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The Synthesis of a New Class of Highly Fluorescent Chromones via an Inverse-Demand Hetero-Diels–Alder Reaction

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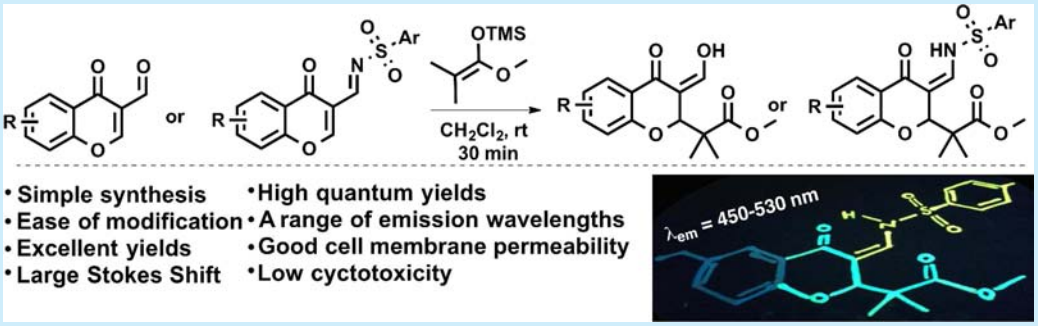
Supporting Information



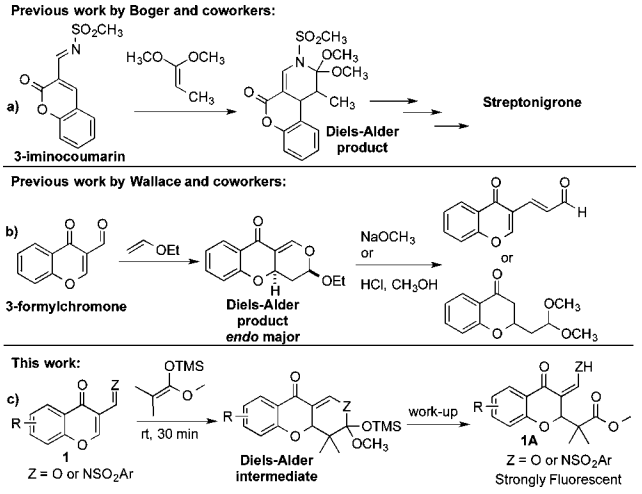
ABSTRACT: A new class of fluorophores has been developed utilizing an inverse-demand hetero-Diels–Alder reaction with silyl enol ethers and substituted 3-formylchromones. These compounds yield blue to green fluorescence with quantum yields up to 73%. They also exhibit good potential for use as fluorescent probes in biological systems, as they are cell membrane permeable with low cytotoxicity.

Fluorescence has been a very popular topic of study and has found many applications such as lasers, biological probes, and nonlinear optics.[1] Fluorescent applications for biological measurements have received much attention over the past decade, especially through the discovery of new organic fluorophores.[2] The use of small molecule dyes for the preparation of biological fluorescent probes can be advantageous because their structures can be tuned to optimize parameters such as solubility and fluorescence intensity.[3] Although significant progress has been achieved in this field, there is still a high demand for new fluorophore systems that allow for easy modifications and good photophysical properties.[4]

Fluorescent dyes based upon the coumarin skeleton have been extensively studied over the years.[5] Additionally, the flavonoids have been broadly studied with regard to their biological and fluorescent properties;[6] however, other chromone frameworks have received little attention with regard to fluorescence.[7] The heterocyclic, conjugated infrastructure of chromones and their derivatives offers a wide range of synthesis opportunities for derivatization. Herein, we report the development of a new fluorophore system using an inverse-demand hetero-Diels–Alder (HDA) reaction with silyl enol ethers and substituted 3-formyl chromones.

In the early 1990s Boger and co-workers reported two studies involving inverse-demand HDA reactions between alkyl enol ethers and conjugated enones.[8] One of the reports utilized this transformation on a coumarin diene as a key step for a total synthesis project (Scheme 1a).[8b] Another report by Wallace showed that formyl chromones underwent fast hetero-Diels–Alder reactions with alkyl enol ethers, and upon treatment with a

Scheme 1. Coumarins and Chromones in Inverse-Demand Hetero-Diels–Alder Reactions



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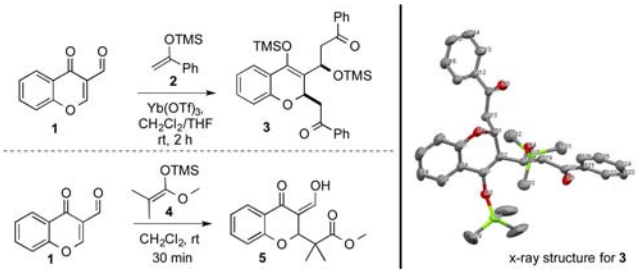
base or acid, these Diels–Alder products rearranged to give aldehyde or acetal compounds (Scheme 1b).[9] Based on these two previous accounts and our ongoing interest in the chromone skeleton,[10] we set out to study the use of various chromones in an HDA reaction. While Snider reported the use of alkyl enol ethers in inverse electron demand Diels–Alder reactions in 1980,[11] we were interested in using silyl enol ethers as the electron-rich dienophile due to the potential ease of removal of the silicon group postreaction (Scheme 1c). We knew this was an important feature since Wallace and co-workers demonstrated that the ring opening of the HDA products containing alkyl groups on the acetal moiety could result in a complex mixture of products.[9]

Our studies began with the reaction of 3-formylchromone (**1**) with the trimethylsilyl (TMS) enol ether of acetophenone (**2**) (Scheme 2). Initially, we found that addition of a Lewis acid

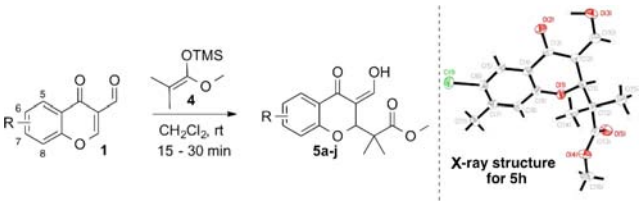
Scheme 2. Reaction of Silyl Enol Ethers with 3-Formyl Chromone



($Yb(OTf)_3$, 25 mol %) greatly enhanced the reaction rate; however, the major product was the tandem Mukaiyama aldol/Michael addition adduct **3** rather than the desired HDA adduct. Although this was an interesting result, we wanted to focus on the potential Diels–Alder pathway. Therefore, we investigated other silyl enol ethers and found that dimethylsilyl ketene acetal (**4**) reacted very rapidly with enone **1** to give product **5** in excellent yield. Analysis of the crude reaction mixture via ^1H NMR revealed the absence of an enol proton at ~14 ppm. The lack of an enol proton and the absence of a Lewis acid catalyst suggested that the Diels–Alder product was initially formed; however, after chromatography the cyclic product hydrolyzed to give the open conjugated enol compound **5**. Interestingly, it was discovered that enol **5** strongly fluoresced blue upon UV irradiation (365 nm). This result sparked our interest since blue and green emitters represent a significant area of research and many of the known compounds have lengthy or low yielding synthetic routes.[12] We also discovered that a simple structure search on these new fluorophores showed the enol moiety in **5** resembles a similar feature found in epicocconone (see Figure S1 in Supporting Information for structural similarities), a natural product isolated from the fungus *Epicoccum nigrum*,[13] that has found wide biological application as a reversible-covalent latent fluorophore.[14]

To examine the fluorescent properties resulting from substitution on the benzene ring of the 3-formyl-chromones, we set out to synthesize a small library of these enol products (Table 1). The reaction proceeds very rapidly and with good to excellent yields in the presence of both electron-donating and -withdrawing groups on the chromone aryl ring. It was observed that the dichloro and dibromo chromones gave lower yields. An X-ray structure for compound **5h** was also obtained which clearly shows the internal hydrogen-bonded conjugated enol functionality.

Table 1. Synthesis of Fluorescent Enol Chromone Derivatives

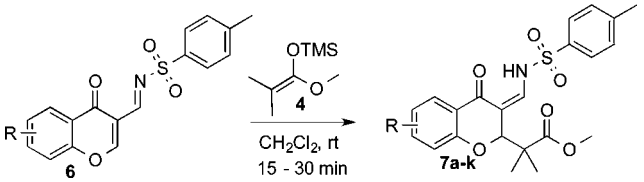


entry	product	R	yield (%)[a]
1	**5a**	6-H	77
2	**5b**	6-F	87
3	**5c**	6-Cl	82
4	**5d**	6-Br	87
5	**5e**	6-CH_3	78
6	**5f**	6-Et	91
7	**5g**	6-OCH_3	82
8	**5h**	6-Cl, 7-Me	77
9	**5i**	6,8-Cl	71
10	**5j**	6,8-Br	72

[a]Isolated yields.

Next, we focused on modifying the aldehyde moiety on the chromone. It was discovered that reacting 3-formylchromone with *p*-toluene sulfonamide in the presence of PPTS gave the sulfonamide Schiff base product **6**. These sulfonamide derived starting materials also underwent fast HDA reactions with silyl enol ether **4** (see Table 2). Again, the yields were good to

Table 2. Synthesis of Fluorescent Sulfonamido-Chromone Derivatives



entry	product	R	yield (%)[a]
1	**7a**	6-H	78
2	**7b**	6-F	90
3	**7c**	6-Cl	80
4	**7d**	6-Br	88
5	**7e**	6-CH_3	80
6	**7f**	6-Et	70
7	**7g**	6-iPr	93
8	**7h**	6-OCH_3	97
9	**7i**	6-H	72[b]
10	**7j**	6-CH_3	76[b]
11	**7k**	6-OCH_3	77[b]
12	**7l**	6-OCH_3	72[b,c]

[a]Isolated yields. [b]*p*-Methoxy substitution on a sulfonamide aryl ring. [c]TBS-silyl enol ether of methyl acetate used as dienophile.

excellent with a variety of substitutions on the chromone aryl ring. The reaction also worked well using the TBS-silyl enol ether derived from methyl acetate (entry 12, Table 2). The resulting conjugated sulfonamido-chromone products **7** gave intense green fluorescence under UV irradiation at 365 nm.

With a series of new fluorophore compounds in hand, absorption and emission maxima along with quantum yields were measured (Table 3). The enol compounds (**5**) gave emission maxima from 446 to 486 nm resulting in blue fluorescence.

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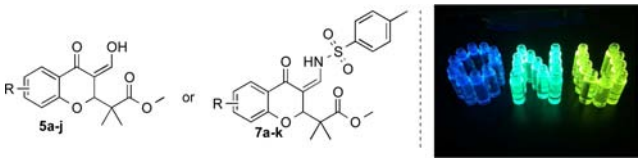
Table 3. Spectroscopic Properties of Fluorophores 5 and 7



entry	product	λ_{abs} [a] (nm)	λ_{em} [b] (nm)	Stokes shift (nm/cm^{-1})	Φ_F [c] (%)
1	**5a**	362	446	84/5203	6
2	**5b**	364	486	122/6896	2
3	**5g**	383	482	99/5363	20
4	**5j**	371	451	80/4781	3
5	**7a**	358	479	121/7056	37
6	**7b**	367	492	125/6923	33
7	**7c**	367	483	116/6544	39
8	**7d**	343	483	140/8451	34
9	**7e**	367	492	125/6923	70
10	**7e**	380	468	88/4948	8[d]
11	**7e**	364	479	115/6596	27[e]
12	**7f**	370	490	120/6619	73
13	**7f**	369	496	127/6939	16[d]
14	**7f**	364	481	117/6683	30[e]
15	**7g**	368	490	122/6766	71
16	**7h**	390	529	139/6737	66
17	**7i**	358	479	121/7056	38
18	**7j**	367	492	125/6923	72
19	**7k**	392	527	135/6535	69
20	**7l**	386	529	143/7003	67

[a]Absorption maximum. [b]Emission maximum. [c]Absolute quantum yields determined by an integrating sphere system in CH_2Cl_2. [d]Absolute quantum yield in CH_3CN. [e]Absolute quantum in yield cyclohexane.

The sulfonamido-chromone products (**7**) were a more visibly blue-green to green color of fluorescence with emission maxima ranging from 479 to 529 nm. Another general trend observed was that the enol products had much lower quantum yields (Φ_F = 2–20%, CH_2Cl_2) as compared to the sulfonamido-chromone compounds (Φ_F = 33–73%, CH_2Cl_2). Electron-donating groups (EDGs) located on the 6-position of the chromone red-shifted the emission (compare entry 5 with 9, 14, 15, and 16). It was also observed that EDGs increased the quantum yields (for example, compare entry 1 with 3 and entry 5 with 12). A strong EDG on the sulfonamide aryl ring did not affect the emission maximum or quantum yield (compare entries 16 and 19). It was also observed that the quantum efficiency in both nonpolar and polar solvents was lower for compound **7e** (Φ_F = 70% in CH_2Cl_2, 8% in CH_3CN and 30% in cyclohexane, entries 9, 10, and 11). A similar trend for compound **7f** was also observed.

Next, we wanted to investigate the possible application of these new chromone fluorophores as potential biological imaging agents. Initially we incubated compound **5g** (150 mg/mL) with A375 cells. Figure 1c shows the intense green fluorescence inside the cells with excitation at 358 nm. This result indicates that compound **5g** has good cell membrane permeability. Next, we examined sulfonamido-chromone substrates **7e** and **7k** under identical conditions. It was observed that **7k** readily penetrated the cell membrane and emitted strong green fluorescence in living cells (Figure 1b). Interestingly, compound **7e** resulted in almost no fluorescence indicating that **7e** most likely has poor membrane permeability since it exhibits a large quantum yield (Φ_F = 69%, CH_2Cl_2). This poor penetration into cells may be due to the lipophilic properties of **7e**. A simple calculation shows that the clog P for **7e** is 4.5 while compounds **5g** and **7e** are

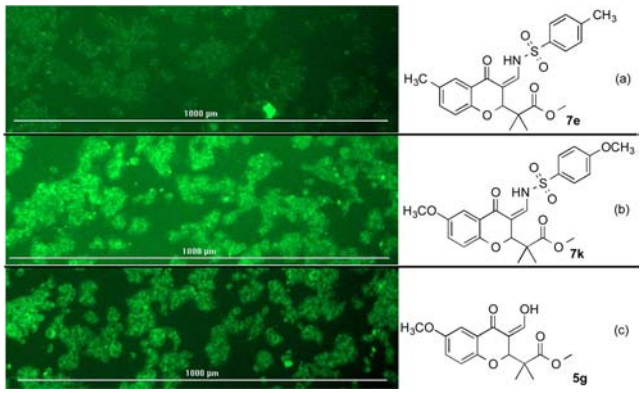


Figure 1. Fluorescent images of A375 cells with compounds **5g**, **7e**, and **7k**.

2.7 and 3.8 respectively.[15] Next, we evaluated the cytotoxicity of **5g**, **7e**. and **7k** in three different cell lines. The IC_{50} values for each compound in all cell lines were relatively large (83 to >250 μM in A375 cells)[16] which demonstrates low cytotoxicity.

In conclusion, we have developed a new chromone-derived fluorescent scaffold using an efficient synthetic scheme that allows for ease of modification. By utilizing simple substituent changes to chromones or sulfonamide moieties, a range of blue to green fluorescence can be achieved with quantum yields up to 73%. This new class of fluorophores has also been shown to permeate living cell membranes with low cytotoxicity. This may allow them to be used as biological dyes or sensors in future applications. Experiments focusing on chemical sensor applications for this new fluorophore are currently underway and will be published in due course.

■ ASSOCIATED CONTENT

Supporting Information

Detailed synthetic and experimental procedures, characterization data, and CIF for all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01417.

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Notes

The authors declare no competing financial interest.

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(15) These clog P values were calculated using ChemBioDraw 14.0.
(16) See Supporting Information for cell culture details.

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