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Dehydrogenative Heck coupling of biologically relevant N-heteroarenes with alkenes: discovery of fluorescent core frameworks†

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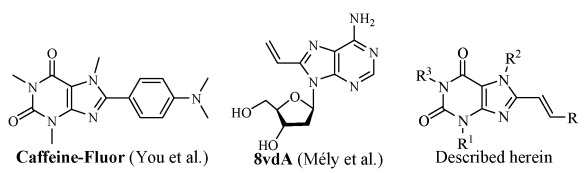
A Pd/Cu-catalyzed dehydrogenative Heck coupling is established that allows direct alkenylation of various biologically relevant N-heteroarenes with alkenes. The resulting π-extended alkenylated N-heteroarenes exhibit interesting fluorescent properties and have proven to be potentially useful fluorescent probes for bioimaging.

Small organic fluorophores have been widely applied in various research fields. In drug discovery, biological science and biomedical study, molecular fluorescent imaging techniques have enabled researchers to identify potential targets through intracellular visualization.[1] The autofluorescing biologically active molecules maintain their molecular recognition capabilities and thus should be an ideal candidate to develop specific imaging probes.[2] Purines and xanthines are vital imidazole-bearing N-heterocycles that are frequently found in synthetic compounds, biological systems, and pharmacophores, which would be valuable designer molecules for bioimaging probes. However, parent purines and xanthines have poor or no fluorescence characteristics. Recent examples have demonstrated that the extension of their π-conjugated systems may yield highly emissive species through the introduction of a vinyl or aromatic group (Scheme 1).[3,4] Inspired by the pioneering work, we herein wish to assemble a small fluorescence library of various biologically relevant alkenylated N-heteroarenes with tunable emission wavelength. However, conventional routes to such molecules usually involve the multistep synthesis of the alkaloid skeleton or the transition metal-catalyzed C–X/C–M cross-coupling reaction. The tedious multistep operation and prefunctionalization may restrict a rapid survey for molecular diversity. In this context, developing a concise and clean approach to alkenylated alkaloids is highly warranted for the rapid assembly of a structurally diverse fluorescence library.



Scheme 1 Selected examples of fluorescent xanthines and purines.

The Heck coupling reaction is one of the most reliable tools for the construction of heteroaryl alkenes through the palladium-catalyzed C–C cross-coupling of heteroaryl halides with alkenes. Over the past few years, transition metal-catalyzed direct C–H alkenylation of heteroarenes with vinyl halides has been well developed.[5] Clearly, the dehydrogenative Heck reaction (DHR) for the transition metal-catalyzed oxidative C–H/C–H cross-coupling of heteroarenes with alkenes would be the most ideal approach to alkenylated heteroarenes, which eliminates the complications associated with the use and preparation of both vinyl halides and heteroaryl halides.[6,7] Despite significant progress in the dehydrogenative Heck coupling of arenes and heteroarenes with alkenes, there are fewer examples describing the Pd-catalyzed oxidative C–H/C–H cross-coupling reactions between azoles and alkenes, which mainly refer to the alkenylation of C2-substituted azoles at the C4- or C5-position (probably due to the problematic oxidative homocoupling and decomposition of C2-unsubstituted azoles under the oxidative reaction conditions).[8] Following our continuing interest in the direct (hetero)arylation of xanthines and purines,[4c,9] we herein wish to describe the dehydrogenative Heck coupling of various biologically relevant N-heteroarenes (*e.g.*, xanthines, purines, and indolizines) with alkenes.[10]

8-Vinyl xanthines are known as selective antagonists for the human A_{2A} adenosine receptors.[11] Thus, our investigation started with the dehydrogenative coupling of caffeine **1a** with *n*-butyl acrylate **2a** as a model reaction to optimize the reaction conditions. After screening a number of parameters (Tables S1 and S2, ESI†), we found that Cu(I) salts, additives, oxidants, and solvents all played critical roles. The best result was obtained when the reaction was performed in the presence of $Pd(OAc)_2$ (2.5 mol%), CuCl (15 mol%), $Cu(OAc)_2{\cdot}H_2O$ (1.5 equiv.) and pyridine (1.0 equiv.) in DMA at 120 °C for 20 h. Under the optimized reaction conditions, a relatively broad range of olefins were then tested. As shown in Table 1, activated alkenes such as α,β-unsaturated esters and amides smoothly coupled with caffeines to give the corresponding alkenylated products in satisfactory yields (Table 1, **3a–d**). In addition, various aryl alkenes with both electron-rich and

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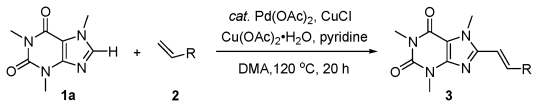
Table 1 Pd/Cu-catalyzed dehydrogenative alkenylation of caffeine with alkenes[a,b]



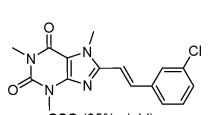
Product	R	λ_{ex}/nm	λ_{em}/nm	Yield[c] (%)
3a	$CO_2{}^nBu$	353	422	81
3b	CO_2Me	353	423	66[d]
3c	$CO_2{}^tBu$	348	420	62
3d	$CONMe_2$	348	402	84
3e	C_6H_5	354	417	68
3f	4-F–C_6H_4	353	417	82
3g	4-Cl–C_6H_4	358	423	70
3h	4-Me–C_6H_4	355	418	62
3i	4-OAc–C_6H_4	356	421	58

[a] Conditions: caffeine (0.5 mmol), alkene (3.0 mmol), $Pd(OAc)_2$ (2.5 mol%), CuCl (15 mol%), $Cu(OAc)_2{\cdot}H_2O$ (0.75 mmol), and pyridine (0.5 mmol) in a 0.6 M DMA solution at 120 °C for 20 h. [b] Absorption and emission data in CH_2Cl_2. [c] Isolated yield. [d] Methyl acrylate (4.5 mmol) was used owing to its volatility. DMA: *N*,*N*-dimethyl acetamide.

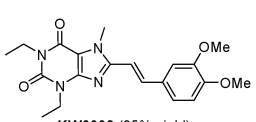
electron-poor substituents also worked well (Table 1, **3e–i**). Notably, the reaction conditions were compatible with functional groups such as esters, amides and halogens, which are useful for further synthetic transformations.

Besides caffeine derivatives, this protocol was applicable to other xanthines to prepare 8-alkenylated theophylline and theobromine derivatives (Table 2, **4a–d**). For example, the N1-benzylic theobromine coupled with *n*-butyl acrylate **2a** to give **4b** in 58% yield. The benzyl group can be conveniently removed through hydrogenation to give 8-alkenylated free (NH)-xanthines. It is worth noting that the N1-allyl-substituted theobromine could undergo the coupling reaction to form the desired product **4c** in 51% yield. The competitive Heck reaction of the allylic group was not yet observed. In addition, the free (NH)-1,3-diethylxanthine coupled with an alkene without the need for protecting the N1–H group of imidazole (Table 2, **4d**). We were delighted to find that purine nucleobases could also be alkenylated in good yields (Table 2, **4e–h**). Interestingly, the alkenylation of electron-rich indolizines selectively afforded the linear β-products rather than the branched α-products developed by Zhang and co-workers (Table 2, **4i–m**).[7g]

Utilizing this double C–H activation strategy, the pharmaceutically active molecules **CSC** and **KW6002**, which have been applied in clinical trials, were synthesized in a single step from the readily available xanthine derivatives in 65% and 95% yields, respectively.[11] Thus, this concise and efficient synthesis may provide an opportunity to rapidly assemble a combinatorial library for drug screening.



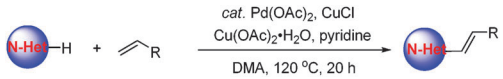
CSC (65% yield)
$\lambda_{ex}/\lambda_{em}$ = 357/425 nm (in DCM)

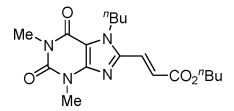


KW6002 (95% yield)
$\lambda_{ex}/\lambda_{em}$ = 362/428 nm (in DCM)

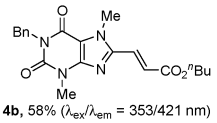
With these fluorescent scaffolds in hand, we investigated in detail their fluorescent properties and found that these alkenylated compounds exhibited strong fluorescence both in solution and in the solid state. Clearly, the fluorescence emission wavelengths were

Table 2 Pd/Cu-catalyzed dehydrogenative alkenylation of biologically relevant N-heteroarenes with alkenes[a,b,c]

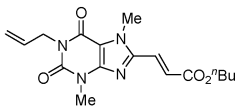




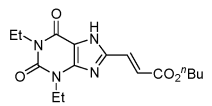
4a, 83% ($\lambda_{ex}/\lambda_{em}$ = 349/421 nm)



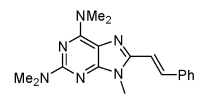
4b, 58% ($\lambda_{ex}/\lambda_{em}$ = 353/421 nm)



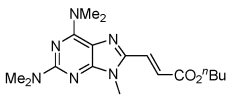
4c, 51% ($\lambda_{ex}/\lambda_{em}$ = 351/422 nm)



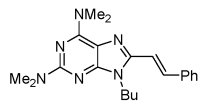
4d, 71% ($\lambda_{ex}/\lambda_{em}$ = 349/417 nm)



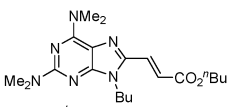
4e, 92% ($\lambda_{ex}/\lambda_{em}$ = 395/483 nm)



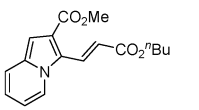
4f, 75%[d] ($\lambda_{ex}/\lambda_{em}$ = 417/510 nm)



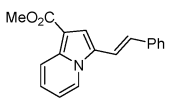
4g, 90% ($\lambda_{ex}/\lambda_{em}$ = 395/482 nm)



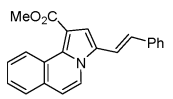
4h, 75%[d] ($\lambda_{ex}/\lambda_{em}$ = 417/508 nm)



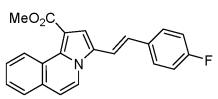
4i, 60%[e] ($\lambda_{ex}/\lambda_{em}$ = 394/446 nm)



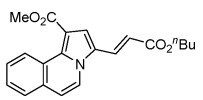
4j, 47%[e] ($\lambda_{ex}/\lambda_{em}$ = 368/461 nm)



4k, 64%[e] ($\lambda_{ex}/\lambda_{em}$ = 367/435 nm)



4l, 62%[e] ($\lambda_{ex}/\lambda_{em}$ = 365/434 nm)



4m, 68%[d,e] ($\lambda_{ex}/\lambda_{em}$ = 381/433 nm)

[a] Conditions: N-heteroarene (0.5 mmol), alkene (3.0 mmol), $Pd(OAc)_2$ (2.5 mol%), CuCl (15 mol%), $Cu(OAc)_2{\cdot}H_2O$ (0.75 mmol), and pyridine (0.5 mmol) in a 0.6 M DMA solution at 120 °C for 20 h. [b] Absorption and emission data in CH_2Cl_2. [c] Isolated yield. [d] $Cu(OAc)_2{\cdot}H_2O$ (1.5 mmol, 3 equiv.) was used. [e] $Pd(dppf)Cl_2$ instead of $Pd(OAc)_2$.

related to the electronic nature and π-extended molecular framework. As shown in Fig. 1, the emission colors in CH_2Cl_2 could be tuned from blue to yellowish green by modifying the N-heteroarene cores and the substituents at the C=C double bonds. The fluorophore **4f** showed the longest emission wavelength (λ_{em} = 510 nm, CH_2Cl_2), which indicated the existence of an intramolecular charge transfer (ICT) from the electron donor (NMe_2) to the electron acceptor ($CO_2{}^nBu$) upon excitation by light. Compared with **4j**, **4k** exhibited a hypsochromic shift from 461 to 435 nm, although its π-conjugated system is larger than **4j** (Table 2). As illustrated in Fig. 1b and d, these alkenylated N-heteroarenes also exhibited strong solid-state fluorescence properties, and the emission wavelengths covered the range of 458–566 nm.

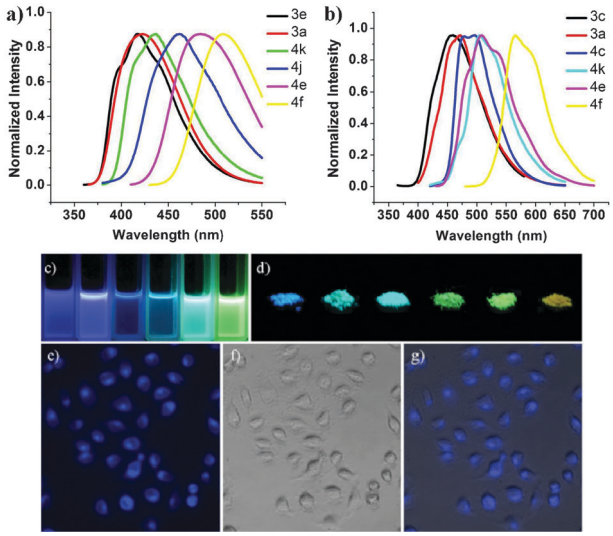


Fig. 1 (a) Fluorescent spectra of alkenylated N-heteroarenes **3** and **4** in CH_2Cl_2 (signal intensities have been normalized). From left to right: **3e**, **3a**, **4k**, **4j**, **4e**, and **4f**. (b) Solid-state fluorescent spectra of **3** and **4** in powder (signal intensities have been normalized). From left to right: **3c**, **3a**, **4c**, **4k**, **4e**, and **4f**. (c) Fluorescence images in CH_2Cl_2, irradiated at 365 nm. From left to right: **3e**, **3a**, **4k**, **4j**, **4e**, and **4f**. (d) Fluorescence images of powder samples (λ_{ex} = 365 nm). From left to right: **3c**, **3a**, **4c**, **4k**, **4e**, and **4f**. (e) Fluorescence image of SMMC-7721 cells incubated with **4f** (5 μM). (f) Bright-field transmission image of SMMC-7721 cells incubated with **4f** (5 μM). (g) Overlay of the fluorescence and bright-field transmission images of SMMC-7721 cells incubated with **4f** (5 μM).

Given that nucleobase analogues are capable of undergoing base pairing, live cell imaging experiments were performed with the alkenylated purine **4f** as a representative example. The human hepatocellular carcinoma cells (SMMC-7721) were incubated with **4f** in a physiological saline solution containing 1% DMSO for 40 min at 37 °C after being cultured in DMEM Dulbecco's minimum essential medium (containing 10% fetal bovine serum (FBS), 100 IU mL^{-1} penicillin, and 100 mg mL^{-1} streptomycin). As shown in Fig. 1e–g, **4f** successfully marked SMMC-7721. In addition, the MTT assays on HepG2 cells and A549 cells exhibited low toxicity of **4e**, **4f** and **3g** to cultured cells (Fig. S1 and S2, ESI†). These primary results demonstrated that the biologically relevant alkenylated N-heteroarenes would be valuable sources of imaging probes for biomarker discovery.

In summary, we have developed an efficient and versatile palladium/copper bimetallic catalyst system for the dehydrogenative Heck coupling of biologically relevant N-heteroarenes with alkenes, and elucidated how this strategy rapidly transforms the alkaloid scaffolds into fluorescent molecules. These resulting π-extended conjugated N-heteroarenes exhibit interesting fluorescent properties and have proven to be potentially useful reagents for biological imaging.

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