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ESIPT-Mediated Photocycloadditions of 3-Hydroxyquinolinones: Development of a Fluorescence Quenching Assay for Reaction Screening

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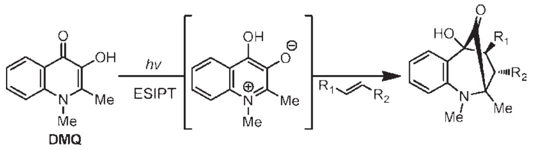
ABSTRACT



Irradiation of 1,2-dimethyl-3-hydroxyquinolinone (DMQ) leads to excited state intramolecular proton transfer (ESIPT) generating a 3-oxidoquinolinium species which undergoes [3 + 2] photocycloaddition with dipolarophiles. A parallel, fluorescence quenching assay using a microplate format has been developed to evaluate fluorescence quenching of this species with a range of dipolarophiles.

We have reported the excited state intramolecular proton transfer (ESIPT)[1] of 3-hydroxyflavone (3-HF) derivative **1** and photochemical [3 + 2] cycloaddition of the derived oxidopyrylium[2] species **2** with methyl cinnamate **3** (Figure 1).[3] The resulting cycloadduct **4** was subsequently transformed to the natural product methyl rocaglate **5**. Nitrogen analogues of 3-hydroxyflavone including 1,2-dimethyl-3-hydroxyquinolinone (DMQ) (**6**)[4] have been reported to undergo ESIPT to afford 3-oxidoquinolinium **7***.[5] This precedent prompted us to investigate the feasibility for photocycloaddition of oxidoquinolinium intermediates such as **7** with a variety of dipolarophiles as reaction partners.

In order to study the cycloaddition reactivity of DMQ **6** under UV irradiation in the presence of various dipolarophiles, we considered a parallel screening approach. A number of recent reports have outlined use of fluorescent probes for high throughput reaction development.[6] Our

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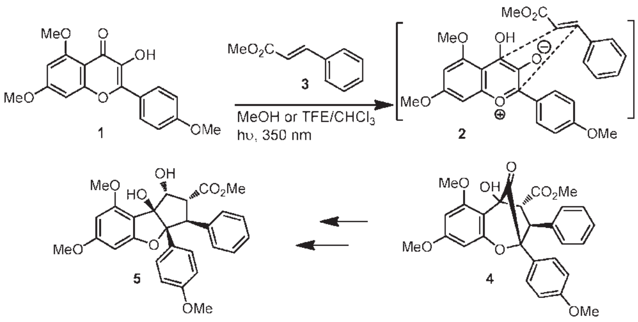

Figure 1. Photochemical cycloaddition of **3** and 3-hydroxyflavone **1** to access methyl rocaglate **5**.

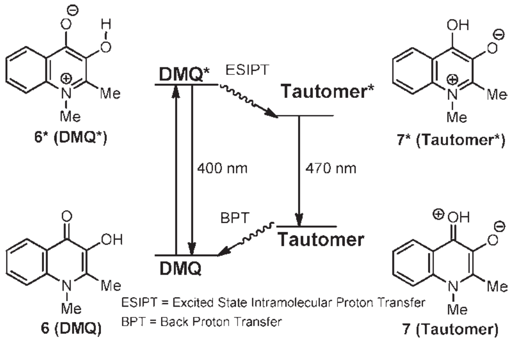

Figure 2. ESIPT of 3-hydroxyquinolinone **6** (**DMQ**).

initial plan was to take advantage of the inherent photochemical behavior of **6** to implement a parallel, fluorescence quenching assay. Under UV irradiation, 3-hydroxyquinolinone **6** undergoes rapid proton transfer (ns time scale) from its first excited state **6*** leading to the formation of phototautomer species **7*** which decays to **7** *via* fluorescence emission at 470 nm (Figure 2).[7] We envisioned that quenching of the fluorescence of transient intermediate **7*** in the presence of a selection of dipolarophiles should provide an indication of photocycloaddition reactivity.[8] In this paper, we report development of a parallel, fluorescence quenching assay using a microplate format to evaluate fluorescence quenching of this species with a range of dipolarophiles and correlation of the photophysical data with preparative photocycloadditions.

We first investigated the photophysical properties of 3-hydroxyquinolinone **6**. UV/vis spectra of **6** in various solvents showed an absorbance maximum at 370 nm (Figure 3)[8] which was well separated from the absorption bands of most commonly encountered dipolarophiles such that selective light absorption by the heterocycle could be easily accomplished. Upon excitation at 350 nm, dual fluorescence associated with normal excited state **6*** (400 nm) and tautomeric excited state **7*** (470 nm) was observed. The latter emission related to ESIPT was characterized by an unusually large Stokes shift as expected and previously reported (Figure 3).[4d] The origin of this ESIPT emission was also confirmed by excitation spectra recorded at 470 nm and were found to be identical to the corresponding absorption spectra.[8] The species **7*** was also observed to have a fluorescence lifetime of 16.8 ns and a very high quantum yield of 0.65 in 1,4-dioxane.[8] The latter lifetime should make **7*** a trappable species in comparison to **6*** whose lifetime is only 0.23 ns due to a very fast ESIPT rate.[2,4b,4d] The relatively long absorption wavelength of DMQ **6** and its inherent fluorescence properties were highly favorable for fluorescence quenching studies.

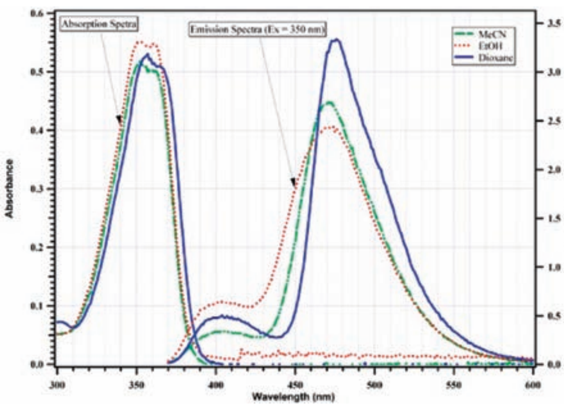

Figure 3. Absorption and normalized fluorescence spectra of **6** (concentration is 45 μM; for fluorescence spectra, λ_{ex} = 350 nm).

To establish a relationship between photoreactivity and quenching behavior,[9] we investigated the fluorescence quenching of **7*** by various dipolarophiles using a 96-well glass microtiter plate and a microplate reader. Microplate fluorescence measurements were performed in triplicate in black 96-well plates, using a SpectraMax Gemini XS fluorescence plate reader (Molecular Devices) (excitation wavelength: 350 nm; emission wavelength: 470 nm with a filter cutoff of 455 nm). The addition of 17 quenchers (Figure 4) to 1,4-dioxane solutions of **6** (the final concentrations of **6** and quenchers were 0.08 and 50 mM, respectively) resulted in the quenching of the fluorescence emission of **7*** to varying extents.

A summary of the fluorescence quenching results provided in the form of a "heat map" (Figure 4) shows that dipolarophiles can be categorized into three categories: strong quenchers (light green, **3a**−**3e**), moderate quenchers (dark green, **3f**−**3k**), and poor quenchers (brownish red, **3l**−**3q**). The quenching behavior can be roughly considered as an indicator for the efficiency of bimolecular interactions and therefore potential for

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[8] See Supporting Information for complete experimental details.

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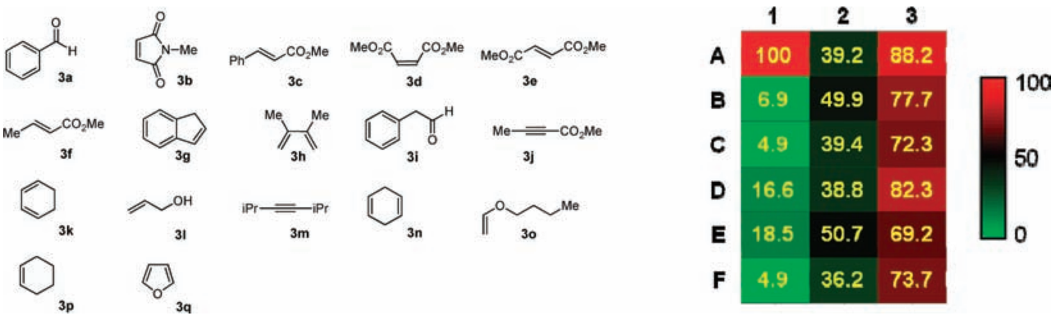
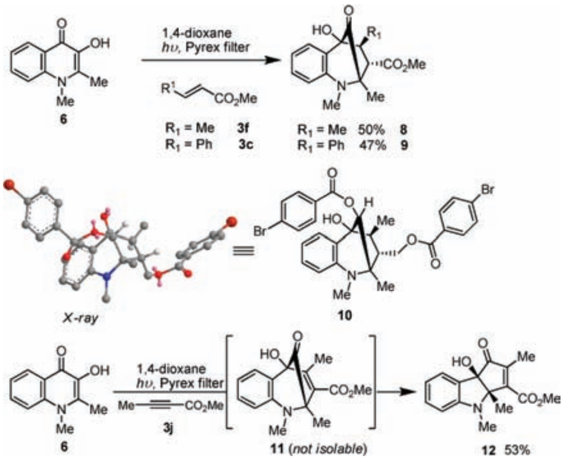


	1	2	3
A	100	39.2	88.2
B	6.9	49.9	77.7
C	4.9	39.4	72.3
D	16.6	38.8	82.3
E	18.5	50.7	69.2
F	4.9	36.2	73.7

Figure 4. List of dipolarophiles used in fluorescence quenching and “heat map” of fluorescence measurements using a microplate reader. Fluorescence quenching data presented are an average of three data points[8] and have been normalized to a scale from 0 to 100%, where 100% represents the fluorescence emission of DMQ in the absence of dipolarophile. **A1** represents the emission of 80 μM **DMQ** in 200 μL of dioxane: Fluorescence emissions reported in the presence of 0.05 M dipolarophile were normalized according to **A1**. **3a**: B1; **3b**: C1; **3c**: D1; **3d**: E1; **3e**: F1; **3f**: A2; **3g**: B2; **3h**: C2; **3i**: D2; **3j**: E2; **3k**: F2; **3l**: A3; **3m**: B3; **3n**: C3; **3o**: D3; **3p**: E3; **3q**: F3. Measurements were performed at an excitation wavelength of 350 nm and an emission wavelength of 470 nm with filter cutoff at 455 nm.

Scheme 1. Photocycloaddition of 3-Hydroxyquinolinone **6** and Electron-Deficient Dipolarophiles[10]



photocycloaddition. Based on the analysis of the quenching rates and considering that there might be a number of pathways to quench fluorescence, our expectation was that “poor quenchers” would not likely show photocycloaddition reactivity. Next, a photochemical reactivity screen with the series of dipolarophiles was conducted.[10] We noticed no photoreactivity for poor quenchers and that about two-thirds of strong and moderate quenchers showed photoreactivity. It should be noted that a number of strong quenchers (*e.g.*, **3a**, **b**, **d**, and **e**) did not afford photocycloadducts. We hypothesized that this lack of reactivity in photocycloaddition may be attributed to quenching *via* charge-transfer mechansims in which the photoexcited aromatic **7*** serves as a charge donor to a carbonyl quencher.[11] Other electron deficient-alkenes which were found as reaction partners including methyl cinnamate **3c**, methyl crotonate **3f**, and methyl butynoate **3j** possess electronic properties similar to those of methyl cinnamate, a workable dipolarophile in ESIPT-mediated photocycloaddition with 3-hydroxyflavones.[3] When subjected to irradiation in 1,4-dioxane, dipolarophiles **3c** and **3f** showed photoreactivity with **6** (Scheme 1) to afford moderate yields of cycloadducts **8** and **9** along with unknown polar byproducts.[12] The structure of **8** was supported by X-ray crystal structure analysis of the derived *bis-p*-bromobenzoate **10**.[8] Using methyl butynoate **3j** as a dipolarophile, the presumed [3 + 2] cycloadduct **11** was not detected but the isomeric ketol **12** was formed, presumably because the doubly conjugated enone provides a strong, thermodynamic driving force for α-ketol rearrangement.[13]

Under similar photochemical reaction conditions, two major products were isolated from the photocycloaddition of **6** and electron-rich dipolarophiles including indene **3g**, 2,3-dimethylbutadiene **3h**, and cyclohexadiene **3k** in 1,4-dioxane (Scheme 2). The regio- and stereochemistry of *endo* cycloadducts **13–18** were deduced from spectroscopic analysis, including advanced NMR experiments (HMQC, HMBC, and NOESY).[8] Chemical modification of products also provided further proof for structure determination. For example, careful hydrogenation (monitored by NMR to avoid over-reduction of the ketone moiety) of a mixture of cycloadducts **17** and **18** yielded a single saturated compound **19**. This result further confirmed that the isomers share the same carbon skeleton and configuration (*endo* diastereoisomer). No [4 + 3]

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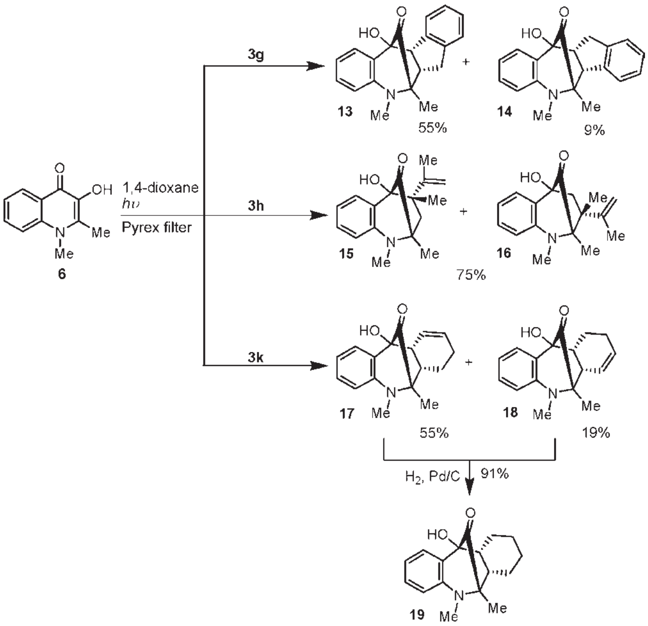
[10] *Experimental procedure*: A solution of 3-hydroxyquinolinone **6** (0.25 mmol) and dipolarophile (20 equiv) of dipolarophile in 6 mL of anhydrous 1,4-dioxane was degassed with argon for 5 min and then irradiated (Hanovia UV lamp, Pyrex filter) using an ethylene glycol cooling system for 14 h. The solution was concentrated *in vacuo* to afford an orange-brown oil. Purification *via* flash chromatography on silica gel (50:50 hexanes/CH_2Cl_2 to 100% CH_2Cl_2) afforded the cycloadduct.

[11] For quenching of photoexcited aromatics by charge transfer, see: Busch, D.; Dahm, L.; Siwicke, B.; Ricci, W. R. *Tetrahedron. Lett.* **1977**, *51*, 4489–4492.

[12] During reactions, decomposition of starting materials or unidentified reaction pathways may lead to the formation of polar byproducts which were difficult to fully characterize.

[13] For a review on the α-ketol rearrangement, see: Paquette, L. A.; Hofferberth, J. E. *Org. React.* **2003**, *62*, 477–567.

Scheme 2. Photocycloaddition of 3-Hydroxyquinolinone **6** and Electron-Rich Dipolarophiles[10]



photocycloaddition[14] products were isolated in photocycloadditions with diene reaction partners **3h** and **3k**.

To establish a more quantitative relationship between photocycloaddition reactivity and quenching behavior, Stern−Volmer analyses[15] were also examined. Data were plotted using Stern−Volmer analysis ($F_0/F = 1 + kq\,\tau[\mathrm{Q}]$) where F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively, and [Q] is the concentration of quencher. Slopes ($kq\ \tau$) were calculated by least-squares analysis; τ is the measured singlet lifetime of **7***. The calculated quenching constant kq (Table 1) can again be divided into three groups: strong quenchers (10^9−10^{10}, light green block in the "heatmap" (Figure 4)), moderate quenchers (~10^8, dark green block in "heatmap"), and poor quenchers (10^6−10^7 brown/red blocks in the "heatmap"), values which are in overall agreement with our plate-reader data (Figure 3).

Table 1. Rate Constants for the Quenching of **7*** and Diverse Dipolarophiles[a]

quencher kq ($\mathrm{S^{-1}\,M^{-1}}$)	**3a** 3.8×10^{10}	**3b** 3.8×10^{10}	**3c** 1.0×10^{10}	**3d** 0.8×10^{10}	**3e** 0.5×10^{10}
3f	**3g**	**3h**	**3i**	**3j**	**3k**
9.5×10^8	3.8×10^8	2.5×10^8	1.3×10^8	1.3×10^8	1×10^8
3l	**3m**	**3n**	**3o**	**3p**	**3q**
2.4×10^7	1.7×10^7	1.4×10^7	1.2×10^7	1×10^7	0.5×10^7

[a] Data were plotted using Stern−Volmer analysis: $F_0/F = 1 + kq\,\tau[\mathrm{Q}]$, [**DMQ**] = 15 μM.

In conclusion, we have expanded our ESIPT/photocycloaddition methodology to the use of 3-hydroxyquinolinone substrates. A parallel, fluorescence quenching assay using a microplate format has been developed to evaluate fluorescence quenching of a range of dipolarophiles. Data from photophysical studies have been correlated with preparative photocycloadditions which have demonstrated access to novel nitrogen-containing, bicyclic frameworks. Further studies involving photocycloadditions of 3-hydroxyquinolinones and reaction screening of photochemical transformations are currently in progress and will be reported in due course.

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Supporting Information Available. Experimental procedures, compound characterization data and X-ray crystallographic information files. This material is available free of charge via the Internet at http://pubs.acs.org.

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