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ARTICLE

6-Arylcoumarins: Versatile Scaffolds for Fluorescent Sensors

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Fluorescent sensors are used in many fields of scientific research. Here, we proposed that 6-arylcoumarins could be scaffolds for various types of fluorescent sensor. From our newly synthesized 6-arylcoumarins, 6-(*p*-cyanophenyl)-7-hydroxycoumarin was found as a ratiometric viscosity sensor, that is, it exhibits two fluorescent peaks in low-viscosity solution, but only one in high-viscosity solution. In addition, 6-arylcoumarins bearing a 7-methoxy or 7-hydroxyl group exhibit large shifts of fluorescence maximum wavelength that are dependent upon the 6-aryl substituents. So we considered that these compounds would be good candidates as scaffolds for fluorescent sensors that would utilize the shift of fluorescence maximum wavelength for analyte detection. To validate this idea, we designed, synthesized and characterized two sodium ion sensors, in which a 15-crown-5 ether moiety is attached to the 6-phenyl group of these scaffolds as the recognition site; these sensors exhibit a blue shift of the fluorescence maximum wavelength in the presence of sodium ion. Our results suggest that 6-arylcoumarins are available as versatile scaffolds for various types of fluorescent sensors.

Introduction

Fluorescent sensors, whose fluorescence properties are altered by a specific analyte, have been widely utilized in various fields of scientific research.[1] For development of novel sensors, knowledge of the structure-fluorescence relationship of the scaffold is important. For example, in the case of fluorescein, derivatives with an electron-donating group (such as an amino group) attached to the benzoic acid moiety are weakly fluorescent, whereas those with a non-electron-donating group (such as an amido group) are strongly fluorescent.[2] Thus, substituents can be used to regulate the fluorescence intensity with little change of the fluorescence maximum wavelength, and this has enabled development of a range of sensors for various analytes.[3] Our group has synthesized various 6-aryl-7-diethylaminocoumarins (**1**) (Figure 1) and 6-aryl-3-triazoylarylcoumarins, and based on a study of their fluorescence properties, we have developed a number of sensors, including nitric oxide sensors and multi-analyte sensor candidates, whose modes of fluorescence change vary depending upon the analyte.[4] In addition, a wide diversity of the structure and fluorescent property of fluorescent compounds could be utilized to develop a novel sensor, especially that with unpredictable function. Such a diversity-oriented fluorescence library approach (DOLFA)[5] has been applied to various dyes, including styryl dyes,[6] benzimidazole dyes[7] and xanthene dyes,[8] and has yielded fluorescent sensors for various ions, proteins, organ or specific types of cells.[9] As this approach, we also constructed a library of coumarin derivatives, and discovered fluorescent bioactive compound, *i.e.,* a progesterone receptor (PR) ligand,[10] whose fluorescence intensity was increased upon binding to PR, and fluorescent sensor that responds to specific range of pH.[11] In the present work, we set out to further expand the library of coumarin derivatives, focusing on 7-substituted 6-arylcoumarins, with the aim of discovering suitable scaffolds for novel fluorescent sensors.

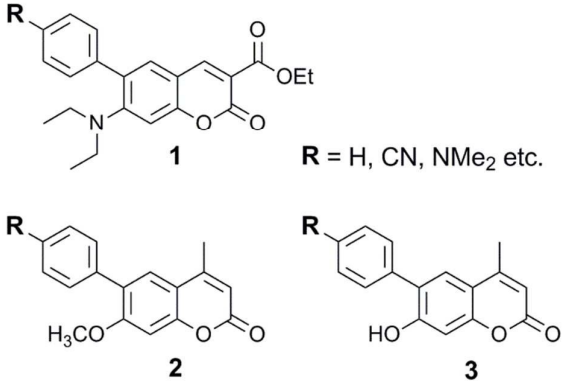

Figure 1. Structures of 6-arylcoumarins.

RESULTS AND DISCUSSION

Synthesis of 6-aryl-7-methoxy- and 6-aryl-7-hydroxycoumarins

Our group previously reported the synthesis of various 6-aryl-7-diethylaminocoumarin derivatives (**1**) via Suzuki-Miyaura coupling between 6-bromocoumarin and various arylboronic acid derivatives. The fluorescence intensity of

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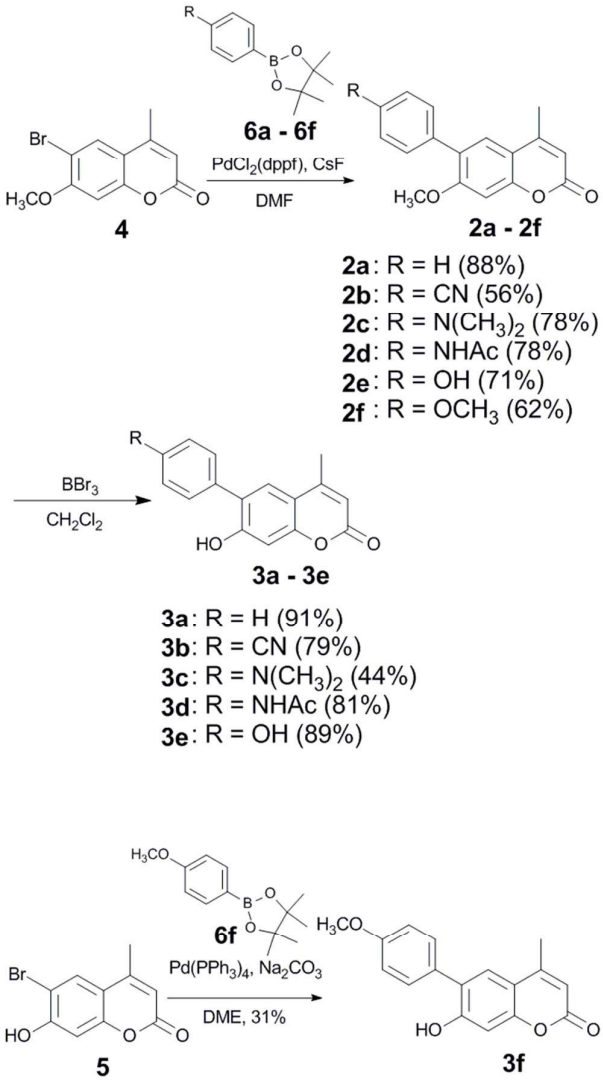
these compounds is greatly influenced by the substituent group (**R**) on the 6-aryl ring, although the shift of the fluorescence maximum wavelength is relatively small (less than 20 nm).[4a] On the other hand, the 7-substituent can influence both the fluorescence intensity and the fluorescence maximum wavelength via photophysical processes such as intermolecular charge transfer (ICT) and twisted intermolecular charge transfer (TICT).[1a,12] Because 6-aryl ring and 7-positions could interact sterically, we were interested in further investigating the fluorescence properties of 7-substituted 6-arylcoumarins. In this work, we synthesized a series of 6-aryl-7-methoxycoumarins (**2**) and 6-aryl-7-hydroxycoumarins (**3**), as shown in Scheme 1. Compounds **2a** - **2f** were derived from 6-bromo-7-methoxycoumarin (**4**) and arylboronic acid derivatives **6a - 6f**. Demethylation of **2a** – **2e** yielded **3a** - **3e**, and **3f** was synthesized from 6-bromo-7-hydroxycoumarin (**5**) and **6f**.



Scheme 1. Synthesis of 6-aryl-7-methoxy- and 6-aryl-7-hydroxycoumarins **2** and **3**.

Fluorescence properties of 6-aryl-7-methoxy- and 6-aryl-7-hydroxycoumarins

The fluorescence properties of **2a - 2f** and **3a - 3f** were studied in acetonitrile and methanol (Table 1), and typical absorption and fluorescence spectra are shown in Figure S1 and Figure S2. In 6-aryl-7-methoxycoumarins (**2**), the effect of substituents at the 6-aryl group on the absorption maximum wavelength was relatively small, whereas the effect on the fluorescence maximum wavelength was large, up to 76 nm (**2b** vs **2d** in methanol). 6-Aryl-7-hydroxycoumarins (**3**) showed similar behavior in acetonitrile, and the range of fluorescent maximum wavelength was up to 57 nm (**3b** vs **3e**). Interestingly, **3b** showed two fluorescence maxima at 390 and 498 nm in methanol. Dimethylamino-substituted compounds **2c** and **3c** showed little fluorescence in either of these solvents. Such weak fluorescence of compounds bearing an electron-donating group was also observed among our previously reported 6-aryl-7-diethylaminocoumarin derivatives (**1**), as well as in other fluorophores such as xanthene derivatives,[13] and is presumably due to a photo-induced electron transfer (PeT) process.

Compared with our previously reported 6-aryl-7-diethylaminocoumarins (**1**), in which the change of fluorescence maximum was less than 20 nm, 6-arylcoumarins with 7-methoxy or 7-hydroxy group (**2**, **3**) showed a large dependence of the fluorescence maximum wavelength on the substituent groups in the 6-aryl ring. Therefore, these compounds might be useful as scaffolds of fluorescent sensors that would utilize the shift of fluorescence maximum wavelength for analyte detection.

Table 1. Photophysical properties of **2** and **3**.

	in acetonitrile			in methanol		
Comp. (R)	Abs_{max} (nm)	Em_{max} (nm)	Q.Y.[c]	Abs_{max} (nm)	Em_{max} (nm)	Q.Y.[c]
2a (H)	328	399	0.028	330	403	0.15
2b (CN)	328	381	0.029	330	385	0.18
2c (NMe_2)	-[a]	n.d.[b]	n.d.[b]	-[a]	n.d.[b]	n.d.[b]
2d (NHAc)	331	436	0.066	334	461	0.13
2e (OH)	332	441	0.047	335	452	<0.01
2f (OMe)	331	435	0.13	334	454	0.16
3a (H)	329	395	0.031	334	407	0.24
3b (CN)	330	384	0.043	334	390, 498	0.27
3c (NMe_2)	342	n.d.[b]	n.d.[b]	345	n.d.[b]	n.d.[b]
3d (NHAc)	331	436	0.12	336	457	0.14
3e (OH)	332	441	0.11	339	469	<0.01
3f (OMe)	332	433	0.048	337	452	0.34

[a]Not determined. [b]No fluorescence peak was detected. [c]Quantum yields of fluorescence were determined using that of quinine sulfate in 0.1 M H_2SO_4 (0.577) as a standard.[1a]

Development of sodium ion sensors utilizing the shift of fluorescence maximum wavelength

To confirm the suitability of 7-methoxy or 7-hydroxy-6-arylcoumarins as scaffolds for fluorescent sensors we utilized them to design candidate sensors for sodium ion, **2g** and **3g** (Figure 2), in which a 15-crown-5 ether moiety is attached to the 6-phenyl group as the recognition site.

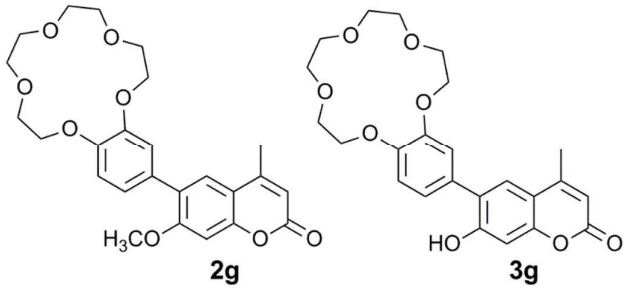
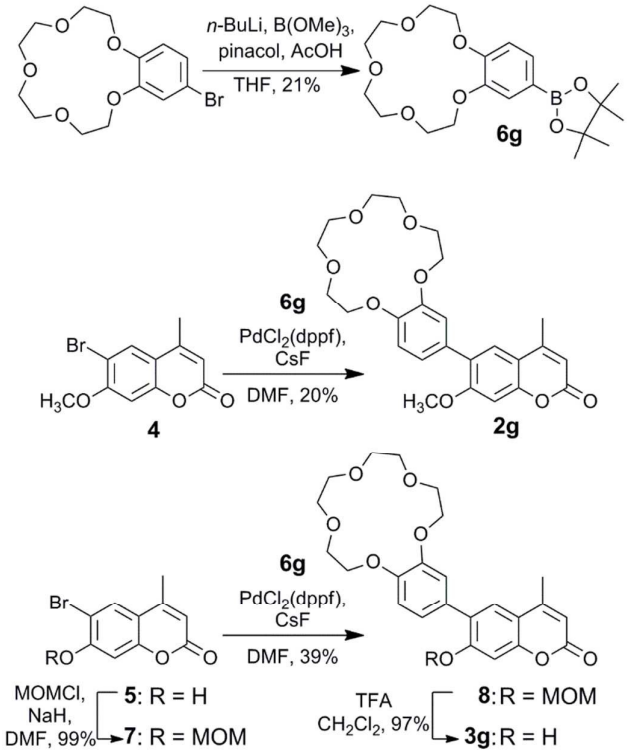


Figure 2. Structure of sodium ion sensors.

These compounds were synthesized as shown in Scheme 2. An arylboronic acid derivative bearing 15-crown-5 ether (**6g**) was prepared from 4-bromobenzo-15-crown 5-ether, and coupled with 6-bromo-7-methoxycoumarin (**4**) to afford compound **2g**. On the other hand, coupling between 6-bromo-7-hydroxylcoumarin (**5**) and **6g** did not proceed. Thus, 6-bromo-7-methoxymethoxycoumarin (**7**) was prepared from **5**, then coupled with **6g** to obtain **8**, which was deprotected to afford compound **3g**.



Scheme 2. Synthesis of sodium ion sensor candidates **2g** and **3g**.

The absorption (Figure S3) and fluorescence spectra (Figure 3) of **2g** and **3g** were examined. For both compounds, the absorption spectra showed almost no change upon addition of sodium ion. On the other hand, the fluorescence maximum was shifted from 466 nm to 436 nm for **2g** and from 467 nm to 433 nm for **3g**. These data indicate that **2g** and **3g** could be utilized as sodium ion sensors, utilizing the shift of fluorescence maximum in the presence of sodium ion.

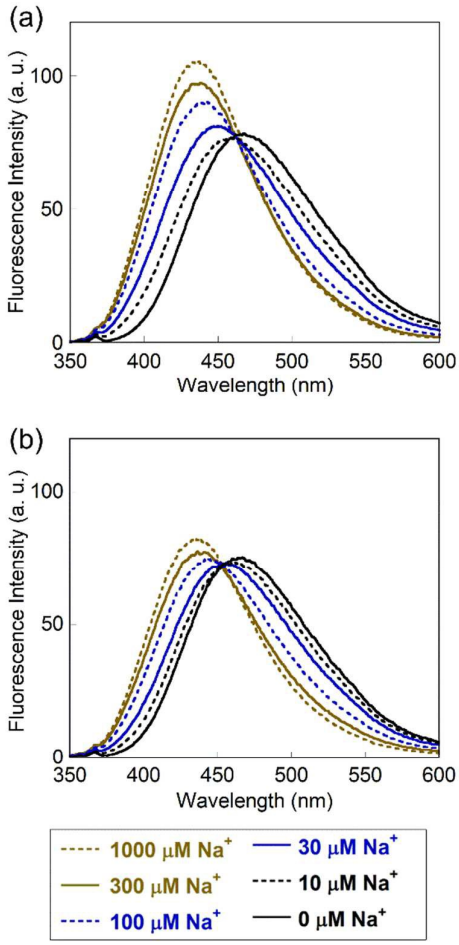


Figure 3. Fluorescence spectra of sodium ion sensors. Fluorescent spectra excited at 330 nm of (a) **2g** and (b) **3g** with sodium perchlorate (0 μM ~ 1000 μM) were measured in acetonitrile (0.3% DMSO as a cosolvent).

Development of viscosity sensor utilizing viscosity-sensitive fluorescence change

Variations in the viscosity in biological systems are related to physiological functions at both microscopic and macroscopic levels.[14] At the cellular level, membrane and cytoplasmic viscosity can influence signaling pathways via modulation of the activity or intermolecular interaction of biomolecules.[15] In the level of the organism, changes of viscosity of blood, plasma or lymphatic fluid occur in cardiovascular diseases, diabetes and aging.[16] Therefore, monitoring viscosity with high spatial and temporal resolution is expected to be useful in cellular biology studies and for clinical diagnosis, and several fluorescent viscosity sensors such as 9-(dicyanovinyl)julolidine (DCVJ) have been developed.[17] To see whether our compounds **2** and **3** would also be applicable for this purpose, we examined their fluorescence properties in various mixtures of glycerol and ethylene glycol (0 : 100, 30 : 70, 60 : 40), which would exhibit marked changes of viscosity (Figure 4).

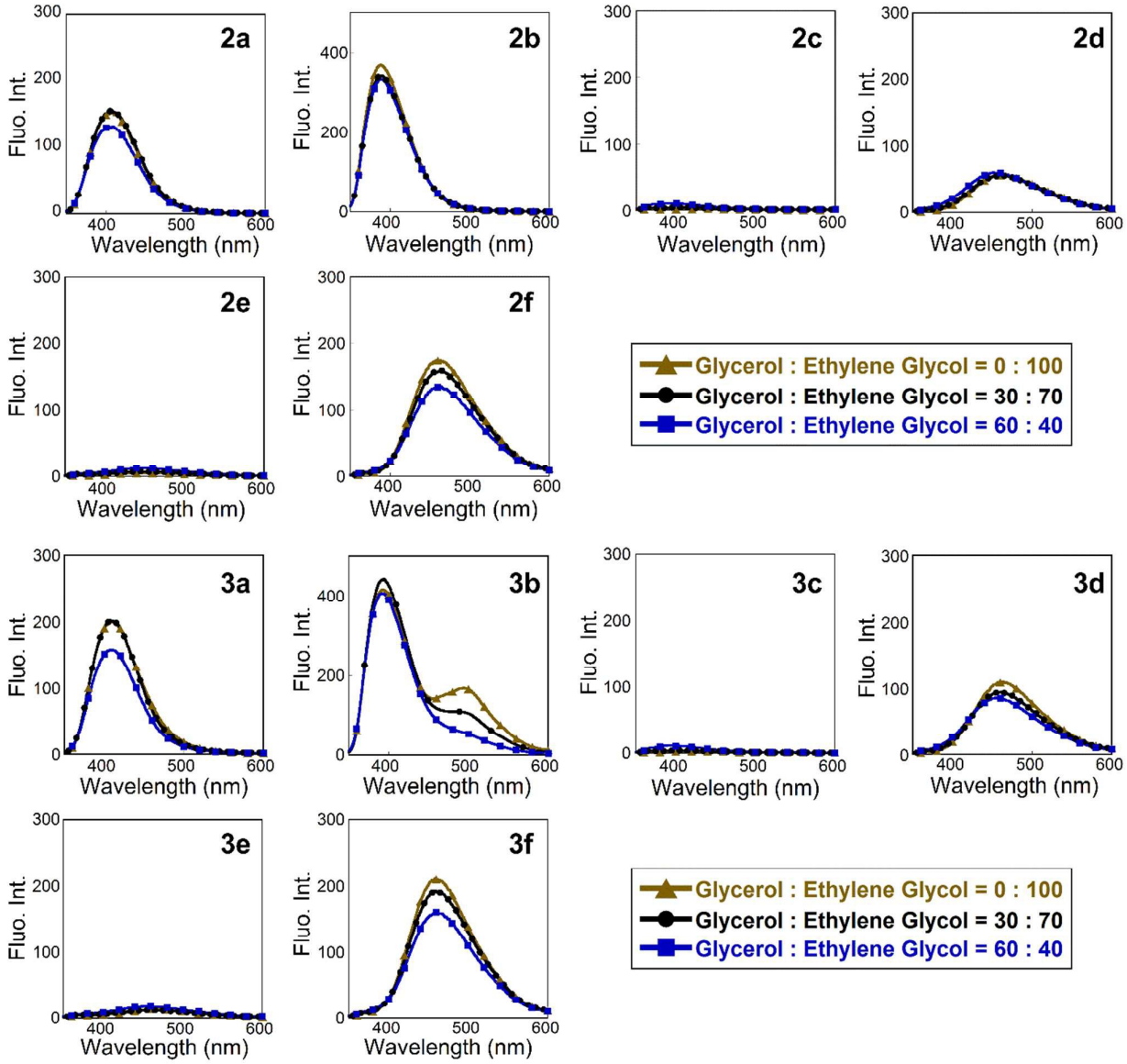


Figure 4. Fluorescence spectra of compounds **2** and **3** in mixtures of glycerol and ethylene glycol.

Among these compounds, **3b** showed two fluorescent peaks at 395 nm and 492 nm in low-viscosity solution (ethylene glycol : glycerol = 0 : 100), but only one peak at 395 nm in high-viscosity solution (ethylene glycol : glycerol = 60 : 40). The related compound **2b**, which has a 7-methoxy group instead of the 7-hydroxyl group of **3b**, did not show this feature. Compounds with a dimethylamino- or hydroxyl group in the 6-aryl ring (**2c**, **2e**, **3c**, **3e**) showed weak fluorescence, whereas **2a**, **2d**, **2f**, **3a**, **3d** and **3f** showed only small fluorescence changes in response to viscosity change.

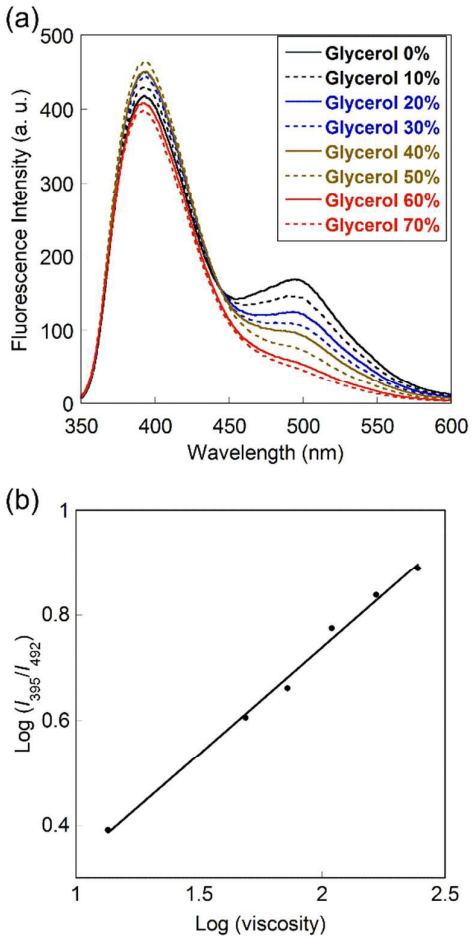


Figure 5. Fluorescence properties of compound **3b**. (a) Fluorescence spectra excited at 330 nm of **3b** (5 μM) in mixtures of ethylene glycol and glycerol (0.3% DMSO as a cosolvent). (b) Regression line between log(I_{395}/I_{492}) and log(viscosity) (R^2 = 0.994, x = 0.406).

Ratiometric measurement of fluorescence intensities at two wavelengths is a useful technique to minimize assay variability due to factors such as differences of sensor concentration and photobleaching. However, only a few ratiometric fluorescent sensors for viscosity change have been reported.[18] Since our compound **3b** showed enhancement of fluorescence intensity at 492 nm with little change at 395 nm in response to a decrease of viscosity, it may be a good candidate as a ratiometric sensor. Therefore, we examined the spectra of **3b** in mixtures of glycerol / ethylene glycol with 10% increments of ethylene glycol (0% ~ 70%). The absorption spectra (Figure S4) showed almost no change, while the fluorescence spectra (Figure 5) gradually changed as expected from the preliminary study. The logarithmic value of the ratio of fluorescence intensities at 395 nm and 492 nm (log(I_{395}/I_{492})) showed a good linear relationship with log(viscosity) (R^2 = 0.994), in accordance with the Förster–Hoffmann equation,[19] logI_f = C + $x$logη. In this equation, I_f is the fluorescence intensity, C is a constant depending on the measurement conditions, such as concentration and temperature, x is the sensitivity of the dye response to viscosity change, and η is the viscosity. Compared with previously reported viscosity sensors such as DCVJ (x = 0.542),[20] the value of x (0.406) is sufficiently large, suggesting that compound **3b** could be practically useful as a fluorescent sensor for ratiometric measurement of solvent viscosity. The 7-methoxy compound (**2b**), did not show viscosity-dependent fluorescence change (Figure 4), so the viscosity sensitivity appears to depend upon the nature of the 7-substituent. If this is so, it should be possible to develop a range of related viscosity sensors, whose function could be activated only in specific cells or subcellular compartments like recently reported lysosomal viscosity sensor.[21] In the case of **3b**, a little enhancement of fluorescence at basic conditions was observed in aqueous solution, however, the intensity was relatively weak compared with that in mixtures of ethylene glycol and glycerol (data not shown). The elucidation of the mechanism of fluorescent change and the work for the development of novel functional sensor are in progress.

Conclusions

We synthesized 6-arylcoumarins bearing a 7-methoxy or 7-hydroxyl group, and found that they exhibit large shifts of fluorescence maximum wavelength that are dependent upon the nature of the 6-aryl substituent. We considered that these compounds would be good candidates as scaffolds for fluorescent sensors. To validate this idea, we designed, synthesized and characterized two sodium ion sensors bearing a 15-crown-5-ether recognition site in the aryl group and confirmed that they exhibit a blue shift of the fluorescence maximum in the presence of sodium ion. In addition, we found that 6-(*p*-cyanophenyl)-7-hydroxycoumarin **3b** exhibits two fluorescent peaks in low-viscosity solution, but only one in high-viscosity solution, and we show that this compound can be used as a ratiometric solvent viscosity sensor. Our results indicate that 7-methoxy and 7-hydroxy- 6-arylcoumarin, as well as our previously reported 7-diethylamino derivatives, are promising scaffolds for design of various types of fluorescent sensors.

EXPERIMENTAL SECTION

General: All reagents were purchased from Sigma-Aldrich Chemical, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku. Silica gel for column chromatography was purchased from Kanto Kagaku. Preparative GPC (gel permeation chromatography) was carried out using an LC-9201 instrument (Japan Analytical Industry). NMR spectra were recorded on Bruker AVANCE 400 or Bruker Advance 500 spectrometer. Mass spectral data was obtained on Brucker Daltonics microTOF-2focus in the positive and negative ion detection modes. Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. UV

spectra were recorded with JASCO V-550, and fluorescence spectra were recorded with JASCO FP-6600.

Synthesis

General procedure for preparation of 6-aryl-7-methoxycoumarins (2) (2a as an example)

A solution of 6-bromo-7-methoxy-4-methylcoumarin (**4**) (99.5 mg, 0.370 mmol), **6a** (218 mg, 1.07 mmol), cesium fluoride (284 mg, 1.87 mmol) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (60.3 mg, 0.0738 mmol) in dry *N,N*-dimethylformamide (7.0 ml) was stirred for 3 h at 60°C under an atmosphere of argon. Then, the reaction mixture was cooled to room temperature and saturated aqueous ammonium chloride was added. The mixture was extracted with dichloromethane. The organic solution was washed with brine and concentrated. Purification of the residue by column chromatography (silica gel) gave **2a** (86.5 mg, 88%) as yellow crystals. A small amount of the compound was recrystallized to give an analytical sample. Mp 213.1-215.9°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51-7.36 (m, 6H), 6.91 (s, 1H), 6.18 (d, *J* = 1.1 Hz, 1H), 3.88 (s, 3H), 2.42 (d, *J* = 1.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.2, 159.6, 154.7, 152.6, 137.1, 129.5, 128.2, 128.0, 127.5, 126.3, 113.4, 112.2, 99.4, 56.1, 18.7; Anal. Calcd for $C_{17}H_{14}O_3\cdot 1/5\ H_2O$: C, 75.65; H, 5.38. Found: C, 75.77; H, 5.39; HRMS (ESI) calcd for $C_{17}H_{14}O_3$ $(M+H)^+$ 267.1016. Found 267.1021.

Other compounds (**2b – 2f**) were prepared from **4** and the corresponding arylboronic acids (**6b – 6f**). Analytical data are given below.

2b: Mp 287.1-289.5°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 8.2 Hz, 2H), 7.46 (s, 1H), 6.93 (s, 1H), 6.20 (s, 1H), 3.90 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.0, 159.6, 155.6, 152.4, 142.1, 132.2, 130.5, 126.5, 126.2, 119.1, 113.9, 112.9, 111.4, 100.0, 56.4, 18.9; Anal. Calcd for $C_{18}H_{13}NO_3\cdot 1/4\ H_2O$: C, 73.09; H, 4.60; N, 4.74. Found: C, 73.22; H, 4.65; N, 4.76; HRMS (ESI) calcd for $C_{18}H_{13}NO_3$ $(M+H)^+$ 292.0968. Found 292.0964.

2c: Mp 278.5-283.7°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (s, 1H), 7.40 (dd, *J* = 6.8 Hz, 2.1 Hz, 2H), 6.88 (s, 1H), 6.80 (dd, *J* = 6.8 Hz, 2.1 Hz, 2H), 6.16 (d, *J* = 1.0 Hz, 1H), 3.88 (s, 3H), 3.00 (s, 6H), 2.41 (d, *J* = 1.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.7, 160.0, 154.3, 153.0, 150.2, 130.4, 128.4, 125.9, 125.0, 113.6, 112.4, 112.2, 99.5, 56.3, 40.8, 19.0; Anal. Calcd for $C_{19}H_{19}NO_3$: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.67; H, 6.20; N, 4.81; HRMS (ESI) calcd for $C_{19}H_{19}NO_3$ $(M+H)^+$ 310.1438. Found 310.1442.

2d: Mp 279.9-284.0°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.99 (s, 1H), 7.61 (d, *J* = 8.7 Hz, 2H), 7.57 (s, 1H), 7.44 (d, *J* = 8.6 Hz, 2H), 7.13 (s, 1H), 6.22 (d, *J* = 1.1 Hz, 1H), 3.85 (s, 3H), 2.43 (d, *J* = 1.1 Hz, 3H) , 2.05 (s, 3H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.8, 160.6, 159.8, 154.5, 154.1, 139.0, 131.8, 130.2, 127.1, 126.8, 119.1, 113.4, 111.8, 100.1, 56.8, 24.5, 18.6; Anal. Calcd for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.38. Found: C, 70.35; H, 5.38; N, 4.35; HRMS (ESI) calcd for $C_{19}H_{17}NO_4$ $(M+H)^+$ 324.1230. Found 324.1227.

2e: Mp 271.0-274.8°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.38 (dd, *J* = 6.5, 2.1 Hz, 2H), 6.91 (dd, *J* = 6.6, 2.2 Hz, 2H), 6.90 (s, 1H), 6.17 (d, *J* = 1.2 Hz, 1H), 4.82 (s, 1H), 3.88 (s, 3H), 2.41 (d, *J* = 1.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.3, 159.6, 155.1, 154.5, 152.6, 130.9, 129.6, 127.5, 126.0, 115.2, 113.4, 112.2, 99.4, 56.1, 18.7; Anal. Calcd for $C_{17}H_{14}O_4\cdot 1/3\ H_2O$: C, 70.82; H, 5.13. Found: C, 70.90; H, 5.08; HRMS (ESI) calcd for $C_{17}H_{14}O_4$ $(M+H)^+$ 283.0965. Found 283.0959.

2f: Mp 220.8-222.9°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.43 (dd, *J* = 6.8, 2.1 Hz, 2H), 6.98 (dd, *J* = 6.7, 2.1 Hz, 2H), 6.90 (s, 1H), 6.17 (d, *J* = 1.2 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 2.41 (d, *J* = 1.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.3, 159.6, 159.1, 154.4, 152.6, 130.6, 129.4, 127.6, 126.0, 113.7, 113.3, 112.2, 99.3, 56.1, 55.3, 18.7; Anal. Calcd for $C_{18}H_{16}O_4\cdot 1/4\ H_2O$: C, 71.87; H, 5.53. Found: C, 72.09; H, 5.25; HRMS (ESI) calcd for $C_{18}H_{16}O_4$ $(M+H)^+$ 297.1121. Found 297.1126.

General procedure for preparation of 6-aryl-7-hydroxycoumarins (3) (3a as an example)

Boron tribromide (1 M solution in dichloromethane, 3.4 ml, 3.4 mmol) was added to a solution of **2a** (46.5 mg, 0.175 mmol) in dry dichloromethane (2.5 ml) at -78°C under an atmosphere of argon. The reaction mixture was stirred for 21 h at room temperature, and added to iced water. The mixture was extracted with dichloromethane. The organic solution was washed with brine and concentrated to give **3a** (40.3 mg, 91%) as white cubes. A small amount of the compound was recrystallized to give an analytical sample. Mp 235.3-239.7°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56-7.45 (m, 6H), 6.96 (s, 1H), 6.18 (d, *J* = 1.1 Hz, 1H), 5.66 (s, 1H), 2.40 (d, *J* = 1.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.3, 156.0, 154.6, 152.5, 135.5, 129.6, 129.2, 128.6, 126.1, 125.8, 114.0, 112.4, 103.7, 18.7; Anal. Calcd for $C_{16}H_{12}O_3\cdot 1/3\ H_2O$: C, 74.41; H, 4.94. Found: C, 74.16; H, 4.98; HRMS (ESI) calcd for $C_{16}H_{12}O_3$ $(M+H)^+$ 253.0859. Found 253.0864.

Other compounds (**3b – 3e**) were prepared from the corresponding 7-methoxycoumarins (**2b – 2e**). Analytical data are given below.

3b: Mp 290.8-294.9°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.89 (dd, *J* = 6.6 Hz, 1.8 Hz 2H), 7.82 (dd, *J* = 6.7 Hz, 1.8 Hz, 2H), 7.67 (s, 1H), 6.88 (s, 1H), 6.18 (d, *J* = 1.0 Hz, 1H), 2.42 (d, *J* = 0.9 Hz, 3H) ; ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.2, 158.6, 154.7, 153.9, 142.3, 132.1, 130.4, 127.5, 123.9, 119.2, 112.6, 110.9, 109.7, 103.0, 18.3; Anal. Calcd for $C_{17}H_{11}NO_3$: C, 73.64; H, 4.00; N, 5.05. Found: C, 73.36; H, 4.25; N, 5.13; HRMS (ESI) calcd for $C_{17}H_{11}NO_3$ $(M+H)^+$ 278.0812. Found 278.0815.

3c: Mp 238.8-242.1°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (s, 1H), 7.31 (dd, *J* = 6.6 Hz, 2.1 Hz, 2H), 6.94 (s, 1H), 6.85 (dd, *J* = 6.8 Hz, 1.9 Hz, 2H), 6.15 (d, *J* = 1.2 Hz, 1H), 5.73 (s, 1H), 3.03 (s, 6H), 2.39 (d, *J* = 1.2 Hz, 3H) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.4, 156.2, 154.2, 152.6, 150.6, 129.9, 126.0, 125.7, 122.2, 113.8, 113.1, 112.1, 103.2, 40.4, 18.7; Anal. Calcd for $C_{18}H_{17}NO_3\cdot 2/3\ H_2O$: C, 70.34; H, 6.01; N, 4.56. Found: C,

70.23; H, 5.83; N, 4.57; HRMS (ESI) calcd for $C_{18}H_{17}NO_3$ $(M+H)^+$ 296.1281. Found 296.1284.

3d: Mp 243.2-247.1°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H), 7.61 (d, *J* = 8.7 Hz, 2H), 7.55 (s, 1H), 7.51 (d, *J* = 8.7 Hz, 2H), 6.85 (s, 1H), 6.14 (d, *J* = 1.1 Hz, 1H), 2.41 (d, *J* = 1.0 Hz, 3H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 160.4, 158.3, 153.9, 153.7, 138.5, 131.9, 129.7, 126.8, 125.5, 118.8, 112.4, 110.7, 102.7, 24.2, 18.3; HRMS (ESI) calcd for $C_{18}H_{15}NO_4Na$ $(M+Na)^+$ 332.0893. Found 322.0894.

3e: Mp 284.8-289.2°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.6 (brs, 1H), 9.44 (s, 1H), 7.50 (s, 1H), 7.39 (dd, *J* = 6.6, 2.0 Hz, 2H), 6.83 (s, 1H), 6.80 (dd, *J* = 6.7, 2.0 Hz, 2H), 6.14 (d, *J* = 1.2 Hz, 1H), 2.40 (d, *J* = 1.0 Hz, 3H) ; ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.4, 158.2, 156.7, 153.9, 153.4, 130.5, 127.9, 126.5, 125.9, 115.0, 112.4, 110.6, 102.6, 18.3; Anal. Calcd for $C_{16}H_{12}O_4 \cdot 1/6\ H_2O$: C, 70.84; H, 4.58. Found: C, 71.10; H, 4.76; HRMS (ESI) calcd for $C_{16}H_{12}O_4$ $(M+H)^+$ 269.0808. Found 269.0811.

Preparation of 3f

A solution of 6-bromo-7-hydroxy-4-methylcoumarin (**5**) (49.0 mg, 0.192 mmol), **6f** (81.7 mg, 0.538 mmol) and $Pd(PPh_3)_4$ (42.1 mg, 0.0364 mmol) in dimethoxyethane (3.5 ml) was mixed with 2 M sodium carbonate in water (2.8 ml), then stirred for 3.5 h at 60°C under an atmosphere of argon. The reaction mixture was cooled to room temperature, and saturated aqueous ammonium chloride was added. The mixture was extracted with dichloromethane. The organic solution was washed with brine and concentrated. Purification of the residue by column chromatography (silica gel) gave **3f** (16.7 mg, 31%) as a white powder. A small amount of the compound was recrystallized to give an analytical sample. Mp 243.5-246.2°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (s, 1H), 7.38 (dd, *J* = 6.6, 2.0 Hz, 2H), 7.06 (dd, *J* = 6.7, 2.1 Hz, 2H), 6.96 (s, 1H), 6.17 (d, *J* = 1.1 Hz, 1H), 5.70 (s, 1H), 3.88 (s, 3H), 2.40 (d, *J* = 1.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.2, 159.9, 156.0, 154.5, 152.4, 130.4, 127.4, 125.9, 125.4, 115.0, 113.9, 112.4, 103.5, 55.4, 18.7; Anal. Calcd for $C_{17}H_{14}O_4 \cdot 1/3\ H_2O$: C, 70.82; H, 5.13. Found: C, 70.63; H, 4.88; HRMS (ESI) calcd for $C_{17}H_{14}O_4$ $(M+H)^+$ 283.0965. Found 283.0957.

Preparation of 6g

n-Butyllithium (1.6 M solution in hexane, 2.3 ml, 3.7 mmol) was added to a solution of 4'-bromobenzo-15-crown 5-ether (1.00 g, 2.88 mmol) in dry tetrahydrofuran (30 ml) at -78°C under an atmosphere of argon. The reaction mixture was stirred for 10 min, and trimethyl borate (0.40 ml, 3.7 mmol) was added. Stirring was continued for another 30 min at -78°C, and then the mixture was allowed to warm to room temperature. Stirring was continued for 1.5 h, then a solution of pinacol (429 mg, 3.63 mmol) in dry tetrahydrofuran (5 ml) was added, and the mixture was further stirred for 5 h at room temperature. Acetic acid (0.20 ml, 3.7 mmol) was added, and after further stirring for 13 h, the solvent was removed. The residue was diluted with 2 M aqueous sodium hydroxide, and washed with dichloromethane. The aqueous layer was extracted with ethyl acetate. The organic solution was washed with brine and concentrated to give **6g** as a white solid (237 mg, 21%). **6g** was used without further purification for the next reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.29 (d, *J* = 1.2 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 4.17 (m, 4H), 3.90 (m, 4H), 3.76 (m, 8H), 1.33 (s, 12H).

Preparation of 2g

Compound **2g** was prepared from **4** and **6g** according to the procedure described for **2a** (yield 20%, yellow solid). Mp 208.3-211.5°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 7.02 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 6.89 (s, 1H), 6.17 (d, *J* = 1.2 Hz, 1H), 4.19 (m, 4H), 3.94 (m, 4H), 3.87 (s, 3H), 3.77 (m, 8H), 2.42 (d, *J* = 1.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.2, 159.6, 154.4, 152.6, 148.9, 148.7, 130.3, 127.6, 126.0, 122.5, 116.1, 113.8, 113.3, 112.2, 99.4, 71.1 (2C), 70.6 (2C), 69.7, 69.6, 69.5, 69.2, 56.1, 18.7; Anal. Calcd for $C_{25}H_{28}O_8$: C, 65.78; H, 6.18. Found: C, 65.78; H, 5.98; HRMS (ESI) calcd for $C_{25}H_{28}O_8$ $(M+Na)^+$ 479.1676. Found 479.1681.

Preparation of 7

Chloromethyl methyl ether (0.15 ml, 2.0 mmol) was added to a solution of **5** (346 mg, 1.36 mmol) and NaH (72.1 mg, 3.00 mmol) in dry *N,N*-dimethylformamide (3.7 ml) at 0°C under an atmosphere of argon. The mixture was stirred for 5 h at room temperature, and saturated aqueous ammonium chloride was added. The mixture was extracted with AcOEt. The organic solution was washed with brine, dried over Na_2SO_4, and concentrated. Purification of the residue by open column chromatography (silica gel) gave **7** (384 mg, 99%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.14 (s, 1H), 6.18 (d, *J* = 1.2 Hz, 1H), 5.31 (s, 2H), 3.52 (s, 3H), 2.39 (d, *J* = 1.2 Hz, 3H).

Preparation of 8

Compound **8** was prepared from **7** and **6g** according to the procedure described for **2a**. Yield 39% (yellow solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.16 (s, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 7.02 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 6.19 (d, *J* = 0.9 Hz, 1H), 5.20 (s, 2H), 4.19 (m, 4H), 3.94 (m, 4H), 3.78 (m, 8H), 3.42 (s, 3H), 2.41 (d, *J* = 0.8 Hz, 3H).

Preparation of 3g

Trifluoroacetic acid (0.10 ml, 1.3 mmol) was added to a solution of **8** (20.5 mg, 0.0421 mmol) in dichloromethane (0.6 ml) at 0°C. The reaction mixture was stirred for 1 h at room temperature, and water was added. The mixture was extracted with dichloromethane. The organic solution was washed with brine and concentrated. Purification of the residue by r-GPC gave **3g** (18.1 mg, 97%) as a brown solid. Mp 180.1-181.9°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (s, 1H), 7.01-6.95 (m, 4H), 6.16 (s, 1H), 5.95 (brs, 1H), 4.20 (m, 4H), 3.95

(m, 4H), 3.78 (m, 8H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.5, 156.6, 154.3, 152.8, 149.4, 149.1, 128.8, 125.7, 122.1, 115.0, 114.3, 113.6, 112.0, 103.6, 70.9 (2C), 70.3 (2C), 69.4, 69.0, 68.9, 18.7; HRMS (ESI) calcd for $C_{24}H_{26}O_8$ $(M+Na)^+$ 465.1520. Found 465.1526.

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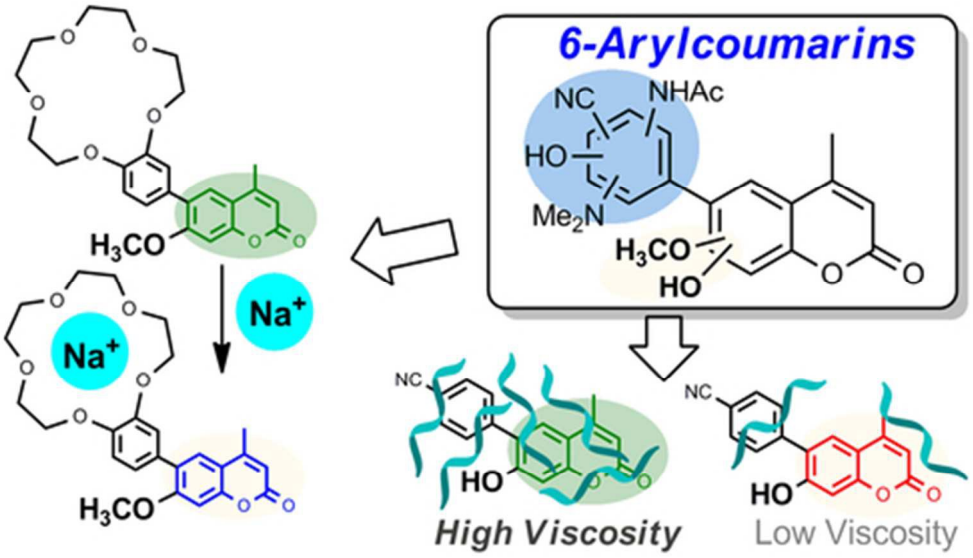

6-arylcoumarins are available as versatile scaffolds for various types of fluorescent sensors like those for cation and viscosity.
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