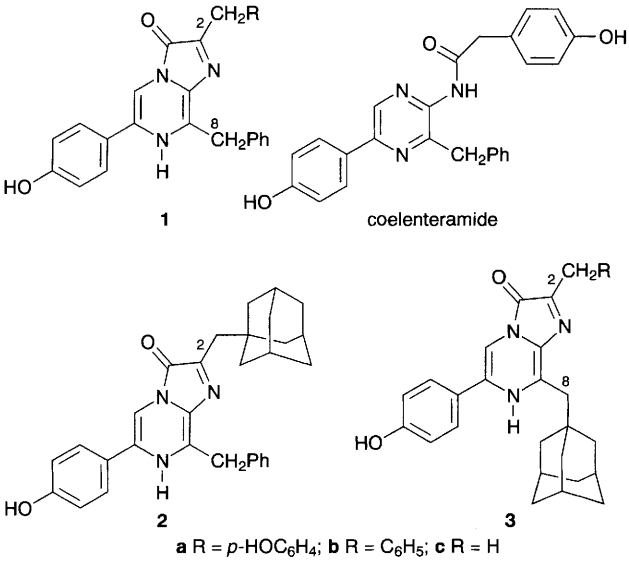
The chemi- and bio-luminescent properties of coelenterazine analogues possessing either 2- or 8-adamantylmethyl groups are studied and it is found that the bioluminescence intensity of semi-synthetic AQ and AQC145,152,180S containing an 8-adamantylmethyl coelenterazine analogue is stronger than that of natural coelenterazine.

Coelenterazine **1a** is a luminescent chromophore of a photoprotein aequorin (AQ) isolated from the jellyfish *Aequorea victoria*, which forms AQ with apoaequorin (apoAQ, apoprotein) and molecular oxygen (Scheme 1).[1] In the presence of Ca^{2+}, AQ emits light with a quantum yield of *ca.* 23%,[2] giving a blue fluorescent protein (BFP) which consists of coelenteramide and apoAQ.[3] Coelenterazine **1a** also luminesces in organic solvents under O_2 giving coelenteramide as the oxidized product. However, the quantum yield of chemiluminescence is lower than that of AQ bioluminescence.[4] The hydrophobic character of apoAQ is expected to give good conditions for obtaining efficient luminescence of **1a**.[5] Replacing a substituent of **1a** with a hydrophobic group will intramolecularly affect the chemi- and bio-luminescent properties of **1a**. Herein, we describe the synthesis of coelenterazine analogues **2** and **3a–c**, which have an adamantylmethyl group as the hydrophobic substituent, and our investigations into their chemi- and bio-luminescent properties, as compared with those of **1a**.

2-Adamantylmethyl analogue **2** was prepared by the condensation of keto acetal **4** with coelenteramine[6] under acidic conditions [3% HCl–dioxane (3:4), 100 °C] in 44% yield as shown in Scheme 2. Similarly, analogue **3a** was obtained by coupling keto acetal **5** with 3-adamantylmethyl coelenteramine analogue **6** in 63% yield (Scheme 2). Compounds **3b** and **3c** were prepared by the same procedure. Keto acetal **5** was found to be an useful reagent for preparing coelenterazine **1a** in the place of 4-acetoxybenzylglyoxal.[7]

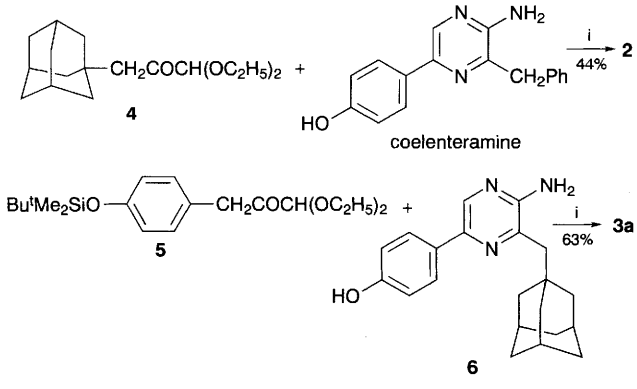


Scheme 1



When solutions of the analogues **2** or **3a** in methanol (1.8×10^{-3} mol dm^{-3}, 100 μl) were mixed with 2 ml of Me_2SO or diglyme containing 0.20 mol dm^{-3} acetate buffer (pH 5.6, 0.66% *v/v*) under air, the solution began to emit light.† The chemiluminescence properties of **2** and **3a** in Me_2SO were similar to those of **1a**.[8–10] In diglyme–acetate buffer, the chemiluminescence spectra of **2** and **3a** were different from that of **1a** as shown in Fig. 1, although the chemiluminescence quantum yields of **1a**, **2** and **3a** were similar. The emission of **2** with the maxima at 405 and 445 nm came from the excited states of both the neutral and the amide anion forms of the coelenteramide analogue, respectively, and that of **3a** came only from the excited neutral form, although **1a** chemiluminesced from the excited amide anion of coelenteramide. This result suggests that the excited state of the amide anion of coelenteramide analogue may be destabilized intramolecularly, by a hydrophobic adamantylmethyl group, accelerating the rate of protonation to the amide anion to give the excited state of the neutral form.[11]

The bioluminescent properties of **2** and **3a–c** were investigated by preparing semi-synthetic AQ according to the method of Shimomura *et al.*[12] Analogues **2** and **3a–c** as well as coelenterazine **1a** and its analogues **1b,c**[13] were incubated with recombinant wild-type apoAQ in 0.030 mol dm^{-1} Tris–HCl buffer (pH 7.6) containing EDTA (0.010 mol dm^{-3}) and dithiothreitol (0.002 mol dm^{-3}) for 3 h in an ice bath under air.[14] To compare the nature of the active site of wild-type apoAQ with that of the modified apoAQ with the cysteine residues 145, 152 and 180 replaced by serine (apoAQC145,152,180S), semi-synthetic AQC145,152,180S



Scheme 2 *Reagents and conditions*: i, 3% aq. HCl–dioxane (3:4), 100–110 °C

was also prepared by the same incubation procedure.[15] The maximum light intensities were measured by adding solutions of $CaCl_2$ in Tris–HCl buffer to the regeneration mixtures, and the relative luminescence intensities are summarized in Table 1. Analogue **3a** gave the best bioluminescence intensities both of semi-synthetic AQ and of semi-synthetic AQC145,152,180S, although **2** gave no activity. Replacement of the 2-(4-hydroxyphenyl)methyl group of **3a** with a benzyl or methyl group reduced the bioluminescence intensity. The intensity ratio of **3a** : **3b** : **3c** in AQ bioluminescence matches that of **1a** : **1b** : **1c**, indicating that the side-chains at the C2 and C8 positions of coelenterazine independently determined the bioluminescence activity. These ratios also resemble those of the bioluminescence of AQC145,152,180S, showing that replacing cysteine in apoAQ with serine does not change the properties of the active site for recognizing the structure of coelenterazine. In order to obtain information on the lack of activity of compound **2**, analogue **2** was incubated with apoAQ for 3 h, followed by the addition of **1a** to the regeneration mixture. After an additional 3 h, a solution of $CaCl_2$ was added and no bioluminescence activity of AQ was observed. This result indicates that **2** effectively binds to the active site of apoAQ irreversibly and inhibits the regeneration of AQ from **1a** and apoAQ.

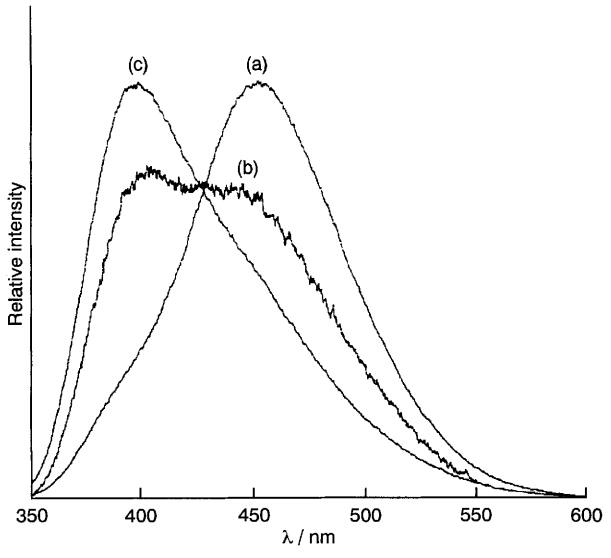


Fig. 1 Chemiluminescence spectra of (a) **1a**, (b) **2** and (c) **3a** in diglyme containing acetate buffer (pH 5.6) under air

Table 1 Relative intensities of bioluminescence of coelenterazine **1a** and its analogues **1b**, **c**, **2** and **3a–c**

	Relative bioluminescence intensity[a]	
Compound	AQ	AQC145,152,180S
1a	1.0	1.0
1b	0.35	0.14
1c	0.01	0.01
2	0	0
3a	2.0	2.4
3b	0.73	0.67
3c	0.02	<0.01

[a] A solution (20 μl) of coelenterazine analogue (2.4×10^{-3} mol dm^{-3}) in methanol was added to a solution (1.0 ml) of recombinant apoAQ and apoAQC145,152,180S in 0.030 mol dm^{-3} Tris–HCl buffer (pH, 7.6) containing dithiothreitol (0.002 mol dm^{-3}) and EDTA (0.010 mol dm^{-3}), and was allowed to stand in an ice bath for 3 h. To 20 μl of the regeneration mixture was injected a solution (20 μl) of 0.030 mol dm^{-3} $CaCl_2$ in Tris–HCl buffer (pH, 7.6), and the initial maximum light intensity was measured with a lumiphotometer [Labo Science (Tokyo) TD-1000].

The bioluminescence emission spectra of **3a** in semi-synthetic AQ and AQC145,152,180S showed maxima at 445 and 442 nm, respectively, which were at shorter wavelengths than those of **1a**. Shimomura *et al.* have already reported that 8-cycloalkylmethyl coelenterazine analogues in semi-synthetic AQ emit light with maxima around 445 nm.[12] These results indicate that 8-adamantylmethyl and 8-cycloalkylmethyl groups intramolecularly destabilize the excited state of coelenteramide and give a blue-shifted emission.

In conclusion, the results that **3a** gives the most active semi-synthetic AQ and that **2** inhibits the regeneration of wild-type AQ indicate that the adamantylmethyl group helps **2** and **3a** to occupy the active centre of apoAQ by hydrophobic interactions. For efficient bioluminescence activity, the 8-adamantylmethyl group of **3a** fixes the coelenterazine skeleton with the most suitable configuration at the active site of apoAQ. On the other hand, the hydrophobic interaction may destabilize the excited state of the coelenteramide analogue, causing a blue-shifted emission.

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Footnote

† The chemiluminescence spectra of **1a** and its analogues in diglyme are strongly dependent on the pH value and the volume of acetate buffer added in diglyme. The data in our previous paper (ref. 13) were obtained in diglyme containing 0.1 mol dm^{-3} acetate buffer (pH 5.6, 2.6% *v/v*).

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