Tetrahedron Letters 53 (2012) 5114–5118

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

New red-shifted coelenterazine analogues with an extended electronic conjugation

Germano Giuliani [a,*], Paola Molinari [b], Giulia Ferretti [a], Andrea Cappelli [a], Maurizio Anzini [a], Salvatore Vomero [a], Tommaso Costa [b]

[a] Dipartimento Farmaco Chimico Tecnologico and European Research Centre for Drug Discovery and Development, University of Siena, Via A. Moro 2, 53100 Siena, Italy
[b] Dipartimento di Farmacologia, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy

ARTICLE INFO

Article history:
Received 23 May 2012
Revised 6 July 2012
Accepted 10 July 2012
Available online 20 July 2012

Keywords:
Bioluminescence
Coelenterazine
Red-shift
2Y-System
Renilla Luciferase

ABSTRACT

A new promising approach to the development of red-shifted coelenterazine analogues was described. In order to alter the photochemical properties of native coelenterazine, we have designed and synthesized analogues bearing a new electron-rich structure. The spectroscopic results obtained, in the presence of the target enzyme (Renilla Luciferase), show a bathochromic emission shift of the entire class of new derivatives. Among them, the 2-benzyl-8-(4-chlorophenylthio)-6-(4-hydroxyphenyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one (**8**) shows an emission at 510 nm and uncommon slow kinetic decay.

© 2012 Elsevier Ltd. All rights reserved.

The use of bioluminescent enzymes as gene reporters in cell culture and small-animal imaging[1,2] has become a useful technological tool for life science research and drug discovery. Most of the bioluminescence systems investigated require a luciferase (enzyme), a luciferin (substrate), and molecular oxygen to produce visible light.[3] Enzymatic degradation of luciferin components produces intermediates bearing an electron in an excited state. More in detail, the luminescence reaction is initiated by the binding of O_2 at the 2-position of the coelenterazine molecule, giving a peroxide. The peroxide then forms a four-membered ring 'dioxetanone',[4] that promptly decomposes producing CO_2 and the amide anion of coelenteramide in its excited state. The excited state of the amide anion of coelenteramide emits light when its energy level falls to the ground state, resulting in the emission of blue light (λ_{max} 450–470 nm).[5] Clearly, the energy of the emitted photons depends on the difference between the two energy states. For imaging experiments in intact animals, photon emissions in the red to near-infrared wavelengths (600–900 nm) are required, since the tissue absorption of photons is reduced in this region of the spectrum.[6] For this reason the firefly (beetle) luciferase that uses D-luciferin **1** (Fig. 1) as substrate (emission spectra in the range of 540–615 nm) is the preferred bioluminescent system in small-animal imaging applications. Unfortunately, the Mg^{2+}- and ATP-

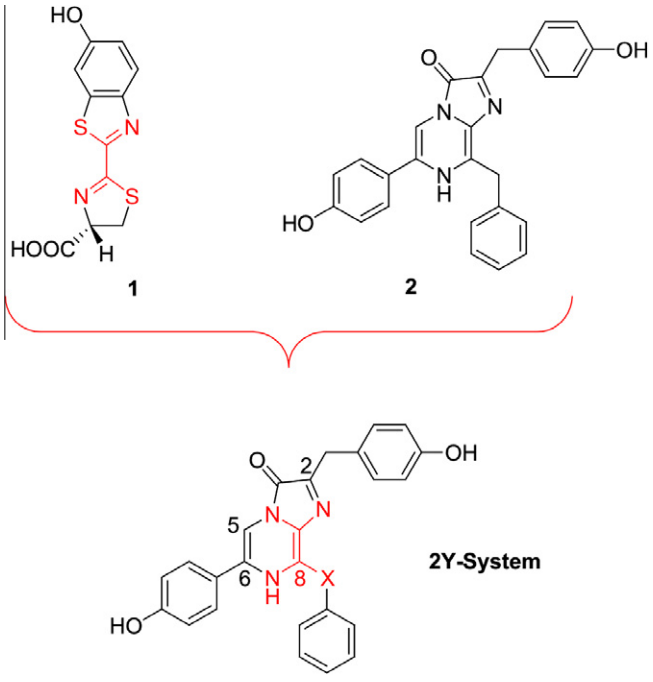


Figure 1. General formula of the derivatives, where X is a not specified heteroatom.

* Corresponding author. Tel.: +39 0577 234359; fax: +39 0577 234333.
E-mail address: giuliani5@unisi.it (G. Giuliani).

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.07.041

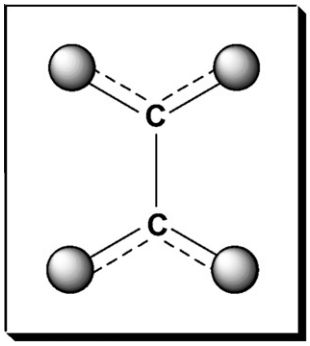


Figure 2. The spheres represent generic heteroatoms, in the 2Y-System in which three of them are nitrogen atoms: N-1, N-4, and N-7 of the original imidazo[1,2-a]pyrazin-3(7*H*)-one nucleus.

dependence of the reaction and the poor thermal stability of some of these enzymes limit their versatility.

On the other hand, the reaction of imidazopyrazinone-type luciferases that use coelenterazine **2** as the substrate [e.g. *Renilla* (RLuc), *Oplophorus*, and *Gaussia* luciferases], only require is molecular oxygen. Thus, they do not suffer from such problems, but the emission range of these enzymes is in the blue region (450–475 nm) of the visible spectrum. To overcome such a limitation, the development of red-shifted and stable variants of RLuc obtained via random mutagenesis[7] used in combination with red-shifted analogues of coelenterazine,[8] has proven to be a promising approach.[9] While it seems to be difficult to achieve additional red-shifting by modifications of the enzyme at the current state of knowledge, the synthesis of new coelenterazine analogues with better bathochromic emission shifts may offer some room for improvement. In fact, despite the large number of coelenterazine analogues generated during the past forty years of research, only few red-shifted substrates have been reported thus far. One of such molecules, is *v*-coelenterazine, which displays a 35 nm bathochromic shift and high quantum yield with RLuc, but not *Oplophorus* luciferase.[8,9] Additional coelenterazine analogues with a conjugated group at the C-8 position of the imidazopyrazinone ring displayed large bathochromic shifts (50–110 nm) of chemiluminescence in polar aprotic solvent, but their bioluminescence in the Rluc reaction was negligibly low.[10]

On confronting **1** and **2**, we note that in the D-luciferin molecule N and S heteroatoms exist in a particular arrangement (see Fig. 2). We suspect that this electron-rich configuration may play a fundamental role in the formation of the multiple oxyluciferin excited states that result in a broad range of photon-emission colors.[11] Thus, we hypothesize that building a similar heteroatomic configuration in the imidazopyrazinone scaffold (which we name 2Y-System, for heteroatoms stand symmetrically at the four tips of a scaffold resembling an upside-down linked double Y) might enhance the stability of lower-energy emitting species of coelenterazine.

As a first step in this direction, we replaced with an S heteroatom the methylene group bonded to the C-8 of the imidazo[1,2-*a*]pyrazin-3(7*H*)-one nucleus of **2**, thus generating a series of hybrid compounds between the D-luciferin **1** and coelenterazine **2** (Fig. 1). By means of this simple structural modification, we assumed to modulate the photoemission of the analogues, maintaining the enzymatic binding properties of the native substrate. A number of derivatives (**3**–**6**, Fig. 3) were made using *bis*-deoxycoelenterazine **3** as the reference, because this compound represents a simpler coelenterazine analogue, which generates a lower-efficiency blue-shifted luminescence (~400 nm) with Rluc. Together with heteroatom interchange, *S*-phenyl halogen substitution was

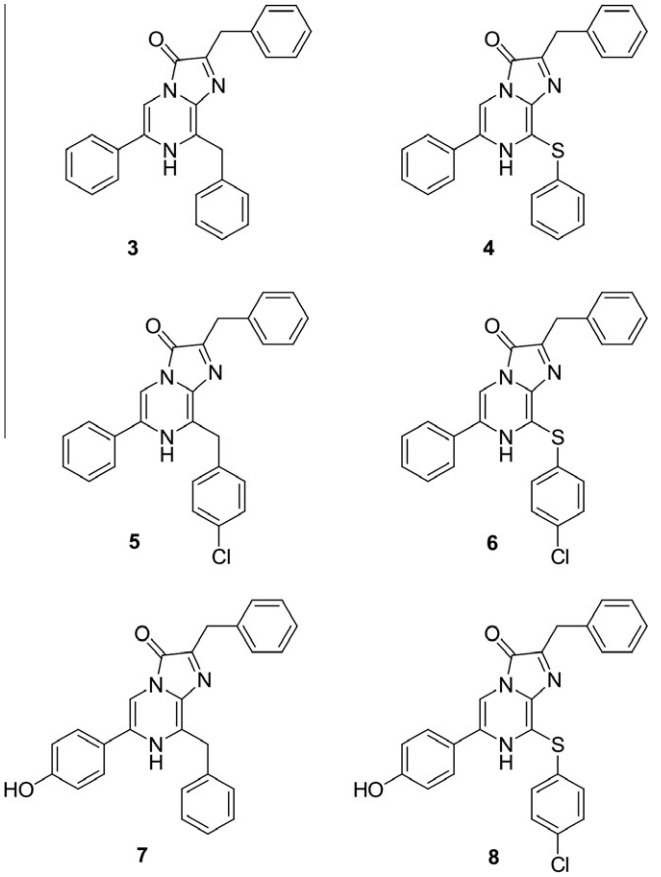


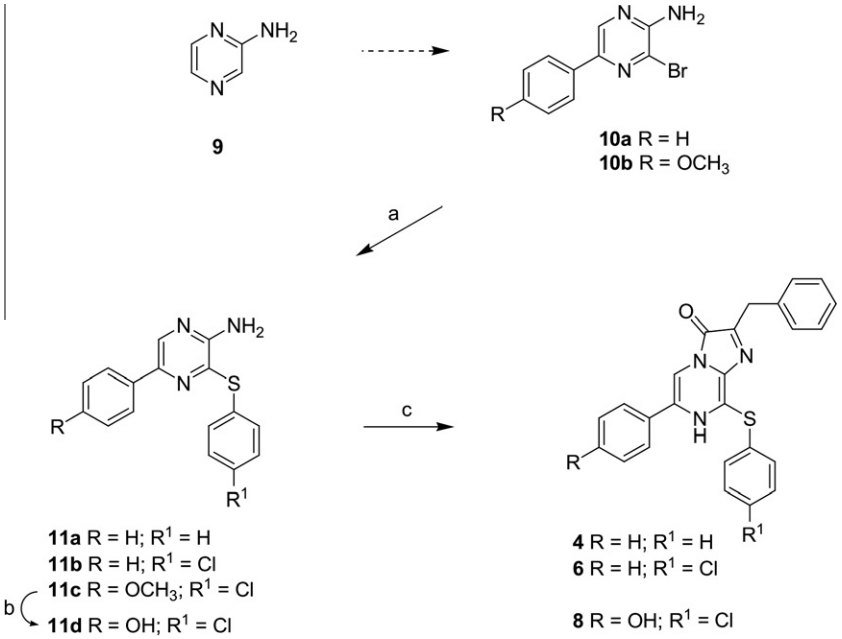
Figure 3. Structures of the newly-synthesized coelenterazine analogues compared to those of reference compounds **3** and **7**.

also explored, in the attempt to further modulate the electron density of the 2Y-System. Similar substitutions were also made on *h*-coelenterazine (**7**–**8**, Fig. 3), which has Rluc bioluminescent properties indistinguishable from the natural substrate.

The synthesis of the analogues **4**,**6**,**8** with sulfur heteroatom was performed from commercially available 2-aminopyrazine (**9**) by means of the procedure reported in Scheme 1 based on the Suzuki coupling [12]. Key pyrazine intermediates **11a–c**[13] were obtained by reaction of bromoderivatives **10a**,**b**[14] with the appropriate thiol in dry DMF or acetonitrile in the presence of sodium hydride as the base.[15] Demethylation of compound **11c** was performed with pyridine hydrochloride (Py×HCl)[12d] at 190 °C to obtain the corresponding hydroxyl derivative **11d**.[13]

The final step of the convergent synthesis of the coelenterazine analogues **4**,**6**,**8**[16] was achieved by condensation of intermediates **11a**,**b**,**d**, with diethoxy derivative[17] in ethanol in the presence of concentrated HCl.[10]

The introduction of the sulfur heteroatom in place of the methylene bridge in **3** leading to compound **4** produces a significant (40 nm) bathochromic shift of the chemiluminescence spectrum measured in neutral DMSO (Table 1). The *p*-chlorophenyl substituent did not change the chemiluminescence spectrum in the absence of heteroatom (compound **5**[18]) nor did alter the red-shifting effect of sulfur replacement (**6**). A similar red-shift (47 nm) of chemiluminescent emission was observed in the *h*-coelenterazine derivative (**8**, Table 1). This overall bathochromic shifting effect of C-8 heteroatom replacement was also maintained in the bioluminescent reaction with Rluc. The bioluminescence spectra of **4** displayed a 30 nm red-shift compared to **3** (Fig. 4). Likewise, both the minor shoulder (λ_{max} ~387 nm) and the main peak (λ_{max} 471 nm) in the bioluminescent emission of **7** (believed to represent the neutral and the



Scheme 1. Reagents: (a) C_6H_5SH or 4-ClC_6H_4SH, NaH, dry CH_3CN; (b) Py·HCl; (c) 1,1-diethoxy-3-phenylacetone, concd HCl, H_2O, EtOH.

Table 1
Chemiluminescence, bioluminescence, and relative quantum yields of coelenterazine analogues

Compd	Chemilum. λ_{max}	Bioluminescence[a]		RQY[b](biolum.)	RQY[c](chemilum.)
		$\lambda_{max}{}^1$	$\lambda_{max}{}^2$		
3	457	390	—	1	1
4	497	419	—	3.6	1.83
5	456	382	432	1.3	1.80
6	499	469	—	0.2	0.92
7	460	471	387	688	0.96
8	507	509	432	23	0.42

[a] Main peak ($\lambda_{max}{}^1$) and minor shoulder ($\lambda_{max}{}^2$) in the bioluminescent emission of analogues.
[b] Bioluminescence quantum yield, photons emitted per molecule of luciferin reacted.
[c] Chemiluminescence quantum yield, photons emitted per molecule of luciferin reacted.

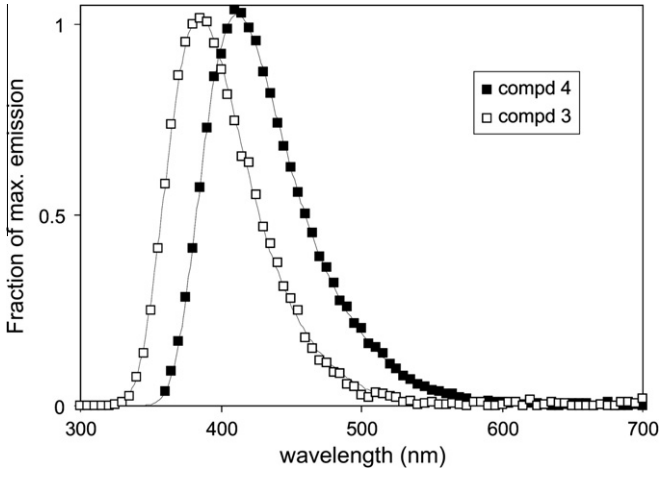


Figure 4. Effect of the structural modifications on the corrected bioluminescence emission spectra. Comparison between compounds **3** and **4**.

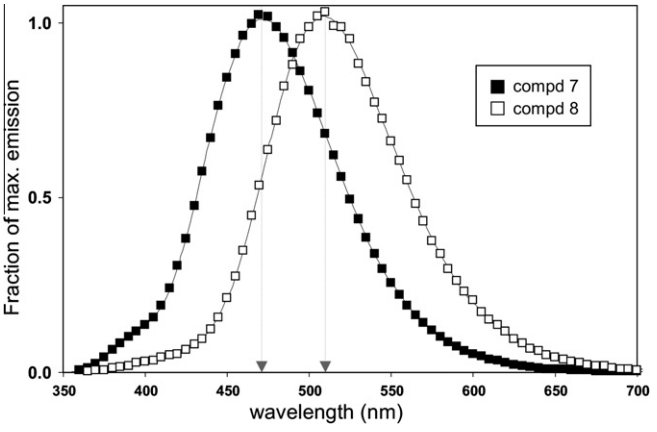


Figure 5. Effect of the structural modifications on the corrected bioluminescence emission spectra. Comparison between compounds **7** and **8**.

amide anion emitting species, respectively) were red-shifted in compound **8** (Fig. 5 and Table 1). However, while replacement of only S in the *bis*-deoxycoelenterazine scaffold (**3**) resulted in enhancement (~fourfold) of bioluminescence efficiency, the combined substitutions of both S and Cl caused a fivefold decrease. Similarly S and Cl substitutions in *h*-coelenterazine (7) resulted in a 30-fold lower relative quantum yield (Table 1).

Interestingly, unlike chemiluminescence, the bioluminescence of *bis*-deoxycoelenterazine derivatives was affected by chlorine substitution of the S-linked phenyl ring. In fact, the red-shift

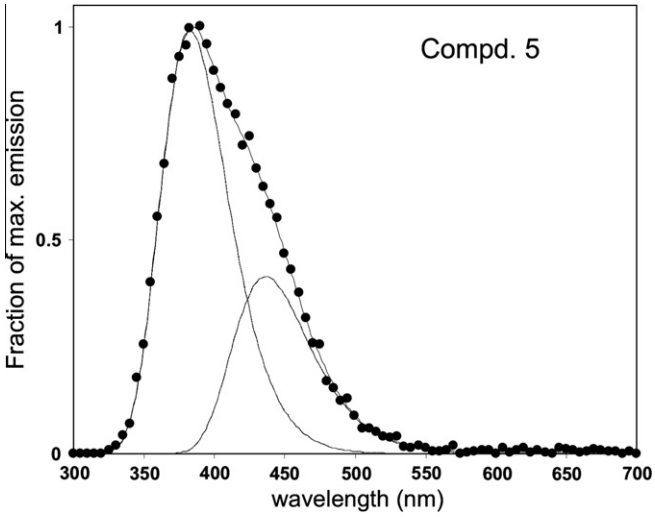


Figure 6. Bioluminescence spectrum of compound **5**. The dotted lines indicate the two emitting components resolved by the fitting procedure (see Section 4 of Supplementary data).

(~80 nm) with both Cl and S substitutions (**6** vs **3**, Table 1) was significantly greater than that produced by only heteroatom replacement (**4**). Moreover, Cl substitution in the absence of S (**5**) determined a broadening of the bioluminescence spectrum, which could be resolved as two components of higher and lower energy (Fig. 6), suggesting that this compound generates two stable emitters during the enzymatic reaction. Unfortunately, compound **6** also suffered a fivefold drop of bioluminescence efficiency compared to **3**; consequently the lower quality of spectral data did not allow further analysis (Fig. S1).[18]

An additional interesting finding was only observed in the *h*-coelenterazine derivative **8**, and involved a sharp change of bioluminescence kinetics.[18] Unlike **7**, which shows the typical flash-like kinetics of coelenterazines, with an immediate burst to peak emission rate followed by steady decay, the derivative **8** displayed a more complex pattern. The initial burst was followed by a second slower increase of emission rate, which lasted 5–7 s before giving rise to the decay phase (Fig. S2). This peculiar difference in kinetics was not observed in the chemiluminescent reaction (Fig. S3), which suggests that the phenomenon is caused by different interactions of the two molecules within the binding pocket of the enzyme.

The results of this study appear to support the idea that the insertion of a C-8 bonded S atom, (thus achieving partial mimicry of the 2Y heteroatomic pattern in D-luciferin), can favor the emergence of lower energy emitters in coelenteramide. This bathochromic effect was evident in the presence of either the phenyl or the phenol ring in C-6, indicating that the red shift can occur regardless of whether the main emitter is the neutral or the amide-anion form of the molecule. It also occurred in both chemiluminescence and bioluminescence, suggesting that it likely results from an intrinsic change in the electronic properties of the excited states of the molecule, independently of how they are produced. Fluorescence studies of coelenteramide analogues and model compounds suggest that the lowest energy emitting species (530–550 nm) formed during coelenterazine oxidation is the pyrazine-N(4) anion.[19] We may speculate that S replacement and the highly conductive 2Y pattern that it generates may favor the formation of this anionic species. However, further work is necessary to verify this possibility.

In conclusion, we describe here a new series of red-shifted coelenterazine analogues and a novel approach to alter the photochemical properties of the light-emitter intermediates. Further exploitation of these findings may extend understanding on the underlying mechanisms of bioluminescence in this class of molecules.

Acknowledgments

We thank Professor P. Knochel and Dr. T. Bresser (Dep. Chemie & Biochemie, LMU, Munchen, Germany) for their experimental support and encouragement.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.07.041.

References and notes

1. (a) Rowe, L.; Dikici, E.; Daunert, S. *Anal. Chem.* **2009**, *81*, 8662–8668; (b) Michelini, E.; Cevenini, L.; Mezzanotte, L.; Ablamsky, D.; Southworth, T.; Branchini, B.; Roda, A. *Anal. Chem.* **2008**, *80*, 60–67; (c) De, A. *Curr. Pharm. Biotechnol.* **2011**, *12*, 558–568; (d) Menon, V.; Ranganathn, A.; Jorgensen, V. H.; Sabio, M.; Christoffersen, C. T.; Uberti, M. A.; Jones, K. A.; Babu, P. S. *Assay Drug Dev. Technol.* **2008**, *6*, 787–793.
2. (a) Watts, J. C.; Giles, K.; Grillo, S. K.; Lemus, A.; DeArmond, S. J.; Prusiner, S. B. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 2528–2533; (b) Patel, M. R.; Chang, Y. F.; Chen, I. Y.; Bachmann, M. H.; Yan, X.; Contag, C. H.; Gambhir, S. S. *Cancer Res.* **2010**, *70*, 10141–10149.
3. Campbell, A. K. *Chemiluminescence, principles and applications in biology and medicine*; Ellis Horwood Ltd: Chichester (England), 1988. pp. 189–218.
4. (a) Shimomura, O. *Bioluminescence, chemical principles and methods*; World Scientific Publishing Co. Ltd: Singapore, 2006. pp. 159–176; (b) Liu, F.; Liu, Y.; De Vico, L.; Lindh, R. *J. Am. Chem. Soc.* **2009**, *131*, 6181–6188; (c) Isobe, H.; Yamanaka, S.; Okumura, M.; Yamaguchi, K. *J. Phys. Chem. A* **2009**, *113*, 15171–15187.
5. Hori, K.; Wampler, J. E.; Cormier, M. J. *Chem. Commun.* **1973**, 492–493.
6. Weissleder, R. *Nat. Biotechnol.* **2001**, *19*, 316–317.
7. Loening, A. M.; Wu, A. M.; Gambhir, S. S. *Nat. Methods* **2007**, *4*, 641–643.
8. Inouye, S.; Shimomura, O. *Biochem. Biophys. Res. Commun.* **1997**, *233*, 349–353.
9. Dragulescu-Andrasi, A.; Chan, C. T.; De, A.; Massoud, T. F.; Gambhir, S. S. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 12060–12065.
10. Wu, C.; Nakamura, H.; Murai, A.; Shimomura, O. *Tetrahedron Lett.* **2001**, *42*, 2997–3000.
11. Naumov, P.; Ozawa, Y.; Ohkubo, K.; Fukuzumi, S. *J. Am. Chem. Soc.* **2009**, *131*, 11590–11605.
12. (a) Nakamura, H.; Aizawa, M.; Murai, A. *Synlett* **1996**, 1015–1017; (b) DeBie, D. A.; Ostrowicz, A.; Guertsen, G.; VanDerPlas, H. C. *Tetrahedron* **1988**, *44*, 2977–2983; (c) Jones, K.; Keenan, M.; Hibbert, F. *Synlett* **1996**, 509–510; (d) Adamczyk, M.; Johnson, D. D.; Mattingly, P. G.; Pan, Y.; Reddy, R. E. *Org. Prep. Proced. Int.* **2001**, *33*, 477–485.
13. *General procedure for the synthesis of **11a–c***: To a cooled solution of the appropriate thiophenol (1.0 equiv) in dry DMF, sodium hydride (1.2 equiv) was added and the resulting mixture stirred at 5–10 °C for 15 min. Bromo derivative **10a,b** (1.0 equiv) was then added and the mixture was heated at 80 °C for 17–20 h. The reaction mixture was quenched with water and extracted with EtOAc. The combined extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification of the residue by flash chromatography gave the target intermediates **11a–c** (yield 74–80%). *Compound* **11a**: mp: 110–112 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.37 (s, 1H, Ar-H), 7.78 (d, J = 7.2, 2H, Ph-H), 7.53–7.50 (m, 2H, Ph-H), 7.41–7.29 (m, 6H, Ph-H), 5.02 (br s, 2H, NH_2). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 151.8, 143.0, 137.8, 137.0, 136.6, 132.7, 130.7, 129.3, 128.8, 128.3, 125.5. MS (ESI) m/z: 280 [M+H$^+$]. *Compound* **11b**: mp: 157–158 °C. ^{1}H NMR (400 MHz, CD_3OD, 25 °C, TMS): δ = 8.29 (s, 1H; Ar-H), 7.68 (d, J(H;H) = 7.2 Hz, 2H, Ph-H), 7.51 (d, J(H;H) = 8.4 Hz, 2H, Ph-H), 7.43 (d, J(H;H) = 8.8 Hz, 2H, Ph-H), 7.32 (t, J(H,H) = 7.4 Hz, 2H, Ph-H), 7.24–7.27 (m, 1H, Ph-H). ^{13}C NMR (400 MHz, CD_3OD, 25 °C, TMS): δ = 152.7, 140.0, 137.4, 136.8, 136.7, 135.3, 133.7, 129.8, 129.7, 129.1, 128.2, 125.1. MS (ESI) m/z: 314 [M+H$^+$]. *Compound* **11c**: Purification of the residue by flash chromatography using Al_2O_3, with CH_2Cl_2·EtOAc (9:1 v/v) as the eluent, afforded compound **11c**. mp: 154–155 °C. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 8.28 (s, 1H; Ar-H), 7.68 (d, J(H,H) = 8.8 Hz, 2H; Ph-H), 7.43 (d, J(H,H) = 8.8 Hz, 2H; Ph-H), 7.34 (d, J(H,H) = 8.8 Hz, 2H; Ph-H), 6.91 (d, J(H,H) = 8.8 Hz, 2H; Ph-H), 4.90 (br s, 2H; NH_2), 3.82 (s, 3H; CH_3). ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 160.0, 151.0, 143.1, 136.9, 136.4, 134.4, 134.0, 129.3, 129.1, 126.7, 114.2, 55.4. MS (ESI) m/z: 366 [M+Na$^+$]. *Compound* **11d**: To a Schlenk's tube containing pyridine hydrochloride (20 equiv) heated at 190 °C, compound **11c** (1 equiv) was added. The resulting mixture was stirred at 190 °C for 2 h under an argon atmosphere, cooled at RT, and quenched with a saturated solution of $NaHCO_3$. The resulting mixture was extracted with EtOAc and the combined extracts were dried and concentrated under reduced pressure. Purification of the residue by flash chromatography using Al_2O_3, with CH_2Cl_2·EtOAc (1:1 v/v) as the eluent,

afforded compound **11d** (yield 55%) as a yellow solid. mp: 133–134 °C. ^{1}H NMR (400 MHz, CD_3OD, 25 °C, TMS): δ = 8.18 (s, 1H; Ar-H), 7.52 (d, *J*(H,H) = 8.8 Hz, 2H; Ph-H), 7.47 (d, *J*(H,H) = 8.4 Hz, 2H; Ph-H), 7.39 (d, *J*(H,H) = 8.4 Hz, 2H; Ph-H), 6.74 (d, *J*(H,H) = 8.8 Hz, 2H; Ph-H). ^{13}C NMR (400 MHz, CD_3OD, 25 °C, TMS): δ = 157.6, 151.2, 142.1, 137.3, 134.9, 134.6, 134.2, 129.4, 128.9, 127.9, 126.2, 115.1. MS (ESI, negative ions) *m*/*z*: 328 [M−H$^+$].

14. Sato, N. *J. Heterocycl. Chem.* **1978**, *15*, 665–670.
15. Shimazaki, M.; Hikita, M.; Hosoda, T.; Ohta, A. *Heterocycles* **1991**, *32*(5), 937–947.
16. *General procedure for the synthesis of* ***4***,***6***,***8***: A mixture of the appropriate pyrazine derivative (**11a,b,d** 1 equiv), 1,1-diethoxy derivative **12** (1.4 equiv), 36% aqueous HCl (13 equiv) and ethanol (10 mL) was refluxed for 5–8 h. After cooling to RT, the reaction mixture was concentrated under reduced pressure and the residue obtained was purified by flash chromatography with CH_2Cl_2·MeOH (95:5 v/v) as the eluent to obtain the corresponding coelenterazine derivative (**4**,**6**,**8,** yield 25–45%). Note: final compounds should be protected from the exposure to air, light and stored at −18 °C. *Compound* **4**: mp: 115–117 °C. ^{1}H NMR (400 MHz, CD_2Cl_2, 25 °C, TMS): δ = 8.12 (s, 1H; Ar-H), 7.65–7.59 (m, 4H; Ph-H), 7.49–7.45 (m, 2H; Ph-H), 7.32–7.11 (m, 9H; Ph-H), 4.10 (s, 2H; CH_2). ^{13}C NMR (400 MHz, CD_2Cl_2/CD_3OD (0.4 mL/0.1 mL), 25 °C, TMS): *d* = 139.9, 136.8, 136.2, 130.0, 129.7, 129.2, 129.1, 129.0, 128.9, 128.2, 126.8, 126.2, 108.5, 32.1. MS (ESI) *m*/*z*: 410 [M+H$^+$]. *Compound* **6**: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 8.13 (s, 1H; Ar-H), 7.53–7.50 (m, 2H; Ph-H), 7.35–7.27 (m, 7H; Ph-H), 6.97 (d, *J*(H,H) = 6.8 Hz, 2H; Ph-H), 6.90–6.81 (m, 3H; Ph-H), 4.00 (s, 2H; CH_2). ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C, TMS): *d* = 146.5, 142.1, 140.4, 138.6, 138.5, 136.8, 135.9, 135.8, 129.4, 128.9, 128.3, 128.2, 126.3, 125.8, 108.2, 31.5. MS (ESI, negative ions) *m*/*z*: 442 [M-H$^+$]. *Compound* **8**: mp: 166–167 °C. ^{1}H NMR (400 MHz, CD_3OD, 25 °C, TMS): δ = 8.07 (s, 1H; Ar-H), 7.58 (d, *J*(H,H) = 8.4 Hz, 2H; Ph-H), 7.47–7.44 (m, 4H; Ph-H), 7.27–7.12 (m, 5H; Ph-H), 6.71 (d, *J*(H,H) = 8.8 Hz, 2H; Ph-H), 4.10 (s, 2H; CH_2). ^{13}C NMR (400 MHz, CD_3OD, 25 °C, TMS): δ = 159.4, 149.1, 147.3, 143.5, 140.6, 138.2, 136.7, 130.3, 129.5, 129.5, 128.4, 128.2, 127.9, 127.3, 116.4, 107.6, 97.6, 32.0. MS (ESI) m/z: 460 [M+H$^+$].
17. Adamczyk, M.; Johnson, D. D.; Mattingly, P. G.; Pan, Y.; Reddy, R. E. *Synth. Commun.* **2002**, *32*, 3199–3205.
18. Synthetic scheme and procedure of compound **5**, material and methods for luminescent measurement are reported in detail in the Supplementary data. For bioluminescent spectra of compound **6**, kinetic plots of **7** and **8** see section 7 of the Supplementary data.
19. Shimomura, O.; Teranishi, K. *Luminescence* **2000**, *15*, 51–58.