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Naked-eye fluorescent sensor for Cu(II) based on indole conjugate BODIPY dye

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ABSTRACT

A novel on/off fluorescent indole/BODIPY-based Cu^{2+} chemosensor (**3**) was synthesized by Knoevenagel condensation of BODIPY derivative **1** with 2-methyl-indole-3-carbaldehyde **2**. The identity of compound **3** was confirmed by ^{1}H NMR, ^{13}C NMR, FT-IR, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and single crystal X-ray diffraction techniques. Fluorescent chemosensor (**3**) is found to be highly selective and sensitive for detection of Cu^{2+} with a color change from purple to yellow. The optical sensor features for the Cu^{2+} complex of **3** were investigated by UV–Vis and fluorescence spectroscopy. The addition of Cu^{2+} caused quenching of fluorescence intensity and the detection limit was calculated to be 0.124 μM. Also, the stoichiometry ratio of **3**+$\mathbf{Cu^{2+}}$ was obtained 2:1 by Job's plot. Live cell image, flow cytometry and cytotoxic properties of compound **3** were examined.

1. Introduction

The design and construction of fluorescent chemosensors with high selectivity and sensitivity through metal ions such as iron, aluminium and copper has been the subject of intense study because of their potential application in supramolecular chemistry, organic chemistry, drug delivery, biological chemistry and environmental research [1–3]. Also, they have a significant sensitivity and selectivity for detecting metal ions in both aqueous and non-aqueous media [4–7] many chemosensors have been developed specific for Ca^{2+}, Hg^{2+}, Cu^{2+}, Zn^{2+} or other transition metals [8–13]. The selective recognition and sensing of soft metal ions is quite important as these cations play an important role in various biological processes, including iron absorption, haemopoiesis, various enzyme-catalyzed and redox reactions [14]. Among these transition metals, copper is known as third abundant transition metal element in human body and generally found as Cu(II) in natural water [15]. It is one of the most essential trace elements of importance for both physical and mental health and serves as a key co-factor for a wide variety of enzymes in living organism [16–19]. Copper overload can lead to vomiting, lethargy, increased blood pressure and respiratory rates, acute haemolytic anaemia, liver damage, neurotoxicity and neurodegenerative disease [20,21]. Furthermore, it can disrupt natural ecosystems due to their adverse effects on microorganism [22]. Recently, some fully water soluble fluorescence probes have been reported here represents the first example of cell-elective imaging. [23–25]. Consequently, much attention has been drawn to the development of highly selective fluorescent chemosensor for Cu^{2+} to satisfy the biological and environmental applications [26]. The limit for copper in drinking water, as set by the US Environmental Protection Agency (EPA) is 1.3 ppm (20 μM). Also, the average concentration of blood copper in the normal group is 5.7–23.6 μM.

Among the fluorescent sensors, boradiazaindacene (BODIPY) is one of the most important highly fluorescent dyes and extensively studied due to its excellent photo physical properties such as high fluorescence quantum yield, large extinction, high photo-stability, long absorption and fluorescence wavelengths [27]. These dyes are widely used as optical chemosensor, fluorescent bio-labelling reagents, light harvesting materials and photodynamic therapy reagents have been broadly investigated [28–31]. In particular, BODIPY-based metallic cation sensors have attracted great deal of attention [32]. BODIPY based fluorescent chemosensor derivatives for selective detection of metal ions such as Fe^{3+}, Al^{3+} and Cu^{2+} was recently reported [33,34].

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Indole is an aromatic heterocycle that is widely distributed in nature [35]. There has been a significant recent interest in synthesizing indole and its derivatives because of the utility of these compounds as fluorescence probe and also displayed wide range of biological activity such as anti-fungal, anti-inflammatory and anti-tumor properties [36–40].

Herein, we reported the facile synthesis of a new fluorescence chemosensor based on indole-conjugate mono-stryl BODIPY **3** which was served as reliable, more sensitive, more selective and more soluble for Cu^{2+} sensor. Compound **3** and Cu^{2+} ion complex were detected by UV–Visible absorption and fluorescence emission methods. Also, properties of compound **3** in live cells, flow cytometry and bacterial and mammalian cells were investigated.

2. Experimental

2.1. Materials

The deuterated solvent ($CDCl_3$) for NMR spectroscopy, silica gel, dichloromethane and metal chlorides were provided from Merck. Following chemicals were obtained from Sigma Aldrich; 2,4-dimethylpyrrole, trifluoroacetic acid, 2,3-dichloro-5,6-dicyano-1, 4-benzoquinone, trimethylamine, boron trifluoride diethyl etherate, glacial acetic acid, piperidine, 2-methylindole, phosphorus(V) oxychloride, *N*,*N*-dimethylformamide, benzene and 1,8,9-anthracenetriol for the MALDI matrix was obtained from Fluka. All other chemicals used for the synthesis were reagent grade unless otherwise specified.

2.2. Equipment

Electronic absorption spectra were recorded with a Shimadzu 2101 UV spectrophotometer in the UV–Visible region. Fluorescence excitation and emission spectra were recorded on a Varian Eclipse spectrofluorometer using 1 cm pathlength cuvettes at room temperature. The fluorescence lifetimes were obtained using Horiba- Jobin-Yvon-SPEX Fluorolog 3-2iHR instrument with Fluoro Hub-B Single Photon Counting Controller at an excitation wavelength of 470 nm. Signal acquisition was performed using a TCSPC module. Mass spectra were acquired in linear modes with average of 50 shots on a Bruker Daltonics Microflex mass spectrometer (Bremen, Germany) equipped with a nitrogen UV-Laser operating at 337 nm. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions on a Varian 500 MHz spectrometer. Analytical thin layer chromatography (TLC) was performed on silica gel plates (Merck, Kieselgel 60 Å, 0.25 mm thickness) with F_{254} indicator. Column chromatography was performed on silica gel (Merck, Kieselgel 60 Å, 230–400 mesh). Suction column chromatography was performed on silica gel (Merck, Kieselgel 60 Å, 70–230 mesh).

2.3. X-ray data collection and structure refinement

Unit cell measurements and intensity data collection were performed on an Bruker APEX II QUAZAR three-circle diffractometer using monochromatized Mo Kα X-radiation (λ = 0.71073 Å). Indexing was performed using APEX2 [41]. Data integration and reduction were carried out with SAINT V8.34A [42]. Absorption correction was performed by multi-scan method implemented in SADABS V2014/5 [43]. The structures were solved and refined using the Bruker SHELXTL Software Package [44]. All non-hydrogen atoms were refined anisotropically using all reflections with $I > 2\sigma(I)$. The C-bound H atoms were positioned geometrically and refined using a riding mode. The N-bound H atoms were located from the difference Fourier map and restrained to be 0.89 Å from N atom using DFIX and their position were constrained to refine on their parent N atoms with *U*iso(H) = 1.2*U*eq(N). Crystallographic data and refinement details of the data collection are given in Table 1. The final geometrical calculations and the molecular drawings were carried out by PLATON (version 1.17) and MERCURY CSD (version 3.5.1) program [45,46].

2.4. Live cell imaging and flow cytometry

Bacterial and mammalian cells were analysed by Nikon Eclipse 80i fluorescence microscopy utilizing TritC filter (Ex: 540/25 nm and Em:605/55 nm) and mammalian cells were analysed by BD Accuri C6 flow cytometer. For bacterial imaging Bacillus thuringiensis (Bt) vegetative cells and for mammalian cell imaging HuH7 cells were stained by 0.1 μM of compound **3** for 15 min. Flow cytometry analysis of HuH7 performed after staining of cells with 0.1 and 5 μM of compound **3** for 15 min.

2.5. Cytotoxicity assays

Bacterial toxicity of 0.1 μM of compound **3** was analysed against 4 different bacterial cells namely, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*. Colony forming units calculated and compared to DMSO treated control bacterial cells after 24 h incubation. The viability of HuH7 cells were examined by The CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega, USA) in 96-well culture plate format. HUH7 cells were treated with DMSO control and various concentrations (0.1, 0.5, 1, 5, 100 μM) of compound **3**. Absorbance was recorded at 490 nm using Varioskan™ Flash Multimode Reader (Thermo Scientific, USA).

3. Synthesis

Compound **1** was prepared with a minor revision by the procedure described previously [47]. The synthesis method of compound **2** was as follows based on literature [48] (Scheme 1).

Table 1
Crystal data and refinement parameters for **3**.

Formula	$C_{29}H_{26}N_3BF_2$
Formula weight (g mol^{-1})	465.34
Temperature (K)	293(2)
Wavelength (Å)	0.71073
Crystal system	triclinic
Space group	$P\bar{1}$
a (Å)	9.9821(5)
b (Å)	11.4520(5)
c (Å)	12.5022(5)
α (°)	85.206(3)
β (°)	67.687(3)
γ (°)	67.139(3)
Crystal size (mm)	0.072 × 0.144 × 0.305
V (Å^3)	1214.89(10)
Z	2
ρ_{calcd} (g cm^{-3})	1.272
μ (mm^{-1})	0.085
F(000)	488
θ range for data collection (°)	3.42–25.68
h/*k*/*l*	−12/12, −13/13, −15/15
Reflections collected	16342
Independent reflections (R_{int})	4603 (0.1028)
Absorption correction	Multi-scan
Data/restraints/parameters	4603/0/324
Goodness-of-fit on F^2	1.008
Final *R* indices [$I > 2\sigma(I)$]	R_1 = 0.0587, wR_2 = 0.1263
R indices (all data)	R_1 = 0.1242, wR_2 = 0.1528
Largest diff. peak and hole (e Å^{-3})	0.281 and −0.270