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Suzuki–Miyaura coupling for general synthesis of dehydrocoelenterazine applicable for 6-position analogs directing toward bioluminescence studies

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ABSTRACT

Synthesis of coelenterazine analogs is in recent demand to supply more luminescent compounds with reasonable stability as substrate for the photoprotein manipulated in a living cells or particular organelle. There are limited methods for the synthesis of 6-substituted coelenterazine due to the route and instability of the compounds under the existing conditions. This paper describes six examples including Suzuki–Miyaura cross coupling reaction with reactive triflate (unstable) and stable tosylate intermediates of the imidazo[1,2-*a*]pyrazin-3-one. Five examples of 2-amino-3-benzyl-5-*O*-Tf-pyrazine are also discussed. The product coelenterazine analogs are obtained in the form of dehydrocoelenterazine, which is the substrate of a squid photoprotein, *symplectin*.

1. Introduction

Bioluminescence of marine organisms has widely been recognized among those people, who visit the seashore to observe strong emission of blue or green light. Coelenterazine **1** has been one of the most popular substrates for the marine bioluminescent systems,[1] which was found in organisms such as jellyfish *Aequoria victoria*,[2] sea cactus *Cavernularia obesa*,[3] sea pansy *Renilla reniformis*[4–6], deep sea shrimp *Oplophorus gracilirostris*,[7–9] obelin, *Obelia longissima*,[10] and oceanic squids *Symplectoteuthis oualaniensis*.[11,12] Some examples as *C. obesa* or the tiny squid *Watasenia scintillans* uses the corresponding sulfate of **1**.[3,11–15] Dehydrocoelenterazine (DCL) **2**, however, is an oxidized chromophore of coelenterazine **1**. But **2** is not luminescent by itself under chemiluminescence condition (strong alkaline in dipolar aprotic solvent) due to the oxidation stage. We have reported that DCL **2** shows strong bioluminescence as a quite unique chromophore in the photoprotein, *symplectin* of the squid, *S. oualaniensis* (Tobi-Ika, Japanese name meaning flying squid).[16–19] We have elucidated the chromophore structure as **2** but not **1** due to the fact that only **2** gives the bioluminescence with its *apo-symplectin*. The molecular mechanism of the bioluminescent system in *symplectin* has been solved in such a way that DCL or its ^{13}C analog binds with an HS-residue of cysteine to form a conjugate adduct **3** as reported by Isobe's group (Scheme 1).[20–22] In 2008, the active center cysteine was determined to be 390-Cys in the 501 amino acids of *symplectin*.[22] Recently, Kuse independently reported that a commercially available photoprotein pholasin, from *Pholas dactylus*, showed an increase in the bioluminescence by addition of DCL.[23]

Synthesis of coelenterazine or dehydrocoelenterazine has been well established since 1990 until recent 2009.[18,20,24–33] This is largely due to the fact that increase of sample-request has become much more in order to supply as the substrates for the photoproteins, which has been expressed on living cells or organelle through gene transcription for chemical biology studies.[34] A more stable coelenterazine, a fluorinated-analog, was selected to exhibit stronger light amount at plant organelle, on which aequorin is often expressed to monitor increasing amount of Ca^{2+} ions in the living cells.[35] Besides these applications, fluorinated-analog has been playing important roles to elucidate the molecular mechanism of the bioluminescence of *symplectin* photoprotein.[22]

Many important syntheses of coelenterazine **1** and its analogs have been reported by several research groups including ours. However, most of the synthetic routes are focused on either modifying the aryl group on the 2-position (R^1) or introducing only limited diversified groups at the 6-position (R^2) and the 8-position (R^3) starting from 2-aminopyrazine analogs as shown in Scheme 2. This fact largely depends on the synthetic route, which allows finalizing the synthesis by condensing aminopyrazine (e.g., **G**) with ketoaldehyde equivalent as shown in Scheme 2. There still exists limitation

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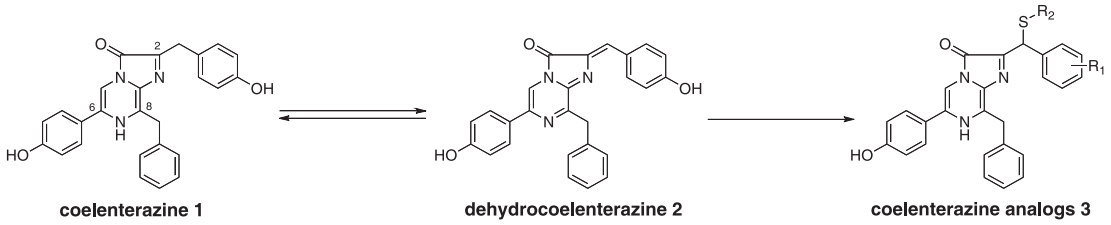
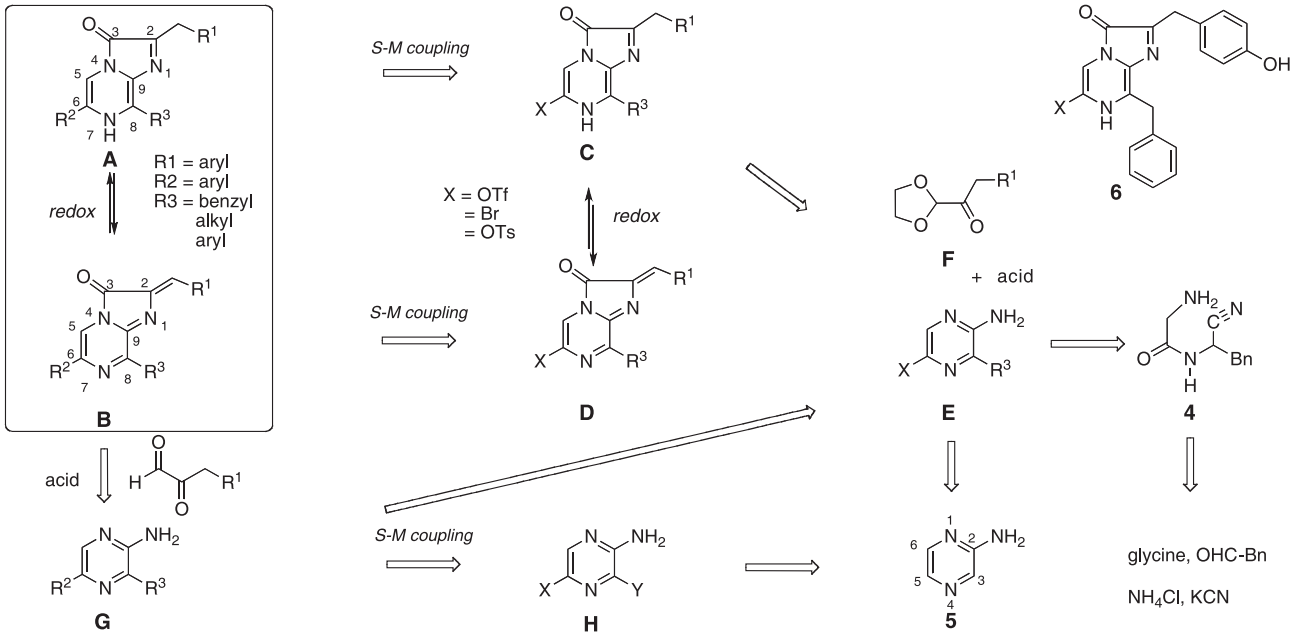


Scheme 1. Coelenterazine **1** and dehydrocoelenterazine **2** with its protein bound form substructures with *Symplectin*.



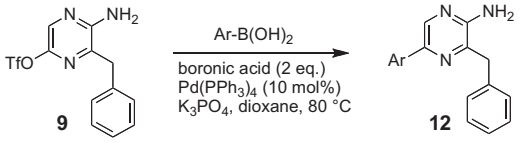
Scheme 2. General retro-synthetic analysis of coelenterazine analogs.

for synthesizing diversified R^2 analogs due to most of the routes, which starts from introduction of 4-methoxyphenyl group at the R^2 from the beginning. Nakamura et al.[36] in 2001 and Adamczyk et al.[37,38] in 2003 reported the synthesis of analogs of **1** from 3, 5-dibromo-2-aminopyrazine on the basis of Suzuki–Miyaura and Negishi coupling. In 2009, Knochel reported an elegant synthesis of **1** on the basis of the Pd-mediated multiple cross coupling reactions starting from 2,5-dichloropyrazine in eight steps.[32] These new synthetic routes donated advantages for synthesizing analogs of R^2 and R^3 groups, but we still have limitation for diversified synthesis. Herein we want to report a new route, which enables the easy synthesis of unstable coelenterazine analogs.

In 2004, we reported a new route, which may allow the diversified synthesis of R^2 at the 6-position of coelenterazines **A**. This had been left unexplored. Instead of the fact that R^2 had to be selectively introduced at the early stage of the synthesis, the attempted R^2 introduction at a relatively later stage was achieved to provide two kinds of coelenterazine analogs.[39] The coupling of 5-*O*-triflyl-3-benzyl-2-aminopyrazine **E** (X=OTf) from **4** as a precursor for cross coupling (**G** from **E**) at the corresponding position.[39] In this paper, we report more examples of the cross coupling reaction with 2-amino-3-benzyl-5-*O*-triflate to form five aromatic compounds as summarized in Table 1.

For the purpose of the long time awaited synthesis route for **A** via the direct introduction of the R^2 group into imidazopyrazinone **6** (X=OTf) at the last step of the synthesis for CL, amino-*O*-triflylpyrazine **E** was condensed with the ketoaldehyde to afford **6**. The attempted R^2 introduction was implemented with six kinds of aromatic system, and the results are summarized in Table 2. In addition, the product was not **A**, but **B** from **C**.

Table 1
Suzuki-Miyaura coupling of 2-aminopyrazin-5-triflate to various aryl analogs



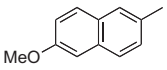
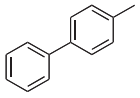
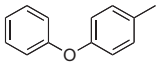
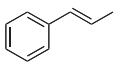
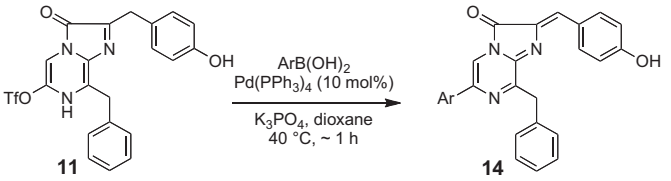
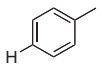
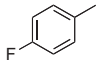
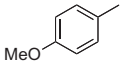
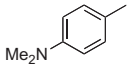
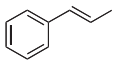
Entry	–Ar	Yield %
1		**12a** 83
2		**12b** 85
3		**12c** 70
4		**12d** 76
5		**12e** 72

Table 2
Suzuki-Miyaura coupling of dihydroimidazopyrazinone-6-triflate to various aryl analogs



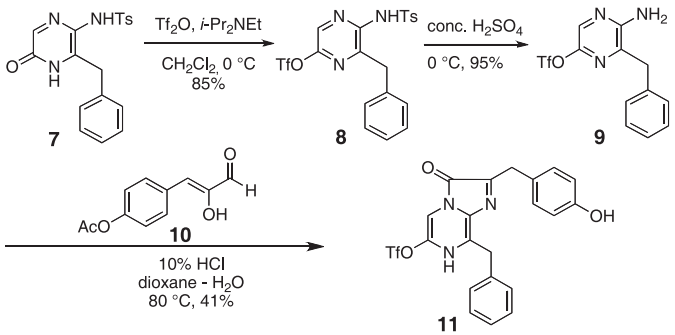
Entry	Ar	Yield %
1		**14a** 75
2		**14b** 61
3		**14c** 93
4		**14d** 72
5		**14e** 67
6		**14f** 54

These successful couplings were eventually found to be very inconvenient for extremely low stability of **6**, so that one have to finish the cross coupling reaction of the triflate **6** (X=*O*-Tf) within 24 h to have good yields even if **6** was stored in a freezer at −30 °C. To solve such a problem, we prepared the corresponding tosylate **6** (X=*O*-Ts) with better stability. So we went back to explore the coupling of 5-imino-*O*-tosy-3-benzyl-2-aminopyrazine **E** (X=*O*-Ts) for the Suzuki–Miyaura reaction for variety of the R^2 with organoboron reagents. This iminotosylate showed good results having electron donating and electron withdrawing group attached to the aromatic ring of the boronates. In 2009, Makarasen and Isobe reported the palladium-mediated cross coupling reaction with 2-amino-3-benzyl-5-*O*-tosyl-pyrazine **E** (X=OTs, R^3=Bn).[31] This product aminopyrazine **G** has to be further derivatized via the acid-mediated condensation with aryl-α-ketoaldehyde in acetal form **F** to obtain the DCL analogs. We now envision that the Suzuki–Miyaura reaction could be achieved even in much later stage; thus, Suzuki–Miyaura coupling (S–M-coupling) of imidazopyrazinone heterocyclic systems (**C** or **D**) at the last step of the synthesis to **A** or **B**. We describe the details of the results as follows first with **6** (X=*O*-triflyl) and then with **6** (X=*O*-tosyl).

2. Results and discussion

2.1. Preparation of imidazopyrazinone 6-*O*-triflate 11

As summarized in the retrosynthesis routes in Scheme 2, the challenging issue is the synthesis of **A** from **C** for providing diversified analog syntheses. The necessary triflate **9** was prepared in accordance to our previous paper; thus, 5-pyrazinone **7** was converted to **8** and *N*-tosyl group was hydrolyzed to amino-benzyl-pyrazin-*O*-triflate **9**.[39] To this aminopyrazine, a ketoaldehyde

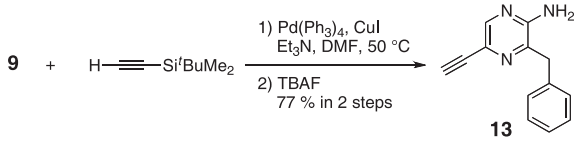


Scheme 3. Synthesis of the triflate aminopyrazine **9** and imidazopyrazinone **11**.

equivalent **10** was subjected for condensation to obtain imidazopyrazinone 6-*O*-triflate **11** (Scheme 3).

2.2. Pd-mediated cross coupling reactions with benzyl-aminopyrazine 5-*O*-triflate 9

The 2-amino-3-benzyl-5-*O*-triflylpyrazine was subjected to the various aromatic boronic acids [Ar-B(OH)$_2$]. This coupling was first achieved with the *N*-tosylamide-triflate **8** and 4-methoxyphenylboronate to give *aryl aminopyrazine* in high yield as reported prevously.[39] Here, we first confirmed that the same S–M-couplings were achievable between the free aminopyrazin-*O*-triflate **9** and various aryl boronates, such as the thiophyl, naphtyl, biphenyl, 4-phenoxyphenyl, and stylenyl in good yields as shown in Table 1. Incidentally, the aminopyrazinetriflate **9** also showed good reactivity even in the Sonogashira reaction to give the following cross coupling product **13**.



2.3. Suzuki–Miyaura cross coupling with the imidazo[1,2-*a*]pyrazine 6-*O*-triflate 11

The S–M coupling was also examined with the triflate **11** under the similar condition as aminopyrazinetriflate case. The yield, however, was lower with phenylboronate to show only 35% after heating at 80 °C for 2 h, while starting material disappeared. Heating at lower temperatures at 50 °C and 40 °C for 1 h afforded 64% and 75%, respectively. This difference was due to the instability of the triflate **11**. So the results of the S–M-coupling with various aryl boronates were carried out right after the triflate **11** was prepared under the condition at 40 °C for 1 h. However, lower yields were observed after the storage due to partial decomposition. The results are summarized as shown in Table 2. The products of the S–M coupling were not the reduced form as **A** but the oxidized form **14** as shown below.

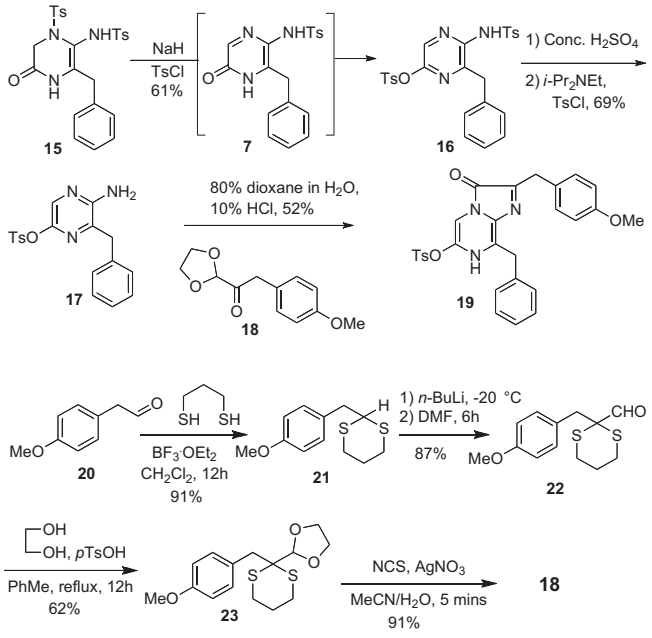
The details of the oxidation are not known why the products **14** were always obtained as dehydrocoelenterazine form either during the reaction in a reductive elimination step of palladium or after the reaction due to the air-oxidation during work-up. It may be necessary to monitor the color change during the reaction via the colorimetric analysis. But this result is not bad for the product **14** being the substrate for the *symplectin* bioluminescence. On the other hand, the Sonogashira reaction between **11** and trimethylsilylacetylene, for example, was not successful, although the same reaction with **9** afforded **13** in 77% yield in two steps. This is also

due to the instability of the triflate **11** under the homogeneous basic condition. So we went on the more stable but less reactive tosylate.

2.4. Preparation of imidazopyrazinone 6-*O*-tosylate 19 and Suzuki–Miyaura cross coupling reaction of the imidazo[1,2-*a*] pyrazine 6-*O*-tosylate 24

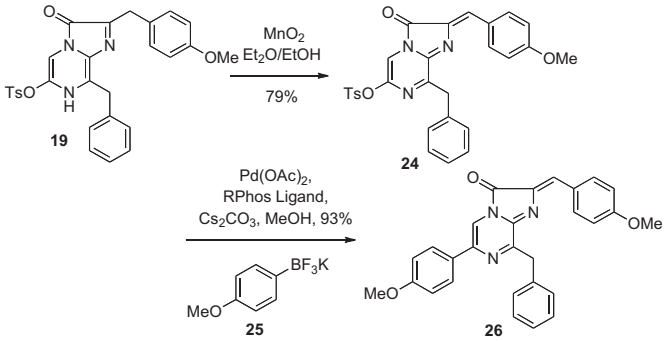
The *N*-tosylpyrazine *O*-tosylate **16** was synthesized directly from the *N*,*N*′-ditosyl compound **15** in one-pot through intermediate **7**. Namely, stirring **15** with sodium hydride was followed by addition of *p*-toluenesulfonyl chloride to give **16** in 61% yield. Treatment of this product *N*,*O*-ditosyl pyrazine **16** with concd sulfuric acid was only the method to remove the *N*,*O*-ditosyl groups to afford 2-amino-3-benzyl-5-hydroxypyrazine, which was selectively *O*-tosylated providing **17** in 69% yield. Condensation of this aminopyrazine **17** with ketoacetal **18** was examined in various solvents such as *N*,*N*-dimethylformamide (DMF), *n*-butanol/water and dioxane/water. The best solvent was 1,4-dioxane/water (4:1), which facilitated the condensation under 10% HCl, and the product was purified by reverse phase Cosmosil open column chromatography to provide the imidazopyrazinone *O*-tosylate **19** in 52% yield.

The ketoacetal **18** was synthesized during the current works via an alternative route from phenylacetaldehyde **20**. The reason of developing new route was to avoid utilization of diazomethane as a carbon elongation reagent. It is, however, a potentially explosive reagent, so that some research groups tried safer preparation routes. Adamczyk reported a coupling method between benzylmagnesium bromide and ethyl diethoxyacetate.[37] Knochel reported an alternative route by DMSO oxidation of the ketonitrate.[32] We have established the third route, which would be applicable for analogous compounds as shown in Scheme 4. In this case, one carbon elongation was achieved via the lithiated dithiane **21**, which was formylated by trapping with *N*,*N*-dimethylformamide (DMF). And manipulation of the protective groups of the carbonyl provided the ketoacetal **18**. The aldehyde group of **22** was subsequently protected to the corresponding acetal **23**. Selective hydrolysis of the thioketal acetal moietie of **23** was achieved under *N*-chlorosuccinimide and silver nitrate in acetonitrile for a very short reaction period providing α-keto acetal **18** in high yield.



Scheme 4. Preparation of the 6-*O*-tosylate **19** and new route of ketoaldehyde **18**.

Attempted coupling of the tosylate **19** with methoxyphenylboronate, or pinacol aryl boronates was not quite successful under the various conditions including the coupling employed in the aminopyrazine tosylate.[31] Though tosylate **19** was more stable than the corresponding triflate **11**, it is still not stable enough to survive the basic conditions during the cross coupling. We decided to oxidize the tosylate **19** in order to compare the stability and reactivity (Scheme 5).



Scheme 5. Oxidation of **19** to the corresponding 6-*O*-tosylate of dehydroimidazopyrazinone **24** and cross coupling reaction.

Oxidation of **19** was carried out with MnO_2, providing *O*-tosyl dehydroimidazopyrazine **24** in 79% yield. This compound is much more stable to indicate a single spot on TLC for a few weeks after storage in a refrigerator at −30 °C. Interestingly, when **24** was subjected to the Suzuki–Miyaura cross coupling condition, the reaction was proceeded in the presence of cesium carbonate in methanol solvent to yield dehydrocoelenterazine **26** in 93% yield.

3. Conclusion

We have established a successful route to synthesize dehydrocoelenterazine analogs at the 6-position at the last step of the synthesis through the Suzuki–Miyaura reaction with the 6-*O*-triflate and 6-*O*-tosylate with the arylborone compounds. Due to the unstable nature of the final coelenterazine-equivalent compounds, the cross coupling with the tosylate was better implemented with the oxidized heteroaromatic system through the most stable synthetic intermediates. Further synthetic studies are in progress to prepare analogs with functional groups at the 8-position of dehydrocoelenterazine, which would be useful for the biological studies with the photoprotein, *symplectin*, and other luciferases.

4. Experimental

4.1. General procedures

UV–vis spectra were obtained on a JASCO V-570 spectrometer. Fluorescence spectra and chemiluminescence spectra were measured with a JASCO FP-777 spectrometer. IR spectra were recorded on a JASCO FT/IR 6100 spectrometer. Proton NMR spectra were recorded on a JEOL GSX 270 for 270 MHz and a JEOL A 400 for 400 MHz. Chemical shift (δ) are given in parts per million relative to DMSO-d_6 (δ 2.49) or CD_3OD (δ 3.30) as internal standard and coupling constants (J) in hertz. Carbon NMR spectra were recorded on a JEOL A 400 for 400 MHz and a JEOL A 600 for 600 MHz. Chemical shift (δ) are given in parts per million relative to DMSO-d_6 (δ 45.0) or CD_3OD (δ 77.0) as internal standard. Low-resolution EI mass spectra and FAB mass spectra were measured with a JEOL JMS-700. High-resolution (HR) mass spectra were measured with a JEOL JMS-700. Light yields of bioluminescence of reconstructed *symplectin* were determined with a ATTO Luminescencer-PSN AB-2200. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride. Pyridine was dried over NaOH pellet and used without distillation.

The other solvents were of reagent grade. Analytical thin-layer chromatography (TLC) was conduced on precoated TLC plates: silica gel 60 F-254 [E. Merk (Art 5715) Darmstadt, Germany], layer thickness 0.25 nm. Silica gel column chromatography utilized Silica Gel 60 (spherical) 40–50 mm [KANTO CHEMICAL CO., INC].

*4.1.1. 8-Benzyl-2-(4-hydroxybenzyl)-3-oxo-3,7-dihydroimidazo[1,2-a]pyrazin-6-yl trifluoromethanesulfonate (**11**).* A solution of aminopyrazinetriflate (**9**) (200 mg, 0.601 mmol) and ketoaldehyde (**10**) (247 mg, 1.20 mmol) in 15 mL of 30% water/dioxane was degassed. To this solution was added 5 mL of 10% HCl. This solution was stirred under argon atmosphere at 80 °C for 3 h. After cooling, to this solution was added water at 0 °C. The reaction mixture was extracted with AcOEt (×3). The combined organic layer was washed with brine and dried over Na_2SO_4, then concentrated under reduced pressure. Purification of crude oil by silica gel chromatography provided 118 mg of coelenterazinetriflate (**11**) as a yellow solid (41% yield). IR (KBr) ν_{max} 3365, 1566, 1514, 1454 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz), δ 3.96 (2H, s, CH_2Ph), 4.25 (2H, s, CH_2Ph), 6.58 (2H, d, J=8.2 Hz, *Ph*OH), 6.95 (2H, d, J=8.2 Hz, *Ph*OH), 7.07–7.25 (5H, m, 6-Ph), 8.02 (1H, s, C*H*-5) ppm. ^{13}C NMR (CD_3OD, 150 MHz), δ 31.7, 39.1, 106.7, 116.3, 116.4, 119.0, 121.1, 125.5, 127.8, 129.4, 130.2, 130.5, 130.9, 131.2, 133.2, 146.0, 157.0, 190.0 ppm. FAB-MS (NBA) m/z 480 (MH^+). HRMS (FAB/NBA) calcd for $C_{21}H_{17}N_3O_5F_3S$ 480.0841, found 480.0871 (MH^+).

*4.1.2. 3-Benzyl-5-(thiophen-3-yl)pyrazin-2-amine (**12a**).* A mixture of aminopyrazinetriflate (**9**) (50 mg, 0.150 mmol) and boronic acid (40 mg, 0.300 mmol) and $Pd(PPh_3)_4$ (17 mg, 0.0150 mmol) and K_3PO_4 (64 mg, 0.300 mmol) in dioxane (1 mL) was heated to 80 °C for 4 h under argon atmosphere. The product was extracted with AcOEt (×3), washed with brine, and dried over Na_2SO_4. After evaporation, the residue was purified by column chromatography on silica gel with AcOEt/hexane (1:2) to give aminopyrazine (**2b**) (33 mg, 83%) as a pale yellow solid. ^1H NMR ($CDCl_3$, 400 MHz), δ 4.08 (2H, s, CH_2Ph), 4.36 (2H, s, NH_2), 7.16–7.26 (5H, m, Ph), 7.31 (1H, dd, J=5.0, 3.0 Hz, thiophene-5), 7.51 (1H, dd, J=5.0, 1.2 Hz, thiophene-4), 7.68 (1H, dd, J=5.0, 3.0 Hz, thiophene-2), 8.20 (1H, s, C*H*-6) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz), δ 41.1, 121.1, 125.4, 126.3, 127.0, 128.5, 129.0, 136.7, 137.1, 139.5, 139.7, 140.6, 151.4 ppm. FAB-MS (NBA) m/z 268 (MH^+). HRMS (FAB/NBA) calcd for $C_{15}H_{14}N_3S$ 268.0908, found 268.0858 (MH^+).

*4.1.3. 3-Benzyl-5-(6-methoxynaphthalen-2-yl)pyrazin-2-amine (**12b**).* A mixture of aminopyrazinetriflate (**9**) (68.8 mg, 0.207 mmol) and boronic acid (83 mg, 0.413 mmol) and $Pd(PPh_3)_4$ (24 mg, 0.0207 mmol) and K_3PO_4 (88 mg, 0.413 mmol) in dioxane (1.5 mL) was heated to 80 °C for 2 h under argon atmosphere. The mixture was treated with 1 N NaOH aq (1 mL) and 30% H_2O_2 (0.5 mL) for 1 h at room temperature to oxidize the residual borane. To this mixture, 1 N HCl aq (1 mL) was added for neutralization. The product was extracted with AcOEt (×3), washed with brine, and dried over Na_2SO_4. After evaporation, the residue was purified by column chromatography on silica gel with AcOEt/hexane (1:2) to give aminopyrazine (**2c**) (60 mg, 85%) as a pale yellow solid. ^1H NMR ($CDCl_3$, 400 MHz), δ 3.93 (3H, s, OCH_3), 4.22 (2H, s, CH_2Ph), 4.47 (2H, s, NH_2), 7.18–7.35 (7H, m, Ph+naphthalene-1,8), 7.82 (1H, d, J=8.4 Hz, naphthalene-4), 7.84 (1H, s, naphthalene-3), 8.06 (1H, dd, J=8.4, 1.6 Hz, naphthalene-5), 8.34 (1H, d, J=1.6 Hz, naphthalene-7), 8.50 (1H, s, C*H*-6) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz), δ 41.2, 55.3, 105.7, 119.1, 124.3, 124.4, 127.0, 127.3, 128.6, 129.0, 129.1, 129.9, 132.5, 134.3, 136.8, 137.5, 140.6, 142.7, 151.6, 157.9 ppm. FAB-MS (NBA) m/z 342 (MH^+). HRMS (FAB/NBA) calcd for $C_{22}H_{20}N_3O$ 342.1606, found 342.1590 (MH^+).

*4.1.4. 3-Benzyl-5-(biphenyl-4-yl)pyrazin-2-amine (**12c**).* A mixture of aminopyrazinetriflate (**9**) (115 mg, 0.345 mmol) and boronic acid (137 mg, 0.691 mmol) and $Pd(PPh_3)_4$ (40 mg, 0.0345 mmol) and K_3PO_4 (146 mg, 0.691 mmol) in dioxane (3 mL) was heated to 80 °C for 2 h under argon atmosphere. The mixture was treated with 1 N NaOH aq (1 mL) and 30% H_2O_2 (0.5 mL) for 1 h at room temperature to oxidize the residual borane. To this mixture, 1 N HCl aq (1 mL) was added for neutralization. The product was extracted with AcOEt (×3), washed with brine and, dried over Na_2SO_4. After evaporation, the residue was purified by column chromatography on silica gel with AcOEt/hexane (1:2) to give aminopyrazine (**2d**) (81 mg, 70%) as a pale yellow solid. ^1H NMR ($CDCl_3$, 400 MHz), δ 4.21 (2H, s, CH_2Ph), 4.47 (2H, s, NH_2), 7.24–7.37 (7H, m, aromatic), 7.45 (1H, t, J=7.6 Hz, aromatic), 7.65 (2H, dd, J=8.3, 1.2 Hz, aromatic), 7.69 (2H, d, J=8.5 Hz, aromatic), 8.02 (2H, d, J=8.5 Hz, aromatic), 8.43 (1H, s, C*H*-6) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz), δ 41.2, 55.3, 105.7, 119.1, 124.3, 124.4, 127.0, 127.3, 128.6, 129.0, 129.1, 129.9, 132.5, 134.3, 136.8, 137.5, 140.6, 142.7, 151.6, 157.9 ppm. FAB-MS (NBA) m/z 338 (MH^+). HRMS (FAB/NBA) calcd for $C_{23}H_{20}N_3$ 338.1657, found 338.1663 (MH^+).

*4.1.5. 3-Benzyl-5-(4-phenoxyphenyl)pyrazin-2-amine (**12d**).* A mixture of aminopyrazinetriflate (**9**) (100 mg, 0.300 mmol) and boronic acid (128 mg, 0.600 mmol) and $Pd(PPh_3)_4$ (35 mg, 0.0300 mmol) and K_3PO_4 (127 mg, 0.600 mmol) in dioxane (3 mL) was heated to 80 °C for 2 h. The mixture was treated with 1 N NaOH aq (2 mL) and 30% H_2O_2 (1 mL) for 1 h at room temperature to oxidize the residual borane. To this mixture, 1 N HCl aq (2 mL) was added for neutralization. The product was extracted with AcOEt (×3), washed with brine, and dried over Na_2SO_4. After evaporation, the residue was purified by column chromatography on silica gel with AcOEt/hexane (1:2) to give aminopyrazine (**2e**) (81 mg, 76%) as a pale yellow solid. ^1H NMR ($CDCl_3$, 400 MHz), δ 4.18 (2H, s, CH_2Ph), 4.46 (2H, s, NH_2), 7.04–7.14 (3H, m, Ph), 7.10 (2H, d, J=8.8 Hz, Ph), 7.28–7.38 (6H, m, Ph), 7.91 (2H, d, J=8.8 Hz, Ph), 8.36 (1H, s, C*H*-6) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz), δ 41.2, 118.9, 123.3, 127.0, 127.2, 128.5, 129.0, 129.7, 132.5, 136.7, 137.0, 140.6, 142.1, 151.5, 157.1, 157.4 ppm. FAB-MS (NBA) m/z 354 (MH^+). HRMS (FAB/NBA) calcd for $C_{23}H_{20}N_3O$ 354.1606, found 354.1636 (MH^+).

*4.1.6. (E)-3-Benzyl-5-styrylpyrazin-2-amine (**12e**).* A mixture of aminopyrazinetriflate (**9**) (100 mg, 0.300 mmol) and boronic acid (90 mg, 0.600 mmol) and $Pd(PPh_3)_4$ (35 mg, 0.0300 mmol) and K_3PO_4 (127 mg, 0.600 mmol) in dioxane (2 mL) was heated to 80 °C for 6 h. The mixture was treated with 1 N NaOH aq (1 mL) and 30% H_2O_2 (1 mL) for 1 h at room temperature to oxidize the residual borane. To this mixture, 1 N HCl aq (1 mL) was added for neutralization. The product was extracted with AcOEt (×3), washed with brine, and dried over Na_2SO_4. After evaporation, the residue was purified by column chromatography on silica gel with AcOEt/hexane (1:2) to give aminopyrazine (**2f**) (63 mg, 72%) as a pale yellow solid. ^1H NMR ($CDCl_3$, 400 MHz), δ 4.16 (2H, s, CH_2Ph), 4.44 (2H, s, NH_2), 7.10 (1H, d, J=15.8 Hz, C*H*-1′), 7.24–7.38 (6H, m, Ph), 7.50 (1H, d, J=15.8 Hz, C*H*-2′), 7.54–7.56 (4H, m, Ph), 8.02 (1H, s, C*H*-6) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz), δ 41.3, 124.8, 126.7, 127.1, 127.7, 128.5, 128.7, 129.0, 129.6, 136.6, 137.1, 139.4, 141.0, 141.2, 151.7 ppm. FAB-MS (NBA) m/z 288 (MH^+). HRMS (FAB/NBA) calcd for $C_{19}H_{18}N_3$ 288.1561, found 288.1507 (MH^+).

*4.1.7. 3-Benzyl-5-ethynylpyrazin-2-amine (**13**).* To a mixture of aminopyrazinetriflate (**9**) (100 mg, 0.300 mmol), $Pd(PPh_3)_4$ (35 mg, 0.0300 mmol), CuI (14 mg,0.075 mmol), and TBS-acetylene (67 μl, 0.36 mmol) in DMF (5 mL) was added NEt_3 (1 mL) under argon atmosphere. The reaction mixture was stirred at 100 °C for 3 h. After checking TLC, the reaction mixture was cooled to room temperature and treated with TBAF (500 μl). The reaction mixture was diluted with water and extracted with AcOEt (×3). The organic layer was washed with water and brine, dried over Na_2SO_4. After

evaporation to dryness, purification by column chromatography on silica gel with AcOEt/hexane (1:2) provided acetylene (**13**) (58 mg, 77%) as a pale yellow solid. IR (KBr) ν_{max} 3496, 3296, 3175, 1622, 1522 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz), δ 3.20 (1H, s, C—C*H*), 4.12 (2H, s, CH_2Ph), 4.56 (2H, s, NH_2), 7.21–7.36 (5H, m, Ph), 8.13 (1H, s, C*H*-6) ppm. ^{13}C NMR ($CDCl_3$, 150 MHz), δ 41.2, 77.9, 80.9, 127.1, 127.3, 128.4, 129.1, 135.9, 141.3, 144.6, 152.1 ppm. FAB-MS (NBA) *m*/*z* 210 (MH^+). HRMS (FAB/NBA) calcd for $C_{13}H_{12}N_3$ 210.1031, found 250.1033 (MH^+).

Since all spectroscopic data of **1b**, **1c**, **1d**, **1e** were already reported, just ^1H NMR data and MS data were showed.

*4.1.8. 8-Benzyl-2-(4-hydroxybenzylidene)-6-phenylimidazo[1,2-a]-pyrazin-3(2H)-one (**14a**).* A round bottomed flask was charged with coelenterazinetriflate (**11**) (25.0 mg, 0.0522 mmol), boronic acid (14 mg, 0.104 mmol), $Pd(PPh_3)_4$ (6 mg, 0.00522 mmol), and K_3PO_4 (22 mg, 0.104 mmol) and connected to a vacuum/argon line. The flask was evacuated and then filled with argon, this evacuation/filling cycle being conducted three times. These reagents were dissolved in dioxane (1 mL) and then heated to 40 °C for 1 h. The mixture was extracted with AcOEt (×3) and dried over Na_2SO_4. After evaporation, the residue was purified by preparative TLC to give dehydrocoelenterazine (**14a**) (16 mg, 75%) as a purple solid. ^1H NMR ($CDCl_3$, 400 MHz), δ 4.20 (2H, s, CH_2Ph), 6.93 (2H, d, *J*=8.4 Hz, *Ph*—OH), 7.49–7.25 (10H, m, 6-Ph+Ph), 7.75 (1H, s, C*H*-5), 7.94 (2H, d, *J*=8.4 Hz, *Ph*—OH), 8.39 (1H, s, C*H*—Ph—OH) ppm. FAB-MS (NBA) *m*/*z* 406 (MH^+). HRMS (FAB/NBA) calcd for $C_{26}H_{20}N_3O_2$ 406.1556, found 406.1561 (MH^+).

*4.1.9. 8-Benzyl-6-(4-fluorophenyl)-2-(4-hydroxybenzylidene)-imidazo[1,2-a]pyrazin-3(2H)-one (**14b**).* A round bottomed flask was charged with coelenterazinetriflate (**11**) (30 mg, 0.0626 mmol), boronic acid (17 mg, 0.125 mmol), $Pd(PPh_3)_4$ (10 mg, 0.00626 mmol), and K_3PO_4 (27 mg, 0.125 mmol) and connected to a vacuum/argon line. The flask was evacuated and then filled with argon, this evacuation/filling cycle being conducted three times. These reagents were dissolved in dioxane (1 mL) and then heated to 40 °C for 1 h. The mixture was extracted with AcOEt (×3) and dried over Na_2SO_4. After evaporation, the residue was purified by preparative TLC to give dehydrocoelenterazine (**14b**) (16 mg, 61%) as a purple solid. FAB-MS (NBA) *m*/*z* 424 (MH^+). HRMS (FAB/NBA) calcd for $C_{26}H_{19}N_3O_2F_1$ 424.1461, found 424.1490 (MH^+).

*4.1.10. 8-Benzyl-2-(4-hydroxybenzylidene)-6-(4-methoxyphenyl)-imidazo[1,2-a]pyrazin-3(2H)-one (**14c**).* A round bottomed flask was charged with coelenterazinetriflate (**11**) (30 mg, 0.0626 mmol), boronic acid (19 mg, 0.125 mmol), $Pd(PPh_3)_4$ (10 mg, 0.00626 mmol), and K_3PO_4 (27 mg, 0.125 mmol) and connected to a vacuum/argon line. The flask was evacuated and then filled with argon, this evacuation/filling cycle being conducted three times. These reagents were dissolved in dioxane (1 mL) and then heated to 40 °C for 1 h. The mixture was extracted with AcOEt (×3) and dried over Na_2SO_4. After evaporation, the residue was purified by preparative TLC to give dehydrocoelenterazine (**14c**) (24 mg, 93%) as a purple solid. FAB-MS (NBA) *m*/*z* 436 (MH^+). HRMS (FAB/NBA) calcd for $C_{27}H_{22}N_3O_3$ 436.1661, found 436.1673 (MH^+).

*4.1.11. 8-Benzyl-6-(4-(dimethylamino)phenyl)-2-(4-hydroxybenzylidene)imidazo[1,2-a]pyrazin-3(2H)-one (**14d**).* A round bottomed flask was charged with coelenterazinetriflate (**11**) (30 mg, 0.0626 mmol), boronic acid (21 mg, 0.125 mmol), $Pd(PPh_3)_4$ (10 mg, 0.00626 mmol), and K_3PO_4 (27 mg, 0.125 mmol) and connected to a vacuum/argon line. The flask was evacuated and then filled with argon, this evacuation/filling cycle being conducted three times. These reagents were dissolved in dioxane (1 mL) and then heated to 40 °C for 1 h. The mixture was extracted with AcOEt (×3) and dried over Na_2SO_4. After evaporation, the residue was purified by preparative TLC to give dehydrocoelenterazine (**14d**) (20 mg, 72%) as a purple solid. FAB-MS (NBA) *m*/*z* 449 (MH^+). HRMS (FAB/NBA) calcd for $C_{28}H_{25}N_4O_2$ 449.1978, found 449.1956 (MH^+).

*4.1.12. 8-Benzyl-2-(4-hydroxybenzylidene)-6-(thiophen-3-yl)-imidazo[1,2-a]pyrazin-3(2H)-one (**14e**).* A round bottomed flask was charged with coelenterazinetriflate (**11**) (25 mg, 0.0507 mmol), boronic acid (15 mg, 0.114 mmol), $Pd(PPh_3)_4$ (7 mg, 0.00507 mmol), and K_3PO_4 (22 mg, 0.114 mmol) and connected to a vacuum/argon line. The flask was evacuated and then filled with argon, this evacuation/filling cycle being conducted three times. These reagents were dissolved in dioxane (1 mL) and then heated to 40 °C for 1 h. The mixture was extracted with AcOEt (×3) and dried over Na_2SO_4. After evaporated, the residue was purified by preparative TLC to give dehydrocoelenterazine (**14e**) (15 mg, 67%) as a purple solid. IR (KBr) ν_{max} 3604, 1725, 1565 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), δ 4.31 (2H, s, CH_2Ph), 6.95 (2H, d, *J*=8.8 Hz, *Ph*—OMe), 7.22–7.50 (5H, m, 6-Ph), 7.51 (1H, s, C*H*—Ph—OMe), 7.62 (1H, dd, *J*=4.8, 2.8 Hz, thiophene-5), 7.70 (1H, dd, *J*=4.8, 0.8 Hz, thiophene-4), 7.92 (1H, dd, *J*=4.8, 2.8 Hz, thiophene-2), 8.10 (1H, s, C*H*-5), 8.31 (2H, d, *J*=8.8 Hz, *Ph*—OMe) ppm. ^{13}C NMR (DMSO-d_6, 150 MHz), δ 38.9, 110.8, 116.4, 121.9, 125.1, 125.6, 127.3, 128.3, 129.5, 130.5, 133.8, 135.6, 136.2, 136.7, 138.0, 140.2, 157.3, 161.7, 165.9 ppm. FAB-MS (NBA) *m*/*z* 412 (MH^+). HRMS (FAB/NBA) calcd for $C_{24}H_{18}N_3O_2S$ 412.1120, found 412.1075 (MH^+).

*4.1.13. 8-Benzyl-2-(4-hydroxybenzylidene)-6-styrylimidazo[1,2-a]-pyrazin-3(2H)-one (**14f**).* A round bottomed flask was charged with coelenterazinetriflate (**11**) (25 mg, 0.0507 mmol), boronic acid (15 mg, 0.114 mmol), $Pd(PPh_3)_4$ (7 mg, 0.00507 mmol), and K_3PO_4 (22 mg, 0.114 mmol) and connected to a vacuum/argon line. The flask was evacuated and then filled with argon, this evacuation/filling cycle being conducted three times. These reagents were dissolved in dioxane (1 mL) and then heated to 40 °C for 1 h. The mixture was extracted with AcOEt (×3) and dried over Na_2SO_4. After evaporated, the residue was purified by preparative TLC to give dehydrocoelenterazine (**14f**) (14.0 mg, 54%) as a purple solid. IR (KBr) ν_{max} 3552, 1710, 1562, 1513, 1405 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz), δ 4.37 (2H, s, CH_2Ph), 6.76 (2H, d, *J*=8.4 Hz, *Ph*—OMe), 7.17–7.54 (10H, m, Ph), 7.33 (1H, d, *J*=14.3 Hz, C*H*-1′), 7.44 (1H, d, *J*=14.3 Hz, C*H*-2′), 7.59 (1H, s, C*H*-5), 7.71 (2H, d, *J*=8.4 Hz, *Ph*—OMe) ppm. ^{13}C NMR (pyridine-d_5, 100 MHz), δ 39.9, 113.4, 116.8, 117.4, 123.0, 124.0, 124.3, 126.7, 127.0, 127.1, 127.2, 128.1, 128.8, 129.2, 130.3, 130.4, 132.7, 133.4, 137.0, 137.4, 137.7, 158.1, 163.4, 166.0 ppm. FAB-MS (NBA) *m*/*z* 432 (MH^+). HRMS (FAB/NBA) calcd for $C_{28}H_{22}N_3O_2$ 432.1712, found 432.1666 (MH^+).

*4.1.14. 6-Benzyl-5-(4-methylphenylsulfonamido)pyrazin-2-yl 4-methylbenzenesulfonate (**16**).* To a solution of sodium hydride (31 mg, 0.65 mmol) in tetrahydrofuran (5 mL) was added di-tosylamidine **15** (100 mg, 0.20 mmol) dissolved in tetrahydrofuran (10 mL) at 0 °C and then continuously stirred for 45 min. The resulting mixture was slowly added tosyl chloride (41 mg, 0.215 mmol) dissolved in tetrahydrofuran (5 mL) at 0 °C and then raised a temperature to refluxing condition for 2 h. The resulting mixture was quenched with ammonium chloride solution and extracted with dichloromethane (3×mL). Combined organic phases were washed with water and brined, dried over $MgSO_4$, filtered, concentrated under reduce pressure to give brown solid, which was purified by column chromatography using 35% ethyl acetate in hexanes as eluant to furnish ditosylate pyrazine **16** (0.0610 g) in 61% yield. ^1H NMR ($CDCl_3$, 400 MHz), δ 2.38 (3H, s, CH_3—Ts), 2.43 (3H, s, CH_3—Ts), 3.99 (2H, s, CH_2Ph), 7.02–7.09 (2H, m, Ph), 7.20 (2H, d, *J*=8.0 Hz, Ts, Ph), 7.27–7.29 (3H, m, Ph), 7.27 (2H, d, *J*=8.4 Hz, Ts Ph), 7.29 (1H, s,

C*H*═N), 7.65 (2H, d, *J*=8.4 Hz, Ts Ph), 7.75 (2H, d, *J*=8.0 Hz, Ts Ph), 7.95 (1H, s, N*H*Ts).

4.1.15. 5-Amino-6-benzylpyrazin-2-ol. Ditosylated pyrazine **16** (350 mg, 0.69 mmol) was added concd Sulfuric acid at 0 °C and then continuously stirred for 30 min. The resulting mixture was quenched with water and neutralized to pH=6. The neutral solution was extracted with dichloromethane (10×mL). The combined organic layers were dried over $MgSO_4$, filtered, concentrated under reduced pressure to give aminopyrazinol in 85% yield. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 3.89 (2H, s, CH_2Ph), 5.36 (2H, s, NH_2), 7.16–7.28 (5H, m, Ph), 7.40 (1H, s, C*H*═N Ph), 10.05 (1H, s, O*H*). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 38.0, 126.1, 126.6, 128.3, 128.8, 135.8, 138.5, 147.8, 152.3. Mass spectrum: (EI) *m*/*z* (% relative intensity) 202 (M^++1, 12), 201 (M^+, 100), 172 (10), 91 (16). HREIMS calcd for $C_{11}H_1N_3O_1$ $[M+H]^+$: 201.0902. Found: 201.0901.

*4.1.16. 5-Amino-6-benzylpyrazin-2-yl 4-methylbenzenesulfonate (**17**).* To a solution of aminopyrazinol (63 mg, 0.31 mmol) dissolved in dichloromethane (5 mL) was added di-isopropylehtylamine (0.14 mL, 0.36 mmol) and subsequently added tosyl chloride (72 mg, 0.38 mmol) dissolved in dichloromethane at 0 . A resulting mixture was stirred at room temperature for 1.5 h. The reaction was quenched with water and aqueous layer was extracted with dichloromethane (2×mL). The combined organic phases were washed with water and brine, dried over $MgSO_4$, filtered, concentrated under reduced pressure to give a yellow-brown solid, which was washed with cool ethyl acetate to give *O*-tosylate-2-aminopyrazine **17** (90.4 mg) in 81% yield. mp 166.7–167.3 . IR ($CHCl_3$) ν_{max} 3444, 3312, 1644, 1177 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.41 (3H, s, CH_3–Ts), 3.82 (2H, s, CH_2Ph), 6.53 (2H, s, NH_2), 7.04 (2H, d, *J*=8.0 Hz, Ph), 7.17–7.25 (3H, m, Ph), 7.41 (2H, d, *J*=8.4 Hz, Ts Ph), 7.698 (1H, s, C*H*═N), 7.705 (2H, d, *J*=8.4 Hz, Ts Ph). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 21.2, 37.6, 126.3, 128.1, 128.2, 128.8, 130.0, 132.4, 132.7, 137.0, 138.1, 143.4, 145.4, 153.2. Mass spectrum: (EI) *m*/*z* (% relative intensity) 357 (M^++2, 4), 356 (M^++1, 13), 355 (M^+, 55), 200 (96), 172 (61), 130 (90), 91 (100). HREIMS calcd for $C_{18}H_{17}N_3O_3S$ $[M+H]^+$: 355.0991. Found: 355.0991 Anal. Calcd for $C_{18}H_{17}N_3O_3S$: C, 60.83; H, 4.82; N, 11.82. Found: C, 60.98; H, 4.98; N, 11.83.

*4.1.17. 1-(1,3-Dioxolan-2-yl)-2-(4-methoxyphenyl)ethanone (**18**).* This compound was prepared from methoxyacetaldehyde in 4 sequential steps in total 63% yield as follows: To a solution of 2-(4-methoxyphenyl)acetaldehyde **20** (1.000 g, 6.66 mmol), 4 Å MS (6.000 g), and 1,3-propanedithiol (0.8 mL, 7.98 mol) in anhydrous CH_2Cl_2 (20.0 mL) under Argon at 0 °C, boron trifluoride ethyl etherate (3.3 mL, 26.6 mmol) was added. The reaction mixture was stirred for 2 h at 0 °C, and then was allowed to warm to room temperature and stirred for 12 h. The reaction mixture was quenched by $NaHCO_{3(aq)}$, filtered, and extracted with CH_2Cl_2 (10 mL×3). The combined organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to dryness. The crude was purified by column chromatography (EA/hexane=1:8) to give 2-(4-methoxybenzyl)-1,3-dithiane **21** as a yellow-green solid (1.487 g, 93%).

To a solution of 2-(4-methoxybenzyl)-1,3-dithiane **21** (1.000 g, 4.16 mmol) in anhydrous THF (10 mL) at −20 °C, *n*-butyllithium (2.5 M, 1.7 mL, 4.25 mmol) was added. After 30 min, anhydrous DMF (1.6 mL, 20.8 mol) was added, and the reaction mixture was allowed to warm to room temperature and stirred for 6 h. The reaction was quenched by 10% $HCl_{(aq)}$ (30 mL), then extracted with EA (20 mL×3). The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure to dryness. The crude product was purified by column chromatography (EA/hexane=1:8) to give 2-(4-methoxybenzyl)-1,3-dithiane-2-carbaldehyde **22** as a colorless oil (0.970 g, 86%).

To a solution of 2-(4-methoxybenzyl)-1,3-dithiane-2-carbaldehyde **22** (1.000 g, 3.73 mmol) in toluene (20 mL), ethylene glycol (1.0 mL, 17.9 mmol) and *p*-toluenesulfonic acid (6 mg, 1 mol %) were added. The reaction mixture was refluxed for 12 h under Dean–Stark conditions. The reaction was cooled to room temperature, quenched by $NaHCO_{3(aq)}$, and extracted with EA (20 mL×3). The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure to dryness. The crude was purified by column chromatography (EA/hexane=1:8) to give 2-(2-(4-methoxybenzyl)-1,3-dithian-2-yl)-1,3-dioxolane **23** as a colorless oil (1.094 g, 94%).

To a solution of 2-(2-(4-methoxybenzyl)-1,3-dithian-2-yl)-1,3-dioxolane (1.000 g, 3.20 mmol) in the mixed solvent of acetonitrile (100 mL) and water (35 mL) were added *N*-chlorosuccimide (2.128 g, 16.0 mmol) and silver nitrate (2.718 g, 16.0 mmol). The reaction solution was stirred for 5 min then quenched by $Na_2SO_{3(aq)}$ (40 mL), $NaHCO_{3\ (aq)}$ (40 mL), and $NaCl_{\ (aq)}$ (40 mL), filtered, and extracted with EA (100 mL×3). The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure to dryness. The crude was purified by column chromatography (EA/hexane=1:5) to give 1-(1,3-dioxolan-2-yl)-2-(4-methoxyphenyl) ethanone **18** as a colorless oil (1.094 g, 84%).

*4.1.18. 8-Benzyl-2-(4-methoxybenzyl)-3-oxo-3,7-dihydroimidazo[1,2-a]pyrazin-6-yl 4-methylbenzenesulfonate (**19**).* Round bottle flask, flame-dried, charged with 2-aminopyrazine **17** (80 mg, 0.23 mmol) and α-keto acetal **18** (100 mg, 0.45 mmol) was dissolved with 20% water in dioxane (water to dioxane (1 mL:4 mL)) and subsequently added 10% HCl (2 mL). A resulting mixture was raised temperature to 75 °C for 3.5 h. The reaction was quenched with ice water, and extracted with dichloromethane (4×mL). The combine organic layers were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated under reduce pressure to give brown oil, which was purified by column chromatography using 2% methanol in dichloromethane to provide coelenterazine **19** (60 mg) in 52% yield. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.32 (3H, s, CH_3–Ts), 3.49 (3H, s, OCH_3–Ph), 3.69 (2H, s, CH_2Ph), 4.04 (2H, s, CH_2Ph), 6.32 (2H, d, *J*=8.4 Hz, Ph), 6.58 (2H, d, *J*=8.4 Hz, Ph), 6.96–7.00 (5H, m, Ph), 7.13 (2H, d, *J*=8.4 Hz, Ts Ph), 7.17 (1H, s, N*H*), 7.56 (1H, s, C═C*H*–N), 7.65 (2H, d, *J*=8.4 Hz, Ts Ph). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 21.7, 29.7, 38.0, 55.0, 105.5, 113.6, 126.8, 128.3, 128.6, 128.8, 129.0, 129.6, 129.7, 133.1, 136.1, 143.8, 144.8, 145.3, 146.9, 158.1.

*4.1.19. (Z)-8-Benzyl-2-(4-methoxybenzylidene)-3-oxo-2,3-dihydroimidazo[1,2-a]pyrazin-6-yl 4-methylbenzenesulfonate (**24**).* A solution of coelenterazine **19** (25 mg, 0.048 mmol) dissolved ether and ethanol (4 mL:1 mL) was added manganese dioxide (105 ng, 1.20 mmol) at 0 , and then stirred at room temperature for 1 h. The resulting mixture was filtered through pad Celite and concentrated under reduce pressure to provide red solid, which was purified by column chromatography using 2% methanol in dichloromethane to give dehydrocoelenterazine **24** (19.7 mg) in 79% yield as a red solid. IR ($CHCl_3$) 1715, 1636, 1586, 1509 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.40 (3H, s, CH_3–Ts), 3.84 (3H, s, Ph–OCH_3), 4.07 (2H, s, CH_2Ph), 6.93 (2H, d, *J*=8.0 Hz, Ph), 7.18–7.25 (5H, m, Ph), 7.23 (2H, d, *J*=8.0 Hz, Ph), 7.43 (1H, s, C═C*H*–N), 7.72 (2H, d, *J*=8.4 Hz, Ts Ph), 8.18 (2H, d, *J*=8.4 Hz, Ts Ph). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 21.8, 39.0, 55.6, 108.6, 114.8, 127.0, 127.2, 128.4, 128.6, 129.6, 129.8, 132.9, 135.5, 136.16, 136.2, 136.7, 141.3, 145.5, 146.5, 157.8, 163.1, 166.0. Mass spectrum: (EI) *m*/*z* (% relative intensity) 513 (M^+, 13), 359 (M^++1, 68), 358 (M^+, 55), 91 (100). HREIMS calcd for $C_{28}H_{23}N_3O_5S$ $[M+H]^+$: 513.1358. Found: 513.1363.

*4.1.20. (Z)-8-Benzyl-2-(4-methoxybenzylidene)-6-(4-methoxy-phenyl)imidazo[1,2-a]pyrazin-3(2H)-one (**26**).* Two round bottle flask,

flame-dried, charged with dehydrocoelenterazine **24**, potassium fluoroboronate ester **25**, SPhos ligand, $Pd(OAc)_2$, and Cs_2CO_3 was dissolved in dry methanol, and subsequently degassed for three times. The resulting mixture was stirred at 80 °C oil bath for 2 h. When reaction mixture was become to room temperature, it was filtered through pad Celite. The resulting solution was dilute with ethyl acetate (mL) and washed with water followed by brine. The organic phase was dried over MgSO4, filtered, and concentrated under reduce pressure to give red oil, which purified by column chromatography using 2% methanol in dichloromethane to provide dehydrocoelenterazine **26** in 93% yield. IR ($CHCl_3$) 1713, 1637, 1587, 1508 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz), δ 3.82 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 4.36 (2H, s, CH_2Ph), 6.93 (2H, d, J=8.8 Hz, Ph), 6.99 (2H, d, J=8.8 Hz, Ph), 7.23–7.34 (3H, m, Ph), 7.46 (1H, s, C═C*H*–N, Ph), 7.56 (2H, d, J=8.0 Hz, Ph), 7.62 (1H, s, N–C═C*H*), 7.74 (2H, d, J=8.8 Hz, Ph), 8.27 (2H, d, J=8.8 Hz, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 39.8, 55.3, 55.5, 109.1, 114.2, 114.7, 121.0, 126.6, 126.8, 127.5, 128.2, 128.4, 127.7, 134.4, 134.5, 135.9, 136.7, 137.0, 146.9, 157.7, 159.9, 162.7, 166.8. Mass spectrum: (EI) m/z (% relative intensity) 450 (M^++1, 34), 449 (M^+, 100), 421 (85), 379 (37), 91 (90). HREIMS calcd for $C_{28}H_{23}N_3O_5S$ $[M+H]^+$: 449.1739. Found: 449.1730.

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Supplementary data

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