Coumarin Based Highly Sensitive and Selective Ratiometric Fluorescence Sensor for Chromium Ions in Aqueous Media

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***Abstract*: - A fluorescent sensor, 6-(bis((1H-benzo[d]imidazol-2-yl)methyl)amino)-2H-chromen-2-one (BIC), based on 2-chloromethyl benzimidazole with 6-amino coumarin as receptor, was synthesized. Both BIC and its Cr(III) complex are well characterized by different spectroscopic techniques like ^{1}HNMR, QTOF–MS ES^{+}, FTIR and elemental analysis as well. BIC exhibits fluorescence enhancement upon binding Cr(III) in aqueous (water: methanol =7:3, v/v) solutions. Detection limit of the method is 1 x10^{-7} M. Binding constant is estimated with the Benesi-Hildebrand method and the value 1.39 x10^{5} indicates a fairly strong interaction between BIC and Cr(III) . There has no fluorescence response seen towards other competing cations. BIC a water soluble off-on type ratiometric Cr(III) selective fluorescent probe and used for biological applications like living cell imaging at physiological pH using a confocal microscope. The fluorescence enhancement can be ascribed to the CHEF effect associated with better rigidity and planarity of the sensor molecules induced by chelation of Cr(III). The design strategy and remarkable photophysical properties of sensor BIC help to extend the development of fluorescent sensors for metal ions.**

Keywords: **Coumarin, 2-chloromethyl benzimidazole, Turn-on fluorescent probe, Cr(III), living cells.**

I. INTRODUCTION

Chromium is the new entry, after lead, cadmium and mercury in the major toxic metal series. Chromium exists in the environment mainly in two stable oxidation states, Cr(III) and Cr(VI). This two species differ in charge, physicochemical properties and in chemical and biological reactivity. Trivalent chromium is relatively immobile and less toxic because its compounds are usually weakly soluble in aquatic media and it also forms stable complex with soil minerals. Cr(III) is an essential micro nutrient for maintenance of "glucose tolerance factor" whereas excess Cr(III) is harmful to human health [1]. The National Research Council has recommended 50–200 μg d^{-1} as the safe and proper quantity of Cr(III) .On the other hand, Cr(VI) is extremely toxic and potentially carcinogenic [2]. WHO states that the guideline values of 50 μg L^{-1} Cr(VI) are considered to be too high compared with its genotoxicity [3]. Higher oxidation potential and relatively smaller size of Cr(VI) enables it to penetrate biological cell membranes. Cr(III) has great impacts on the metabolism of carbohydrates, fats, proteins and nucleic acids by activating certain enzymes and stabilizing proteins and nucleic acids [4]. Insufficient dietary intake of Cr(III) leads to increases in risk factors associated with diabetes and cardiovascular disease, including elevated levels of circulating insulin, glucose, triglycerides and total cholesterol, and impaired immune function [5]. Chromium can be also released to the environment from anthropogenic sources because it is widely used in manufacturing processes such as tanning, steel works, plating, corrosion control, chromate and chrome pigment production [6-8]. At the same time, it is an environmental pollutant that has caused concern in industry and agriculture, [9] and so systematic investigations into the specific detection of Cr(III) still need to be developed, especially for aqueous systems [10]. Severe matrix interferences restrict its direct determination with sufficient sensitivity and selectivity with flame atomic absorption spectrometry (FAAS) [11, 12], graphite furnace atomic absorption spectrometry (GFAAS) [13, 14], inductively coupled plasma atomic emission spectroscopy (ICPAES) [15], X-ray fluorescence spectrometry [16] and electrochemical methods [17-18]. Some forms of preliminary separation and pre-concentration like liquid–liquid extraction [19-20], cloud point extraction [21], ion-exchange [22, 23] and solid phase extraction [24-26] etc. are required.

Fluorescence method has more advantages over all the other mentioned methods due to its operational simplicity, high selectivity, sensitivity, rapidity, nondestructive methodology, enhanced sensitivity, high sampling frequency and low cost of equipment and direct visual perception [27]. For an efficient fluorescent sensor, in addition to high selectivity towards the target ion, a significant change in the fluorescence intensity and /or a spectral change of the probe are essential upon its interaction with the specific analyte [28].

Paramagnetic property of Cr(III) and lack of Cr(III) selective ligand are the two major culprits for the development of suitable fluorescent turn-on sensors for monitoring intracellular Cr(III). Samanta et al. [29] reported di-(2-ethylsulfanylethyl)amine as a Cr(III) selective receptor in tetrahydrofuran. Liu et al. [30] reported two fluorescent sensors capable of discriminating Fe(III) and Cr(III) . 8-hydroxyquinoline containing rhodamine B derivative [31] is used for bio-imaging of Cr(III) in contaminated cells. Zhou et al. [32] reported a ratiometric FRET-based fluorescent probe

for imaging Cr(III) in living cells. A Dansyl-based Cr(III) selective fluorescent chemosensor is reported by Wu et al. [33].

On the other hand, coumarin derivatives are well known to have diverse applications such as anticoagulants, spasmolytics, anticancer drugs or as plant growth regulating agents [34-35]. Coumarin and its derivatives [36] possess antibacterial [37], antithrombotic and vasodilatory [38], antimutagenic [39], lipoxygenase and cyclooxygenase inhibition [40] properties.

From the above discussion, it is evident that the use of coumarin derivative as a fluorescent probe for trace level determination of Cr(III) and monitoring of intracellular Cr(III) in infected cells might be an important area of research. Considering all these facts, herein we report the synthesis, characterizations and cell imaging studies of a new Cr(III) selective fluorescent probe containing coumarin and 2-chloromethyl benzimidazole units. Our present method is much more superior to our earlier one because it is a fluorescence enhancement method while the earlier one was based on fluorescence quenching. More over the present probe can detect trace level Cr(III) in contaminated living cells under fluorescence microscope.

II. EXPERIMENTAL

2.1. Materials and Methods

2-chloromethyl benzimidazole and coumarin were purchased from Aldrich (USA) and S. D. Fine Chem. Ltd., India respectively. 6-aminocoumarin was synthesized starting from coumarin by following a published procedure [41]. Analytical reagent grade chemicals and spectroscopy grade solvents were used. Milli-Q 18.2 MΩ cm^{-1} water was used throughout all the experiments. The sources of Mn(II), Cr(III), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II)and Pb(II) ions are either their chloride, nitrate or perchlorate salts.

2.2. Physical measurements

^{1}HNMR spectra were recorded in $CDCl_3$ with a Bruker Advance 300 MHz using tetramethylsilane as the internal standard. Absorption and fluorescence spectra were recorded on Shimadzu Multi Spec 1501 absorption spectrophotometer and Hitachi F-4500 fluorescence spectrophotometer equipped with a temperature controlled cell holder, respectively. Mass spectrum was recorded in QTOF Micro YA 263 mass spectrometer in ESI positive mode. IR spectra were recorded on a Perkin Elmer FTIR spectrophotometer (model: RX-1). Micro analytical data (C, H, and N) were collected on Perkin Elmer 2400 CHNS/O elemental analyzer. The fluorescence imaging system was comprised of an inverted fluorescence microscope (Leica DM 1000 LED), digital compact camera (Leica DFC 420C), and an image processor (Leica Application Suite v3.3.0). The microscope was equipped with a mercury 50 watt lamp.

*2.3. Synthesis of (6E)-6-((anthracen-9-yl) methyleneamino)-2H- chromen-2-one) (**BIC**)*

6-Aminocoumarine (0.5 g, 3.1mmol) and 2-chloromethyl benzimidazole (1.26 g, 3.1 mmol), $NaHCO_3$ (1.26 mg, 3.1 mmol) were added to an anhydrous DMF (10 mL), then the suspended solution was refluxed for 24 h under a N_2 atmosphere (the reaction was monitored by thin-layer chromatography and ceased after the starting material disappeared). After the removal of DMF, the solid was extracted with chloroform. The organic layer was dried over anhydrous sodium sulfate. The product was further purified on a chromatography column (basic silica gel, ethanol and $CHCl_3$ as eluent) and identified by ^{1}HNMR and ESIMS. Yield 90%; m.p.112±2°C; ^{1}HNMR (300MHz, $CDCl_3$) (supplementary, Fig.S-1), δ: 4.53(s,2H), 4.84(s,2H), 6.16(d,1H), 6.45(d,1H), 6.66(s,1H), 6.84(d,1H), 7.26(m,4H), 7.36(d,1H), 7.53(d,4H), 7.98(s,2H). QTOF–MS ES+, (supplementary, Fig.S-2) displays two signals of m/z 422.03 and 443.98, which can be assigned as the signals for $[M+H]^+$ and $[M+Na]^+$, respectively; FT-IR (supplementary, Fig S-3) (KBr, νcm^{-1}) ν(COO), 1707; ν(C=N), 1572; UV-Visible spectrum (supplementary, Fig.S-4) in aqueous (water: acetonitrile = 7:3, v/v) solutions at 298K (λ_{max}, nm (ε, $10^3 M^{-1}$ cm^{-1}), 228(1.659), 243(2.061), 263(1.465), 275(1.600), 331(0.236), 362(0.259); Microanalytical data, Calculated for $C_{25}H_{19}N_5O_2$: C, 71.25; H, 4.54; N, 16.62,found: C, 71.23; H, 4.51; N, 16.60.

2.4. Synthesis of [Cr complex]

A 10 mL methanolic solution of $Cr(NO_3)_3$,$9H_20$(0.025 g, 0.062 mmol) was added slowly to a magnetically stirred 10 mL methanolic solution of the ligand (**BIC**) (0.028 g, 0.062 mmol). The mixture was stirred in air for 2 hr and then refluxed for 15 min whereby a clear solution was formed. Complex was obtained by evaporating the solvent. This was characterized from QTOF –MS ES^+, UV-Vis, IR spectroscopy and also by CHN analysis. ESI-TOF–MS ES^+, (supplementary, Fig S-5) $[M+H]^+$ = 681.99; FTIR(supplementary, Fig S-6) (KBr, υ cm^{-1}): ν(COO), 1718; ν(C=N), 1572; ν(NO_3),1385; UV-Visible spectrum (supplementary, Fig S-4) in aqueous (water: acetonitrile = 7:3, v/v) solutions at 298K (λ_{max},nm(ε,10^3 $M^{-1}cm^{-1}$), CH_3OH) 233(2.295), 241(2.424), 261(1.660), 270(1.847), 273(1.803), 276(1.885), 327(0.232), 363(0.272); Microanalytical data, Calculated for $C_{24}H_{18}CrN_2O_8$ C, 56.04; H, 3.53; N, 5.45; found: C, 56.12; H, 3.50; N, 5.54.

2.5. Measurement procedures

A 1×10^{-5} M solution of Cr(III) was obtained by serial dilution of the stock solution. A 10^{-5} mol L^{-1} stock solution of **BIC** was prepared by dissolving appropriate amount of **BIC** in aqueous (water: methanol =7:3, v/v) solutions. The aforementioned solutions of these metals and **BIC** were mixed separately in different ratios for subsequent fluorescence measurement. 1.00 cm quartz cell was used for fluorescence measurement.

III. RESULT AND DISCUSSION

3.1 Spectral characteristics

The mode of coordination of **BIC** with Cr(III) was investigated by spectrophotometric titration at 25°C in aqueous (water: methanol =7:3, v/v) solutions. Fig.S-4 (Supplementary) illustrates a typical UV-vis. titration curve of **BIC** as a function of externally added Cr(III). UV-vis spectrum of the **BIC**-Cr(III) system at 375 nm gradually decrease with the increasing addition of Cr(III) .

Upon excitation of the **BIC** at 350 nm, the emission spectrum of the **BIC** showed the maximum intensity at 510 nm with a low quantum yield value 0.022(details were shown ESI). The emission intensity of the **BIC** (at 510 nm) increased gradually on addition of Cr(III) (0.1 μM to 3 μM) (Fig.1) and the fluorescence quantum yield of the system increased to a value of 0.046(λex = 350 nm). The inset plot of emission intensity as a function of externally added Cr(III) concentration reveals that after a certain amount of externally added Cr(III), there is no further change in the emission intensity of the system. Fig. 2 showed the stoichiometry (**BIC**: Cr(III) = 1:1) of the complex formed between **BIC** and Cr(III) ion as evaluated by the method of continuous variation (Job's plot),which was in agreement with the mass spectral data.

3.2 Calculation of binding constant

The relative binding affinities **BIC** towards Cr(III) was quantified based on fluorescence titration data following the modified Benesi-Hildebrand equation (Fig. 3)[42].

$(1/\Delta F) = 1/\Delta F_{max} + (1/K[C]^n)(1/\Delta F_{max})$. Here $\Delta F = (F_x - F_0)$ and $\Delta F_{max} = F_\infty - F_0$, where F_0, F_x, and F_∞ are the emission intensities of **BIC** considered in the absence of Cr(III), at an intermediate Cr(III) concentration, and at a concentration of complete interaction, respectively, and where K is the binding constant and [C] the Cr(III) concentration and n is the number of these metal ions bound per ligand (here n= 1). The value of K extracted from the slope is 1.39×10^5 M.

One of the most important parameters in cation sensing is the detection limit. For many practical purposes, it is important to sense cations at extremely low concentrations. **BIC** could detect as low as 0.1 μM Cr(III) in aqueous (water: methanol =7:3, v/v) solutions. Fig. 4 showed the plot of variation of emission intensities of **BIC** as a function of added [Cr(III)], which could also be used for determination of unknown [Cr(III)] in a sample. Up to 20 times (2 μM) of the externally added Cr(III) ion, we observed linearity. One can easily find out the concentration of any unknown Cr(III) species in aqueous solution.

3.3 Selectivity

The fluorescence enhancement can be ascribed to the CHEF effect associated with better rigidity and planarity of the sensor molecules induced by chelation of Cr(III). Changes in [Cr(III)] under physiological conditions will exhibit "turn-on" type fluorogenic behavior, which can be detected by measuring the ratio of green fluorescence intensity with good sensitivity and selectivity. Selectivity is another major issue in the field of cation sensing.

The selectivity of **BIC** for Cr(III) over other common accompanying metal ions was examined in aqueous (water: methanol =7:3, v/v) solutions. Fig. 5 indicated that only Cr(III) enhanced the fluorescence intensity of **BIC** whereas other metal ions in the 3d series played no role to coordinate with the imines nitrogen atoms of the compound **BIC**. Furthermore, the enhanced extents were in good proportion to the Cr(III) concentrations in a certain range, and other physiologically important cations, even if their concentrations were 10 times higher than that of Cr(III), and only Cr(III) ion can effectively enhance the fluorescence of **BIC**. It was interesting that the enhanced extents of the fluorescence were in good proportion to the concentrations of Cr(III), indicating that an assay of Cr(III) in this way to be good practice; and, the distinct discrimination between Cr(III) and other ions made it possible for the **BIC** to be used for the analysis of Cr(III) in the presence of other ions in aqueous system.

Interferences from some of the common alkali, alkaline earth and transition metal ions on the emission intensity of the [**BIC**-Cr(III)] system were presented in Fig.6. The fluorescence intensity of the complex in the presence of Cr(III) remain almost unchanged in the presence of the common alkali, alkaline earth and transition metal ions (Na(I), K(I), Mg(II), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II) and Pb(II)).

3.4. Preparation and imaging of cells Studies

Bacillus sp. has long been used as bio-pesticide for controlling looper killer at tea plantation. These cells from 24 h culture medium have been treated with aqueous solution of chromium nitrate (1 mg mL^{-1}) for 1h, washed with normal saline and photographed under 100X fluorescence microscope after adding **BIC**(Fig.7b). Fig.7a shows the control without adding Cr(III) salt but incubated with **BIC**. Similarly, fluorescence image of *Candida sp.* cells (Fig.7d), freshly collected pollen grains of *Allamanda puberula* (Aapocynaceae, Fig.7f) and Arctic fungal strain ASF-11 (Fig.7h) have been collected, while their respective controls are presented in Fig.7c, Fig.7e and Fig.7g respectively.

Photographs indicate that the **BIC** is easily permeable to all types of living cells tested and harmless (as the cells remain alive even after 30 minutes exposure to the **BIC** at 10 μM concentration). Intensity of the fluorescence is proportional to the concentration of Cr(III) present in the cell. **BIC** may be used to detect intracellular Cr(III) in living cells. Thus **BIC** will be useful for studying bioactivity or toxicity of Cr(III) in living cells.

3.5. ***Comparison of the probe with other reported Cr(III) selective turn-on fluorescent probes***

Comparison of the present probe with other existing Cr(III) sensitive turn-on fluorescent probes is presented in Table 1 .

Amongst the reported fluorescent probes, only one probe [44] has better LOD while our probe is least expensive as it involves a facile one step reaction with commercially available much cheaper chemicals. Mao et al. [44] did not have cell imaging of the probe. To the best of our knowledge, this is the second example of a ratiometric fluorescent probe for monitoring Cr(III) in living cells after [32]. But [32] have lower binding constant than our present ligand. Thus, our probe turns out to be more promising and much greener one.

Table 1

Comparison of the present method with the reported turn-on Cr(III) selective fluorescence sensor in the literature.

Type of sensor	selectivity	LOD	association constant / Binding constant	Application	Interferences	Ref.
Turn on	selectivity to Fe(III) and Cr(III) in aqueous solution	-	41600 M^{-1}	-	Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Ag^{+}, Pb^{2+}, Ba^{2+}, Mg^{2+}, Ca^{2+}, K^{+} and Na^{+} displayed little interference	[30]
Turn on	selectivity only Cr(III) in ethanol/H_2O(1:1, v/v, pH 7.4) solution	-	K_a= 7.5 x 10^3 M^{-1}	Cell imaging	Only Hg^{2+} elicited a slight fluorescence intensity enhancement, while the other alkali, alkaline earth, and transition metal ions did not cause any discernible changes	[31]
FRET-based ratiometric	selectivity only Cr(III) in ethanol–water (2 : 1, v/v)	-	K_a=9.4 x$10^3$$M^{-1}$	Cell imaging	Na^{+}, K^{+}, Mg^{2+}, Ca^{2+} gave no interference at a 100-fold excess concentration, and Zn^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, Hg^{2+}, Ag^{+}, Pb^{2+} gave a weak response.	[32]
Turn on	selectivity only Cr(III) in (DMF/H2O (9:1),	-	K_{ass} = 6.07 ±0.10 x10^7 M^{-2}	-	No significant spectral changes observed in the presence of Li^{+}, Na^{+} and K^{+}. Mg^{2+} and Ca^{2+}, and Mn^{2+}, induced a slight increase in fluorescence. The presence of 0.2 mM Zn^{2+}, Cd^{2+}, Fe^{3+}, Hg^{2+} and Pb^{2+} just caused slight fluorescence quenching.	[33]
Turn off	selectivity only Cr(III) in DMF:water (9:1, v/v) solution	9 x 10^{-6} mol L^{-1}	8.1378 x 10^4 M^{-1}	-	Na^{+}, K^{+}, Mg^{2+},Ca^{2+}, Cd^{2+}, Zn^{2+}, Hg^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Co^{2+}, Pb^{2+}, Ni^{2+} show insignificant positive interferences. Common anions including oxalate, dithionite and dithionate have no interference	[43]
Turn on	selectivity only Cr(III) in aqueous media	1.6×10^{-8} mol L^{-1}	-	-	Having no interference of biologically relevant metal ions including Cr^{6+}, Al^{3+}, Fe^{3+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Mg^{2+}, Ba^{2+}, Pb^{2+},Na^{+}, and K^{+}.	[44]
Turn on	selectivity only Cr(III) in acetonitrile-water (1:1, (v/v)) medium of pH 6.00	2.5µg l^{-1}	*K* = 4.7 x 10^5 l mol^{-1}	determination of Cr(III) and total chromium in domestic and industrial waste water samples	Foreign ions e,g. Na^{+}, K^{+}, NH_4^{+}, Cl^{-}, NO_3^{-}, CH_3COO^{-}, SO_4^{2-}, $C_2O_4^{2-}$ have more interference.	[45]

Turn on	selectivity only Cr(III) in CH_3CN–HEPES buffer (0.02 M, pH 7.4) (4:6, v/v) medium	1 x 10^{-6} M	K = 8 x 10^4 M^{-1}	Cell imaging	Fe^{3+} and Cu^{2+} interfered to some extent while Co^{2+}, Ni^{2+} and Pb^{2+} interfered to a negligible extent	[46]
Ratiometric	selectivity only Cr(III) in aqueous (water: methanol =7:3, v/v)	1 x10^{-7} M.	K_a=1.39 x 10^5 M^{-1}	Cell imaging	Na^+, K^+, Mg^{2+}, Ca^{2+} gave no interference and Fe^{2+}, Cu^{2+}, gave a weak response.	present

IV. APPLICATIONS

4.1. Estimation of Cr(III) and Cr(VI) from the binary synthetic mixtures

In different sets (each set in duplicate) different amounts of Cr(III) and Cr(VI) were mixed in a total volume of 100 mL. Direct estimation of Cr(III) was performed using our developed method. Reduction of Cr(VI) to Cr(III) by equivalent amount of oxalic acid was performed and total Cr(III) in the solution was estimated as mentioned above. The difference gave free Cr(VI) present in the solution. The results are presented in Table 1.

Table 1

Separation of Cr(III) and Cr(VI) in binary synthetic mixtures.

No. of observations	Amount taken (μg)	Amount found (μg)	Error (%)
1	Cr(III)—50 Cr(VI)—50	Cr(III)— 48.9±0.6 Cr(VI) —49.4±0.06	1.1 0.6
2	Cr(III)—40 Cr(VI)—50	Cr(III)—38.4±0.5 Cr(VI)—48.5±0.6	1.2 1.5
3	Cr(III)—65 Cr(VI)—25	Cr(III)—64.1±0.03 Cr(VI)—26.2±0.09	0.9 1.2
4	Cr(III)—30 Cr(VI)—45	Cr(III)— 31.3±0.5 Cr(VI)— 44.2±0.7	1.3 0.8
5	Cr(III)—30 Cr(VI)—40	Cr(III)—29.1±0.5 Cr(VI)—40.4±0.7	0.9 0.4

4.2.Real samples analysis

The waste water samples from different sources (three samples from tannery industrial area, Kolkata and three samples from Durgapur Industrial belt, West Bengal, India) were filtered through a 0.45μm Milipore membrane filter. They were analyzed as described in the previous section. The results are compared with a reference method [18] and a good agreement is found between the two (t-test, P = 0.06). Results are presented in Table 2.

Table 2

Level of Chromium species in environmental samples as determined by present method.

Sample no.	Present method		Reference method [18]	
	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
1[a]	682.2 ± 0.2	595.9 ± 2	681.2 ± 0.2	595 ± 2
2[a]	573.6 ± 3	511.5 ± 4	575 ± 3	512 ± 4
3[a]	470 ± 1	413.2 ± 3	467 ± 1	411 ± 3
4[b]	64.3 ± 3	53.5 ± 1	65.3 ± 3	54.3 ± 1
5[b]	42.8 ± 3	43.2 ± 4	43.1 ± 3	41.2 ± 4
6[b]	34.8 ± 4	36.5 ± 5	35.5 ± 4	37.5 ± 5

[a] Tannery water. [b] Industrial water.

V. CONCLUSION

In conclusion, we report four easily synthesized highly sensitive and selective ‘‘turn-on’’ fluorescent sensors (**BIC**) for trace level selective determination of Cr(III) based on the CHEF mechanism. Both the probe and its Cr(III) complex were well characterized by different spectroscopic techniques. The reagent possesses strong binding affinity towards Cr(III) in evident from binding constant data of complexes. The method is free from interferences of common accompanying cations. It almost fulfils the demand of a green analytical method. Finally, the probe could detect trace level Cr(III) in living cells under fluorescence microscopy. These sensors may be further developed as a novel type of readily synthesized, high performance fluorescent Cr(III) sensor with the practical applicability for Cr(III) imaging in living cells and Cr(III) sensing in relevant aqueous (water: methanol =7:3, v/v) systems.

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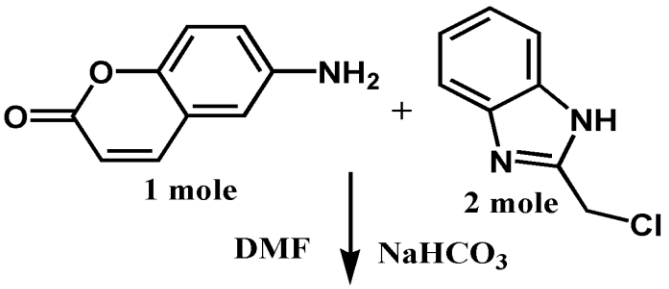
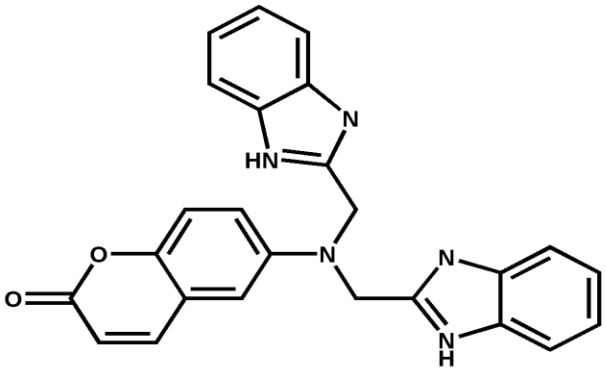




6-(bis((1*H*-benzo[*d*]imidazol-2-yl)methyl)amino)-2*H*-chromen-2-one

Scheme 1

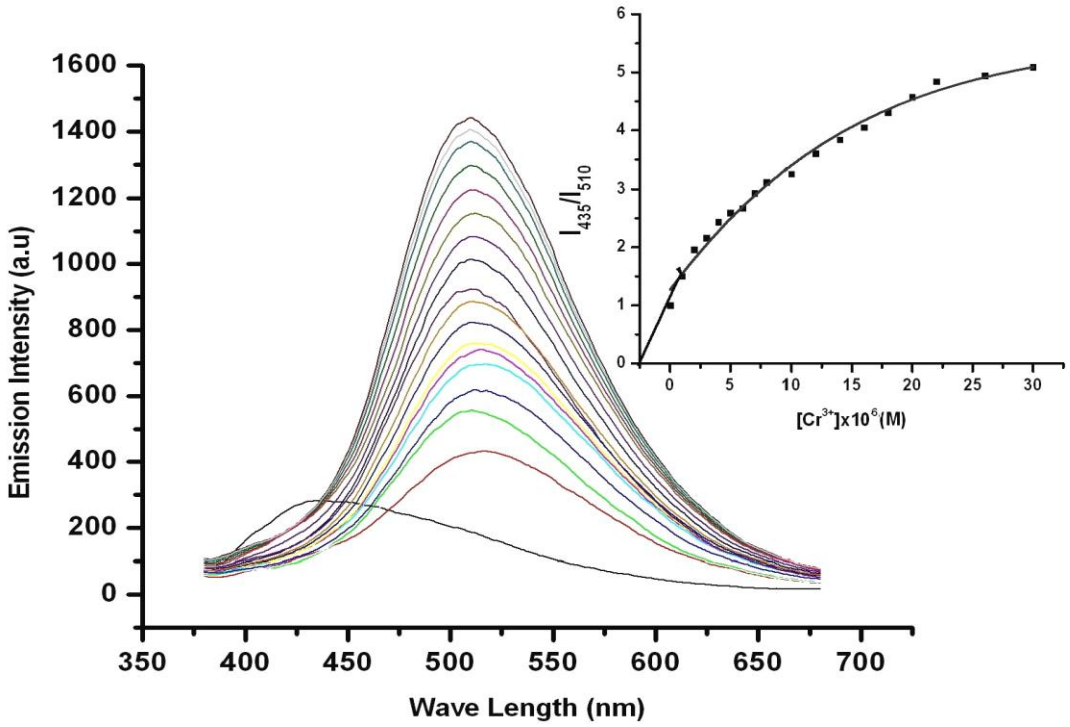


Fig.1

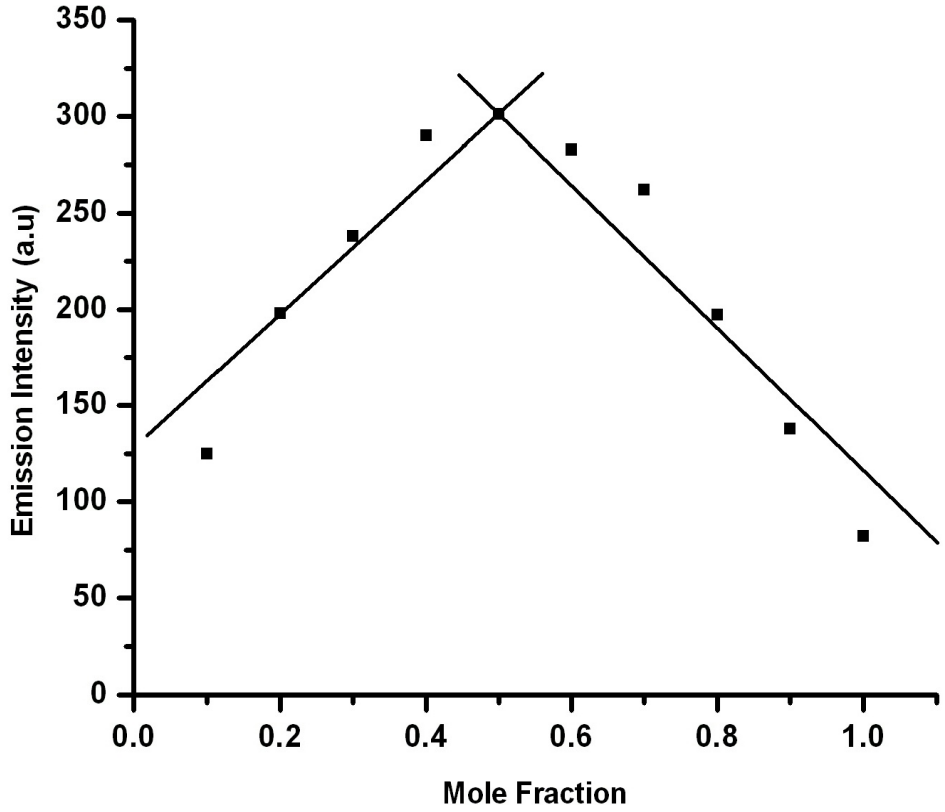

Emission Intensity (a.u)
Mole Fraction
0
50
100
150
200
250
300
350
0.0
0.2
0.4
0.6
0.8
1.0

Fig 2

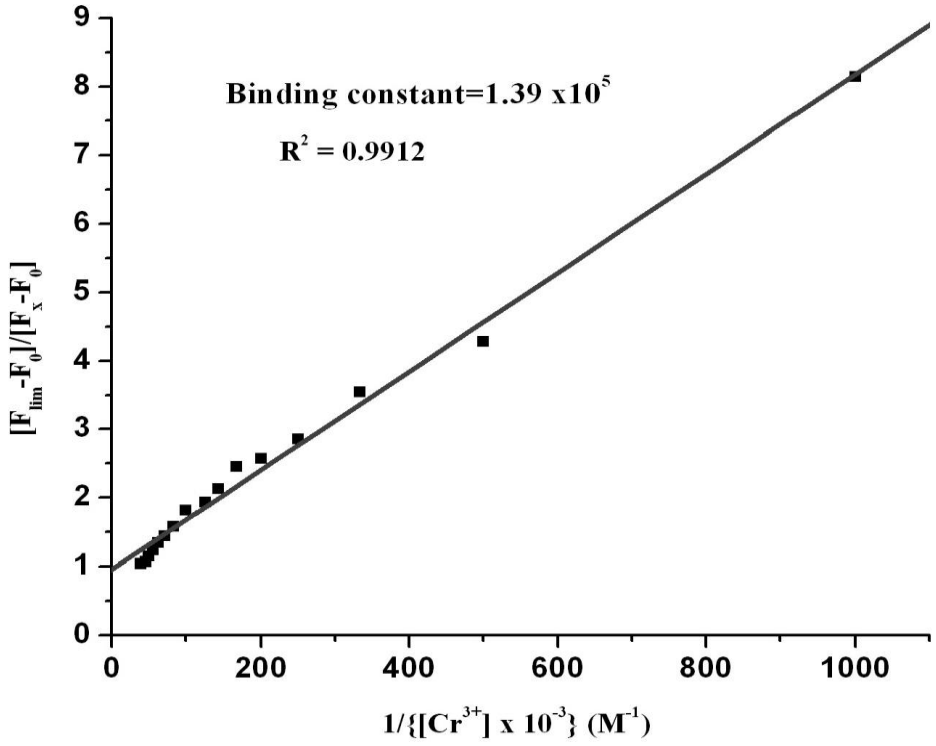

Binding constant=1.39 x10^5
R^2 = 0.9912
[F_lim-F_0]/[F_x-F_0]
1/{[Cr^3+] x 10^-3} (M^-1)
0
1
2
3
4
5
6
7
8
9
0
200
400
600
800
1000

Fig 3

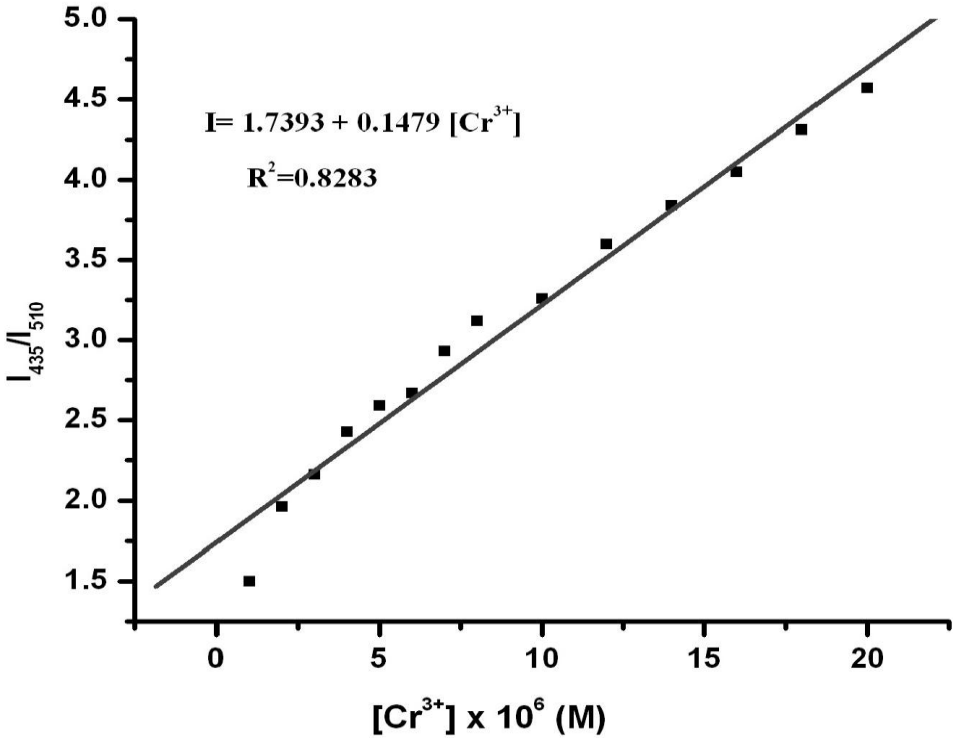

I= 1.7393 + 0.1479 [Cr3+]
R2=0.8283
I435/I510
[Cr3+] x 10^6 (M)

Fig. 4

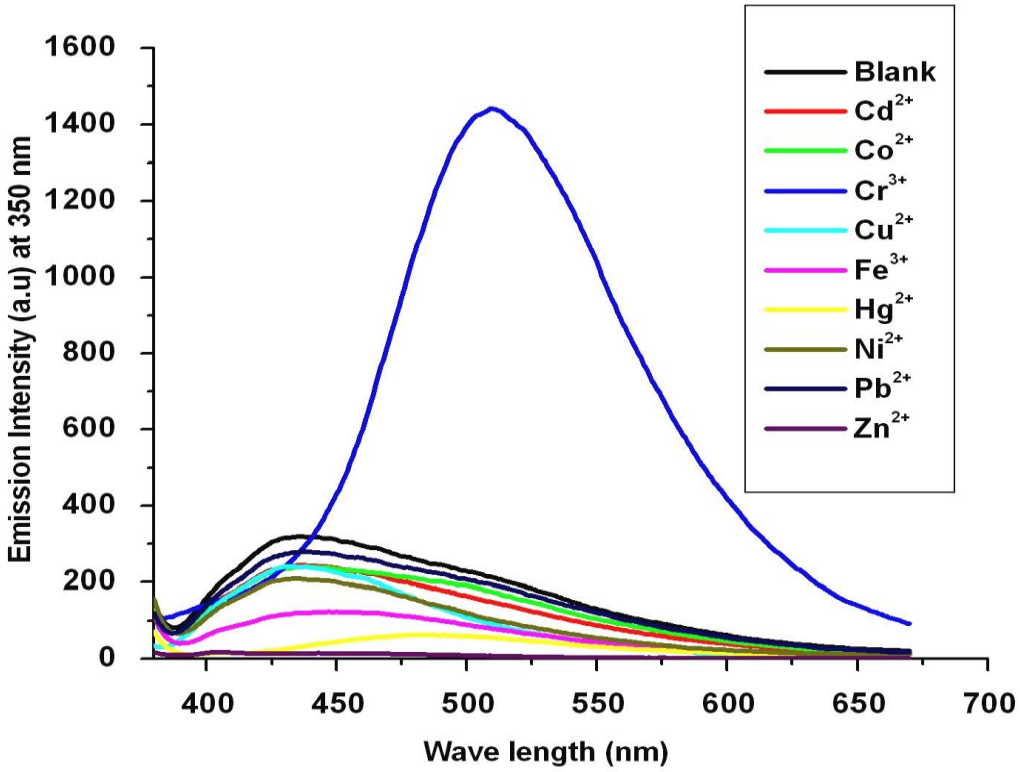

Blank
Cd2+
Co2+
Cr3+
Cu2+
Fe3+
Hg2+
Ni2+
Pb2+
Zn2+
Emission Intensity (a.u) at 350 nm
Wave length (nm)

Fig. 5

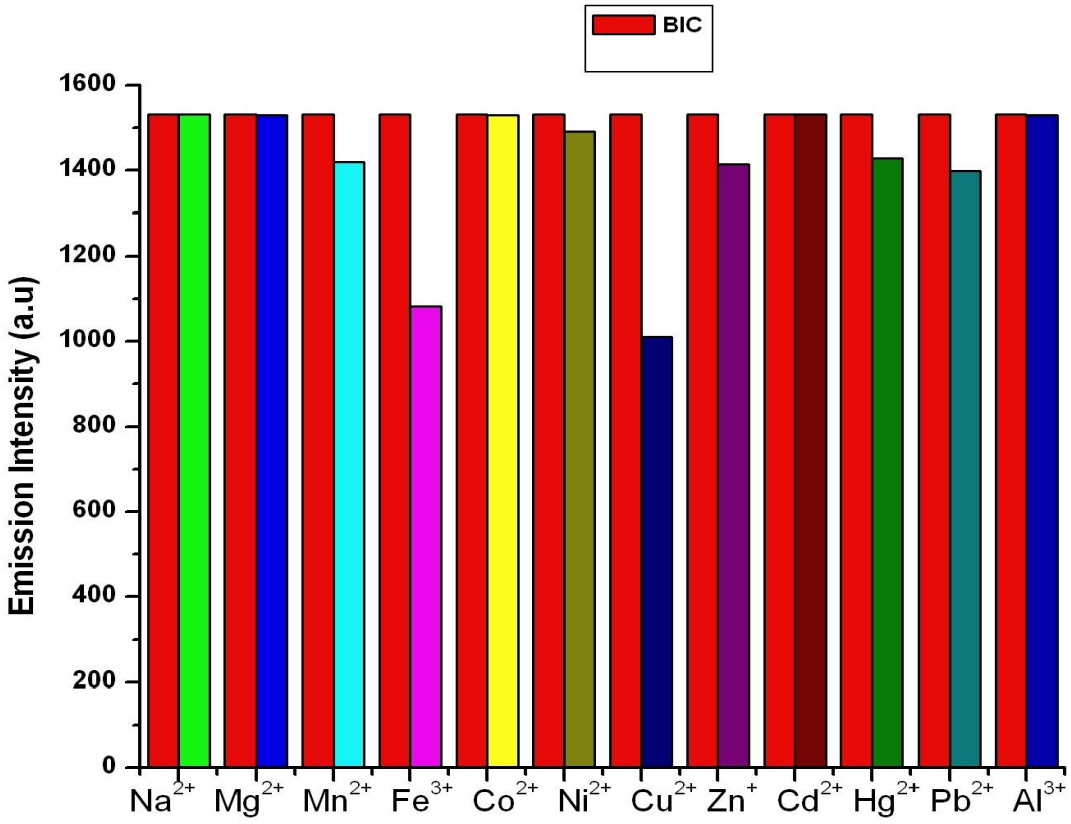

BIC
Emission Intensity (a.u)
0
200
400
600
800
1000
1200
1400
1600
Na2+
Mg2+
Mn2+
Fe3+
Co2+
Ni2+
Cu2+
Zn+
Cd2+
Hg2+
Pb2+
Al3+

Fig.6

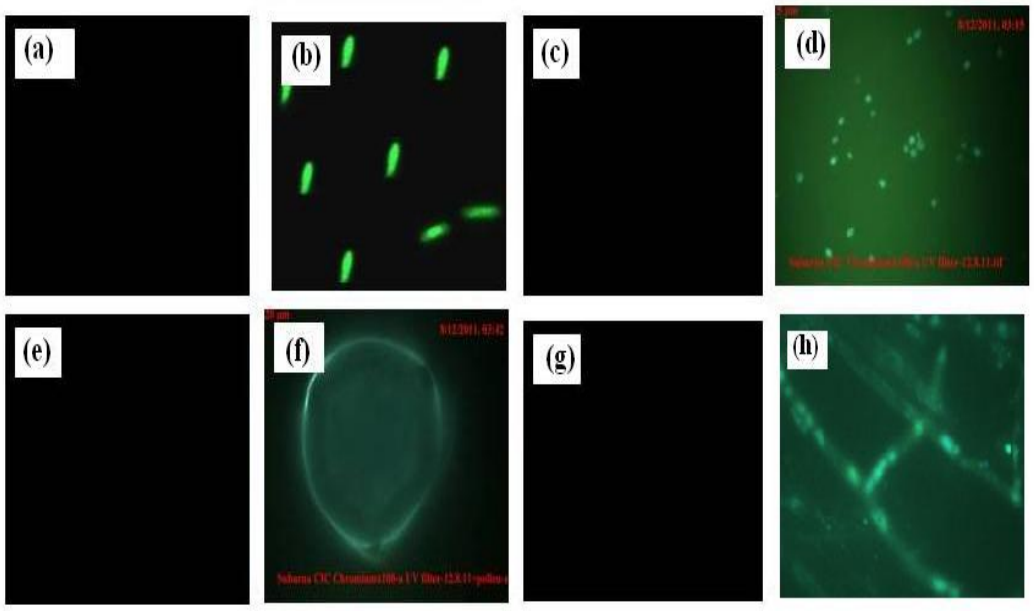
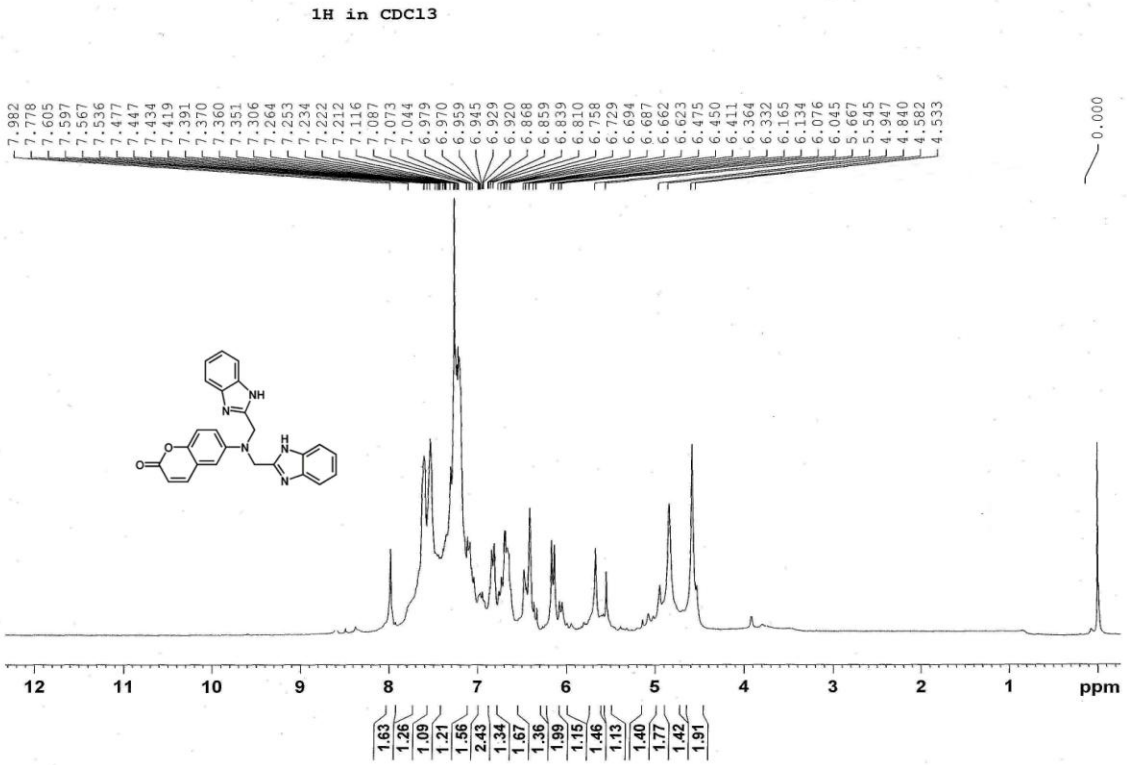
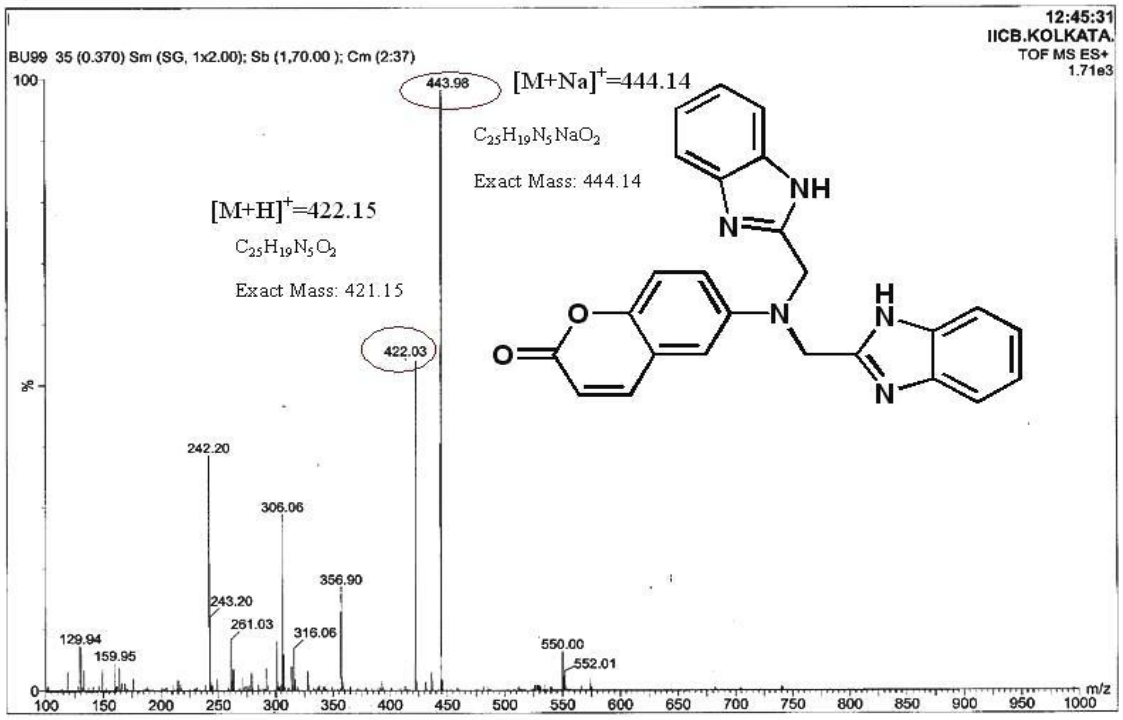

(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)

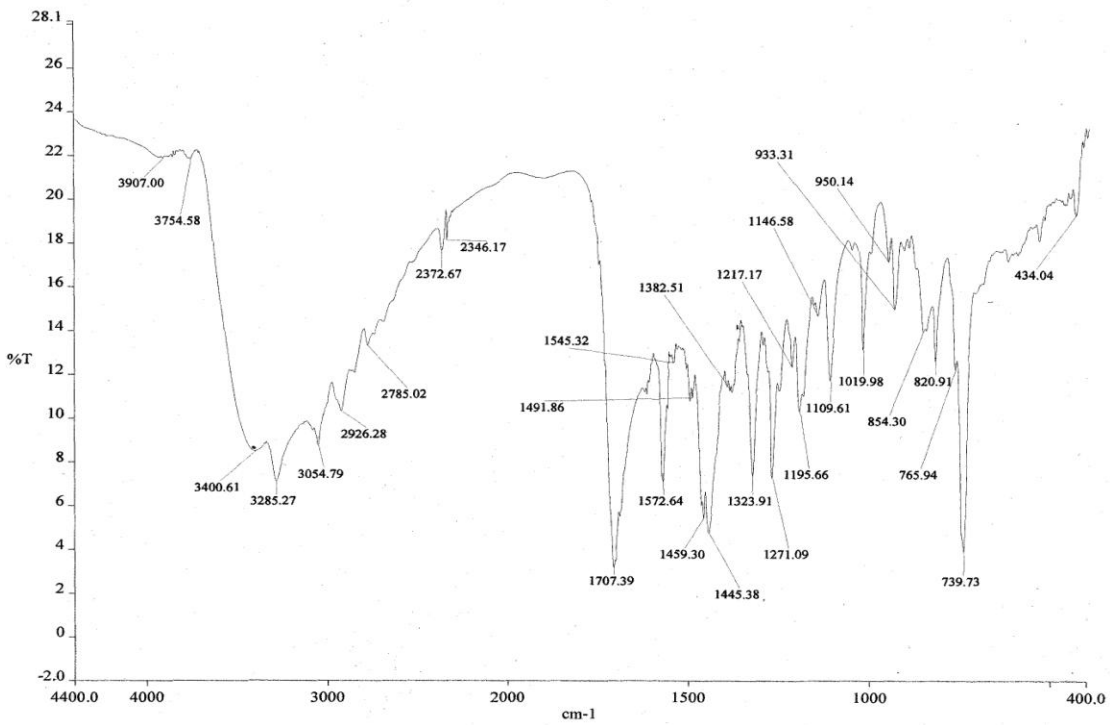
Fig.7



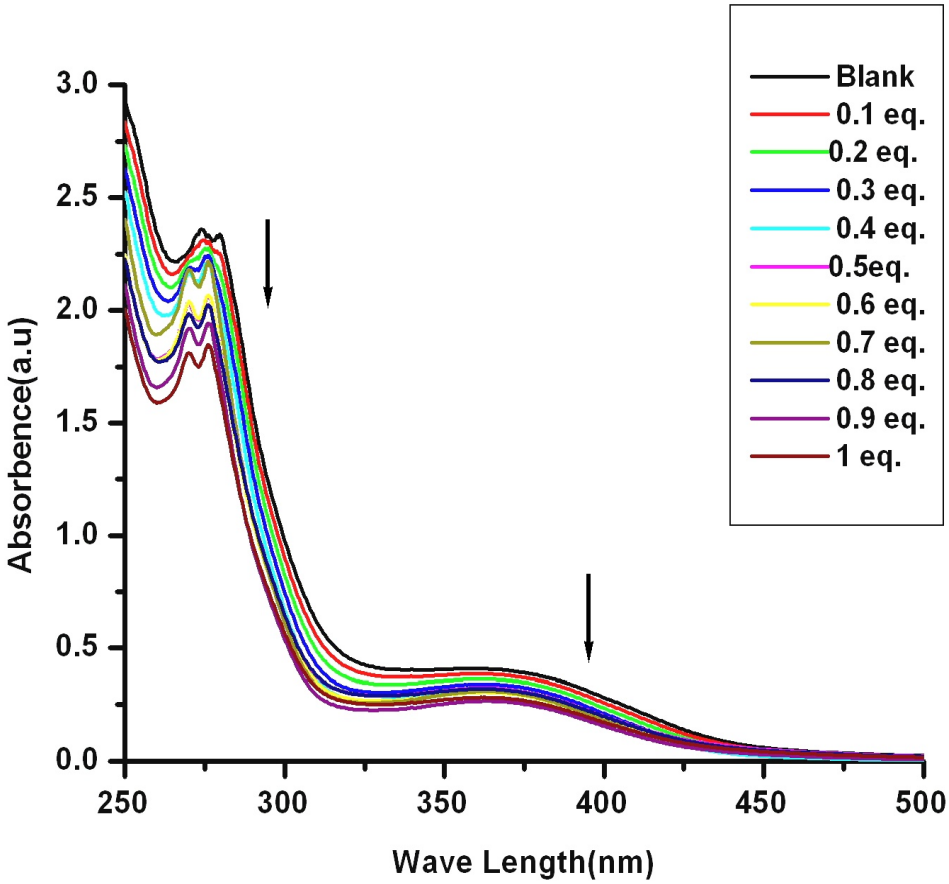
S-1. ^{1}H-NMR spectra of BIC



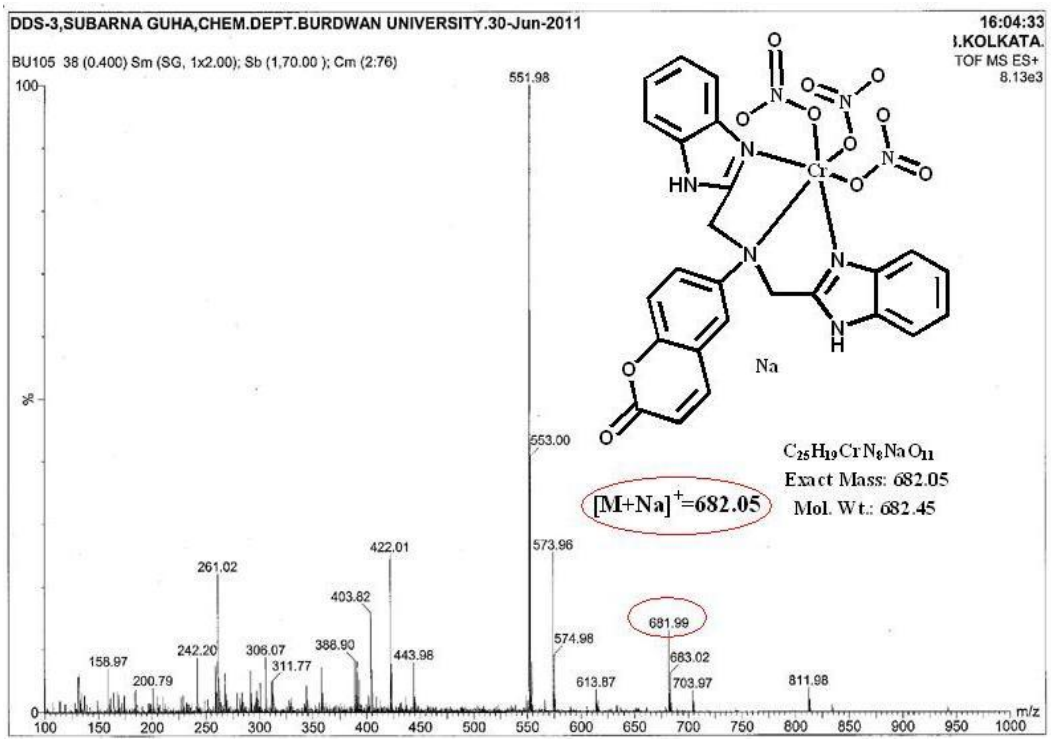
S-2. TOF MS ES (+) mass spectra of BIC



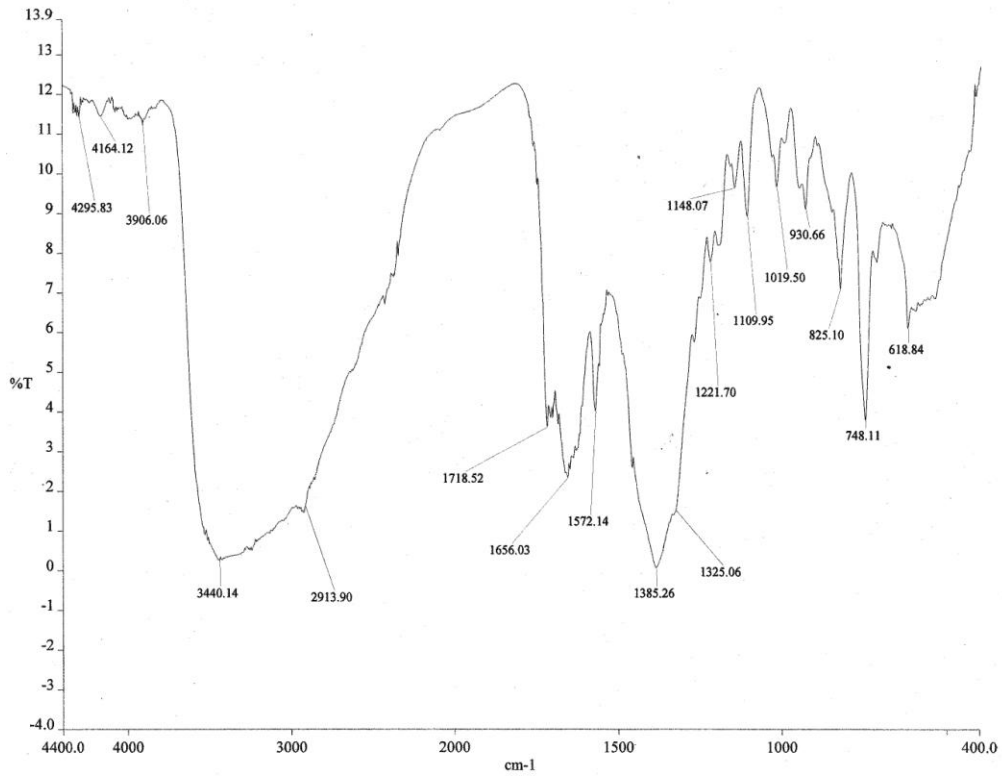
S-3. FTIR spectra of BIC



S-4. UV –Vis spectra of the ligand BIC and BIC+Cr(III) complex in aqueous (water: methanol =7:3, v/v) solutions ([BIC] = [Complex] = 10 μM)



S-5. TOF MS ES (+) mass spectra of BIC+Cr(III)



S-6. FTIR spectra of the BIC+Cr(III)complex

Quantum yield measurement

The fluorescence quantum yield of the complex was determined using anthracene as a reference with a known ϕ_R value of 0.27 in ethanol [1]. The complex and the reference dye were excited at same wavelength (350 nm), maintaining nearly equal absorbance (0.1) and the emission spectra. The area of the emission spectrum was integrated using the software available in the instrument and the quantum yield is calculated according to the following equation:

$$\phi_S/\phi_R = [A_S / A_R] \times [(Abs)_R /(Abs)_S] \times [\eta_S^2/\eta_R^2] \quad (1)$$

Here, ϕ_S and ϕ_R were the fluorescence quantum yield of the sample and reference respectively. A_S and A_R were the area under the fluorescence spectra of the sample and the reference respectively, $(Abs)_S$ and $(Abs)_R$ were the respective optical densities of the sample and the reference solution at the wavelength of excitation, and η_S and η_R are the values of refractive index for the respective solvent used for the sample and reference.

Reference

[1] W. H. Melhuish, J. Phys. Chem. 65 (1961) 229-235.

Legends to figures and tables:

Scheme1. Synthesis of fluorescent sensor of 6-(bis((1H-benzo[d]imidazol-2-yl) methyl)amino)-2H-chromen-2-one (BIC).

Figure 1. Fluorescence spectral changes of BIC (10 μM) up on addition of 1, 2, 3, 4, 5, 6, 7, 8,10,12,14,16,18,20,22,26,30 μM of Cr(III) ion. (λex = 350 nm, λem = 525 nm, slit width, 5/5).

Figure 2. Jobs plot for the determination of stoichiometry of [BIC-Cr(III)] in aqueous (water: methanol =7:3, v/v) solution.

Figure 3. Determination of binding constant of BIC (10 μM) with Cr(III) (10 μM) using Benesi-Hildebrand equation (fluorescence method).

Figure 4. Plot of emission intensities of BIC (10 μM) as a function of externally added [Cr(III)].

Figure 5. Emission intensities of BIC (10 μM) in presence of different metal ions (10 μM).

Figure 6. Interference of different metal ions on the determination of [Cr(III)] with BIC. [BIC] = [Cr(III)] = [foreign cations] = 10 μM.

Figure.7. Fluorescence microscope images of Bacillus sp.; Candida sp.(*Candida albicans)*; pollen grains of *Allamanda puberula* (Aapocynaceae); Arctic fungal strain ASF-11 cells without treated with Cr(III) and BIC-stained with (10 μM) Cr(III) for 30 min under 100X objective lens. Incubation was performed at 40°C.

Electronic supplementary materials (ESI)

Fig. S-1. ^{1}HNMR spectra of BIC

Fig. S-2. TOF MS ES (+) of BIC

Fig. S-3. FTIR spectra of BIC

Fig. S-4. UV –Vis spectra of the ligand BIC and BIC-Cr(III) complex in aqueous (water: methanol =7:3, v/v) solutions at neutral P^H. ([BIC] = [Complex] = 10 μM).

Fig. S-5. TOF MS ES (+) of BIC-Cr(III) complex

Fig. S-6. FTIR spectra of BIC-Cr(III) complex.