Physical properties of coelenterazine analogs

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Glowing Products for Science™

コスモ・バイオ株式会社

Coelenterazine is a substrate for Renilla luciferase. Renilla luciferase, an enzyme derived from sea pansy, catalyzes coelenterazine oxidation by oxygen to produce light, and is used as a reporter gene for luminescence based assays. Coelenterazine native is the natural substrate for Renilla luciferase. However, over a dozen of coelenterazine analogs have been synthesized, many of which are now commercially available from Biotium. These coelenterazine analogs can function as substrates for Renilla luciferase, and have different properties in terms of emission wavelength, cell membrane permeability and quantum efficiency. Coelenterazine also emits light from enzyme-independent oxidation, a process known as autoluminescence. The autoluminescence is enhanced by superoxide anion and peroxynitrite in cells and tissues.

Biotium offers high purity native coelenterazine and a number of coelenterazine analogs. Table 1 summarizes the luminescent properties of coelenterazine derivatives with Renilla luciferase. As the table shows, both native coelenterazine and coelenterazine *e* are good substrates for Renilla luciferase. In addition to consideration of quantum yields, emission wavelength may become important when Renilla luciferase in combination with a fluorescent protein such as GFP is used in bioluminescent resonance energy transfer (BRET), an important application for the studies of protein-protein interactions.

Coelenterazine and its analogs also bind the jellyfish protein apoaequorin to form aequorin, a calcium-sensing photoprotein. Aequorin can be used for bioluminescent detection of calcium with high sensitivity and dynamics. Compared with fluorescent calcium indicators, aequorin has several advantages in detecting calcium. One major advantage is that the aequorin complex can detect a broad range of calcium concentrations, from ~0.1 µM to >100 µM. Another advantage is that the aequorin complex is stably retained inside cells, making it possible to follow calcium concentration changes for hours to days. Table 2 lists the luminescent properties of coelenterazine analogs in complex with apoaequorin.

Aquaphile™ coelenterazines are specially formulated to readily dissolve in water or buffer for in vivo use.

The Coelenterazine Sampler Kit (catalog number 10123) containing 25 ug each of native coelenterazine and coelenterazine analogs cp, f, fcp, h, hcp, i, ip, and n. Biotium also offers Renilla Luciferase Assay Kit (catalog number 30004) and Dual Luciferase Assay for Firefly and Renilla Luciferase (catalog number 30005).

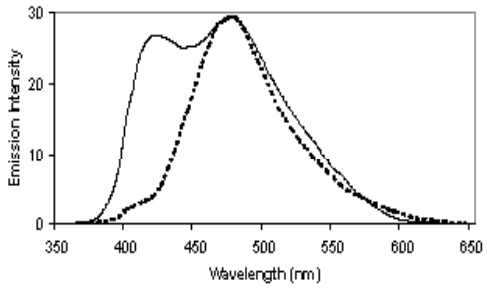

Figure 1. Emission spectra (normalized) of coelenterazine e (solid line) and coelenterazine (native) (dotted line) in the presence of recombinant *Renilla* luciferase.

Table 1. Luminescent Properties of Coelenterazine Analogs with Renilla Luciferase*

Analog	λ_{em} (nm)	Total Light (%)	Initial Intensity (%)
native	475	100	45
400a	400		
cp	470	23	135
e	418, 475	137	900
f	473	28	45
h	475	41	135
n	475	47	900

* Data from Biochem. Biophys. Res. Commun. 233, 349 (1997).

Table 2. Luminescent Properties of Coelenterazine Analogs with Apoaequorin*

Analog	λ_{em} (nm)	Relative luminescence capacity	Relative intensity	Half-rise time (s)
native	465	1.0	1.00	0.4-0.8
cp	442	0.95	15	0.15-0.3
e	405, 465	0.50	4	0.15-0.3
f	473	0.80	18	0.4-0.8
fcp	452	0.57	135	0.4-0.8
h	475	0.82	10	0.4-0.8
hcp	444	0.67	190	0.15-0.3
i	476	0.70	0.03	8
ip	441	0.54	47	1
n	467	0.26	0.01	5

* Data from Biochem. J. 261, 913 (1989).

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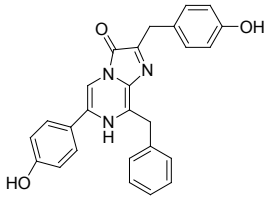
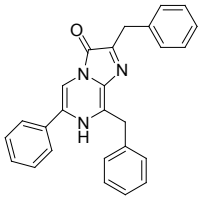
Coelenterazine (also known as native coelenterazine)
Catalog no. 10110



Coelenterazine (native form) is a luminescent enzyme substrate for apoaequorin and Renilla luciferase[1-4]. Renilla luciferase/coelenterazine has also been used as the bioluminescence donor in bioluminescence resonance energy transfer (BRET)[5] to study protein-protein interactions. Other uses of coelenterazine include chemiluminescent detection of superoxide anion and peroxynitrite in cells or tissues.[6-9]

Ref: 1) Meth. Cell Biol. 40, 305 (1994); 2) Meth. Enzymol. 172, 164(1989); 3) J. Cell Biol. 115, 1259 (1991): 4) Cell Calcium, 14, 373 (1993); 5) Proc. Natl. Acad. Sci. USA 96, 151 (1999); 6) Free Radic. Biol. Med. 28, 1232 (2000); 7) Circ. Res. 84, 1203(1999); 8) Immunol. Today 15, 7 (1994); 9) Anal. Biochem. 206, 273 (1992); 10) Biochem. Biophys. Res. Commun. 233, 349 (1997).

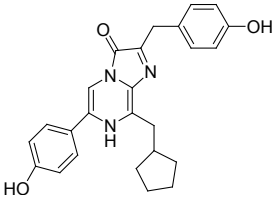
Coelenterazine 400a (also known as DeepBlue C™)
Catalog no. 10124



Colenterazine 400a, also called DeepBlue C™, is a coelenterazine derivative that serves as a substrate for Renilla luciferase (Rluc) and generates an emission peak centered around 400 nm. It is the preferred *Renilla* luciferase substrate for BRET studies because it has minimal interference with the emission of the GFP acceptor.

Ref: 1) Proc. Natl. Acad. Sci. USA 96, 151(1999); 2) Proc. Natl. Sci. USA 97, (7), 3684 (2000).
DeepBlue C is a trademark of Packard BioScience Company.

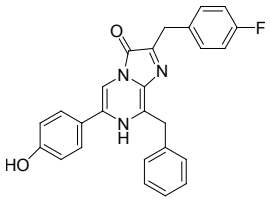
Coelenterazine *cp*
Catalog no. 10112



Coelenterazine cp-aequorin complex generates luminescence intensity 15 times higher and has a faster response time to calcium than the native coelenterazine-aequorin complex.

Ref: 1) Biochem. J. 261, 913 (1989); 2) Cell Calcium 12, 635 (1991); 3) Cell Calcium, 14, 373 (1993).

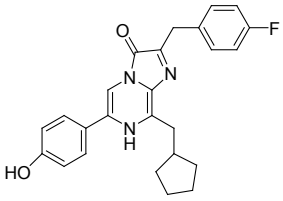
Coelenterazine *f*
Catalog no. 10114



The luminescence intensity of coelenterazine *f*-aequorin complex is almost 20 times higher than that of the aequorin formed from native coelenterazine while its emission maximum is about 8 nm longer than that of the latter.

Ref: 1) Biochem. J. 261, 913(1989); 2) Cell Calcium 12, 635 (1991); 3) Cell Calcium, 14, 373 (1993).

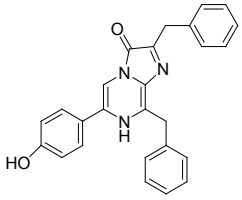
Coelenterazine fcp
Catalog no. 10117



The luminescence intensity of coelenterazine *fcp*-aequorin complex is 135 times higher than that of the aequorin formed from native coelenterazine.

Ref: 1) Biochem. J. 261, 913 (1989); 2) Cell Calcium 12, 635 (1991); 3) Cell Calcium, 14, 373 (1993).

Coelenterazine *h* (2-(4-Dehydroxy) coelenterazine)
Catalog no. 10111



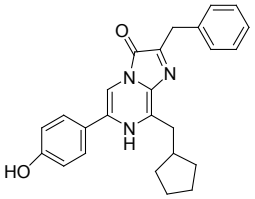
The luminescence intensity of coelenterazine *h*-aequorin complex is more than 10 times higher than that of the aequorin formed from native coelenterazine. Coelenterazine *h* is also a substrate for *Renilla* luciferase.

Ref: 1) Biochem. J. 261, 913(1989); 2) Cell Calcium 12, 635(1991); 3) Cell Calcium, 14, 373 (1993).

Coelenterazine *hcp*
Catalog no. 10113

The luminescence intensity of coelenterazine *hcp*-aequorin complex is 190 times higher than that of the aequorin complex formed from native coelenterazine, with a faster response time.

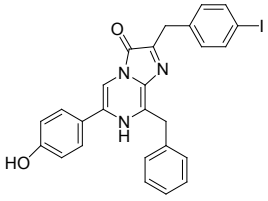
Ref: 1) Biochem. J. 261, 913(1989); 2) Cell Calcium 12, 635(1991); 3) Cell Calcium, 14, 373 (1993).



Coelenterazine *i*
Catalog no. 10121

The luminescence intensity of its coelenterazine *i*-aequorin complex is about 3% that of the aequorin formed from native coelenterazine and the response time to calcium is the slowest of all coelenterazine analogs.

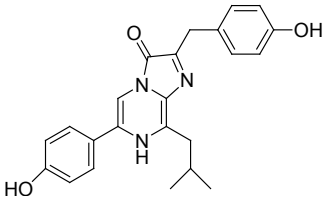
Ref: 1) Biochem. J. 261, 913(1989); 2) Cell Calcium 12, 635(1991); 3) Cell Calcium, 14, 373 (1993).



Coelenterazine *ip*
Catalog no. 10116

The luminescence intensity of coelenterazine *ip*-aequorin complex is almost 50 times higher than that of the aequorin formed from native coelenterazine, while its response time to calcium is much slower than the latter.

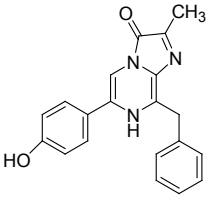
Ref: 1) Biochem. J. 261, 913(1989); 2) Cell Calcium 12, 635(1991); 3) Cell Calcium, 14, 373 (1993).



Coelenterazine, 2-methyl analog (Methyl Coelenterazine)
Catalog no. 10122

Methyl coelenterazine has been reported to be a potent antioxidant against reactive oxygen species (ROS) such as singlet oxygen and superoxide anion.[1] The coelenterazine derivative is membrane-permeant, nontoxic and highly reactive toward ROS. As oxidative stress is believed to be a mediator of apoptosis,[2] methyl coelenterazine may be a useful tool for apoptosis studies.

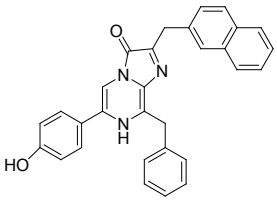
Ref: 1) Biochem. Pharmacol. 60, 471(2000); 2) Immunol. Today 15, 7(1994); 3)Anal. Biochem. 206, 273(1992); 4) Circ. Res. 84, 1203(1999).



Coelenterazine *n*
Catalog no. 10115

The luminescence intensity of Coelenterazine *n*-aequorin complex is the weakest of all coelenterazine analogs and its response time to calcium is also much slower than that of native coelenterazine. Coelenterazine *n* is reported to be a useful low-sensitivity coelenterazine.

Ref: 1) Biochem. J. 261, 913(1989); 2) Cell Calcium 12, 635(1991); 3) Cell Calcium, 14, 373 (1993).



Coelenterazine Sampler Kit
Catalog no. 10123

For researchers wishing to screen coelenterazine derivatives, we a sampler of 25 µg each of nine coelenterazine analogs: coelenterazine, coelenterazine *cp*, coelenterazine *f*, coelenterazine *fcp*, coelenterazine *h*, coelenterazine *hcp*, coelenterazine *i*, coelenterazine *ip*, and coelenterazine *n*.

Aquaphile™ Coelenterazines
Catalog no. 10126 Aquaphile™ Coelenterazine native
Catalog no. 10127 Aquaphile™ Coelenterazine h

Aquaphile™ coelenterazines are specially formulated to readily dissolve in water or buffer for in vivo use.