

Synthesis, UV/Vis and Fluorescence studies of Naphthoquinone Pyridyl Tetrazole-based Chemical Probe

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Balakrishna *et al.*: Fluorescence studies of Naphthoquinone-based Chemical Probe

2-(dimethylamino)-3-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)naphthalene-1,4-dione (2) has been synthesized. The UV/Vis studies of 2-(dimethylamino)-3-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)naphthalene-1,4-dione (2) with different metal ions were performed in aqueous dimethylformamide and were correlated with fluorescence studies of the same. The compound showed higher selectivity and sensitivity for Zn²⁺ ions. Thus this compound could be utilized as a turn-on probe for fluorescent imaging of Zn²⁺ ions in living cells.

Key words: 2,3-dichloronaphthalene-1,4-dione, pyridyl-tetrazole, fluorescence, UV/Vis studies, chemical probe, metal complex

Metals like Na⁺, K⁺ and Mg²⁺ play an important role in biological systems, which enables different processes like transmission of information^[1-5]. d-Block metals such as zinc (Zn²⁺) and copper (Cu²⁺) play important structural and catalytic roles in neurology related diseases like neurophysiology and neuropathology. Accumulation of metals like Cd²⁺ in the brain leads to cancer^[6]. This ion binds in high concentrations in brain systems. In case of brain injury, Zn²⁺ ions induce the selective neuronal cell deaths^[7]. Even though the roles of various metal ions in biological systems are known, the full mechanism of the process is still unknown. Compared to conventional techniques like atomic absorption spectroscopy^[8] and autoradiography^[9], molecular imaging with fluorescent probes, genetically encoded sensors and hybrid probes are playing vital roles to label or sense the presence of metals^[10]. In this view, various chemical probes are developed to study the fluorescence phenomena^[11]. As per the existing literature, probes should not contain their excitation in UV range as they can either damage the cells or emit auto-fluorescence by biological system^[12]. To overcome this defect, various groups developed different chemical probes, which are having excitation in long wavelength region^[13-16].

The work reported by Raulin^[17] and Frederickson *et al.*^[18] left great encouragement for the development of chemical probes especially for the Zn²⁺ ions. Recently

G-quadruplex protein binding probes were developed for the detection of specific biomarkers associated with various human diseases^[19-22]. The use of different fluorophores like pyrazoline, cyanine and rhodamine dyes have been developed as various chemical probes to identify specific metal like Zn²⁺, which is the highest abundant metal after iron in the human body^[23-29]. Bioinformatic researchers have also shown the importance of Zn²⁺ in the biological systems^[30-32]. In the present work, a chemical probe was synthesized, which has an excitation in the visible region. The probe was allowed to complex various metal ions in aqueous dimethylformamide (DMF) and their UV/Vis and fluorescence properties were studied.

All reagents were purchased from Sigma-Aldrich and were used without further purification. DMF (AR grade) was used to perform analytical studies. Metal chloride salts procured from Merck were used as the source for metal ions. The solvents used in the synthesis were distilled before use. Absorption measurements were carried out using a Shimadzu UV-1800 spectrophotometer. Fluorescence spectra were

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recorded on a Perkin-Elmer LS55 spectrophotometer. The slit width was 10 nm for both excitation and emission. All melting points were obtained on an Elico instrument, India (Model MP96) and were uncorrected. Mass spectra (MS) were obtained on a Pexciex API 2000 eV spectrometer; Q1MSQ1/auto injection mass spectra. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Top Spin instrument. Infrared spectra were recorded using an Alpha T OPUS spectrometer. Silica Gel 60 (60-120 mesh) was used for column chromatography.

Synthesis of the compound 2-(1H-tetrazol-5-yl)pyridine (1) was carried out as per the available literature^[33]. Synthesis of 2-(dimethylamino)-3-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)naphthalene-1,4-dione (2) was carried out by adding to a solution of 1 (735 mg, 5 mmol) in DMF (20 ml) 2,3-dichloronaphthalene-1,4-dione (1.1 g, 5 mmol) and K_2CO_3 (272 mg, 2 mmol) and the reaction mixture was stirred for 24 h at 110° . The crude mass was poured over ice and the compound was extracted with ethyl acetate (2×50 ml). The organic layer was dried over magnesium sulphate and evaporated under reduced pressure to yield the crude compound 2, which was purified by column chromatography using hexane and ethyl acetate (2:1 v/v) to afford pure compound 2 (1 g, 2.8 mmol) as a yellowish-brown solid.

Yield of the compound 2 was 60 %; melting point: $87-90^\circ$; FTIR (KBr): ν (cm^{-1}) 2947, 2846, 2753, 1686, 1598, 1254, 1158; ^1H NMR (CDCl_3 , 300 MHz): δ (ppm) 8.81 (d, $J=4.2$ Hz, 1H, pyr- H_2), 8.32 (d, $J=7.8$ Hz, 1H, pyr- H_4), 8.18-8.08 (m, 2H, pyr- $\text{H}_{3,5}$), 7.88 (dt, $J=7.8, 0.75$ Hz, 1H, aromatic H_1), 7.84-7.67 (m, 2H, aromatic $\text{H}_{2,3}$), 7.43 (dd, $J=6.6, 1.5$ Hz, 1H, aromatic H_4), 2.95 (s, 6H, $\text{N}(\text{CH}_3)_2$); ^{13}C NMR (CD_3OD , 75 MHz): δ (ppm) 184.5 (1C, $\text{C}=\text{O}$), 179.5 (1C, $\text{C}=\text{O}$), 165.5 (C-tetrazole), 151.0 (C2, pyr), 147.4 (C6, pyr), 139.3 (C4, pyr), 136.5 (C, benzo), 135.8 (C, aromatic), 135.3 (C, benzo), 134.3 (C, aromatic), 133.5 (C, aromatic), 132.4 (C, aromatic), 129.8 (C, aromatic),

128.2 (C, aromatic), 126.8 (C5, pyr), 124.1 (C3, pyr), 44.0 (aliphatic C2-N); ESI-MS ($\text{M}+1$)=347.

Metal chloride salts (Zn^{2+} , Co^{2+} , Ca^{2+} , Ba^{2+} , Ni^{2+} , Cu^{2+} , Pb^{2+} , Na^+ , K^+ and Mg^{2+}) were used as the source for metal ions. Ten millimolar stock solutions of metal ions were prepared in distilled water. A quantity of 1 mM of chemical probe 2 in DMF/ H_2O was buffered with phosphate buffered saline (PBS, $\text{pH}=7.4$).

The synthetic route started from picolinonitrile by following the method available in the literature to form the tetrazole 1 and the structure was confirmed by comparing the analytical data of 1 with the reported data^[29]. Tetrazole 1 on alkylation with 2,3-dichloronaphthalene-1,4-dione in dry DMF in presence of potassium carbonate at 110° afforded a single isomer by alkylation at N(2) position of tetrazole rings (fig. 1). The ^1H NMR spectra of compound 2 showed characteristic pyridine peaks at δ 8.81, 8.32, 7.88 7.43 and those of naphthaquinone were at δ 8.18-8.08, 7.84-7.67. The six protons for *N,N*-dimethyl group appeared at δ 2.95 as a singlet (fig. 2). The ^{13}C NMR showed a characteristic peak at δ 165.5 (fig. 3) proved the alkylation at N2 position^[29]. The absence of peak at δ 153, which was characteristic of N1 isomer is also proved the formation of N2 isomer. The ESI-MS showed ($\text{M}+1$) peak at 347 (fig. 4), which proved the structure of the compound.

The UV/Vis spectral studies were carried out with and without metal ions such as Zn^{2+} , Co^{2+} , Ca^{2+} , Ba^{2+} , Ni^{2+} , Cu^{2+} , Pb^{2+} , Na^+ , K^+ and Mg^{2+} . The chemical probe 2 contains naphthanone and tetrazole nitrogen atoms, so it can provide a platform for binding metal ions such as Zn^{2+} , Co^{2+} , Ca^{2+} , Ba^{2+} , Ni^{2+} , Cu^{2+} , Pb^{2+} , Na^+ , K^+ and Mg^{2+} . The UV/Vis absorption spectra of chemical probe 2 in the presence of various metal ions Zn^{2+} , Co^{2+} , Ca^{2+} , Ba^{2+} , Ni^{2+} , Cu^{2+} , Pb^{2+} , Na^+ , K^+ and Mg^{2+} using their chloride salts in PBS buffer (20 mM pH 7.4) solutions (DMF:water = 2:8) are shown in fig. 5. The absorption spectrum of chemical probe 2 exhibited

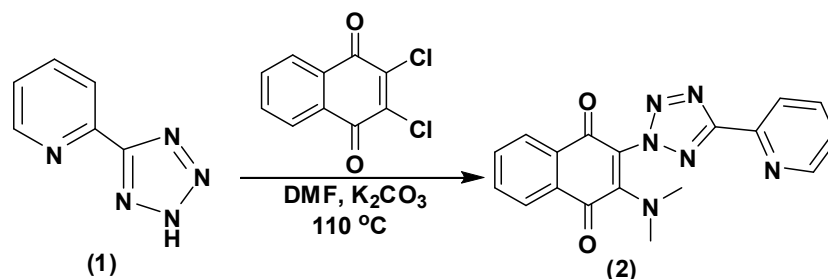


Fig. 1: Synthesis of chemical probe 2
2-(1H-tetrazol-5-yl)pyridine (1); 2-(dimethylamino)-3-(5-(pyridin-2-yl)-1H-tetrazol-1-yl)naphthalene-1,4-dione (2)

a broad band at 436 nm. The absorption maximum changed with the various metal ions such as Zn^{2+} , Co^{2+} , Ca^{2+} , Ba^{2+} , Ni^{2+} , Cu^{2+} , Pb^{2+} , Na^+ , K^+ and Mg^{2+} in aqueous DMF. Except Co^{2+} and Ba^{2+} , the addition of other metal ions to chemical probe 2 showed an increase in absorption. Upon addition of Zn^{2+} ions, the absorption peak of chemical probe 2 at 436 nm was increased to a maximum extent compared to all other metal ions (fig. 5).

The fluorescence properties of chemical probe 2 at 436 nm were investigated in PBS buffer (20 mM pH 7.4) solution (DMF:water = 2:8). The emission maximum for all the complexes was observed at 549 nm. After addition of Co^{2+} , Ca^{2+} , Ba^{2+} , Ni^{2+} , Cu^{2+} , Pb^{2+} , Na^+ and K^+ is not shown any significant change in emission. Whereas Zn^{2+} ions showed the more enhancement in the fluorescence intensity is shown in fig. 6. This observation is revealed that the formation

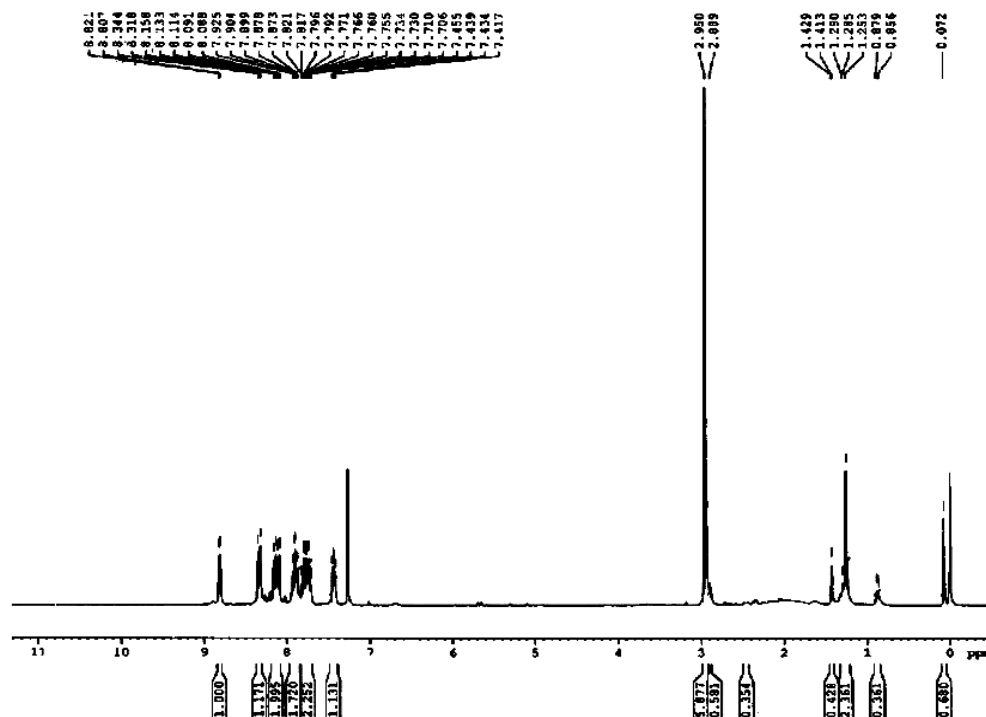


Fig. 2: The 1H -NMR spectrum of chemical probe 2

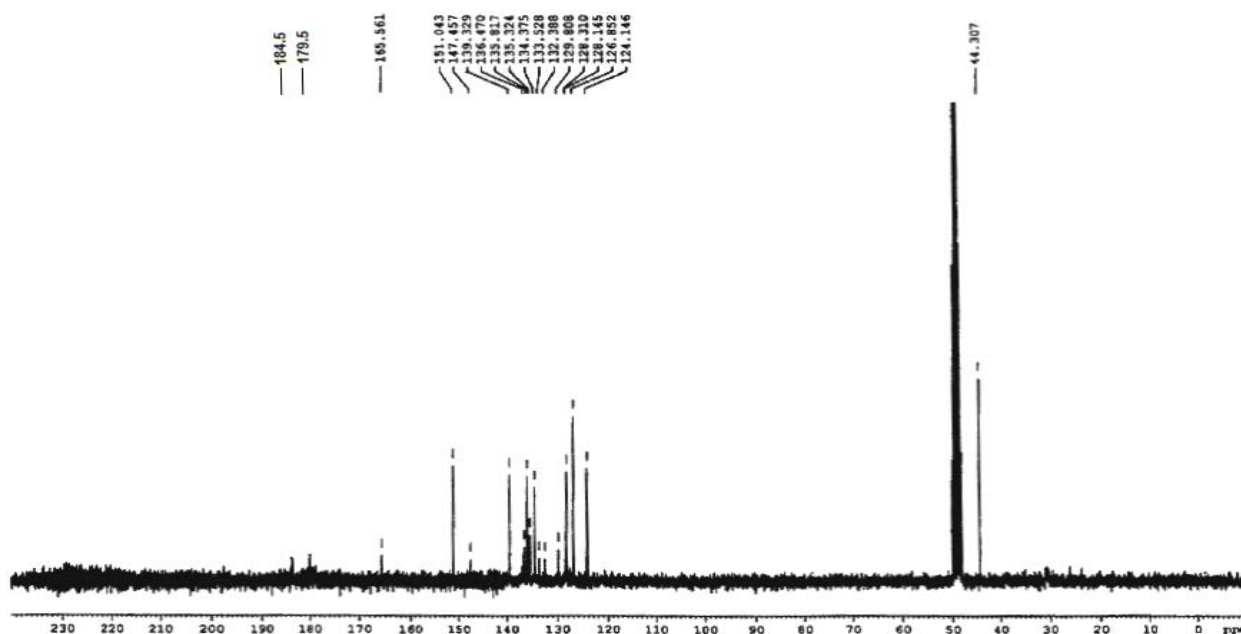


Fig. 3: The ^{13}C -NMR spectrum of chemical probe 2

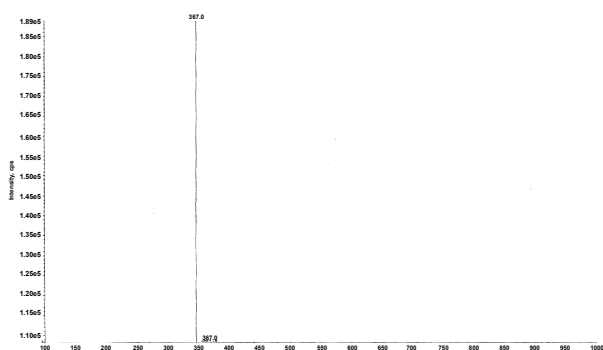


Fig. 4: The mass spectrum of chemical probe 2

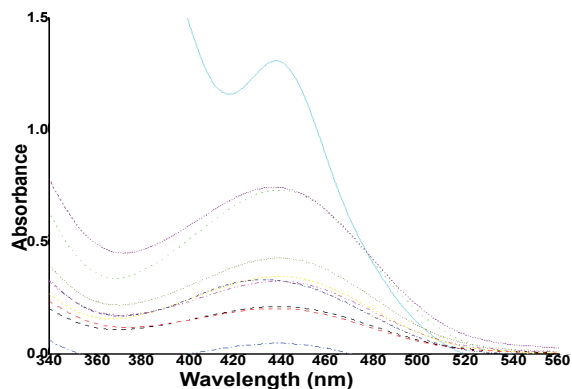


Fig. 5: UV/Vis spectrum of chemical probe 2 in presence of metal ions

UV/Vis spectrum of chemical probe 2 (10 μ M) in presence of Zn^{2+} , Co^{2+} , Ca^{2+} , Ba^{2+} , Ni^{2+} , Cu^{2+} , Pb^{2+} , Na^+ , K^+ and Mg^{2+} (50 μ M) in PBS buffer (20 mM, pH 7.4) solution (DMF:water = 2:8); --- Probe; --- Ba; --- Ca; --- Co; --- Zn; --- K; --- Mg; --- Cu; --- Ni; --- Pb

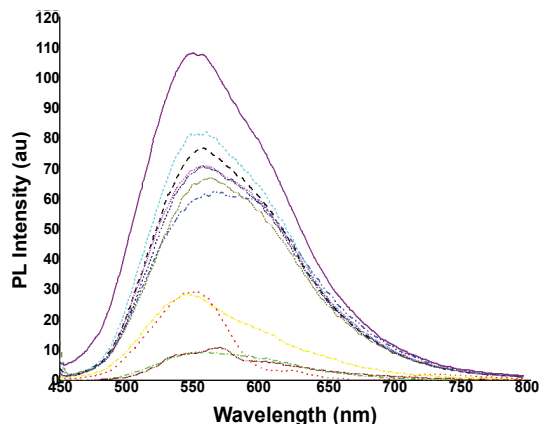


Fig. 6: Fluorescence emission spectrum of chemical probe in presence of metal ions in PBS

Fluorescence emission spectrum of chemical probe (10 μ M) in presence of Zn^{2+} , Co^{2+} , Ca^{2+} , Ba^{2+} , Ni^{2+} , Cu^{2+} , Pb^{2+} , Na^+ , K^+ and Mg^{2+} (50 μ M) in PBS buffer solution; --- Ca; --- Co; --- Cu; --- K; --- Mg; --- Na; --- Ni; --- Pb; --- Probe; --- Zn; --- Fe. Excitation was performed at 425 nm. Slit 5 nm/5 nm

of a complex between chemical probe 2 and Zn^{2+} ions and highest selectivity of chemical probe 2 to Zn^{2+} is disclosed. This can be assigned to $\pi \rightarrow \pi^*$ transition and an intra-molecular charge transfer band from

the naphthoquinone tetrazole moiety. This indicates the selectivity of compound 2 towards specific metal ion (Zn^{2+}) with its high fluorescence emission peak. Therefore the compound 2 can be utilised as a turn-on fluorescent probe for the detection of Zn^{2+} ions in living cells.

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Conflict of interest

The authors declare no conflicts of interest.

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