HETEROCYCLES, Vol. 65, No. 4, 2005, pp. 843 - 856
Received, 25th January, 2005, Accepted, 16th February, 2005, Published online, 16th February, 2005

NOVEL SYNTHETIC ROUTE OF COELENTERAZINES -2-: SYNTHESIS OF VARIOUS DEHYDROCOELENTERAZINE ANALOGS

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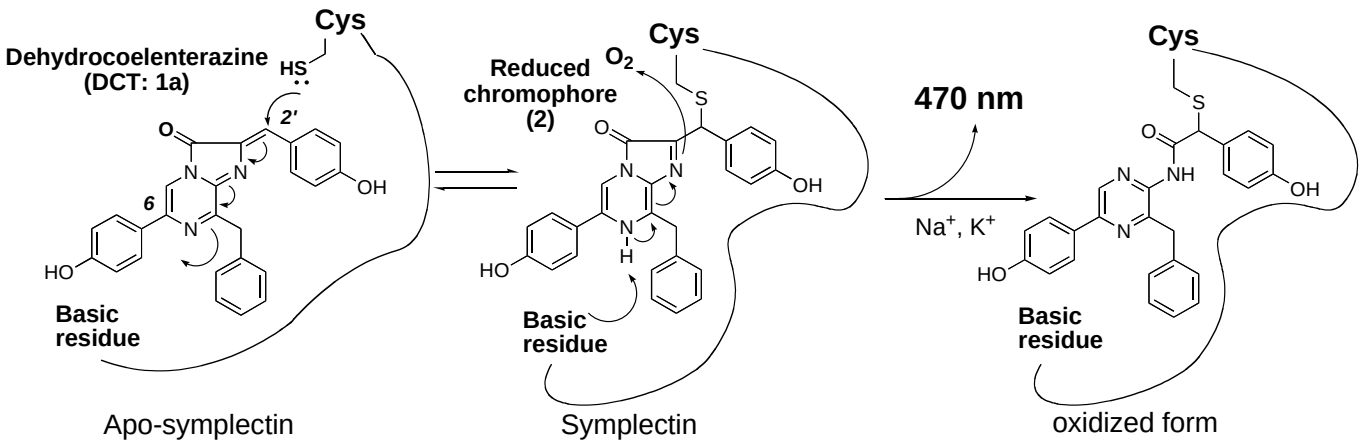
Abstract – The novel synthetic route to introduce various substituents into 5-position of coelenteramine is described. Difficulties, however, are observed in the attempted synthesis of some analogs having labile functional groups. This is due to the strong acid conc. H_2SO_4 after Suzuki-Miyaura coupling, so that the route was limited only to the synthesis for aminopyrazines having acid-stable functional groups. In this report, we describe alternative success in the deprotection of *N*-tosyl-animopyrazine triflate before the cross coupling; thus, we obtained the aminopyrazine triflate in high yield. This compound enables us to synthesize various coelenterazine analogs. This triflate was proven to be so important intermediate that the versatile synthesis for coelenterazine and dehydrocoelenterazine analogs was established through Suzuki-Miyaura or Sonogashira coupling.

INTRODUCTION

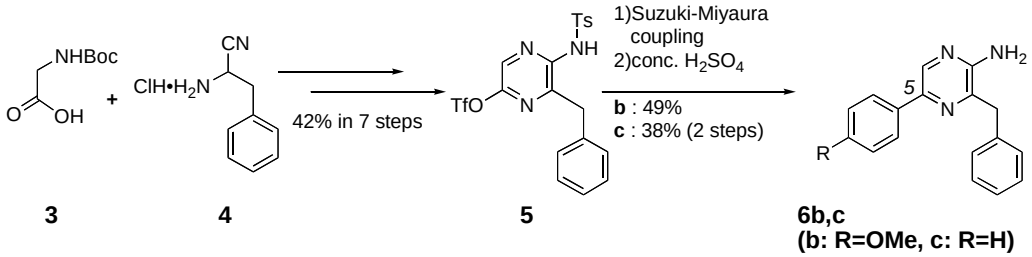
Symplectin is a photoprotein of luminous oceanic squid (*Symplectoteuthis oualaniensis*).[1] Symplectin uses dehydrocoelenterazine (DCT: **1a**) as organic substance for bioluminescence. We have investigated the bioluminescent mechanism of symplectin to demonstrate the

following postulated mechanism: Michael addition of a sulfhydryl group of apo-symplectin to a dehydrocoelenterazine is the initial step in the symplectin bioluminescence to afford a pseudo-reduced chromophore (**2**) having N-H proton (Scheme 1).[2-7]



Scheme 1. Postulated bioluminescent mechanism of *S. oualaniensis*.

Structural change of symplectin by addition of mono-valent ions (Na^+, K^+)[8] initiates the oxidation of the chromophore with molecular oxygen to give the oxidized form with simultaneous blue light luminescence (470 nm). After regeneration of apo-symplectin, dehydrocoelenterazine is reproduced to symplectin. To investigate the three-dimensional structure of symplectin active site, we have focused on the structure and activity relationships between DCT analogs and symplectin. Since we have already reported the substituent effects of 2'-aromatic rings in DCT,[9] we now investigate the substituent effects of 6-aromatic rings in DCT. The Kishi-Goto synthetic route[10,11] is the promising one for DCT, and the Nakamura synthetic method of coelenterazine[12,13] is also applicable to it. In order to obtain various 6-substituted DCT analogs with easier methods, we have to establish a much more convenient route, namely through 2-amino-5-aryl-3-benzylpyrazine as a precursor for imidazopyrazinone (Scheme 2).[14]



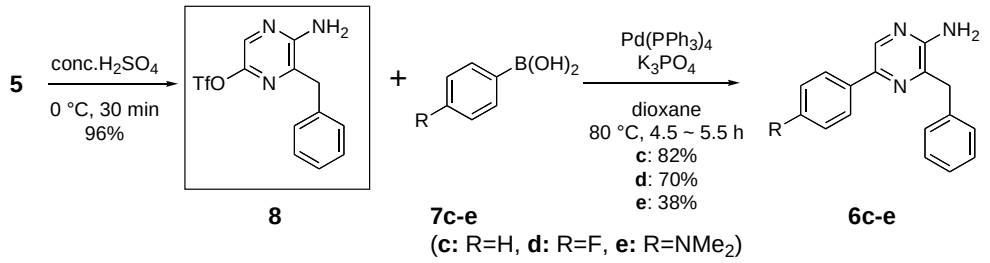
Scheme 2. Previously reported route for coelenteramine synthesis.[14]

In the previous route, deprotection of *N*-tosyl-aminopyrazine with conc. H_2SO_4 was the critical step after Suzuki-Miyaura coupling. Due to the strong acid, the route was limited only to the synthesis for aminopyrazines having acid-stable functional groups. In this report, we describe the improvements in the synthesis of the various coelenterazine analogs.

RESULTS AND DISCUSSION

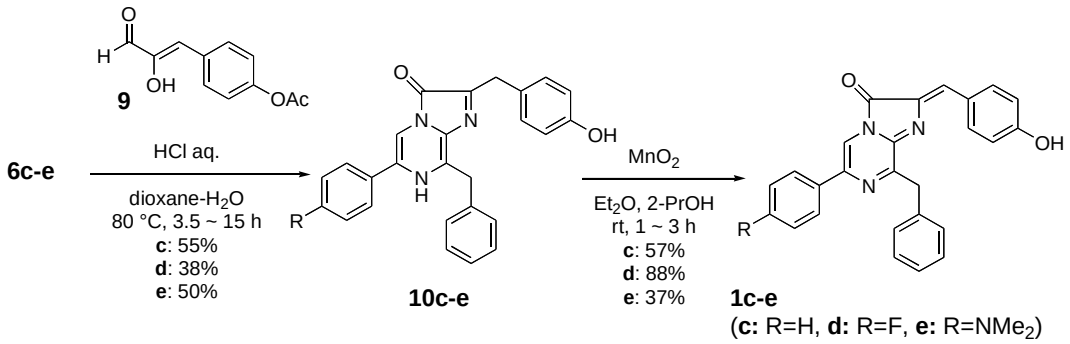
In our previous paper,[14] Strecker type synthesis starting from Boc-glycine (**3**) and aminonitrile (**4**), aminopyrazine was constructed in several steps as shown in Scheme 2. The *N*-tosyl-aminopyrazine triflate (**5**) was subjected to Suzuki-Miyaura coupling[15] with aryl boronic acids to afford coelenteramines (**6b,c**) (Scheme 2). Since many kinds of boronic acids are commercially available, this route was quite efficient for the synthesis of various 5-aryl-aminopyrazines. Furthermore, the merit of Suzuki-Miyaura coupling is convenient usage of boron reagents, compared to the tin reagents in Stille coupling. However, the route contained a critical step in deprotection of the tosyl group with conc. H_2SO_4 after Suzuki-Miyaura coupling. Therefore, the route was limited only to the synthesis for aminopyrazines having acid-stable functionality. Acid-labile functionalized phenyl groups could not be applied in the route, such as phenylacetylene.

To make this route more versatile by removing the limitation, at first, we started to improve the yield in the deprotection step without using conc. H_2SO_4. Although we tried many conditions to deprotect the tosyl group in acidic, basic, and radical cleavage conditions, all attempts failed in improving the yield. Further protection of the sulfonamide with Boc and following treatment with magnesium methoxide was the only condition to provide a coelenteramine (**6b**), though the yield remained low (<25%). Finally, we tried to exchange the reaction steps; deprotection of tosyl group was operated before Suzuki-Miyaura coupling. To our delight, treatment of triflate (**5**) with concentrated sulfuric acid at 0 °C afforded 5-amino-6-benzyl-2-trifluoromethanesulfonyloxypyrazine (**8**) in ideal yield (96%) without any purification (Scheme 3). The stability of the triflate (**8**) in strong acid might contribute to the high yield. This triflate (**8**) was proved to be very important key compound as a precursor of 6-substituted DCT analogs, since many coelenterazine and dehydrocoelenterazine analogs would be prepared through **8**. Furthermore, the yields of every step to prepare the compound (**8**) are quite high starting from inexpensive materials (**3**, **4**).



Scheme 3. Improved synthesis of coelenteramine analogs (**6c-e**) *via* important intermediate triflate (**8**) by using Suzuki-Miyaura coupling.

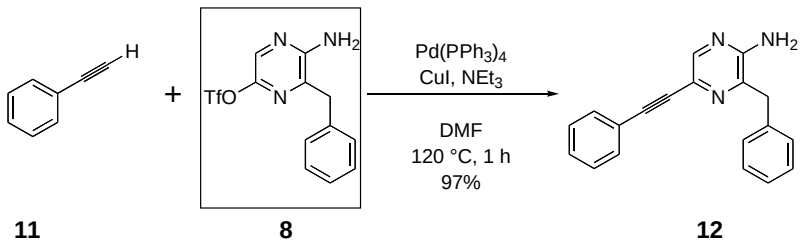
Following the procedure established by Suzuki *et al.*,[16] the triflate (**8**) was coupled with boronic acids by using $Pd(PPh_3)_4$ and K_3PO_4 to afford coelenteramine analogs (**6c-e**) in 38-82% yields. The coupling underwent smoothly, except for the case that 4-dimethylaminophenylboronic acid was used to synthesize coelenteramine analog (**6e**). The low yield was presumably attributed to the dimethylamino moiety, which sometimes decrease yields in palladium-catalyzed reaction. Condensation of coelenteramines (**6c-e**) with keto aldehyde (**9**) in a mixture of 10% aq. HCl and dioxane provided various coelenterazine analogs (**10c-e**) in moderate yields (38-55%) as shown in Scheme 4.



Scheme 4. Novel synthesis of coelenterazine analogs (**10c-e**) and dehydrocoelenterazine analogs (**1c-e**).

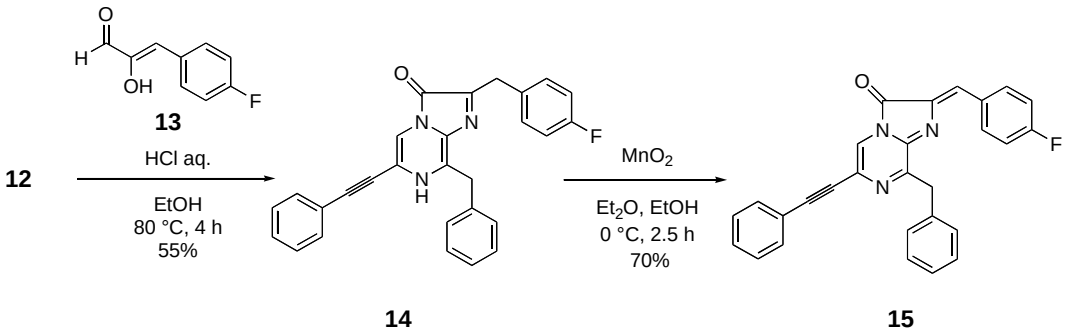
Thus, we have demonstrated that our route is applicable to the synthesis of coelenterazine analogs (**10c-e**), which are candidates for organic substances of luminous jellyfish (*Aequorea victoria*) bioluminescence. Finally, 6-substituted DCT analogs (**1c-1e**) were obtained by oxidation of coelenterazines (**10c-e**) with manganese(IV) oxide in 2-propanol. The weak nucleophilicity of 2-PrOH contributed to the better yields as solvent than MeOH or EtOH. Again, dimethylamino group of **10e** affected the yield (37%) in the step by affording complex mixtures (Scheme 4).

We first chose Suzuki-Miyaura coupling for the synthesis of DCT analogs and succeeded in the coupling of triflate (**8**) with boronic acids (**7b-e**). Because triflates are useful precursors for carbon-carbon bond formation under various coupling conditions, our interests then focused on the reactivity of triflate (**8**) under other coupling conditions. Especially, Sonogashira coupling is promising condition to introduce acetylene[17,18] and is expected to allow us to synthesize not only organic substances for bioluminescence but also new organic electroluminescence materials. Thus, we investigated the introduction of acetylene into aminopyrazine ring starting from triflate (**8**). After many trials, we found that the following procedure was the best condition for coupling triflate (**8**) with phenylacetylene (**11**). To a mixture of triflate (**8**), phenylacetylene (**11**), CuI (0.2 eq.) and $Pd(PPh_3)_4$ (0.1 eq.) in DMF was added NEt_3 at room temperature. Then the whole was stirred at 120 °C for 1 h. Usual work-up provided coelenteramine analog (**12**) in 97% yield as shown in Scheme 5. The addition order of reagents seemed to be important, since the coelenteramine analog (**12**) could not be obtained under the other procedure (phenylacetylene was added in the final step) only to recover triflate **8** (62%).



Scheme 5. Synthesis of coelenteramine analog (**12**) from triflate (**8**) by Sonogashira coupling**.**

The substituent effects on the 6-phenyl ring of DCT for symplectin bioluminescence are quite interesting, therefore, in order to investigate the substituent effects systematically, it is important that the remaining structure of DCT analogs must be the same with natural DCT (**1a**). Therefore, keto aldehyde (**9**) was the best candidate for the condensation with coelenteramine analogs (**6b-e**) (Scheme 4). On the other hand, the structure of coelenteramine analog (**12**) is quite different from natural coelenteramine due to the insertion of acetylene between 5-phenyl ring and pyrazine. Therefore, we planned to prepare a very hydrophobic DCT (**15**) with no hydroxyl group in its structure.



Scheme 6. Synthesis of coelenterazine analog (**14**) and dehydrocoelenterazine analog (**15**).

Condensation of coelenteramine analog (**12**) with *p*-fluorophenylketo aldehyde (**13**)[9] provided coelenterazine analog (**14**) in moderate yield. This reaction progressed in better yield (55%) in ethanol than in aqueous dioxane (37% yield). Finally, DCT analog (**15**) was obtained by oxidation of coelenterazine analog (**14**) by treatment with manganese(IV) oxide (Scheme 6). Although the structure of **15** is quite different from natural DCT (**1a**), by checking its bioluminescence activity, we must obtain more information about symplectin bioluminescent mechanism. Furthermore, we would compare the bioluminescence activity of DCT analog (**15**) with the reported results of 2'-F-DCTs.[9] Investigation of the structure and activity relationship (SAR) between symplectin bioluminescence and DCT analogs (**1c-e, 15**) derived from triflate (**8**) is now underway in our group.

CONCLUSION

We have succeeded in the deprotection of *N*-tosyl-animopyrazine triflate, before cross coupling, to afford aminopyrazine triflate (**8**) in high yield. This compound (**8**) enables us to synthesize various coelenterazine analogs. From the triflate being as important intermediate, the versatile synthesis for coelenterazine and dehydrocoelenterazine analogs has been established through Suzuki-Miyaura or Sonogashira coupling.

EXPERIMENTAL

General

All melting points were measured on Yanaco MP-S3 and uncorrected. UV spectra were obtained on a JASCO U-best 50 spectrophotometer. IR spectra were recorded on a PERKIN ELMER Paragon 1000 FT-IR spectrophotometer. Proton NMR spectra were recorded on a JEOL GSX 270 for 270 MHz, a JEOL JNML-500 for 500 MHz or a Bruker AMX-600 for 600 MHz. Chemical shift (δ) are given in parts per million relative to

tetramethylsilane (δ 0.00) or CD_3OD (δ 3.30) or DMSO-d_6 (δ 2.49) as internal standard. Coupling constants (*J*) are given in Hz. Carbon NMR spectra were recorded on a JEOL GSX 270 for 67.8 Hz, or on a JEOL JNML-500 for 125.7 Hz, or on a Bruker AMX-600 for 150.9 MHz. Chemical shifts are (δ) given in parts per million relative to $CDCl_3$ (δ 77.0) or CD_3OD (δ 49.0) or DMSO-d_6 (δ 45.0) as internal standard. Coupling constants (*J*) are given in Hz. Fluorine NMR spectra were recorded on a JEOL A-400 for 376 MHz or a JEOL JNML-500 for 470 MHz. Chemical shifts are (δ) given in parts per million relative to 1,1,1-trifluorotoluene (δ 0.00) as external standard. Low-resolution EI MS spectra and FAB MS spectra were measured with a JEOL JMS-700. High-resolution (HR) MS spectra were measured with a JEOL JMS-700. Analytical Laboratory of this school performed the combustion elemental analyses by Mr. S. Kitamura. Fluorescence spectra were measured with a JASCO FP-777 spectrometer. All the solvents were of reagent grade. Analytical thin-layer chromatography (TLC) was conducted on precoated tlc plates: silica gel 60 F-254 [E.Merck (Art 5715) Darmstadt, Germany], layer thickness 0.25 mm. Silica gel column chromatography utilized Silica Gel 60 (spherical) 40-50 μm [KANTO CHEMICAL CO., INC].

5-Amino-6-benzyl-2-*O*-trifluoromethanesulfonyloxypyrazine (8).

N-Ts-amidepyrazine (**5**)[14] (49.0 mg, 0.101 mmol) was dissolved in 0.6 mL of conc. H_2SO_4 at 0 °C in an ice bath. After stirring for 30 min at 0 °C, ice was poured into the reaction. The reaction mixture was extracted with CH_2Cl_2 (×2). The organic layer was washed with brine once and then dried over anhydrous Na_2SO_4. Concentration of the solution provided pure aminopyrazine (**8**) (32.3 mg, 96%) as a pale yellow solid without any purification; yellow needles (from ethyl acetate). mp 92-94 °C. UV (MeOH) λ_{max} (log ε), 324 (3.50) nm. FL (MeOH) Em. 401.0 nm (Ex. 350 nm). IR (KBr) ν_{max} 3497, 3312, 3167, 1632, 1544, 1404 cm^{-1}. ^{1}H-NMR (270 MHz, $CDCl_3$) δ 4.05 (2H, s, C*H*$_2$Ph), 4.63 (2H, br s, N*H*$_2$), 7.37-7.18 (5H, m, Ph), 7.93 (1H, s, C*H*-3) ppm. ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ 40.4, 127.4, 128.4, 129.1, 132.5, 135.0, 138.9, 144.9, 152.9 ppm. FAB-MS (NBA) *m/z* 334 (MH^+). HRMS (FAB/NBA) calcd for $C_{12}H_{11}N_3O_3F_3S$ 334.0473, found 334.0469 (MH^+). Anal. Calcd for $C_{19}H_{16}N_3O_5F_3S_2$: C, 43.24; H, 3.02; N, 12.61. Found: C, 43.48; H, 3.19; N, 12.21.

2-Amino-3-benzyl-5-(4-fluorophenyl)pyrazine (6d).

A mixture of triflate (**8**) (105.5 mg, 0.317 mmol), 4-fluorophenylboronic acid (**7d**) (109.1 mg, 0.780 mmol), $Pd(PPh_3)_4$ (18.9 mg, 0.0164 mmol), and K_3PO_4 (133.4 mg, 0.629 mmol) in

dioxane (1.5 mL) was heated to 80 °C for 4 h. The mixture was treated with aqueous 1N aq. NaOH (1 mL) and 30% H_2O_2 (0.5 mL) for 1 h at rt to oxidize the residual borane. After neutralization with 1N aq. HCl, the mixture was extracted with ethyl acetate (×2) and the extract was washed with brine once. The organic layer was dried over anhydrous Na_2SO_4. After evaporated, the residue was purified by column chromatography on silica gel with ethyl acetate/*n*-hexane (1:5) to give coelenteramine (**6d**) as an orange solid (62.1 mg); yellow needles (from ethyl acetate). mp 131-133 °C. UV (MeOH) λ_{max} (log ε), 341 (4.15), 275 (4.42) nm. FL (MeOH) Em. 411.5 nm (Ex. 350 nm). IR (KBr) ν_{max} 3482, 3296, 3136, 1634, 1601, 1541, 1513, 1463 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 4.10 (2H, s, C*H*$_2$Ph), 4.35 (2H, br s, N*H*$_2$), 7.06 (2H, t, *J* = 8.7 Hz, Ph-F), 7.27-7.18 (5H, Ph), 7.84 (2H, dd, *J* = 8.7, 5.3 Hz, Ph-F), 8.27 (1H, s, C*H*-6) ppm. ^{13}C-NMR ($CDCl_3$, 125 MHz), δ 41.2, 115.7 (d, J_{C-F} = 22 Hz), 127.1, 127.5 (d, J_{C-F} = 8.2 Hz), 128.6, 129.0, 133.3, 136.5, 137.2, 140.8, 142.0, 151.8, 163.1 (d, J_{C-F} = 249 Hz) ppm. ^{19}F-NMR ($CDCl_3$, 470 MHz), δ –51.6 ppm. FAB-MS (NBA) *m/z* 280 (MH^+). HRMS (FAB/NBA) calcd for $C_{17}H_{15}N_3F$ 280.1250, found 280.1238 (MH^+). Anal. Calcd for $C_{17}H_{14}N_3F$: C, 73.10; H, 5.05; N, 15.04. Found: C, 72.70; H, 5.09; N, 14.70.

2-Amino-3-benzyl-5-(4-*N,N*-dimethylaminophenyl)pyrazine (6e).

According to the same procedure with **6d** synthesis, coelenteramine (**6e**) was prepared from triflate (**8**) (150.7 mg, 0.452 mmol), 4-dimehylaminophenylboronic acid (**7e**) (127.1 mg, 0.770 mmol), $Pd(PPh_3)_4$ (28.5 mg, 0.0247 mmol), and K_3PO_4 (140.1 mg, 0.661 mmol) in dioxane (3 mL). Column chromatography on silica gel with ethyl acetate/*n*-hexane (1:3) provided coelenteramine (**6e**) as a brown solid (52.6 mg, 38%); brown powder (from ethyl acetate). mp 181-185 °C. UV (MeOH) λ_{max} (log ε), 364 (4.20), 305 (4.56) nm. FL (MeOH) Em. 516.5 nm (Ex. 350 nm). IR (KBr) ν_{max} 3480, 3296, 3133, 1638, 1610, 1522, 1465 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz), δ 3.00 (6H, s, NMe_2), 4.17 (2H, s, C*H*$_2$Ph), 4.30 (2H, br s, N*H*$_2$), 6.81 (2H, dd, *J* = 9.2, 2.4 Hz, Ph-NMe_2), 7.34-7.24 (5H, m, Ph), 7.84 (2H, dd, *J* = 9.2, 2.4 Hz, Ph-NMe_2), 8.31 (1H, s, C*H*-6) ppm. ^{13}C-NMR ($CDCl_3$, 125 MHz), δ 40.5, 41.2, 112.6, 126.6, 126.9, 128.6, 128.9, 136.3, 140.3, 140.7, 143.4, 148.5, 150.6 ppm. EI-MS *m/z* 304 (M^+). HRMS (EI) calcd for $C_{19}H_{20}N_4$ 304.1688, found 304.1669 (M^+). Anal. Calcd for $C_{19}H_{20}N_4 \cdot 1/3CH_3OH$: C, 73.94; H, 6.53; N, 17.84. Found: C, 74.21; H, 6.69; N, 17.53.

8-Benzyl-2-(4-hydroxyphenylmethyl)-6-phenyl-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (10c).

A solution of coelenteramine (**6c**) (89.7 mg, 0.344 mmol) and keto aldehyde (**9**) (100.8 mg, 0.487 mmol) in 2.5 mL of 20% water/dioxane was degassed. To this solution was added 0.5 mL of 10% aq. HCl and the mixture was stirred under argon atmosphere at rt for 5 min, then at 80 °C for 3.5 h. After cooling, to this solution was added water at 0 °C. Precipitate was filtered to provide 72.3 mg (0.163 mmol) of coelenterazine·HCl salt as a yellow solid. The filtrate was extracted with ethyl acetate (×3). The combined organic layer was successively washed with brine once. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness to give 90.0 mg of brown crude oil. Purification by silica gel chromatography with ethyl acetate/*n*-hexane (1:1), then with MeOH/CH_2Cl_2 (1:9) afforded 11.0 mg of coelenterazine (**10c**) as a brown oil (0.027 mmol). Total yield was 83.3 mg (55%); yellow powder (from methanol/ether). mp 143-145 °C (decomp). UV (MeOH) λ_{max} (log ε), 419 (3.67), 346 (3.77), 257 (4.14) nm. FL (MeOH) Em. 517.0 nm (Ex. 350 nm). IR (KBr) ν_{max} 3369, 1566, 1514, 1454 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 600 MHz), δ 4.01 (2H, s, C*H*$_2$Ph), 4.22 (2H, s, C*H*$_2$Ph), 6.58 (2H, d, *J* = 8.4 Hz, Ph-OH), 7.39-7.03 (10H, m, 6-Ph+PhCH$_2$), 7.58 (2H, d, *J* = 8.4 Hz, Ph-OH), 7.80 (1H, s, C*H*-5), 9.10 (1H, br s, O*H*) ppm. ^{13}C-NMR (DMSO-d_6, 150 MHz), δ 38.3, 39.1, 114.8, 114.9, 115.1, 126.2, 126.3, 127.3, 128.0, 128.2, 128.6, 128.7, 128.8, 129.0, 129.5, 138.5, 140.0, 151.8, 155.4, 165.1 ppm. FAB-MS (NBA) *m/z* 408 (MH^+). HRMS (FAB/NBA) calcd for $C_{26}H_{22}N_3O_2$ 408.1712, found 408.1710 (MH^+). Anal. Calcd for $C_{26}H_{21}N_3O_2$: C, 76.64; H, 5.19; N, 10.31. Found: C, 76.47; H, 5.40; N, 10.30.

8-Benzyl-6-(4-fluorophenyl)-2-(4-hydroxyphenylmethyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (10d).

According to the same procedure with **10c** synthesis, coelenterazine (**10d**) was prepared from coelenteramine (**6d**) (22.4 mg, 80.2 μmol) and keto aldehyde (**9**) (23.0 mg, 0.111 mmol) in 0.75 mL of 20% water/dioxane containing 0.15 mL of 10% aq. HCl to give 63.0 mg crude solid. Silica gel column chromatography with MeOH/CH_2Cl_2 (1:10) afforded 12.8 mg of coelenterazine (**10d**) (75.8 mg, 38%) as brown solid; yellow powder (from methanol/ether). mp 138-140 °C (decomp). UV (MeOH) λ_{max} (log ε), 426 (4.05), 353 (3.88), 246 (4.44) nm. FL (MeOH) Em. 527.0 nm (Ex. 350 nm). IR (KBr) ν_{max} 3425, 1598, 1567, 1512, 1455 cm^{-1}. ^{1}H-NMR (CD_3OD, 600 MHz), δ 4.12 (2H, s, C*H*$_2$Ph), 4.46 (2H, s, C*H*$_2$Ph), 6.70 (2H, dd, *J* = 8.4, 3.0 Hz, Ph-OH), 7.12 (2H, dd, *J* = 8.4, 3.0 Hz, Ph-OH), 7.23-7.19 (3H, m, Ph), 7.30-7.27 (2H, m, Ph), 7.38 (2H, dd, *J* = 6.3, 1.5 Hz, Ph-F), 7.85 (2H, m, Ph-F), 8.18 (1H, s, C*H*-5) ppm.

^{13}C-NMR (CD_3OD, 150 MHz), δ 31.3, 37.3, 89.8, 99.0, 110.2, 113.8, 116.4, 116.9 (d, J_{C-F} = 22 Hz), 127.9, 128.2, 129.6 (d, J_{C-F} = 10 Hz), 129.8, 130.0, 130.1, 130.6, 131.3, 137.4,157.3, 161.5, 165.2 (d, J_{C-F} = 210 Hz) ppm. ^{19}F-NMR (CD_3OD, 470 MHz), δ –50.78 ppm. FAB-MS (NBA) *m/z* 426 (MH^+). HRMS (FAB/NBA) calcd for $C_{26}H_{20}N_3O_2F$ 426.1618, found 426.1610 (MH^+). Anal. Calcd for $C_{26}H_{20}N_3O_2F \cdot CO_2 \cdot 3/2H_2O$: C, 65.32; H, 4.67; N, 8.46. Found: C, 65.54; H, 4.61; N, 8.68.

8-Benzyl-6-(*N,N*-dimethyl-4-aminophenyl)-2-(4-hydroxyphenylmethyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (10e).

According to the same procedure with **10c** synthesis, coelenterazine (**10e**) was prepared from coelenteramine (**6e**) (44.6 mg, 0.147 mmol) and keto aldehyde (**9**) (44.8 mg, 0.216 mmol) in 1 mL of 20% water/dioxane containing 0.2 mL of 10% aq. HCl to give 76.5 mg of brown crude oil. Recrystallization with methanol/ether afforded 33.4 mg (50%) of coelenterazine (**10e**) as yellow brown solids. mp 166-167 °C (decomp). UV (MeOH) λ_{max} (log ε), 438 (3.84), 305 (4.22) nm. FL (MeOH) Em. 533.5 nm (Ex. 350 nm). IR (KBr) ν_{max} 3148, 2362, 1611, 1514, 1445 cm^{-1}. ^{1}H-NMR (CD_3OD, 600 MHz), δ 2.86 (6H, s, NMe_2), 3.96 (2H, s, C*H*$_2$Ph), 4.29 (2H, s, C*H*$_2$Ph), 6.58 (2H, d, *J* = 8.8 Hz, Ph-NMe_2), 6.70 (2H, d, *J* = 8.1 Hz, Ph-OH), 7.18-7.03 (5H, m, Ph-CH_2), 7.30 (2H, d, *J* = 8.4, Ph-OH), 7.45 (2H, d, *J* = 8.8 Hz, Ph-NMe_2), 7.64 (1H, s, C*H*-5) ppm. ^{13}C-NMR (CD_3OD, 150 MHz), δ 40.7, 40.8, 48.9, 110.0, 113.3, 113.7, 116.0, 116.4, 127.4, 127.7, 128.5, 129.4, 129.9, 130.6, 130.7, 133.2, 136.8, 143.6, 152.8, 156.6, 166.4 ppm. FAB-MS (NBA) *m/z* 451 (MH^+). HRMS (FAB/NBA) calcd for $C_{28}H_{27}N_4O_2$ 426.1618, found 426.1610 (MH^+). Anal. Calcd for $C_{28}H_{26}N_4O_2 \cdot C_2H_5OH \cdot CO_2$: C, 68.87; H, 5.97; N, 10.36. Found: C, 69.15; H, 5.89; N, 10.50.

8-Benzyl-2-(*p*-hydroxybenzylidene)-6-phenyl-2,3-dyhydroimidazo[1,2-*a*]pyrazin-3-one (1c).

Manganese(IV) oxide (207.8 mg, 2.39 mmol) was added to a solution of coelenterazine (**10c**) (43.0 mg, 0.106 mmol) in ether (6 mL) and 2-PrOH (2 mL) at rt. After stirring for 3 h at rt, the mixture was filtered through a pad of Celite and then concentrated under reduced pressure to dryness. The crude oil was recrystallized with 2-PrOH/ether to provide 9.7 mg of dehydrocoelenterazine (**1c**). Purification of the filtrate by preparative TLC with ethyl acetate/*n*-hexane (1:1) afforded dehydrocoelenterazine (**1c**) (15.0 mg) as a red-purple oil (total 57%); purple amorphous (2-PrOH/ether). mp 146-147 °C (decomp). UV (2-PrOH) λ_{max} (log ε), 560 (3.95), 522 (3.98), 411 (4.21), 343 (4.30), 270 (4.59) nm. IR (KBr) ν_{max} 3406,

1719, 1590, 1514, 1492, 1453 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz), δ 4.20 (2H, s, C*H*$_2$Ph), 6.93 (2H, d, *J* = 8.4 Hz, Ph-OH), 7.49-7.25 (10H, m, 6-Ph+PhCH$_2$), 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), 9.83 (1H, br s, O*H*) ppm. ^{13}C-NMR ($CDCl_3$, 150 MHz), δ 41.2, 110.4, 116.0, 125.3, 125.8, 127.1, 128.1, 128.4, 128.5, 128.8, 129.0, 129.2, 132.4, 136.3, 136.6, 137.1, 142.7, 151.6, 157.8, 166.9 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^+). Anal. Calcd for $C_{26}H_{19}N_3O_2 \cdot CO_2 \cdot 2H_2O$: C, 66.80; H, 4.78; N, 8.66. Found: C, 67.12; H, 4.67; N, 8.73.

8-Benzyl-6-(4-fluorophenyl)-2-(*p*-hydroxybenzylidene)-2,3-dihydroimidazo[1,2-*a*]pyrazin-3-one (1d).

According to the same procedure with **1c** synthesis, dehydrocoelenterazine (**1d**) (15.5 mg, 88%) was purely obtained as a red-brown solid without any purification from manganese(IV) oxide (101.7 mg, 1.17 mmol) and coelenterazine (**10d**) (17.6 mg, 41.4 μmol) in ether (10 mL) and 2-PrOH (2 mL); purple amorphous (2-PrOH/ether). mp 188-190 °C (decomp). UV (2-PrOH) λ_{max} (log ε), 610 (sh) (3.60), 560 (3.84), 522 (3.83), 410 (3.86), 342 (3.87), 273 (4.12) nm. IR (KBr) ν_{max} 3364, 2930, 1693, 1635, 1596, 1574, 1511 cm^{-1}. ^{1}H-NMR (DMSO-*d*$_6$, 600 MHz), δ 2.49 (2H, t, *J* = 1.8 Hz, C*H*$_2$Ph), 6.92 (2H, t, *J* = 9.6 Hz, Ph-OH), 7,49-7.11 (9H, m, Ar), 7.75 (1H, d, *J* = 8.4 Hz, C*H*-5), 7.99 (2H, dd, *J* = 8.4, 5.4 Hz, Ph-F), 8.30 (1H, d, *J* = 7.8 Hz, C*H*PhOH), 10.58 (1H, br s, O*H*) ppm. ^{13}C-NMR (DMSO-*d*$_6$, 150 MHz), δ 25.4, 111.3, 115.5 (d, $J_{C\text{-}F}$ = 22 Hz), 115.8, 125.6, 126.5, 127.2 (d, $J_{C\text{-}F}$ = 8.0 Hz), 128.2, 128.3, 128.4, 128.5, 129.4, 132.0, 133.8, 135.5, 136.1, 136.6, 146.2, 157.1, 162.8 (d, $J_{C\text{-}F}$ = 203 Hz), 163.2, 165.8 ppm. ^{19}F-NMR (DMSO-*d*$_6$, 470 MHz), δ –46.6 ppm. FAB-MS (NBA) *m/z* 424 (MH^+). HRMS (FAB/NBA) calcd for $C_{26}H_{19}N_3O_2F$ 424.1461, found 424.1442 (MH^+). Anal. Calcd for $C_{26}H_{18}N_3O_2F \cdot$2-PrOH$\cdot$1/2AcOEt: C, 71.21; H, 5.27; N, 8.45. Found: C, 71.01; H, 5.21; N, 8.21.

8-Benzyl-6-(*N,N*-dimethyl-4"-aminophenyl)-2-(*p*-hydroxybenzylidene)-2,3-dihydroimidazo[1,2-*a*]pyrazin-3-one (1e).

According to the same procedure with **1c** synthesis, dehydrocoelenterazine (**1e**) was synthesized from manganese(IV) oxide (88.9 mg, 1.02 mmol) and coelenterazine (**10e**) (17.4 mg, 38.6 μmol) in ether (1 mL) and 2-PrOH (2.1 mL). Dehydrocoelenterazine (**1e**) (6.4 mg, 37%) was obtained after purification by silica gel chromatography with 5% 2-PrOH/CH_2Cl_2 as a dark brown solid; purple amorphous (2-PrOH/ether). mp 147-149 °C (decomp). UV (2-PrOH) λ_{max} (log ε), 576 (2.40), 330 (2.17) nm. IR (KBr) ν_{max} 2878, 2806, 1611, 1516, 1456

cm^{-1}. ^{1}H-NMR (DMSO-d_6, 600 MHz), δ 2.87 (6H, s, NMe_2), 4.25 (2H, s, CH_2Ph), 6.68 (2H, d, J = 9.0 Hz, Ph-NMe_2), 6.88, (2H, d, J = 8.1 Hz, Ph-OH), 7.28-7.16 (5H, m, Ph-CH_2), 7.41 (1H, s, CH-5), 7.70 (2H, d, J = 9.0 Hz, Ph-NMe_2), 7.80 (1H, s, CHPh-OH), 8.25 (2H, d, J = 8.1 Hz, Ph-OH), 10.54 (1H, br s, OH) ppm. ^{13}C-NMR (DMSO-d_6, 150 MHz), δ 38.9, 42.8, 112.1, 115.8, 116.3, 121.0, 122.7, 125.8, 126.0, 126.5, 128.3, 129.5, 132.1, 133.4, 133.8, 136.1, 146.4, 150.2, 152.8, 155.1, 166.0 ppm. FAB-MS (NBA) *m/z* 449 (MH^+). HRMS (FAB/NBA) calcd for $C_{28}H_{25}N_4O_2$ 449.1978, found 449.1983 (MH^+).

2-Amino-3-benzyl-5-(phenylethynyl)pyrazine (12).

To a mixture of triflate (**8**) (100 mg, 0.30 mmol), $Pd(PPh_3)_4$ (33.5 mg, 0.029 mmol), CuI (14.1 mg, 0.074 mmol), and phenylacetylene (**11**) (46 mg, 0.45 mmol) in DMF (1.0 mL) was added NEt_3 (0.2 mL) *via* syringe under Ar atmosphere. The reaction mixture was stirred at 120 °C for 1 h. After cooling to rt, the reaction was quenched with water (5 mL), and extracted with ethyl acetate (3×5 mL). The combined organic layer was washed with brine once and dried over anhydrous $MgSO_4$. After evaporation to dryness, purification by column chromatography on silica gel with ethyl acetate/*n*-hexane (1:1) provided 2-amino-3-benzyl-5-(phenylethynyl)pyrazine (**12**) (83.4 mg, 97%) as a yellow solid; brown powder (ethyl acetate). mp 141.5-142.0 °C. UV (MeOH) λ_{max} (log ε), 347 (4.12), 295 (4.27) nm. FL (MeOH) Em. 415.5 nm (Ex. 350.0 nm). IR (KBr) ν_{max} 2350 (weak) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 600 MHz), δ 4.16 (2H, s, CH_2Ph), 4.58 (2H, br s, NH_2), 7.24 (2H, d, J= 7.3 Hz, Ph), 7.27 (1H, t, J = 7.3 Hz, Ph), 7.32 (2H, d, J= 7.7 Hz, Ph), 7.37-7.34 (3H, m, Ph), 7.60-7.59 (2H, m, Ph), 8.20 (1H, s, CH-6) ppm. ^{13}C-NMR ($CDCl_3$, 150 MHz), δ 41.3, 86.6, 89.9, 122.6, 127.3, 128.3, 128.4, 128.5, 128.6, 129.2, 131.8, 136.1, 141.2, 144.5, 151.7 ppm. FAB-MS (NBA) *m/z* 286 (MH^+). HRMS (FAB/NBA) calcd for $C_{19}H_{16}N_3$ 286.1344, found 286.1322 (MH^+). Anal. Calcd for $C_{19}H_{15}N_3$: C, 79.98; H, 5.30; N, 14.73. Found: C, 79.98; H, 5.32; N, 14.78.

8-Benzyl-6-phenylethynyl-2-(4-fluorophenylmethyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (14).

To a solution of coelenteramine analog (**12**) (14.0 mg, 49.1 μmol) and keto aldehyde (**13**)[9] (22.0 mg, 0.132 mmol) in 1 mL of EtOH was added 20 μL of 35% aq. HCl and the mixture was stirred under argon atmosphere at rt for 5 min, then 80 °C for 4 h. After cooling, this solution was diluted with ether (1 mL). The precipitate was filtered to give coelenterazine analog (**14**)·HCl salt (7.7 mg) as a yellow powder. Water was added to the filtrate and the mixture was extracted with CH_2Cl_2 (3×5 mL). The combined organic layer was dried over

anhydrous Na_2SO_4. Concentration of the solution and purification by preparative TLC with ethyl acetate/*n*-hexane (1:1) afforded coelenterazine analog (**14**) (4.7 mg) as a yellow brown solid. Total yield was 12.4 mg (55%); yellow powder (methanol/ether). mp 109-110 °C. ^{1}H-NMR (CD_3OD, 600 MHz), δ 4.26 (2H, s, C*H*$_2$Ph), 4.52 (2H, s, C*H*$_2$Ph), 7.04 (2H, t, *J* = 8.5 Hz, Ar), 7.26 (1H, t, *J* = 7.5 Hz, Ar), 7.34- 7.29 (4H, m, Ar), 7.48-7.39 (5H, m, Ar), 7.61 (2H, dd, J = 8.0, 1.5 Hz, Ar), 8.36 (1H, s, C*H*-5) ppm. ^{13}C-NMR (CD_3OD, 150 MHz), δ 32.3, 36.4, 92.2, 95.5, 116.3 ($J_{C\text{-}F}$ = 21 Hz), 118.1, 122.8, 128.6, 128.7, 129.8, 129.9, 130.0, 130.1, 131.2, 131.5 ($J_{C\text{-}F}$ = 7.4 Hz), 132.9, 134.8, 136.6, 146.6, 159.5, 163.2 ($J_{C\text{-}F}$ = 241 Hz), 163.7 ppm. FAB-MS (NBA) *m/z* 434 (MH^+). HRMS (FAB/NBA) calcd for $C_{28}H_{21}N_3OF$ 434.1669, found 434.1661 (MH^+).

8-Benzyl-6-phenylethynyl-2-(*p*-fluorobenzylidene)-2,3-dihydroimidazo[1,2-a]pyrazin-3-one (15).

Manganese(IV) oxide (126 mg, 1.45 mmol) was added to the solution of coelenterazine analog (**14**) (10.0 mg, 23.1 μmol) in ether (5 mL) and EtOH (1 mL) at 0 °C. The mixture was stirred at 0 °C for 2.5 h. The solution was filtered through a pad of Celite and concentrated under reduced pressure to dryness. The residue was purified by column chromatography on silica gel with 1% MeOH/CH_2Cl_2 to give 6.9 mg (70%) of DCT analog (**15**) as dark purple amorphous; purple amorphous (2-PrOH/ether). mp 139-140 °C (decomp). ^{1}H-NMR (DMSO-d_6, 600 MHz) δ 4.25 (2H, s, C*H*$_2$Ph), 7.23 (2H, d, *J* = 7.5 Hz, Ar), 7.34 (2H, t, *J* = 7.5 Hz, Ar), 7.46-7.34 (6H, m, Ar), 7.57-7.55 (3H, m, Ar), 7.98 (1H, s, C*H*PhF), 8.46 (2H, dd, *J* = 8.5, 6.0 Hz, PhF) ppm. ^{13}C-NMR (DMSO-d_6, 150 MHz) δ 38.8, 85.9, 89.2, 114.9 ($J_{C\text{-}F}$ = 21 Hz), 118.8, 120.2, 121.6, 126.7, 128.3, 128.4, 128.6, 128.8, 129.5, 131.4 ($J_{C\text{-}F}$ = 7.4 Hz), 135.8, 135.9, 136.3, 137.6, 147.4, 158.2, 163.8 ($J_{C\text{-}F}$ = 251 Hz), 165.5 ppm. FAB-MS (NBA) *m/z* 432 (MH^+). HRMS (FAB/NBA) calcd for $C_{28}H_{19}N_3OF$ 432.1512, found 432.1495 (MH^+).

ACKNOWLEDGEMENTS

The authors are grateful for the financial supports from MEXT Grant-in-Aid for Specially Promoted Research (16002007), JSPS Grant-in-Aid for Encouragement of Young Scientists (15780085), and SUNBOR Grant. The authors also specially thank to NRCT-JSPS (National Research Council of Thailand-Japan Society for Promotion of Sciences project) Core University Program.

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