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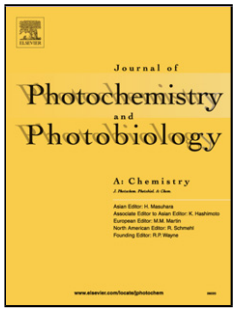

A simple coumarin based “fluorescent On” probe for the selective detection of Al^{3+} along with its application in live cell imaging via AGS cell line

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A simple coumarin based "fluorescent On" probe for the selective detection of Al^{3+} along with its application in live cell imaging via AGS cell line

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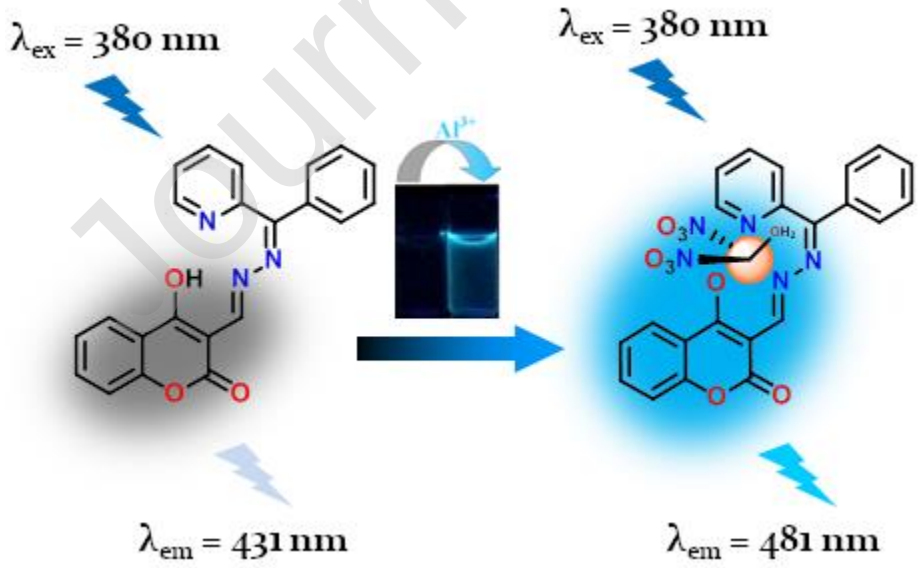
Graphical abstract



Highlights:

- In this article, we report the synthesis and cation sensing properties of a new fluorescence reversible "turn-on" probe.
- The probe showed high selectivity towards Al^{3+} in aqueous methanol solution at physiological pH with an enhancement in emission intensity at 481 nm with a red shift of about 50 nm.
- The optimised geometry, binding mode and change of electronic properties of HCBP with Al^{3+} was modelled by DFT (density functional theory) and TDDFT (time dependent density functional theory) computational calculations.
- The minimum limit of detection (LOD) of the probe was in the order of 10^{-9} M.
- Furthermore, the potentiality of the probe for biological application was confirmed by employing it for fluorescence imaging of Al^{3+} in gastric cancer cells (AGS).

Abstract

A new coumarin based fluorescent switch (HCBP) has been fabricated which displays high selective sensing towards Al^{3+} among other metal cations at physiological pH. On gradual addition of Al^{3+} specifically, HCBP shows a brilliant "turn-on" emission enhancement of about ~13-fold with about 50 nm red shift at 481 nm in MeOH/H_2O (1/1, v/v) solution. The fluorescent switch is proven to be a reversible probe by addition of EDTA gradually into the HCBP-Al^{3+} solution. Detection limit as well as Binding constant values have been calculated and found to be in the order of 10^{-9} M and 10^{3} M^{-1} respectively. DFT and TDDFT studies are conducted with the

probe to establish a similarity between theoretical and experimental outcomes. We can also use this new fluorescent switch as a biomarker kit as it has shown a brilliant potential in the application of live cell imaging using gastric cancer cell (AGS cell).

***Keywords*:** Coumarin based fluorescent probe; Fluorescence "turn-on" switch for Al^{3+}; AGS gastric cancer cell line; Live cell imaging; DFT calculation.

1. Introduction

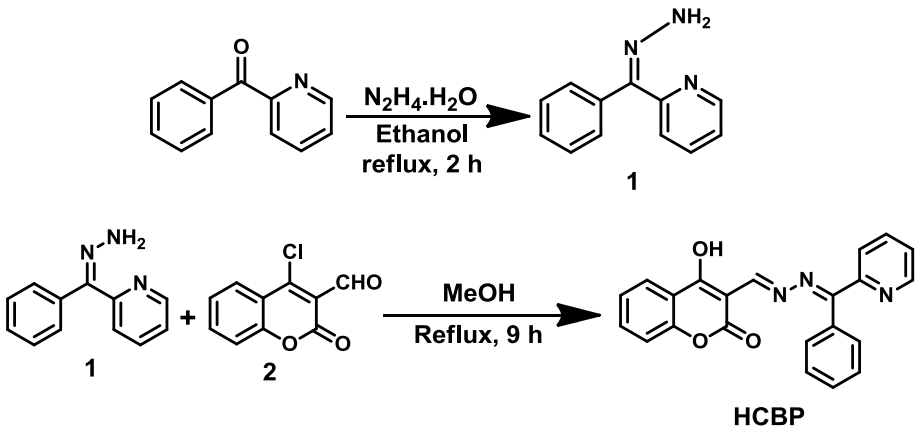
Aluminium being the most frequently found metal in earth's crust is third most abundant metallic element among all metals after oxygen and silicon [1]. This prevalent metal produces phytotoxicity as well as biotoxicity. Aluminum and its alloys cover miscellaneous applications in our daily life, for instance in packaging industry, cooking tools, food additives, water purification, construction materials and clinical medicines [2,3]. It plays an important role in enzyme-catalyzed reactions, biotechnological transformations etc. [4]. But excessive presence of aluminium in human body causes huge damage into our nervous system resulting into several dangerous diseases such as Alzheimer's disease, Parkinson's disease, Osteoporosis, Osteomalacia, gastrointestinal problems, headaches, kidney malfunction, hypochromic anaemia, lateral sclerosis and dementia [5-13]. Besides, the wide presence of aluminium in water as well as in soil basically leads to the obstruction in the growth of the plants [14]. As a result, a small amount of aluminium can be found in almost every food products. It also acts as a potential pollutant in environment and also in soil and water thereby creating a sort of mixed contamination to the environment. According to WHO, the maximum level of intake of aluminium metal in a human body should not exceed 7 mg kg^{-1}. So on account of the various dangerous harms which this metal can cause to the environment, industry as well as in human

health, it is of utmost importance to selectively detect Al^{3+} to control its effect. Fabrication of small organic fluorescent molecules to introduce distinct modification in the spectroscopic properties in presence of particular metal ions is of huge interest for quite a few decades now. The simple-to-synthesize property of these fluorescent chemosensors along with their high sensitivity, non-destructive nature and tunability [15], make them a highly desired chemical tool to detect any particular metal cation selectively from the collection of several other cations. Many fluorescent probes have been reported till now which can detect Al^{3+} selectively [16-32]. A few fluorescent probes were reported with a coumarin framework though, due to its low LOD values, high costing values etc. [33]. Here in this article, we have introduced a very simple design for a fluorescent probe which is based on the coumarin skeleton and specific for sensing only Al^{3+} among other cations with a good detection limit value. So this fluorescent switch, HCBP exhibits a "turn-on" emission response towards Al^{3+} and also shows its reversible nature via EDTA titration.

2. Results and discussion

2.1. Synthesis and spectral characterisation

The synthetic route towards the probe, HCBP was shown in scheme 1. Compound 1 [34] and 2 [35] were synthesized according to the reported procedures in literature. The chemical structure of HCBP was confirmed by ^{1}H and ^{13}C NMR spectroscopy and ESI mass spectrometry. Reflux condensation of compound 1 and 4-chloro-2-oxo-2H-chromene-3-carbaldehyde (2) in methanol for 9 hrs affords the desired fluorescent probe, HCBP with yield of 81%.



Scheme 1. Synthetic scheme of the probe, HCBP

2.2. *Specificity of HCBP towards Al^{3+}*

2.2.1. UV-Vis study

The absorbance properties of HCBP was studied in MeOH/H_2O (1/1, v/v) solution at 25°C under physiological pH (10 mM HEPES buffer, pH=7.2). The UV-vis spectrum of HCBP (10 μM) in MeOH/H_2O (1/1, v/v) showed a moderate absorbance band at 382 with a shoulder at 268 nm. Gradual addition of Al^{3+} into the solution of the probe resulted into a red shift of the former absorbance peak at 407 nm supporting the coordination of Al^{3+} to HCBP (Fig. 1). To ascertain the selectivity of the probe, the absorption spectral changes of the probe were recorded in presence of other metal cations such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+} and Cd^{2+}. Although no absorbance change was observed for any other metals except only Al^{3+} (ESI, Fig. S4).

2.2.2. Emission study showing reversibility property

In the absence of any metal cations, our fluorescent switch, HCBP exhibited a fluorescence band with moderately weak emission intensity with the maxima (F_0) appearing at 431 nm (λ_{ex} = 380 nm) in MeOH/H_2O (1/1, v/v). The emission quantum yield of the probe (HCBP) is very low (φ = 0.01). Upon addition of Al^{3+} to the probe solution, an emission maxima

was observed at 481 nm with a red shift of 50 nm showing a "turn-on" enhancement in emission intensity (φ = 0.34) (Fig. 2). This emission enhancement corresponded to a strong "OFF-ON" category of emission property. Further, on addition of EDTA to the solution of HCBP, the emission intensity at 481 nm reverted back to the original intensity of the free probe (Fig. 3) thereby making HCBP a reversible fluorescent probe. Thus the fluorescent switch here can be called a "OFF-ON-OFF" fluorescence signalling probe. We have also performed the emission study in 99% H_2O (1% MeOH) (ESI, Fig. S5). But the detection of Al^{3+} in this particular solvent is not as much sensitive as in MeOH/H_2O (1/1, v/v). So we have optimized the water and methanol ratio and recorded all spectroscopic studies using MeOH/H_2O (1/1, v/v) .

The sensing capacity of the probe, HCBP was also studied in presence of a group of metal ions such as K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+} and Cd^{2+} to exhibit the discrete sensitivity of HCBP towards only Al^{3+} in MeOH/H_2O (1/1, v/v). It was found that none of the other metal ions caused any significant change in the emission profile of HCBP (ESI, Fig. S6).

An interference experiment was then executed by measuring the fluorescence intensity of HCBP (10 μM) in presence of other metal ions (20 μM) in order to study the specific selectivity of HCBP. It is noted from the experiment that the emission enhancement of HCBP was distinctly specific towards Al^{3+} (Fig. 4) and not at all affected by the presence of other metal cations.

2.2.3. The binding studies of HCBP with Al^{3+}

Now in order to estimate the stoichiometry of the complex of SAPH with Al^{3+}, Job's plot analysis was carried out. The maxima showed the mole fraction at 0.5 for Al^{3+}, which corresponded to the 1:1 complex formation of HCBP and Al^{3+} (ESI, Fig. S7). Mole ratio plot obtained from fluorescence titration indicated to the fact that HCBP showed an increase in

emission intensity till the ratio of HCBP: Al^{3+} reach at ~1, after that there was barely any increase in the emission intensity. The emission intensity of the probe (HCBP) increased linearly at 481 nm with the amount of Al^{3+} added in between 0 to 11 μM (R^2 =0.9946) (ESI,† Fig. S8). The detection limit of HCBP for Al^{3+} was determined to be 3.91×10^{-9} M which was established from the emission spectral change of HCBP upon addition of Al^{3+} following the equation, LOD = K × SD/S where 'SD' is the standard deviation of the blank solution of the probe and 'S' in the slope of the curve (ESI,† Fig. S9). This low LOD value clearly indicated that HCBP has great efficiency in detecting Al^{3+} in very minute level. The association constant (K_a) of HCBP for Al^{3+} was also calculated using Benesi-Hildebrand equation and found to be 9.1×10^3 M^{-1} (ESI, Fig. S10), which suggested that HCBP-Al^{3+} complexation is sufficiently stable. The selective binding of the probe (HCBP) towards Al^{3+} may be attributed to its small size as it fits well to the cavity formed by the N-N-O binding site.

2.2.4. The lifetime decay studies of HCBP with Al^{3+}

To understand the excited state stability of this fluorescent probe, the lifetime measurements were also studied. The fluorescence lifetime of the free probe (HCBP) measured at λ_{em} = 481 nm was found to be 0.19 ns which fitted well with a bi-exponential decay pattern. On the other hand, the average lifetime of HCBP-Al^{3+} moiety showed a noteworthy increase of 2.95 ns owing to the stable complexation of HCBP with Al^{3+} and the pattern of this decay was found to be a mono-exponential one (Fig. S11).

2.2.5. pH study

Now the pH titration experiment of HCBP was performed and the titration revealed the fact that No significant changes have been noticed for HCBP with the increase in pH range at 481 nm thus stating that the probe, HCBP is independent of pH. Now on addition of Al^{3+} in the solution

of HCBP, the emission intensity increased rapidly within the pH range of 3-8 thus indicating that HCBP is capable in detecting Al^{3+} within the aforementioned pH array (ESI, Fig. S12). But on further increase of pH, the emission intensity was diminished. In the basic region, the deprotonation of the -OH group caused the dissociation of the probe which ultimately resulted into the inability of HCBP in sensing Al^{3+} in that pH region. Hence the probe (HCBP) can detect Al^{3+} in the neutral pH range with an outstanding effectiveness.

2.3. Probable sensing mechanism

The enhancement in the emission intensity of the probe (HCBP), on addition of Al^{3+} may be attributed to the CHEF (Chelation induced fluorescence) process. When Al was coordinated to the probe, it formed a tight binding pattern which consequently increased the rigidity of the complex thereby causing enhancement in emission intensity. The red shift of 50 nm in the emission maximum, after addition of Al^{3+} may be due to the ICT (Internal charge transfer) mechanism. A probable binding mode of HCBP with Al^{3+} is shown in the diagram below (Fig. 5).

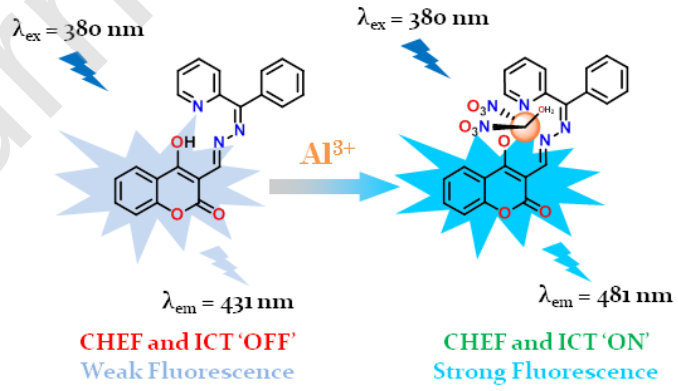


Fig. 5. Probable sensing mechanism of HCBP with Al^{3+}

2.4. Practical Application as Dip-stick experiment: Detection of Al^{3+} using TLC plate

To display some brilliant applications of this probe, we have performed an efficient method named as dipstick experiment in which the probe can act as a fluorescent portable kit showing its sensing property towards particular metal in solid state too. This experiment has an massive significance as it can give some crucial qualitative facts on detecting Al^{3+} without using any instruments. So to execute this experiment, few thin-layer chromatography (TLC) plates were prepared and they were dipped into HCBP solution (2×10^{-4} M) in MeOH and then kept for some time so as to evaporate the solvent. Then the TLC plates were immersed into Al^{3+} (2×10^{-3} M) solution and the solvent was evaporated afterwards in order to dry. Through this experiment, one can easily study the detection of Al^{3+} by bare eye. The colour of the TLC plates showed the change of the colour from colourless to cyan in presence of Al^{3+} in the UV chamber (Fig. 6). So the fluorescent HCBP can be used as a sensing kit for Al^{3+} in solid state.

Fig. 6. Pictures of TLC plates after immersion in HCBP-MeOH solution and in HCBP-Al^{3+}-MeOH solution under UV chamber. [HCBP] = 2×10^{-4} M, [Al^{3+}] = 2×10^{-3} M. Excitation wavelength of the UV light is 380 nm.

2.5. Application in biological field via live cell imaging in human AGS cell

To display some more potential practical applications of the probe (HCBP), the live cell imaging experiment was studied which established its capability in biological field. The MTT experiment established the fact that the probe, HCBP has a very insignificant toxicity at lower concentration levels towards human gastric cancer (AGS) cell lines though it is noticed that HCBP has minor effect on survivability of cells at higher dosage (75 μM) (Fig. 7). The IC_{50} value of HCBP was found to be 69.31 μM and hence we have chosen the dose for the experiment to be 15 μM as the selected dose should be less than the IC_{50} value (Fig. S13). Now the fluorescence imaging study under fluorescence microscope revealed that the treatment of the gastric cancer cells with HCBP itself showed a very weak blue fluorescence while upon incubating the AGS cells with 30 μM of Al^{3+} and HCBP results in a brilliant blue fluorescence in the intracellular region (Fig. 8). The fluorescence image of HCBP with Al^{3+} confirms that the probe can easily penetrate the cell membrane and readily binds with the intracellular Al^{3+}. Further the bright field images showed no physical changes of the cells after incubation with Al^{3+} suggesting that the AGS cells are viable and HCBP is not toxic at that particular concentration. Thus the experiment implied that the probe (HCBP) is cell membrane permeable and can be used as an effective bio-marker to detect Al^{3+} in living gastric cancer cells.

2.6. Computational studies to interprete further structural changes in HCBP

To get a further insight into the structural changes of HCBP and its complex with Al^{3+}, density functional theory (DFT) calculations with the B3LYP/6-31+G(d) method using the Gaussian 09 program were carried out. The optimized structures of HCBP and HCBP-Al^{3+} are shown in Fig. S14 and S15 respectively. Contour plots of some selected molecular orbitals including HOMO and LUMO of HCBP and HCBP-Al^{3+} are also shown in Fig. S16 and S17 respectively. The HOMO-LUMO energy gap of HCBP (3.55 eV) was considerably reduced in HCBP-Al^{3+} (3.26

eV) complex which suggested the shifting of low energy band in the complexes thereby correlating the formation of a new band at longer wavelength for the HCBP-Al^{3+} complex. The energy and % of composition of selected molecular orbitals of HCBP-Al^{3+} are summarized in Table S1. Further, to deduce the electronic transitions, time dependent density functional theory (TDDFT) was performed to the optimized geometries of the compounds. The low energy transition for HCBP at 390 nm ($\lambda_{expt.}$, 382 nm) corresponded to HOMO $\rightarrow$ LUMO transition whereas for HCBP-Al^{3+}, the low energy band shifted to 431 nm ($\lambda_{expt.}$, 407 nm) (Table S2).

3. Conclusions

Thus herein we report the synthesis of a new coumarin-based fluorescent reversible switch which selectively detects Al^{3+} with a brilliant "turn-on" emission response. The probe also exhibits a noteworthy red-shift of about 50 nm at 481 nm with a cyan colored fluorescence under UV-radiation. On addition of EDTA into the probe-metal solution, the emission intensity reverts back to the original intensity of the probe itself thus proving the reversible nature of this new probe. The probe shows high selectivity towards Al^{3+} over other metal cations with satisfactorily low LOD values of the order of 10^{-9} M in physiological pH. The structure of this newly developed fluorescent probe and its probable binding modes with Al^{3+} were thoroughly studied through DFT and TDDFT calculations. The function of this probe in biology as a biomarker tool was also experimented in gastric cancer cell lines (AGS cell).

Author statement

In this communication, we report the synthesis, photophysical properties and live cell imaging studies of a new fluorogenic reversible probe which selectively detects the presence of Al^{3+}. The probe revealed a significant "turn-on" response with a red shift of 50 nm upon addition of Al^{3+}. Other cations showed inertness to perturb the electronic properties of the probe. The probe was also able to detect Al^{3+} in the gastric cancer cells. The complexation of the fluorescent probe with Al^{3+} was modelled by DFT and TDDFT computational calculations.

Author Contributions

S. Gharami executed the experiments and wrote the manuscript. K. Aich and L. Patra helped in the experimental studies for photophysical properties. P. Ghosh and N. Murmu performed the cell imaging and cytotoxicity assay experiments. T. K. Mondal supervised the project. All the authors reviewed the manuscript.

conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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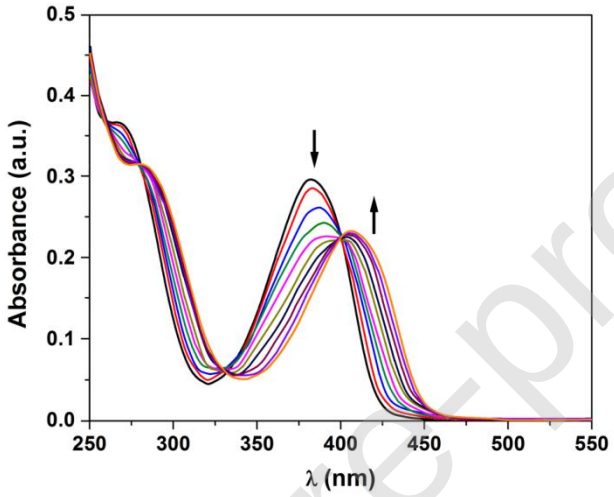

Fig. 1. Change in UV-Vis spectrum of HCBP (10 μM) upon addition of Al^{3+} (0-18 μM) in MeOH/H_2O (1/1, v/v) (HEPES buffer, pH=7.2).

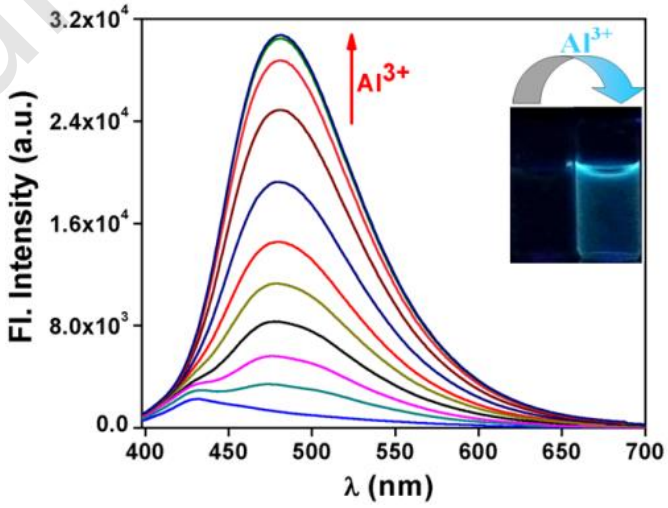

Fig. 2. Change in emission spectra of HCBP (10 μM) upon gradual addition of Al^{3+} (0-18 μM) (Inset shows the change in colour under UV-radiation)

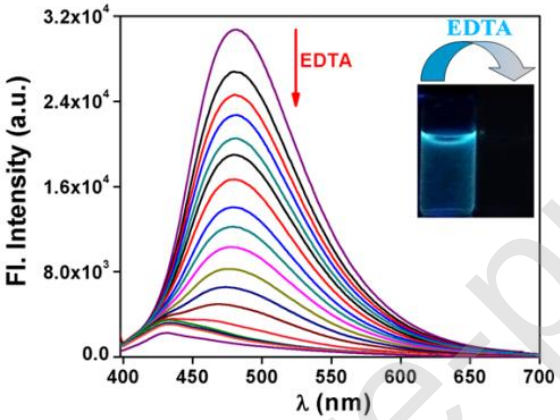

Fig. 3. Change in emission spectra of HCBP (10 μM) upon gradual addition of EDTA (0-20 μM) in MeOH /H_2O (1/1, v/v) (HEPES buffer, pH=7.2). (Inset shows the change in colour under UV-radiation)

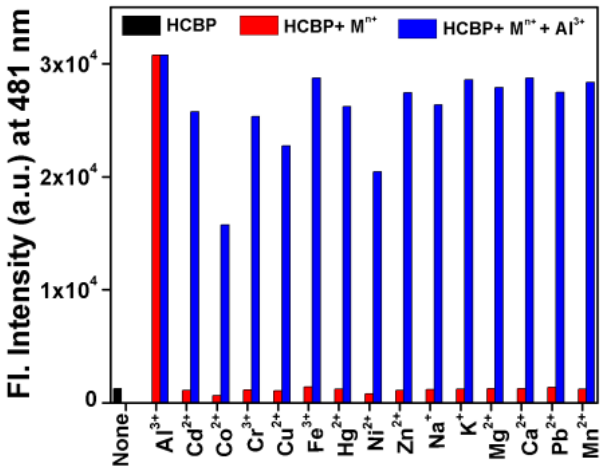

Fig. 4. Bar diagram illustration of the relative emission intensity of HCBP upon addition of various metals (10 μM) in MeOH:H_2O (1:1, v/v) (HEPES buffer, pH=7.2) (red bars) and Al^{3+} (20 μM) in presence of other metal ions (blue bars)

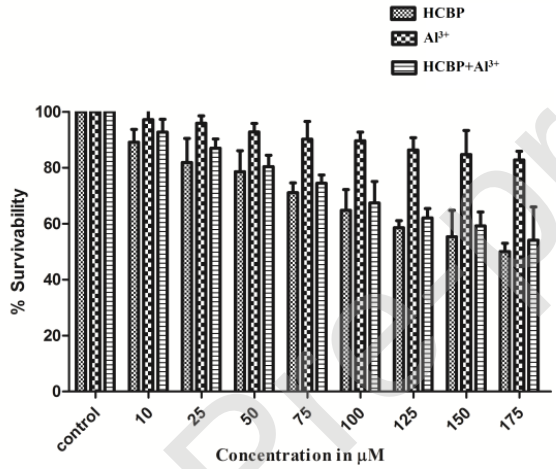

Fig. 7. MTT assay of HCBP, Al^{3+} and HCBP-Al^{3+} complex on AGS cell line.

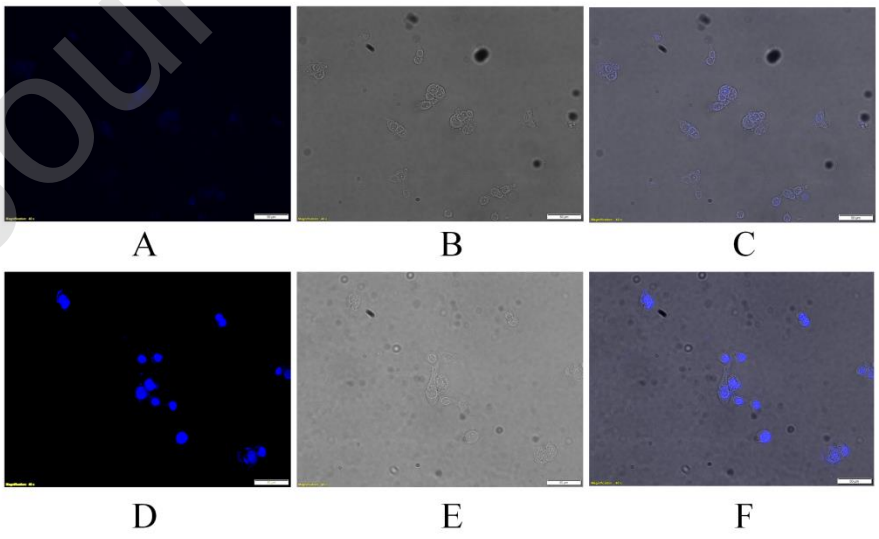

Fig. 8. (A) Fluorescence image of AGS cells after incubation with 15 μM HCBP, respective bright field (B), merged field (C). (D) Flourescence image of AGS cells after incubation with 15 μM HCBP and 15 μM Al^{3+} and its respective bright field (E) as well as merge field (F).