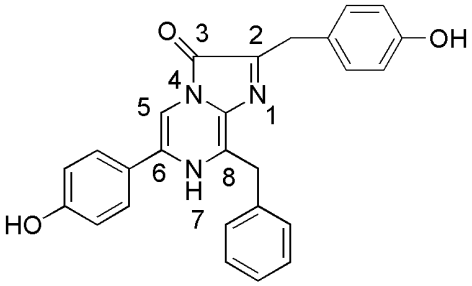
Synthesis, Luminescence, and Applications of Coelenterazine and its Analogs

Reported by Victor M. Gonzalez, Jr. February 26, 2007

INTRODUCTION

The imidazopyrazine coelenterazine **1** is found in many bioluminescent marine organisms, including the sea pansy *Renilla*, the jellyfish *Aequorea*, and the hydroid *Obelia*.[1-4] As with other luciferins, or light-producing compounds, coelenterazine can also take part in chemical reactions outside of biological systems through chemiluminescence. This property has allowed chemists through synthetic methods to learn more about the luminescent properties of coelenterazine, as well as to expand applications of this molecule beyond serving as a light source for marine creatures. This review covers synthetic routes to coelenterazine and its analogues, as well as elaborates on their luminescent properties. Furthermore, applications of coelenterazine are discussed, including its function as a novel antioxidant.



Coelenterazine (1)

Bioluminescence and Chemiluminescence

Chemiluminescence is the generation of light by a chemical reaction. In most cases, a molecule reacts with oxygen, producing an excited intermediate that emits light as it returns to the ground state. When a chemiluminescent reaction occurs in an organism with the aid of an enzyme, known as a luciferase, the reaction is considered to be bioluminescent (Scheme 1). Bioluminescence differs from species to species, but the general mechanism begins with the oxidation of a luciferin by its luciferase

Scheme 1. General bio- and chemiluminescence mechanisms

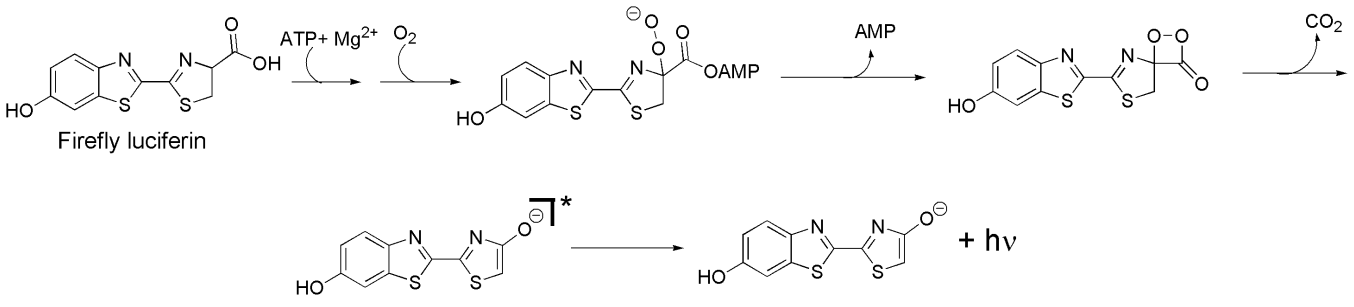
Chemiluminescence

$$\text{Reactant} + O_2 \longrightarrow \text{Peroxide} \longrightarrow \text{Products (excited)} \longrightarrow \text{Products} + h\nu$$

Bioluminescence

$$\text{Luciferin} + \text{Luciferase} + O_2 \longrightarrow \text{Oxyluciferin (excited)} \longrightarrow \text{Oxyluciferin} + h\nu$$

Scheme 2: Firefly luciferin bioluminescent mechanism

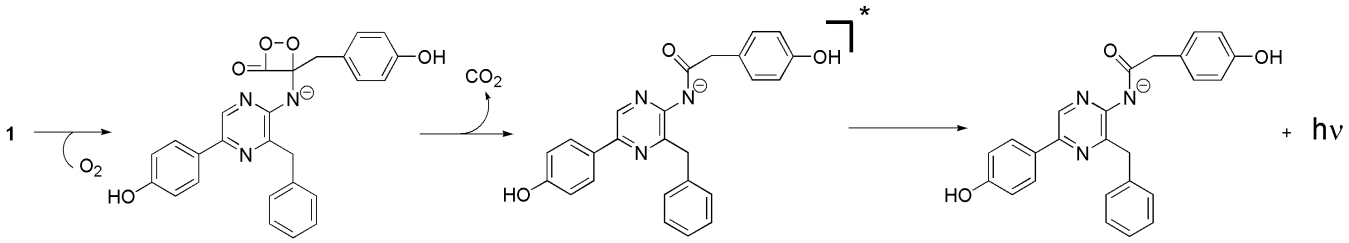


enzyme in the presence of O_2 to form an excited state of the oxidation product, oxyluciferin.[5] The diversity of bioluminescent reactions is evident when comparing the mechanism of coelenterazine with that of the firefly luciferin (Scheme 2). In contrast to the bioluminescent reaction of coelenterazine, which is discussed below, the firefly luciferin mechanism incorporates the use of ATP and Mg^{2+} as cofactors in the reaction with firefly luciferase. The bioluminescent process also affords a higher quantum yield than that of coelenterazine.[5]

BIOLUMINESCENCE AND CHEMILUMINESCENCE OF COELENTERAZINE

In bioluminescent and chemiluminescent reactions, coelenterazine reacts with O_2 to yield a four-membered energy–rich 1,2-dioxetane compound **2** (Scheme 3). Subsequent loss of CO_2 by the dioxetane intermediate yields the coelenteramide anion **3** in the excited state, followed by emission of a photon.[6,7]

Scheme 3: Chemiluminescent mechanism of coelenterazine



Although the bioluminescent and chemiluminescent mechanisms are similar for coelenterazine, the conditions for eliciting each reaction are different. In bioluminescence, coelenterazine is the substrate for the photoprotein aequorin and produces blue light in the presence of Ca^{2+}.[8] Furthermore, the excited state molecule in the bioluminescent reaction is a phenolate anion, unlike the amide anion produced in chemiluminescence. Green light can also be emitted in bioluminescence when the excited phenolate in the photoprotein undergoes radiationless energy transfer to a green fluorescent protein (GFP) that emits light upon relaxation to its ground state.[9] In contrast, chemiluminescent reactions for coelenterazine are typically performed in a purely chemical system with O_2 dissolved in aprotic polar solvents such as dimethylsulfoxide (DMSO), dimethylformamide (DMF), and hexamethylphosphoramide (HMPA) in the presence or absence of bases (e.g., NaOH, t-BuOH, t-BuOK) or acetate buffer .[10-12] It is also important to note that chemiluminescent reactions have a lower luminescence efficiency than bioluminescent reactions, a topic that will be discussed below.

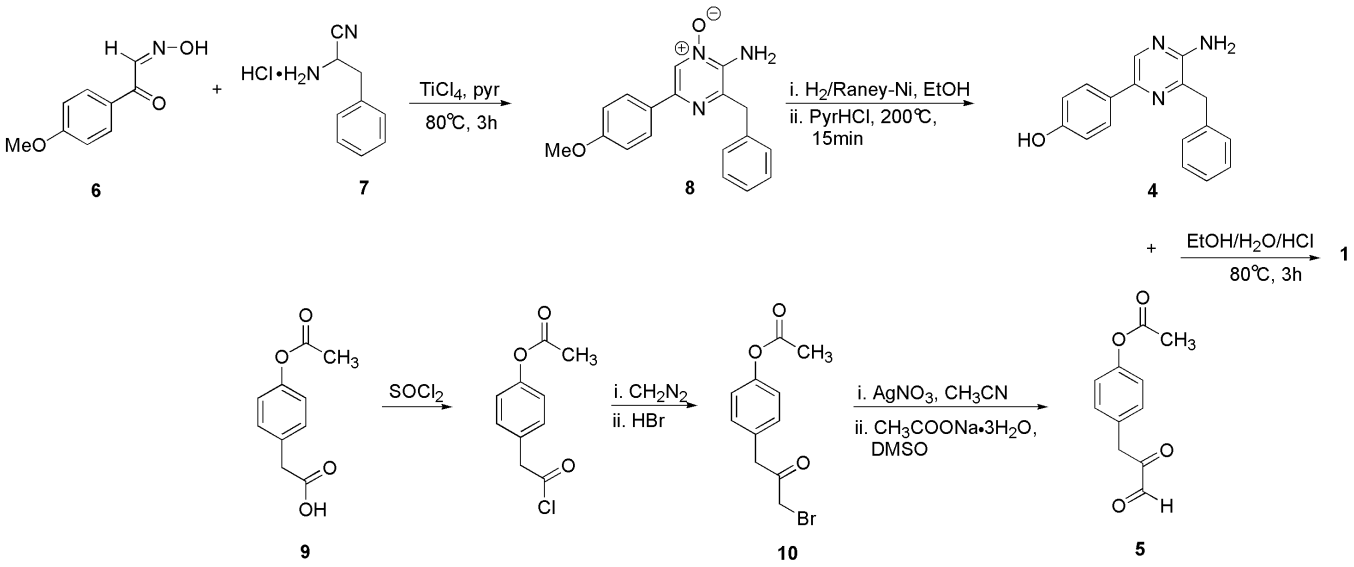
SYNTHESIS OF COELENTERAZINES

Synthetic chemistry has played a key role in studying the luminescence properties of coelenterazine. The two synthetic approaches used to create **1** and its analogues are the "classical" synthesis and a more modern method which utilizes palladium couplings.

"Classical" synthesis

The first synthesis of coelenterazine was completed by Inoue and co-workers in 1975 by condensing 2-aminopyrazine **4** with glyoxal **5** to give coelenterazine in less than 10% yield (Scheme 4).[13] Synthesis of **4** was accomplished by annulating the α-oximinoketone **6** and the α-amino nitrile **7** in

Scheme 4: Classical approach to coelenterazine synthesis



$TiCl_4$ and pyridine. The use of $TiCl_4$ allowed for effective nucleophilic addition to the carbonyl group of **6** while not perturbing the labile amino nitrile of **7**.[14] The resulting pyrazine *N*-oxide **8** was reduced and deprotected to give aminopyrazine **4**. In a parallel synthesis, phenylacetic acid **9** was transformed to the α-bromoketone **10**, which was used to make **5**. Later work demonstrated that the acetyl and methyl protecting groups in starting materials **6** and **9**, respectively, were unnecessary, decreasing the overall steps in the reaction sequence.[15,16] The greatest disadvantage of the classical approach is the difficulty of incorporating substituents on the imidazopyrazinone core. Despite this shortcoming, early work on coelenterazine analogues utilized this synthetic route as there was no other available route.

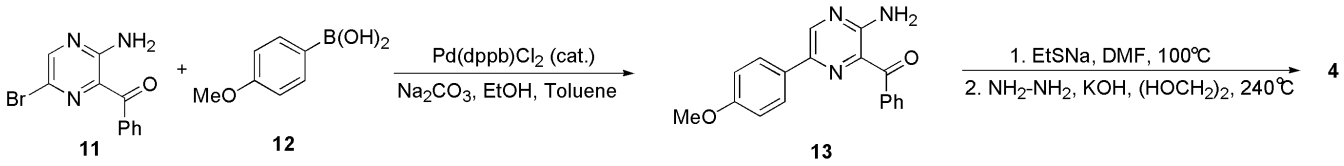
Palladium-coupling syntheses

Developments in palladium coupling chemistry offered new approaches to the synthesis of coelenterazine and its derivatives. Jones and co-workers developed the first coelenterazine synthesis that used a palladium cross-coupling step (Scheme 5).[2,17] Coupling of bromopyrazine **11** and 4-

methoxyphenylboronic acid **12** produced **13** in 92% yield. Deprotection of the phenol followed by Wolff-Kishner reduction of the ketone gave **4**, which was condensed with glyoxal **5** to give coelenterazine. The overall synthesis was accomplished in with a 25% overall yield, an improvement over the classical synthesis.[18]

Formation of **4** using a Suzuki coupling as the key step allowed various coelenterazine

Scheme 5: Synthesis of 4 using Suzuki coupling method



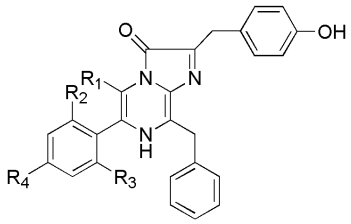
derivatives to be prepared by altering the boronic acid component.[17] The synthetic method of Jones can also be performed with the commercially available 2-amino-3,5-dibromopyrazine to generate coelenterazine derivatives substituted at the 6 and 8 position of the imidazopyrazine core. When dibromopyrazine was used, organozinc reagents selectively attacked the 6 position and thus produced asymmetric imidazopyrazinones.[19]

As an alternative to the Suzuki coupling method, aminopyrazines were also synthesized using tributyltin compounds via the Stille coupling reaction.[20] Nakamura and co-workers cross-coupled various arylstannanes to a 2-amino-5-bromopyrazine to give moderate to good yields of 2-amino-5-arylpyrazines that could serve as synthetic precursors for coelenterazine analogues.

CHEMILUMINESCENT PROPERTIES OF COELENTERAZINES

The synthesis of coelenterazine and its derivatives paved the way for a better understanding of the chemiluminescent properties of this compound. Although many chemiluminescence experiments have been performed since the 1970s, this review focuses primarily on more recent work.

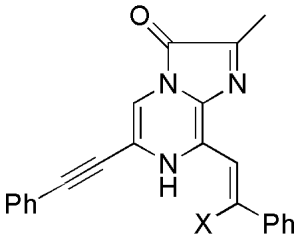
Table 1.[10] Chemiluminescent efficiences of various coelenterazine analogues



Compound	R_1	R_2	R_3	R_4	Φ_{cl} (Relative Light Yield)
1	H	H	H	OH	0.21% (1.0)
14a	-CH_2-		H	OH	0.31% (1.5)
14b	-CH_2CH_2-		H	OH	0.48% (2.3)
14c	-$CH_2CH_2CH_2$-		H	OH	0.31% (1.5)
14d	CH_2CH_2OH	H	H	OH	0.10 % (.46)
14e	-CH_2CH_2-		OH	OH	0.00% (.01)

Chemiluminescence efficiency, or quantum yield, is lower than that of bioluminescence efficiency.[10] Chemiluminescence quantum yields (Φ_{Cl}) for coelenterazine reactions are 0.02 while the

bioluminescence quantum yield is 0.2. The disparity between the chemiluminescent and bioluminescent efficiencies of coelenterazine is attributed to both the conformational stability of the emitter in the protein and the hydrophobic environment surrounding the coelenteramide anion **3**. To test the conformational stability effects on the chemiluminescence of coelenterazine, analogues with rigidifying bridges were synthesized (**14a-c**; Table 1).[10] Other analogues incorporating a hydroxyl group capable of hydrogen bonding to a nitrogen atom in the imidazopyrazine core were also made (**14d,e**).[10] Chemiluminescence experiments using compounds with a rigid *p*-hydroxyphenyl group showed increased Φ_{Cl}, with the six-membered ring derivative **14b** having the highest quantum yield. Intramolecular hydrogen bonding effects between the hydroxyl and nitrogen atoms on the imidazopyrazine core decreased the chemiluminescent efficiencies (**14d,e**), supporting the notion that the bioluminescence reaction takes place in a hydrophobic environment in the luciferase enzyme.[10]



15a: X=Cl
15b: X=H

Coelenterazine analogues have also been modified to produce bimodal chemiluminescent systems. Typically, imidazopyrazinones can emit blue to yellow light from the excited singlet state of the amide anion in both acidic and basic environments.[22] Nakamura and co-workers synthesized a coelenterazine compound that emits orange and yellow light in acidic and basic conditions, respectively. The bimodal analogues created by Nakamura and co-workers have a conjugated system at the 8 position of the imidazopyrazine ring (Figure 1). Compound **15a** exhibited an orange luminescence (580-590 nm) at neutral to acidic conditions. Under basic conditions, **15a** emitted yellow light (545 nm). Analogue **15b** was used to verify that the bathochromic shift was associated with the chlorostyryl group at the 8-position. In basic and acidic environments, **15b** only emitted orange light (583 nm).[23]

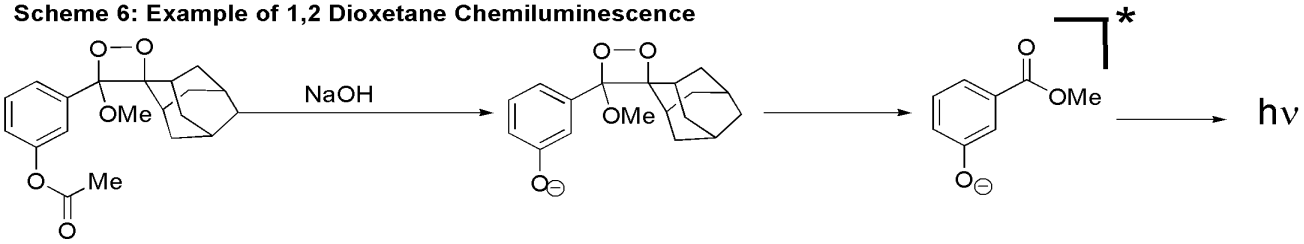
LUMINESCENT APPLICATIONS OF COELENTERAZINE

Coelenterazine has enabled the development of many chemiluminescent and bioluminescent applications. In bioluminescent applications, coelenterazine has been used with the photoprotein aequorin and its recombinant analogues to monitor Ca^{2+} concentrations in mammalian cells.[23] Analogues of coelenterazine are also used with aequorin to study ion channels and tyrosine kinase receptors.[24] Furthermore, fluorescence resonance energy transfer (FRET) assays that use coelenterazine to detect protein-protein interactions have been developed.[25]

Coelenterazine has also influence the field of dioxetane chemistry. One of the important features of the luminescence of coelenterazine and other luciferins is their 1,2-dioxetane intermediate, which has

been used as a model for other chemiluminescent molecules (Scheme 6). Dioxetanes are known to be very efficient chemiluminescent compounds.[26]

Scheme 6: Example of 1,2 Dioxetane Chemiluminescence



COELENTERAZINES AS NOVEL ANTIOXIDANTS

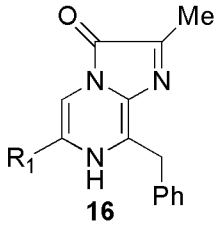
Aside from being a luminescent compound, coeletnerazine has also been studied for its antioxidation properties. Although the connection between luminescence and antioxidation seems to be a coincidence, it is theorized that both might be linked as a result of evolution. Imidazopyrazine luciferins such as coelenterazine are found in a variety of marine organisms, even those that are non-luminescent.[27] The widespread presence of coelenterazine in the marine environment makes it difficult to conclude that the imidazopyrazine had an origin from a bioluminescent ancestor. One theory that could account for the presence of the imidazopyrazine in these organisms is that it served as an antioxidant for protection against the oxygen toxicity in the environment. As these organisms began inhabiting deeper places in the ocean, they escaped the dangers of oxygen toxicity but found themselves in a darker environment. This set the state for evolutionary processes so that coelenterazine could be used by marine creatures as a bioluminescent compound.[28]

Antioxidation Experiments

Various experiments have shown that imidazopyrazines such as coelenterazine can react with reactive oxygen species (ROS) such as superoxide anion (O_2^-), singlet oxygen ($^1O_2$), and peroxynitrite (ONO_2^-). Although ROS are generated in biological systems, excessive concentrations of these species can results in oxidative stress leading to various disorders.[29] The antioxidation properties of coelenterazine and its analogues were tested by measuring the delay of peroxidation of linoleate induced by the radical generator 2,2'-azobis(2-amidopropane)dihydrochloride (AAPH).[30] Coelenterazine slowed the oxidation of linoleate and lowered the propagation rate of oxidation. Coelenteramine, however, did not delay the peroxidation of linoleate but did have a greater effect on lowering the propagation rate of oxidation (Table 2). The decrease in the propagation rate is thought to be a result of coelenteramine's ability to react with a peroxide radical.

With the successful demonstration that coelenterazine is capable of inhibiting the oxidation of linoleate, many other antioxidative uses were explored for this compound. For example, coelenterazine can protect low density lipoproteins (LDL) from oxidative modifications and thus potentially lead to prevention of atherosclerosis.[29] Furthermore, the imidazopyrazine and its derivatives protect neuronal cells against ROS, which may play a role in the development of Parkinson's and Alzheimer's diseases.[29]

Table 2. Effects of coelenterazine and its analogues on the peroxidation of linoleate



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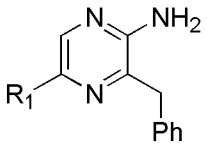

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Analogue	Latency (min)	Propagation rate (mU A234/ min)
Control (AAPH)	0	13.37 ± 0.53
Coelenterazine	75.61 ± 1.43	7.33 ± 0.40
16 (R_1=p-OH-C_6H_5)	37.59 ± 0.64	9.47 ± 0.21
17 (R_1=p-OH-C_6H_5)	4.02 ± 0.54	5.69 ± 0.31

CONCLUSION

The synthesis of coelenterazine and its analogues has been important to understanding this luminescent compound. What was originally viewed solely as a luciferin for bioluminescent marine organisms is a compound that has now found various applications in medicinal chemistry and biochemistry. More recently, coelenterazine has demonstrated antioxidation properties, generating the possibility of another application for the molecule and its derivatives.

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