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Development of a Library of 6-Arylcoumarins as Candidate Fluorescent Sensors

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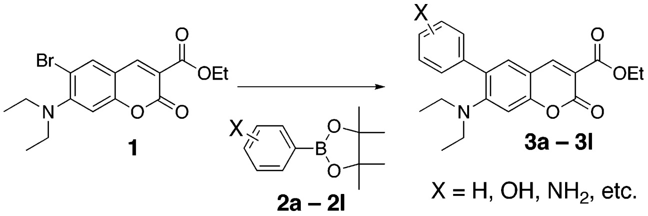
ABSTRACT



X = H, OH, NH$_2$, etc.

A bromocoumarin scaffold (1) was reacted with various boronic acid derivatives (2a–*l*) to afford a library of 6-arylcoumarins (3a–*l*). This library was found to contain candidate fluorescent sensors for peptidase activity and for nitric oxide.

Functional fluorescent molecules are useful in many fields of scientific research, including analytical chemistry or cell biology. For example, fluorescent molecules, whose fluorescence properties can be changed by binding to or reacting with ions, small molecules, or enzymes, enable us to estimate the concentration or activity of the targets.[1,2] Although some theoretical approaches to the design of such sensors have been reported,[2,3] most currently available sensors were developed empirically. Combinatorial synthesis of libraries of fluorescent molecules is an effective approach.[4] Chang et al. have developed libraries of styryl dyes bearing DNA-sensitive fluorescent sensors[5] and β-amyloid sensors,[6] and Finney et al. developed a fluorescent Hg^{2+} sensor via construction of a library.[7]

Coumarins are of interest because of their pharmacological activity[8] and photophysical properties, and they have been applied as laser dyes[9] and fluorophores for fluorescent sensors.[10] Bauerle et al.[11] and Wang et al.[12] reported coumarin libraries with diversity at the 3-position via Suzuki−Miyaura,

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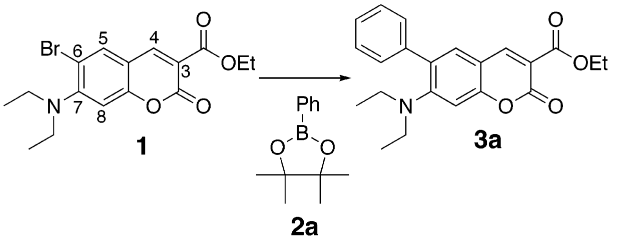
Heck, Sonogashira−Hagihara, or Husigen cycloaddition reactions; they obtained coumarins with a wide range of absorbance, fluorescence wavelength, and fluorescence intensity. Here, we report the development of a coumarin library with diversity at the 6-position via Suzuki−Miyaura coupling.

Compound **1**[13] was expected to be a good scaffold for construction of a 6-arylcoumarin library. The reaction conditions were optimized for the reaction of **1** with unsubstituted phenyl boronic acid pinacol ester **2a** (Scheme 1). Competitive debromination and ester-hydrolysis were considered likely to reduce the yield of the coupling reaction, or to complicate purification. Typical reaction conditions, using $Pd(PPh_3)_4$ as a catalyst and sodium carbonate as a base in THF−H_2O, yielded both undesired byproducts, and the coupling efficiency was relatively low. In an aprotic solvent, such as DMF or DME, no coupling reaction occurred, but the addition of one-third volume of ethanol and the use of a weaker base, CsF, allowed the coupling reaction to proceed, affording **3a** in 50−60% yield, although the debrominated product was also obtained. We examined other Pd catalysts, and found that $PdCl_2$(dppf) is effective in this reaction. Thus, a mixture of **1**, **2a** (1.5 equiv), $PdCl_2$(dppf) (0.1 equiv), and CsF (2.5 equiv) in DMF at 60 °C afforded **3a** in 55% yield without formation of the debrominated molecule. In this case, the addition of ethanol was unnecessary. By increasing the amounts of **2a** to 3.0 equiv, $PdCl_2$(dppf) to 0.2 equiv, and CsF to 5.0 equiv, the yield of **3a** was improved to 72%, and these conditions were adopted for the coupling reaction with other boronic acid derivatives.

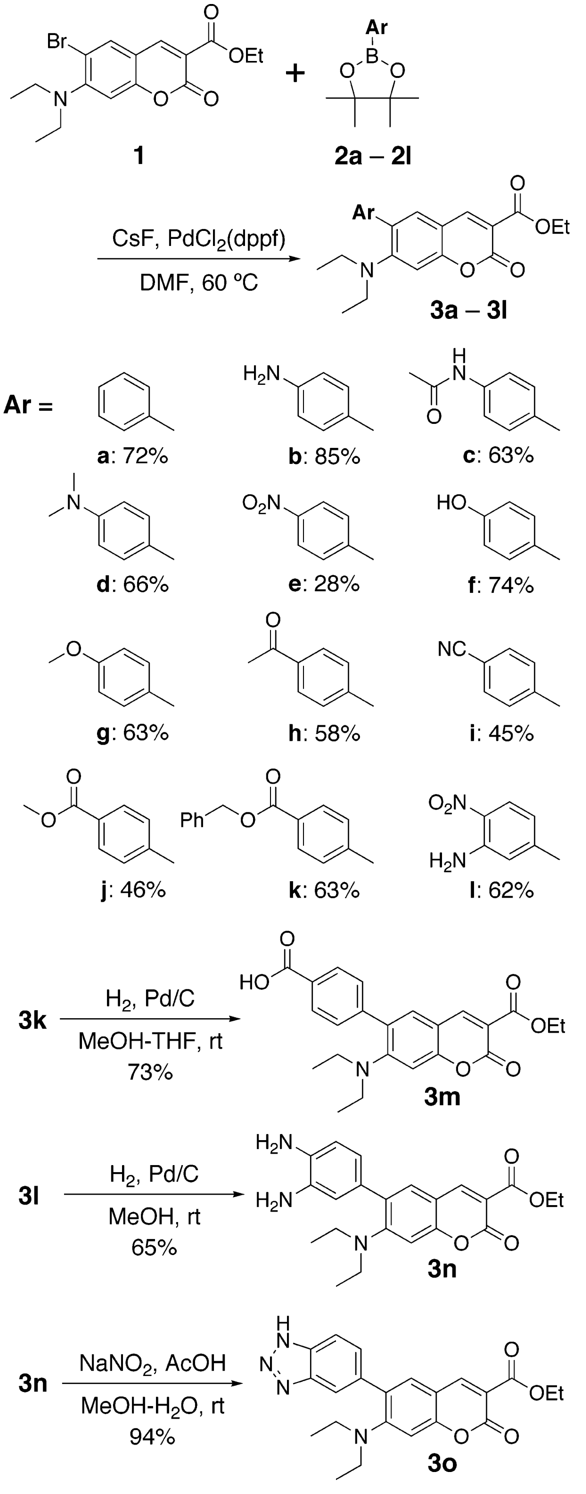
Scheme 1



As shown in Scheme 2, the scaffold **1** was reacted with 12 boronic acid pinacol ester derivatives (**2a**−***l***), yielding the corresponding 6-arylcoumarins, **3a**−***l***. Although the compounds with an electron-withdrawing group, such as nitro (**2e**), cyano (**2i**), or methoxycarbonyl (**2j**), were obtained in relatively low yield (28%, 45%, and 46%, respectively), most of the reactions proceeded in moderate yield. Some functional groups on the phenyl group were rather easily transformed, as observed in the syntheses of **3m**, **3n**, and **3o**.

The fluorescence intensities of these molecules in acetonitrile, methanol, and water (1 mM sodium phosphate buffer at pH 7.4) were measured (Table 1). The absorption maximum wavelength of each compound was around 410 nm in acetonitrile, 415 nm in methanol, and 430 nm in water (Figure 1i−iii; Table S1 in the Supporting Information), while the fluorescence intensities varied. Compound **3j** had the strongest intensity in each solution, whose quantaum yield was similar to that of ethyl 7-diethylaminocoumarin-3-carboxylate (unsubstituted at 6-position), a well-known fluorophore as labeling or sensing biomolecule. Amino-substituted **3b** or *o*-diamino-substituted **3n** had almost no

Scheme 2. Construction of the 6-Arylcoumarin Library



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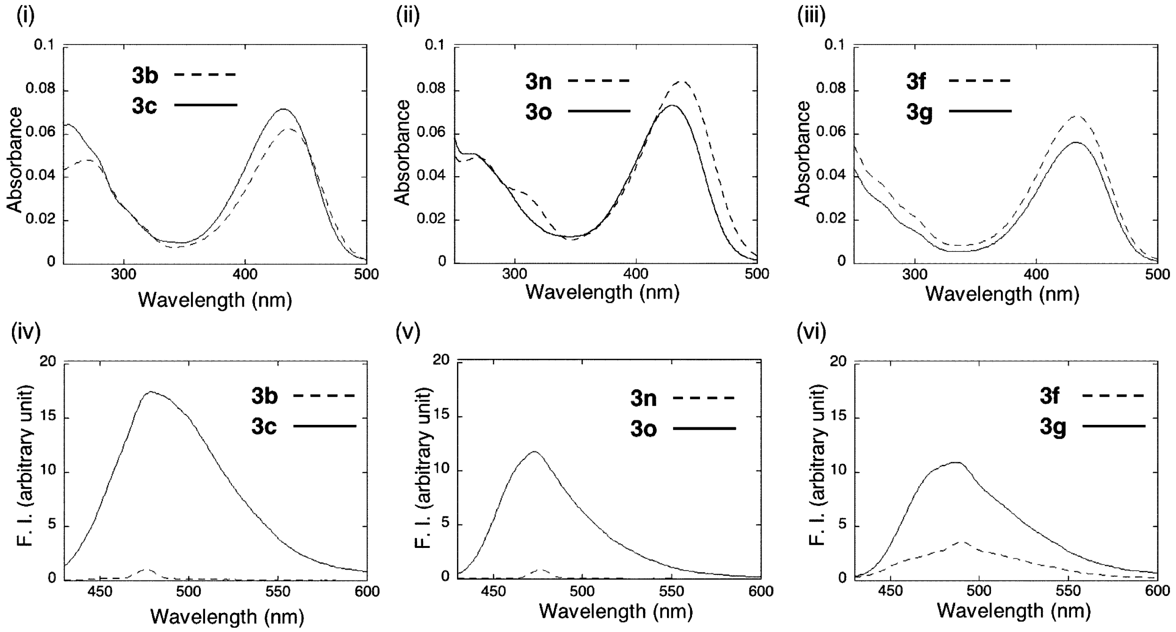

Figure 1. Absorption and emission spectra of 6-arylcoumarin compounds. Absorption spectra of (i) **3b** (dotted line), **3c** (solid line), (ii) **3n** (dotted line), **3o** (solid line), and (iii) **3f** (dotted line), **3g** (solid line) were measured in 3 μM solution in sodium phosphate buffer (pH 7.4, containing 0.3% DMSO as a cosolvent). Fluorescence spectra for (iv) **3b** and **3c**, (v) **3n** and **3o**, and (vi) **3f** and **3g** excited at 420 nm in the same solvent are shown.

fluorescence, while the acetamido **3c** and triazole **3o** compounds had moderate fluorescence. These data suggested that 6-phenylcoumarin would be a good fluorophore for sensors of peptidase activity, which transforms an amido group to an amino group, and of nitric oxide, which reacts with an *o*-diamino group in the presence of oxygen to form triazole[14] (Figure 1iv,v). The transformation of the methoxy group (**3g**) to a hydroxyl group (**3f**) also induced a change of fluorescence intensity, which suggested that this molecule might be useful as a fluorescent sensor for dealkylating enzymes, such as glycosidase (Figure 1vi). Any compound, except **3o**, showed no significant pH-dependency around neutral condition (pH 6.0−8.0). Compound **3o** showed 50% decrease of the fluorescent intensity at pH 8.0 compared with that at pH 6.0 (data not shown). This decrease might be derived from the deprotonation of the triazole ring as shown in the reported *o*-diamine-based fluorescent sensors for nitric oxide, and this pH-dependency might be lost by the monomethylation of one of two amine nitrogens.[14]

Table 1. Fluorescence Intensity of 6-Arylcoumarins[a]

compd	CH_3CN	CH_3OH	water
3a	13.2	8.8	13.6
3b	<1	<1	<1
3c	29.6	30.0	20.4
3d	<1	<1	<1
3e	16.3	15.7	11.4
3f	14.1	7.8	3.7
3g	23.0	17.5	15.8
3h	19.3	6.0	2.7
3i	24.1	11.2	18.2
3j	63.2	48.2	38.2
3l	<1	<1	<1
3m	24.7	18.6	24.1
3n	<1	<1	<1
3o	21.9	15.5	14.0

[a] Fluorescence intensity at the excitation and emission maximum wavelengths of each compound (3 μM) was measured in acetonitrile, methanol, and water (1 mM sodium phosphate buffer (pH 7.4)). All solutions contained 0.3% DMSO as a cosolvent.

The fluorescent property of 7-aminocoumarin derivatives can be controlled partially by intramolecular charge transfer (ICT) and twisted intramolecular charge transfer (TICT).[1,15] In our library, the proximity between aryl ring and diethylamine moiety might influence those mechanisms. In addition, the characteristic of fluorescent intensity is similar to that seen with fluorescein derivatives or Tokyo Green derivatives, whose fluorescent properties are controlled by photoinduced electron transfer (PeT).[3] At present, no direct evidence of such mechanisms has been obtained, and the detailed mechanistic investigations, such as measurements

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by single wavelength excitation, time-resolved measurement of fluorescent decays, and lifetimes, are in progress.

In conclusion, our results indicate that library synthesis of fluorescent molecules is potentially useful for finding lead compounds for the development of functional molecules. Introduction of an aromatic substituent *ortho* to the 7-diethylamino group on the coumarin ring afforded the unique fluorescent molecules that can be applied to some sensors. This strategy may also be applicable to other fluorophores or the chromophores of long-lifetime fluorescent lanthanide complexes.[16] By utilizing the present approach in combination with the introduction of diversity at other sites of the coumarin structure, e.g., at the 3-position,[11,12] it should be possible to construct large libraries of candidate sensors.

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Supporting Information Available: All experimental procedures including synthesis details and identification of each compound. This material is available free of charge via the Internet at http://pubs.acs.org.

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