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Fluorescence and Chemiluminescence Properties of Newly Developed Lophine Analogues

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ABSTRACT

*The fluorescence and chemiluminescence properties of lophine analogues, viz. 2-substituted-4,5-di(2-pyridyl)imidazole, 2-substituted-4,5-di(2-furyl)imidazole and 2-substituted- 4(or 5)-(4-dimethylaminophenyl)-5(or 4)-phenylimidazole, were examined and compared with those of lophine (2,4,5-triphenylimidazole). The fluorescence excitation and emission maxima of the derivatives were at 310–370 nm and 385–565 nm, respectively, in methanol. The compounds carrying a 2-furyl group showed strong fluorescence intensities, while those having 2-pyridyl group gave very weak intensities. The chemiluminescence intensities obtained by the flow-injection method showed that 4-[4(or 5)-(4-dimethylaminophenyl)-5(or 4)phenyl-1H-imidazol-2-yl]benzoyl chloride (**3i**) has the largest intensity; about 40% of that of lophine. 2-(4-Methoxyphenyl)-4,5-di(2-furyl)imidazole (**2b**) and 2-(4-methylphenyl)-4,5-di(2-furyl)imidazole (**2d**) also gave stronger chemiluminescence intensities. Compounds having 2-pyridyl group showed very weak chemiluminescence intensities which were 0.1–0.3% of that of lophine. Chemiluminescence spectra of some compounds which showed relatively large intensities were measured and the wavelengths of the emission maxima were found to be 530–540 nm.* © *1998 Elsevier Science Ltd. All rights reserved*

Keywords: Lophine derivatives, lopine analogues, fluorescence, chemiluminescence, spectra.

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INTRODUCTION

Lophine, 2,4,5-triphenylimidazole, is a well-known potential chemiluminescent (CL) compound [1]. It has been used for analysis of some metal ions [2–4] and chlorinated compounds [5]. However, analytical applications of the compound are quite rare, but several other lophine derivatives have been reported [6]. Lophine derivatives also known to be highly fluorescent (FL), and utilized as labelling reagents for carboxylic acids [7–9]. Recently, we have developed a flow-injection method to evaluate the CL efficiency of lophine derivatives based on their CL reaction with hydroxylammonium chloride, cobalt(II) and hydrogen peroxide in an alkaline solution [10]. The FL efficiencies of fluorophores are known to affect the intensity of CL, and thus, highly efficient FL compounds as enhancers are requisite for increase the sensitivity of peroxyoxalate CL analysis [11].

In this study, we have synthesized and evaluated the FL and CL properties of a variety of lophine analogues having 2-pyridyl or 2-furyl group at both 4- and 5- positions, and 4-dimethylaminophenyl group at 4- or 5-position of 2-(4-substituted)phenyl imidazole derivatives.

EXPERIMENTAL

Lophine was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and other reagents used were of analytical grade.

FL spectra

FL spectra of lophine and its related compounds (Fig. 1) were measured with a Hitachi 650-10S FL spectrophotometer with a band width of 5 nm using 10×10 mm quartz cells. Stock solutions were prepared by dissolving the appropriate amounts of these compounds in methanol to make a 1×10^{-3} M. For the measurement of FL spectra, the stock solution was diluted with methanol, 50% aqueous methanol solution, and n-hexane, respectively, to prepare 1×10^{-6} M of each solution which contains 99% of the diluting solution.

CL intensities and spectra

CL intensities of lophines were obtained with a flow-injection method previously developed in our laboratory [6]. The flow system consisted of three Shimadzu LC-6A HPLC pumps (Tokyo, Japan), a Rheodyne 2175 injector with 20 μl sample loop (Cotati, CA, USA), a Jasco 825-CL detector (Tokyo,

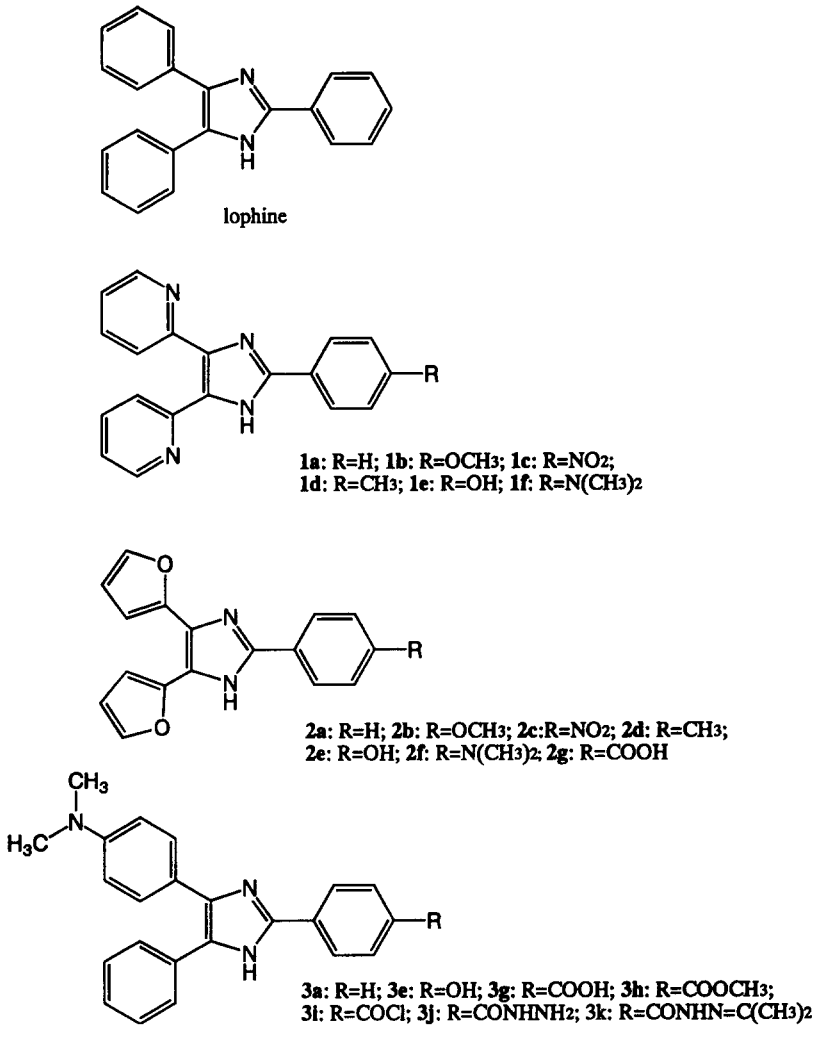

Fig. 1. Structures of lophine and its analogues.

Japan), a Union UNI-1 noise cleaner (Gunma, Japan), and a Rikadenki R101 recorder (Tokyo, Japan). The employed system is shown in Fig. 2. Calibration curves were prepared using standard solutions (1×10^{-4} $\sim1\times10^{-3}$ M) in methanol. The relative CL intensity (RCI) was estimated from the slope of each calibration curve.

CL spectra were measured with a PMA-100 luminescence detector (Hamamatsu Photonics Co. Ltd., Japan) as follows: To 0.1 ml of a mixture of 1.0×10^{-4} M cobalt (II) and 0.2% hydroxylammonium chloride in 75% aqueous methanol solution, 0.1 ml each of 100 mM hydrogen peroxide aqueous solution and sample solution in methanol were added successively,

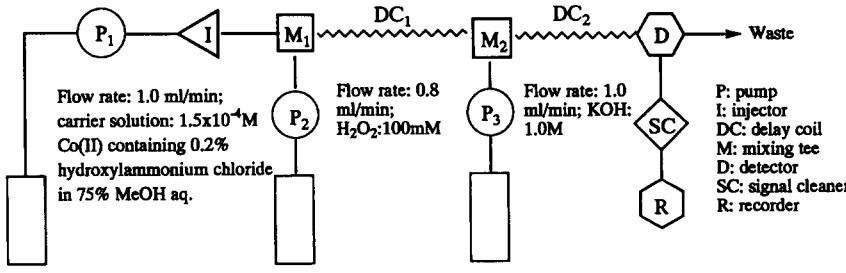

Fig. 2. Flow-injection system for the measurement of CL.

mixed for 5 s, followed by the addition of 0.1 ml of 1.0 M KOH aqueous solution. The CL produced was measured for 20 s.

Synthesis of lophine analogues

Lophine analogues were synthesized by the methods similar to those previously reported [10,12,13]. All melting points were measured on a Yanako micro melting point apparatus and are uncorrected. EI-MS spectra were measured on a JEOL JMS-DX 303 mass spectrometer.

General procedure for synthesis of CL compounds

*2-Substituted-4,5-di(2-pyridyl)imidazole (**1a**~**1f**).* To a mixture of 2,2′-pyridyl (1.0 mmol) and ammonium acetate (8.0 mmol) in 10 ml of acetic acid was added the appropriate aldehyde (1.0 mmol), and refluxed for 5 h. After cooling to room temperature, the mixture was poured into ice water. The solution was neutralized with 10% $NaHCO_3$ aq., and the resultant precipitates were filtered off, and then the filtrate was extracted with chloroform. The organic layer was dried over $MgSO_4$ and condensed to dryness. The residue was purified either by recrystallization or by column chromatography.

Compounds **2a**~**g** and **3a,e,h** were synthesized by a similar process to compounds **1**, using 2,2′-furil and 4-dimethylaminobenzil in the place of 2,2′-pyridl, respectively. Relevant data as outlined below.

*2-Phenyl-4,5-di(2-pyridyl)imidazole (**1a**).* Pale yellow crystals from ethanol; m.p. 191–193°C; yield, 43%; EI-MS (*m/z*), 298 $[M]^+$; Calcd for $C_{19}H_{14}N_4$: C,76.49; H, 4.73; N, 18.78%; Found: C, 76.44; H, 4.92; N, 18.71%.

*2-(4-Methoxyphenyl)-4,5-di(2-pyridyl)imidazole (**1b**).* Orange crystals from acetonitrile; m.p. 178–179°C; yield, 19%; EI-MS (*m/z*), 328 $[M]^+$;

Calcd for $C_{20}H_{16}N_4$: C, 73.15; H, 4.91; N, 17.06%; Found: C, 72.99; H, 5.00; N, 17.22%.

*2-(4-Nitrophenyl)-4,5-di(2-pyridyl)imidazole (**1c**).* Orange crystals from chloroform; m.p. 161–164°C; yield, 25%; EI-MS (*m/z*), 343 $[M]^+$; Calcd for $C_{19}H_{13}N_5O_2 \cdot H_2O$: C, 63.32; H, 4.20; N, 19.44%; Found: C, 63.32; H, 4.02; N, 19.50%.

*2-(4-Methylphenyl)-4,5-di(2-pyridyl)imidazole (**1d**).* Colorless crystals from acetonitrile; m.p. 188–190°C; yield, 34%; EI-MS (*m/z*), 312 $[M]^+$; Calcd for $C_{20}H_{16}N_4$: C, 76.89, H, 5.17; N, 17.94%; Found: C, 76.93, H, 5.27; N, 18.00%.

*2-(4-Hydroxyphenyl)-4,5-di(2-pyridyl)imidazole (**1e**).* Pale yellow crystals from acetonitrile; m.p. 268–271°C; yield, 23%; EI-MS (*m/z*), 314 $[M]^+$; Calcd for $C_{19}H_{14}N_4O$: C, 72.59; H, 4.49; N, 17.83%; Found: C, 72.26; H, 4.70; N, 18.02%.

*2-(4-Dimethylaminophenyl)-4,5-di(2-pyridyl)imidazole (**1f**).* Pale yellow crystals from acetonitrile; m.p. 226–230°C; yield, 13%; EI-MS (*m/z*), 341$[M]^+$; Calcd for $C_{21}H_{19}N_5$: C, 73.87; H, 5.61; N, 20.52%; Found: C, 73.98; H, 5.70; N, 20.58%.

*2-Phenyl-4,5-di(2-furyl)imidazole (**2a**).* Colorless crystals from acetonitrile; m.p. 200–203°C; yield, 17%; EI-MS (*m/z*), 276 $[M]^+$; Calcd for $C_{17}H_{12}N_2O_2$: C, 73.89; H, 4.39; N, 10.14%; Found: C, 73.71; H, 4.51; N, 10.16%.

*2-(4-Methoxyphenyl)-4,5-di(2-furyl)imidazole (**2b**).* Pale greenish yellow crystals from acetonitrile; m.p. 203–207°C; yield, 44%; EI-MS (*m/z*), 306 $[M]^+$; Calcd for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.61; N, 9.15%; Found: C, 70.59; H, 4.74; N, 9.29%.

*2-(4-Nitrophenyl)-4,5-di-(2-furyl)imidazole (**2c**).* Reddish-brown crystals from chloroform; m.p. 233–237°C; yield, 3%; EI-MS (*m/z*), 321 $[M]^+$; Calcd for $C_{17}H_{11}N_3O_4$: C, 63.55; H, 3.45; N, 13.08%; Found: C, 63.43; H, 3.56; N, 12.95%.

*2-(4-Methylphenyl)-4,5-di(2-furyl)imidazole (**2d**).* Colorless crystals from acetonitrile; m.p. 217–223°C; yield, 20%; EI-MS (*m/z*), 290 $[M]^+$; Calcd for $C_{18}H_{14}N_2O_2$: C, 74.46; H, 4.86; N, 9.65%; Found: C, 74.42; H, 4.96; N, 9.68%.

*2-(4-Hydroxyphenyl)-4,5-di(2-furyl)imidazole (**2e**).* Gray crystals from acetonitrile; m.p. 263–267°C; yield, 13%; EI-MS (*m/z*), 292 $[M]^+$; Calcd for $C_{17}H_{12}N_2O_5$: C, 69.85; H, 4.14; N, 9.57%; Found: C, 69.41; H, 4.23; N, 9.67%.

*2-(4-Dimethylaminophenyl)-4,5-di(2-furyl)imidazole (**2f**).* Gray crystals acetonitrile; m.p. 243–250°C; yield, 53%; EI-MS (*m/z*), 319 $[M]^+$; Calcd for $C_{19}H_{17}N_3O_2$: C, 71.45; H, 5.37; N, 13.16%; Found: C, 71.39; H, 5.44; N, 13.25%.

*4-[4,5-Di(2-furyl)-1H-imidazol-2-yl]benzoic acid (**2g**).* Yellow crystals from acetonitrile; m.p. >360°C; yield, 3%; EI-MS (*m/z*), 320 $[M]^+$; Calcd for $C_{18}H_{12}N_2O_4$: C, 66.74; H, 3.79; N, 8.65%; Found: C, 66.79; H, 3.88; N, 8.65%.

*4(or 5)-(4-Dimethylaminophenyl)-2,5(or 4)-diphenylimidazole (**3a**).* Pale yellow crystals from acetonitrile; m.p. 234–237°C; yield, 12%; EI-MS (*m/z*), 339 $[M]^+$; Calcd for $C_{23}H_{21}N_3$: C, 80.53; H, 6.29; N, 12.25%; Found: C, 80.96; H, 6.41; N, 11.80%.

*2-(4-Hydroxyphenyl)-4(or 5)-(4-dimethylaminophenyl)-5(or 4)-phenylimidazole (**3e**).* Colorless crystals from acetone-acetonitrile; m.p. 171–174°C; yield, 8%; EI-MS (*m/z*), 355 $[M]^+$; Calcd for $C_{23}H_{21}N_3O$: C, 75.05; H, 6.13; N, 11.42%; Found: C, 74.94; H, 6.02; N, 11.33%.

*4-[4(or 5)-(4-Dimethylaminophenyl)-5(or 4)-phenyl-1H-imidazol-2-yl]benzoic acid (**3g**).* Compound ***3h*** (1.0 g, 2.5 mmol) was added to 50 ml of 2M KOH, and the mixture stirred for 2 h at room temperature. The mixture was acidified with 1M HCl to give a precipitate which was recrystallized from acetonitrile to give yellow crystals; m.p. 206–209°C; yield, 82%; EI-MS (*m/z*), 383 $[M]^+$; Calcd for $C_{24}H_{21}N_3O_2$: C, 72.12; H, 5.75; N,10.52%; Found: C, 72.31; H, 5.46; N, 10.55%.

*2-Methoxycarbonylphenyl-4(or 5)-(4-dimethylaminophenyl)-5(or 4)-phenylimidazole (**3h**).* Pale yellow crystals from acetone; m.p. 156–158°C; yield, 55%; EI-MS (*m/z*), 397 $[M]^+$; Calcd for $C_{25}H_{23}N_3O_2$: C, 75.54; H, 5.83; N, 10.58%; Found: C, 75.16; H, 5.90; N, 10.55%.

*4-[4(or 5)-(4-Dimethylaminophenyl)-5(or 4)-phenyl-1H-imidazol-2-yl]benzoyl chloride (**3i**).* Compound **3g** (0.75 g, 1.9 mmol) was added to 12 ml of thionyl chloride and refluxed for 2 h. After removing excess thionyl chloride under reduced pressure, the residue was suspended in benzene and filtered.

The resultant yellowish-brown crystals were washed repeatedly with benzene, and used as a reagent in further synthesis without further purification; m.p. > 360°C; crude yield 0.7 g; EI-MS (*m/z*), 366 $[M-Cl]^+$; Calcd for $C_{24}H_{20}N_3OCl$: C, 71.72; H, 5.02; N, 10.46; Cl; 8.82%; Found: C, 69.34; H, 5.61; N, 10.35; Cl, 8.82%.

*2-(4-Hydrazinocarbonylphenyl)-4(or 5)-(4-dimethylaminophenyl)-5(or 4)-phenylimidazole (**3j**).* To the solution of 3 h (0.3 g, 0.75 mmol) in 30 ml of ethanol was added 2.7 ml of hydrazine hydrate and the mixture refluxed for 7 h. The mixture was poured into water and acidified with 1M HCl to give a precipitate which was collected and recrystallized from acetonitrile to give yellowish-green crystals; m.p. 231–235°C; yield, 43%; EI-MS (*m/z*), 397 $[M]^+$; Calcd for $C_{24}H_{23}N_5O$: C, 71.87; H, 5.88; N, 17.47%; Found: C, 71.91; H, 5.85; N, 17.51%.

*Acetone hydrazone of **3j** (**3k**).* Compound **3j** was refluxed in acetone to give (quantitatively) orange crystals; m.p. 262–265°C; EI-MS (*m/z*), 437$[M]^+$; Calcd for $C_{27}H_{27}N_5O$: C, 74.12; H, 6.22; N, 16.01%; Found: C, 74.05; H, 6.29; N, 15.96%.

RESULTS AND DISCUSSION

The FL spectra and intensities of the lophine analogues were measured in methanol, 50% aq. methanol and n-hexane as representative solvents. The FL excitation (Ex) and emission (Em) maxima and intensities are listed in Table 1. The Ex and Em were in the range of 310–370 and 385–565 nm in methanol, 310–370 and 385–490 nm in 50% aq. methanol, and 315–375 and 380–490 nm in n-hexane. The Ex wavelengths were red-shifted in the non-polar solvent, viz. n-hexane, while the Em wavelengths blue-shifted to shorten the Stokes' Shift compared to those in methanol. FL intensities of the furyl derivatives were larger than that of lophine in all solvents, except for **2g** in methanol. Compounds **2a–g** carrying a 2-furyl group showed strong fluorescence intensities, while those having a 2-pyridyl group gave very weak intensities.

As a result, compounds **2**, except for **2g**, could be used as fluorescent enhancers in peroxyoxalate chemiluminescence reaction or candidates as mother compounds for preparing fluorescent labelling reagents.

The CL intensities obtained by the flow-injection method are shown in Fig. 3. Among the newly developed CL compounds, **3i** showed the largest intensity, viz. about 40% of that of lophine. Compounds **2b** and **2d** also gave relatively strong CL intensities. However, compounds having the 2-pyridyl

TABLE 1
Fluorescence Spectra Data for Lophine and its Analogues

Compound	*MeOH*			*50%MeOH aq.*			*n-Hexane*		
	Wavelength (nm)								
	λex	*λem*	*RFI*[a]	*λex*	*λem*	*RFI*	*λex*	*λem*	*RFI*
Lophine	310	385	100.0	310	385	118.9	315	385	103.7
1a	315	400	2.4	310	400	1.8	345	485	2.9
1b	315	415	1.6	325	410	0.7	345	395	2.7
1d	315	400	2.3	310	405	1.3	345	390	3.2
1f	370	480	0.1	370	490	0.1	375	415	3.1
2a	320	410	170.3	320	420	177.3	325	385	167.1
2b	320	395	177.4	320	410	198.2	325	385	182.3
2d	320	400	175.4	320	415	182.9	325	385	175.9
2e	320	390	163.2	320	405	188.5	320	380	147.0
2f	330	400	284.5	335	415	234.4	335	395	244.6
2g	330	465	88.9	330	475	102.0	365	455	119.3
3a	325	450	84.2	320	470	7.3	325	410	121.7
3e	320	425	115.2	320	445	5.2	320	410	62.3
3g	340	500	25.4	320	425	0.2	365	490	50.2
3h	360	560	0.4	335	460	0.7	370	485	92.7
3i	327	460	16.4	320	465	10.5	330	415	32.5
3j	345	535	0.5	335	460	0.1	350	485	73.7
3k	360	565	0.4	325	450	0.3	335	470	27.4

[a]Relative fluorescence intensities: fluorescence intensity of lophine in MeOH (1 μM) was taken as 100.

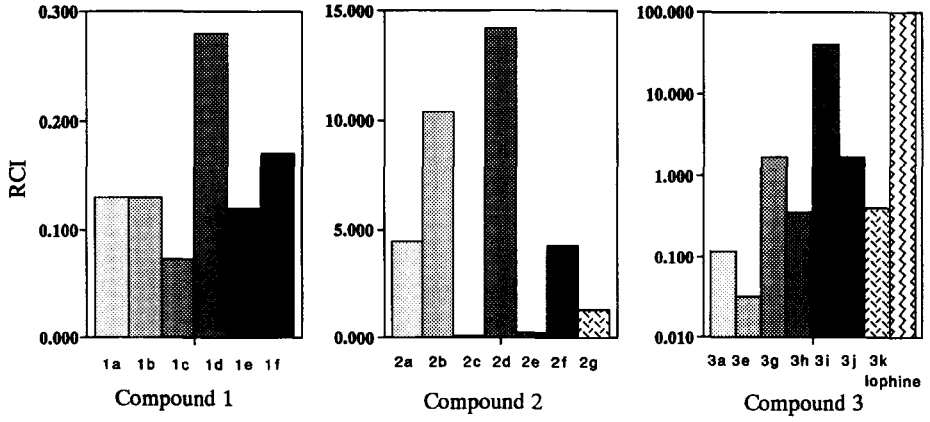

Fig. 3. Comparison of RCI of lophine and its analogues.

group showed very weak CL, i.e. 0.07–0.28% of that of lophine. These results might be based on the low fluorescence quantum yields of the oxidatively decomposed FL compounds from CL compounds, e.g. diaroylamidine derivatives.

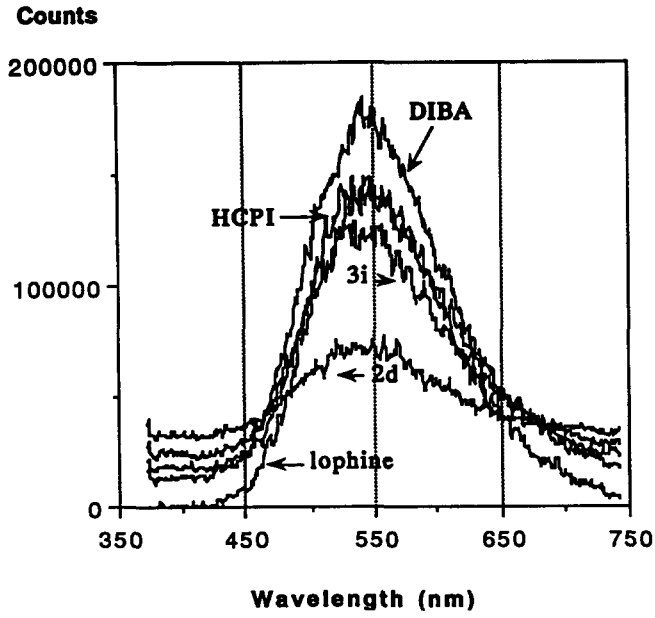

Fig. 4. CL spectra of some lophine analogues. Sample concentration (M): lophine (1 mM); DIBA (1 mM); HCPI (1 mM); **3i** (2 mM); **2d** (5 mM). Other conditions are as described in the Experimental section.

The CL spectra of some compounds which have shown relatively strong CL intensities were measured, i.e. lophine, 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoic acid (DIBA) [10], 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole (HCPI) [10] **2d** and **3i**. As shown in Fig. 4, the wavelengths of the emission maxima were found to be 530–540 nm, and thus there was no remarkable shift in emission wavelength due to the differences of substituents on the imidazole ring.

In conclusion, among the lophine analogues synthesized, compounds **2a–f** showed intense FL. These compounds can thus be considered as potential candidates as FL enhancers in peroxyoxalate CL or as mother compounds for preparing FL labelling reagents.

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