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Fluorescence sensing of caffeine in aqueous solution with carbazole-based probe and imaging application in live cells

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ABSTRACT

The host–guest interaction of caffeine in aqueous solutions has been achieved by using carbazole based imino-phenol receptors with oxopurines influences their fluorescence properties and can be exploited for 'turn-ON' fluorescence sensing of caffeine. This new fluorescent probe with high sensitivity and selectivity for detection and first time imaging of caffeine in living cells was developed in aqueous media.

Caffeine (3) is a widely consumed alkaloidal drug throughout the world.[1] The traditional significant sources of caffeine in our daily lives are coffee, black tea, guarana paste, cola nuts, cocoa beans etc. The alkaloid is also an ingredient of cola beverages and energy drinks.[2] While caffeine is regarded to be harmless for adults in general, there are severe concerns about unfavorable influences of caffeine on young children and pregnant women, including the risk of fetal death.[3] So the extensive and diverse consumption of caffeine requires further analytical tools to be developed for enhanced product safety. For caffeine quantitation, standard chromatographic methods are available such as (HP)TLC, HPLC-UV, LC, MIPs and CE.[4] However all the above methods require a long period of time, so methods requiring shorter time for analysis is needed. This area is left to chemosensors[5], which represent a vivid field in supramolecular chemistry. Several research groups[6–9] including our group[10–13] accomplished the synthesis of artificial caffeine receptors. All these H-bond receptors were only investigated in organic solvents; a significant selectivity and high affinity for the target molecule in aqueous media will be of particular interest for a promising application in caffeine detection.

Caffeine recognition in water has been studied with zinc based porphyrin peptide type receptor.[14] The another receptor cucurbit[7]uril containing macrocyclic host is able to bind caffeine in water using hydrophobic effects.[15] As part of our ongoing research toward the development of fluorophores for xanthine alkaloids,[10–13] here, we report that hydrogen bonding interactions between caffeine and carbazole based imino-phenol can be used for the fluorimetric detection of caffeine in water. To the best of our knowledge, this is the first report, where we explored the possibility of using carbazole based chemosensor (**1**) for the in vivo recognition of the caffeine in living cells using confocal imaging experiments.

It has long been known that caffeine interacts with polyphenolic molecules and these interactions influences the texture, the flavor, and the physiological effects of caffeine-containing beverages. So, our design of a water-soluble carbazole fluorosensor for caffeine possessing two imino-phenol moieties that have an affinity towards the ring oxygen and most basic imidazole 'N' of the caffeine. We chose carbazole as the signal transduction unit because of its chemical stability, large Stokes shift and high fluorescence quantum yield.[16]

Carbazole and its derivatives are an important type of nitrogen containing aromatic heterocyclic compounds, and it is present in a variety of naturally occurring medicinally active substances.[17] These characteristics result in the extensive potential applications of carbazole-based derivatives in the field of medicinal chemistry

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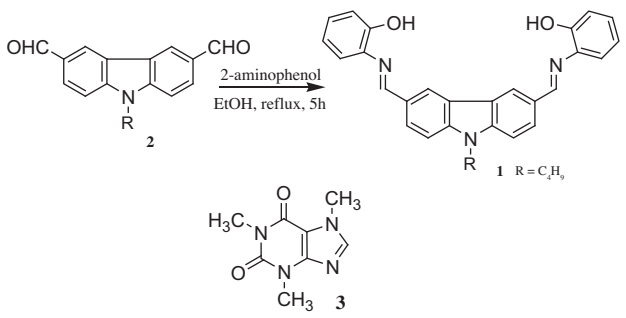
(antitumor, antimicrobial, antihistaminic, antioxidative, anti-inflammatory, psychotropic agents etc.).[18] These characteristics prompted us to investigate carbazole-based imino-phenol as a potential probe for the detection of caffeine in living cell.

Compound **2** was synthesized from carbazole in two steps according to our previous procedure.[19,20] Compound **1** was synthesized (Scheme 1) by condensation of N-butyl- carbazole-dicarbaldehyde **2** and *ortho*-aminophenol in high yield, and its structure has been proved by various spectroscopic characterizations can be found in Supplementary data Figures S1–S5.

The interaction of probe **1** with an aqueous solution of caffeine was investigated by spectrophotometric and spectrofluorimetric titrations in buffered aqueous solution (pH 7.2, 25 mM HEPES buffer) as shown in Figure 1.

During the photometric titration of caffeine to probe **1**, a absorption band at 275 nm was gradually enhanced, while the little intensity of absorption at 343 nm was decreased correspondingly. A clear isosbestic point was observed at 299 nm that indicate that one complex structure is formed. The data of the photometric titration were employed to determine the association constant for the complex of probe **1** with caffeine $(1.75 \pm 0.36) \times 10^3\ M^{-1}$.The interaction of probe **1** with caffeine was also studied by fluorescence spectroscopy. The emission spectra are shown in Figure 2. When caffeine was added to a buffered aqueous solution (pH 7.2, 25 mM HEPES buffer) of probe **1** (5 μM), a pronounced increase in fluorescence intensity was observed (λ_{max} = 431 nm, Fig. 2, inset). The inset shows a linear relationship, which is always important for easy and accurate analysis. A fluorescence titration experiment with caffeine concentrations between 0 and 50 mM was then performed. Judging from the titrations, continuous variation method was used to determine the stoichiometric ratios of the host and the specific guest, which was found to be a 1:1 probe (**1**)-to-caffeine complexation (Fig. S6). For a complex of 1:1 stoichiometry, the association constant $K_a = (1.56 \pm 0.31) \times 10^3\ M^{-1}$ could be determined by non-linear fitting analyses of the titration curves (Figs. S7–S8) according to literature report.[21]

The probe **1** presents some excellent advantages compared to known fluorescent sensor for caffeine[22] due to 'turn-ON' fluorescence response. The 'turn-ON' aqueous sensors has a number of advantages such as (a) it reduces the chance of a false positive, observed in some turn-off probes, (b) allows for the use of multiple probes, selective for different analytes, and (c) is applicable in the analysis of both, aqueous environmental and biological samples. The probe **1** itself is weak emissive because of rapid C=N isomerisation. The C=N isomerisation is the predominant decay process[23] of the excited state. The significant enhancement in fluorescence (10 times at 431 nm) is probably caused by the formation of a1:1 complex of probe **1** with caffeine in which the rotation of acyclic C=N isomerization is prevented upon caffeine binding and hence fluorescence enhancement occurs (Fig. S9). The probe



Scheme 1. Synthesis of Probe **1** and structure of Caffeine **3**.

1 was designed in such way that in which both the optical reporter carbazole and the caffeine binding imino-phenol part were water soluble, biocompatible and stable in buffer solution. So the sensitivity of probe **1** for caffeine was verified in living cells, indicating its potential for application in intracellular imaging of caffeine.

To understand the nature of interaction between probe **1** and caffeine, the fluorescence variation of probe **1** was measured upon the addition of caffeine with buffers adjusted different pH medium. Furthermore, to examine whether the acidic OH groups are plays crucial role during binding event of caffeine, we studied the interaction of caffeine with salt of probe **1**. Fluorescence titration experiments suggest that the emission intensity was affected (Fig. S10). In order to understand the binding potencies and sensing selectivities of probe **1**, parallel investigations were carried out with a variety of biologically relevant analytes such as the physiologically important xanthine derivatives theophylline and theobromine.

Figure 3 displays the changes in fluorescence of probe **1** upon addition various xanthine derivatives. As in the case of caffeine, a marked change of fluorescent emission of probe **1** was observed, but other xanthine derivatives showed negligible effect (however, addition of theobromine leads to a slight increasing). Accordingly, somewhat lower binding constants were obtained from fluorescence titration experiments ($K_a = 148 \pm 6\ M^{-1}$for theobromine and $K_a = 113 \pm 2\ M^{-1}$ for theophylline). None of the other tested analytes gave a pronounced fluorescence response (Fig. 3).This result demonstrated that probe **1** exhibited high selectivity towards caffeine in water.

In order to know more about interactions between caffeine and probe **1**, ^1H NMR titration was also performed in $CDCl_3$ and the results are shown in Figure 4. The phenolic proton and N=CH (imine) proton displayed down field shift with increasing addition of caffeine by 0.55 and 0.15 ppm respectively, indicating the presence of hydrogen bond interaction between acidic OH together with CH and caffeine. In contrast, other hydrogens in the carbazole ring were affected very small. These observations suggested that only the phenolic OH proton of probe **1** participated to complex with caffeine.

Binding of caffeine by probe **1** has also been investigated by quantum chemical calculations at the TDDFT level. The energy minimized structure of the caffeine–probe **1** complex and their HOMO−LUMO energy gap were computed by using Gaussian 2003(B3LYP/6-31G(*d,p*)).[24,25] The result indicates that the most favourable geometry is found for caffeine–probe **1** complex in Figure 5a, which consists of two hydrogen bonds with bond distances 1.89 and 1.84 Å respectively (see Supplementary data). The π electrons on the HOMO of caffeine–probe **1** complex is mainly located on the whole π-conjugated carbazole framework (excluding the butyl group), but the LUMO is mostly positioned at the center of the guest caffeine. Moreover, the HOMO−LUMO energy gap of complex become smaller relative to that of probe **1** in Figure 5b. The energy gaps between HOMO and LUMO in the probe **1** and caffeine complex were 8.6832 eV and 8.3792 eV respectively (see Supplementary data).

To further explored the ability of the probe **1** to image caffeine in living cells (Fig. 6), we carried out experiments in live *Candida albicans* cells. The cells were pre-treated with probe **1** (10 μM in 0.01 M phosphate buffer, pH 7.4) at 37 °C for 30 min and then were incubated with caffeine (initially 5 μM) for 10 min the cells displayed intracellular fluorescence (Fig. 6b), indicating the ability of the probe can penetrate the cell membrane. The cells also exhibited more intense fluorescence when more caffeine was introduced onto the cells externally, and fluorescence responses increase with the increase in caffeine concentration, which could be evident from the cellular imaging (Fig. 6c–f). Moreover, the cells treated with various concentrations of probe **1** for up to 2 h, the result showed no significant

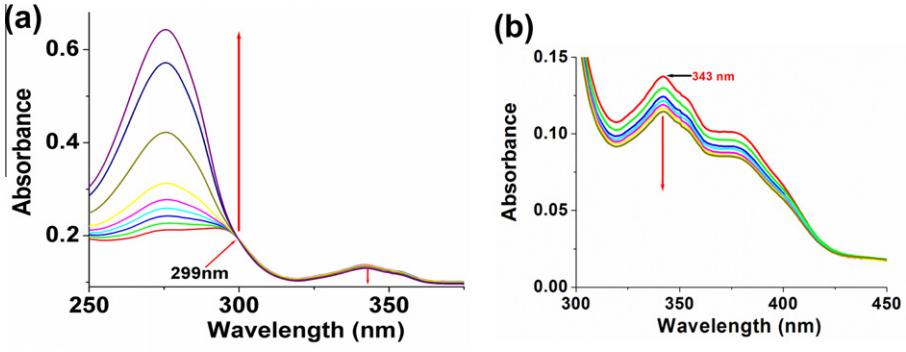


Figure 1. (a) UV–vis spectra of probe **1** ($c = 1.0 \times 10^{-5}$ M) (5 μM) with the increasing concentrations of Caffeine (0–2 equiv) in pH 7.2 HEPES buffer (25 mM , pH 7.2, containing 1.0% methanol). (b) Enhancement of UV–Vis spectra between 300 and 450 nm.

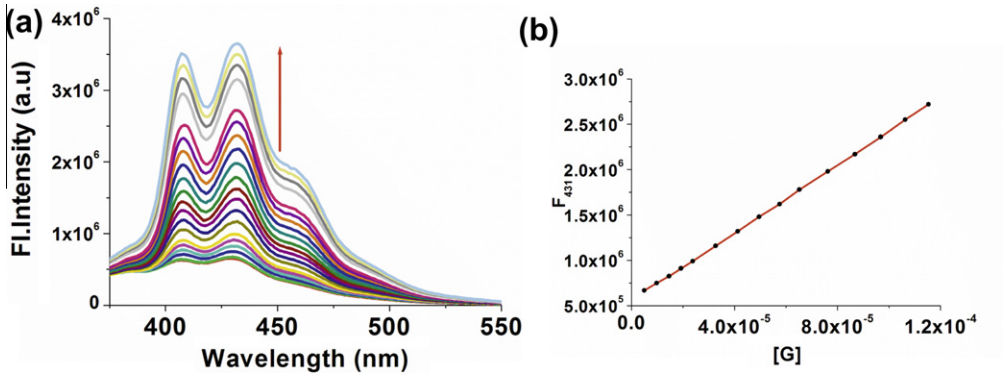


Figure 2. (a) Fluorescence spectra of probe **1** (5 μM) ($c = 1.0 \times 10^{-5}$ M) with the increasing concentrations of Caffeine (0–50 mM) ($c = 5.0 \times 10^{-4}$ M) in pH 7.2 HEPES buffer (25 mM, pH 7.2, containing 1.0% methanol). (b) The fluorescence intensity changes at 431 nm of probe **1** (5 μM) ($c = 1.0 \times 10^{-5}$ M) with the amount of Caffeine ($c = 5.0 \times 10^{-4}$ M).

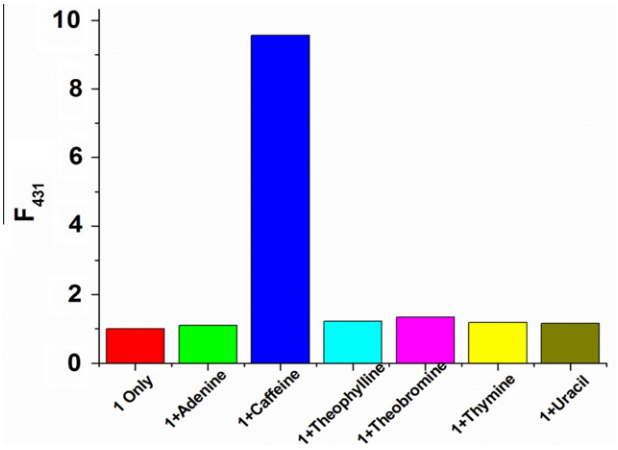


Figure 3. Bar diagram showing interaction of probe **1** (5 μM) ($c = 1.0 \times 10^{-5}$ M) with the increasing concentrations of various xanthine derivatives (0–50 mM) ($c = 5.0 \times 10^{-4}$ M) in pH 7.2 HEPES buffer (25 mM, with tested pH 7.2, containing 1.0% methanol) at 431 nm.

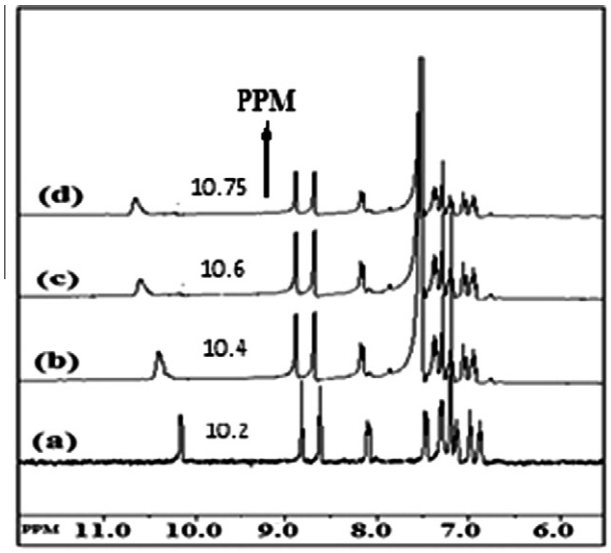


Figure 4. ^{1}H NMR (300 MHz) spectra of Compound **1** (a), **1** + .5 equiv Caffeine (b), **1** + 1 equiv Caffeine (c), and **1** + 1.2 equiv Caffeine in $CDCl_3$(d).

cell death in 2 h incubation, indicating **1** was of low toxicity or non-toxic[26] to cultured cells under the experimental conditions. The results demonstrate for the first time that probe **1** is potentially useful for monitoring caffeine in living organisms.

In conclusion, we present the carbazole based imino-phenol **1** as a promising fluorescent probe for xanthine alkaloids caffeine detection. Probe **1** exhibits a highly sensitive and selective fluorescence enhancement toward the caffeine over other xanthine related alkaloids in aqueous buffer (pH 7.2). It represents one of the few fluorescent probes that allow a selective 'turn-ON' detection of caffeine in water. Probe **1** was successfully expressed in cells, which demonstrates its potential usefulness as a molecular probe in biological systems.

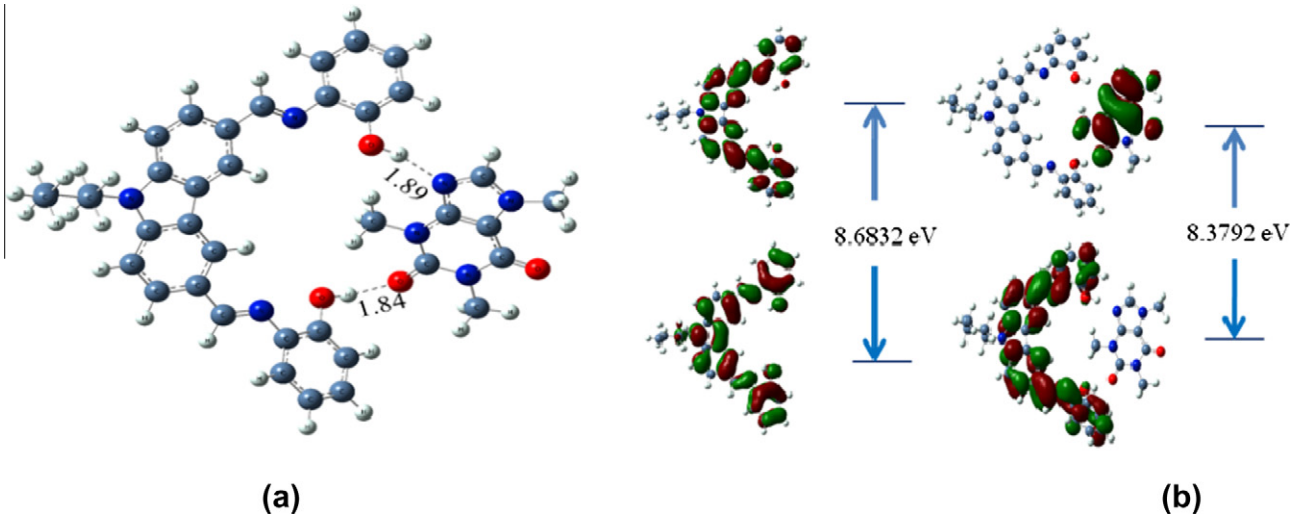


Figure 5. (a) Energy-minimized structure of the Probe **1**-Caffeine complex (b) HOMO and LUMO of Probe **1** and Probe**1**-Caffeine Complex.

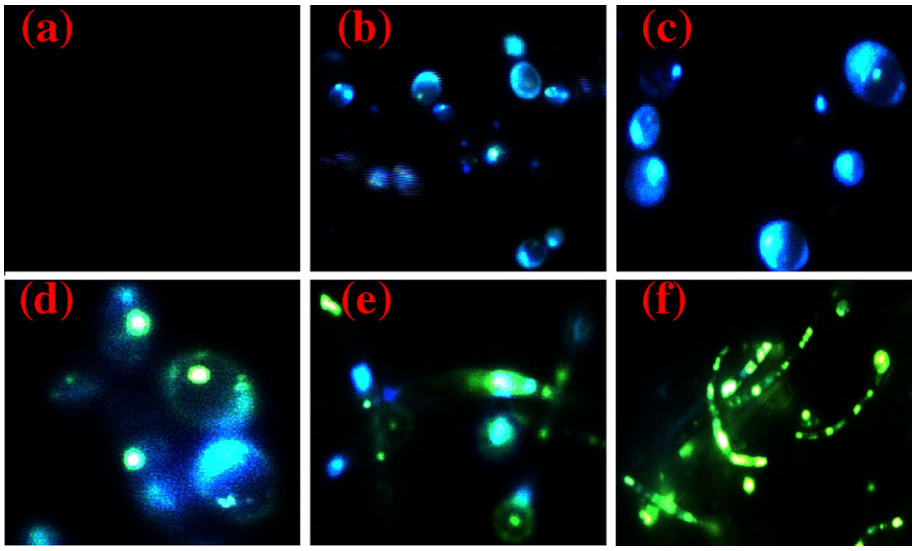


Figure 6. Fluorescence and brightfield images of living cells (a) images of *Candida albicans* cells + probe **1** (10 μM) (b) images of cells + probe **1** + caffeine (5.0 μM), and fluorescence images (c–f) of *Candida albicans* cells incubated with 10 μM, 20 μM, 30 μM, 40 μM of probe **1** for 25 min, respectively.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.07.055.

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