Electronic Supplementary Material (ESI) for Analyst.

Di-oxime based selective fluorescent probe for arsenate and arsenite ions in purely aqueous medium with living cell imaging application and H-bonding induced microstructure formation

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Content:

[1]H-NMR spectrum of DFC-DO :

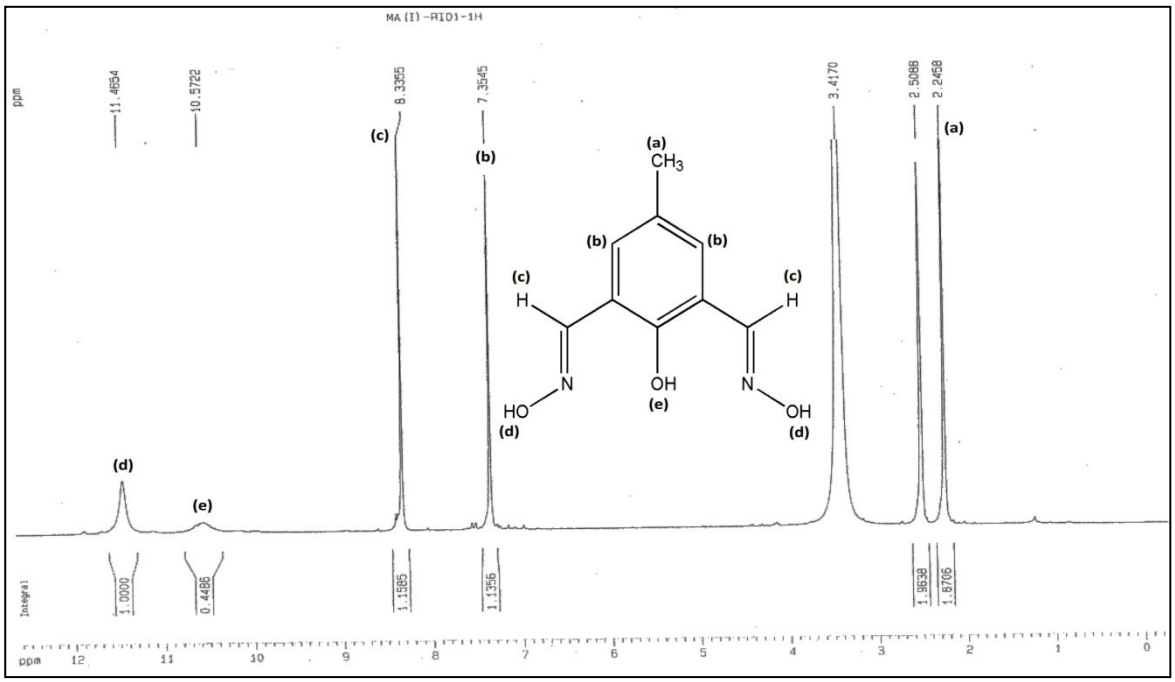


Fig.S1(a).Proton numbering scheme of ligand DFC-DO.

Table I:[1]H NMR shifts for the free ligand DFC-DO:

Ligand Name	δ in ppm
DFC-DO in DMSO-d_6	7.35(2-H, s, aromatic protons), 8.35(2-H, s, CH), 2.24(3-H, s , CH_3), 10.57(1-H,s,- aromatic OH), 11.46(1-H,s, -oxime OH)

Mass spectrum of DFC-DO:

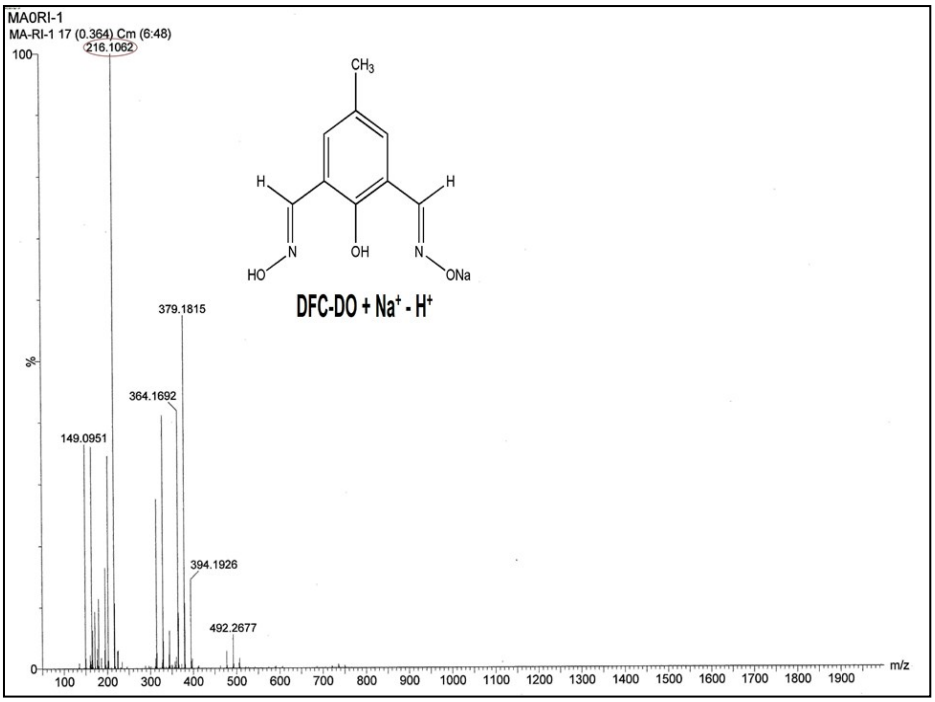


Fig.S1(b) Mass spectrum of DFC-DO in MeOH

Infra-red spectrum (DFC-DO) :

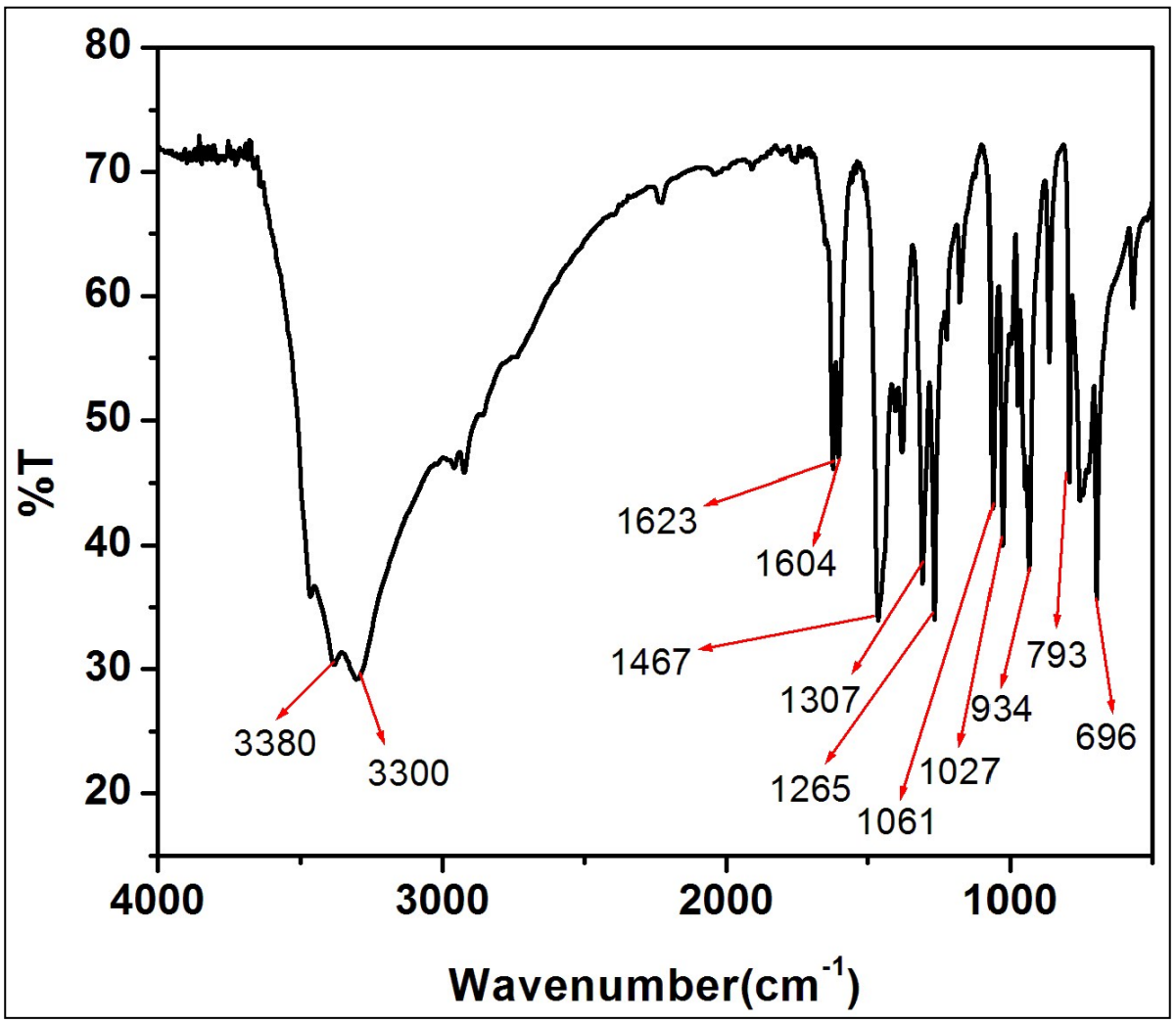


Fig.S1(c) IR spectrum of ligand DFC-DO

Experimental:

Instruments (Physical measurements):

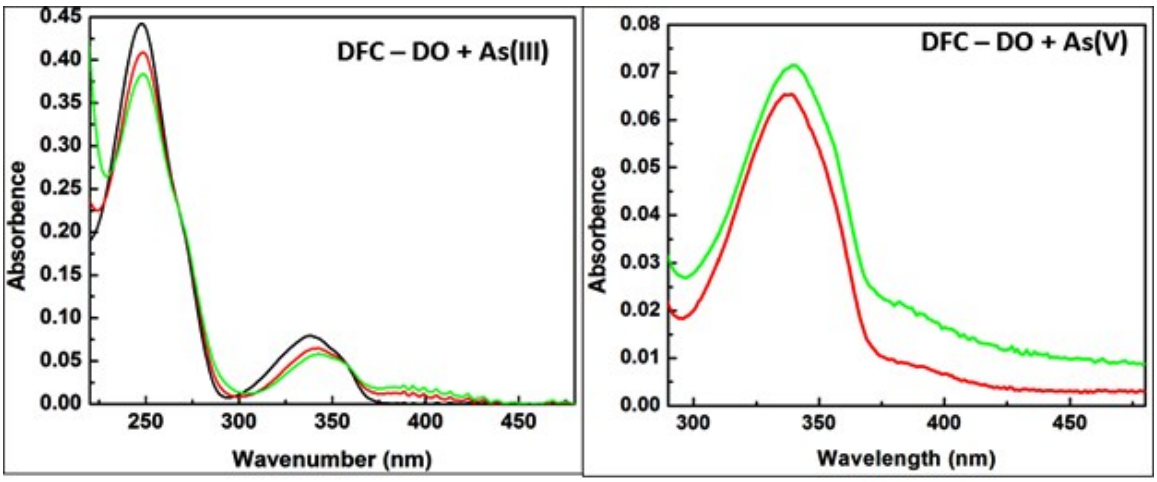
The Fourier Transform Infrared spectra (4000 – 400 cm^{-1}) of the ligands were recorded on a Perkin-Elmer RX I FT-IR spectrophotometer system with solid KBr disc. Electronic spectra were recorded on an Agilent 8453 Diode-array UV-vis spectrophotometer using HPLC grade H_2O as solvent with 1 cm quartz cuvette in the range 200-900 nm. Fluorescence study was done in PTI (Model QM-50) spectrofluorimeter,^{1}H NMR spectrum was recorded on a BRUKER 300MHz FT-NMR spectrometer using trimethylsilane as an internal standard inDMSO (d_6).

Solution preparation for UV-Vis and fluorescence studies :

For both UV-Vis and fluorescence titrations, stock solutions of 1.0 x 10^{-3} M of the probe DFC-DO were prepared by dissolving the L in 0.5 ml MeOH and finally the volume is make up to 10 ml by de-ionised water. Similarly, another 1.0 x 10^{-2} M stock solutions of AsO_2^- and $H_2AsO_4^-$were prepared in de-ionized H_2O. A solution of 10 mM HEPES buffer was prepared and pH was adjusted to 7.24 by using HCl and NaOH. 2.5 ml of this buffered solution was pipetted out into a cuvette to which 20 μM of the probe was added and AsO_2^- or $H_2AsO_4^-$ ions were added incrementally starting from 0 to 560 μM and 0 to 1200 μM respectively in a regular interval of volume and UV-Vis and fluorescence spectra were recorded for each solution.Path length used of the cells for absorption and emission studies was 1 cm.Fluorescence measurements were performed using 5 nm x 3 nm slit width.

UV-Vis absorption studies:

The spectrophotometric titration for the interaction of DFC-DO (20 μM) with AsO_2^-(560 μM)and $H_2AsO_4^-$(1200 μM) at 25 °C in pure aqueous medium at pH 7.24 (10 mM HEPES buffer) reveals that there is small increase in absorption intensity of DFC-DO at 376 nm for AsO_2^-; additionally, there is a red shift with slight decrease in absorbance at 340 nm. For $H_2AsO_4^-$ there are slight increase absorbance at 340 and 376 nm with the increase in the concentration. (Fig.S2)



(a) **(b)**

Fig.S2. Change in absorption spectra of DFC-DO (20 μM) upon addition of **(a)**AsO_2^- (0–560 μM) and **(b)**$H_2AsO_4^-$(0–1200 μM). Conditions:DFC-DO (20 μM), HEPES buffer at pH 7.24 in H_2O at 25 °C.

Fluorescence titration of $H_2AsO_4^-$:

The fluorescence titration for the interaction of DFC-DO with $H_2AsO_4^-$ in the same solvent system as used in UV-Vis. titration reveals that the addition of $H_2AsO_4^-$ leads to an increase in fluorescence intensity at around 460 nm.

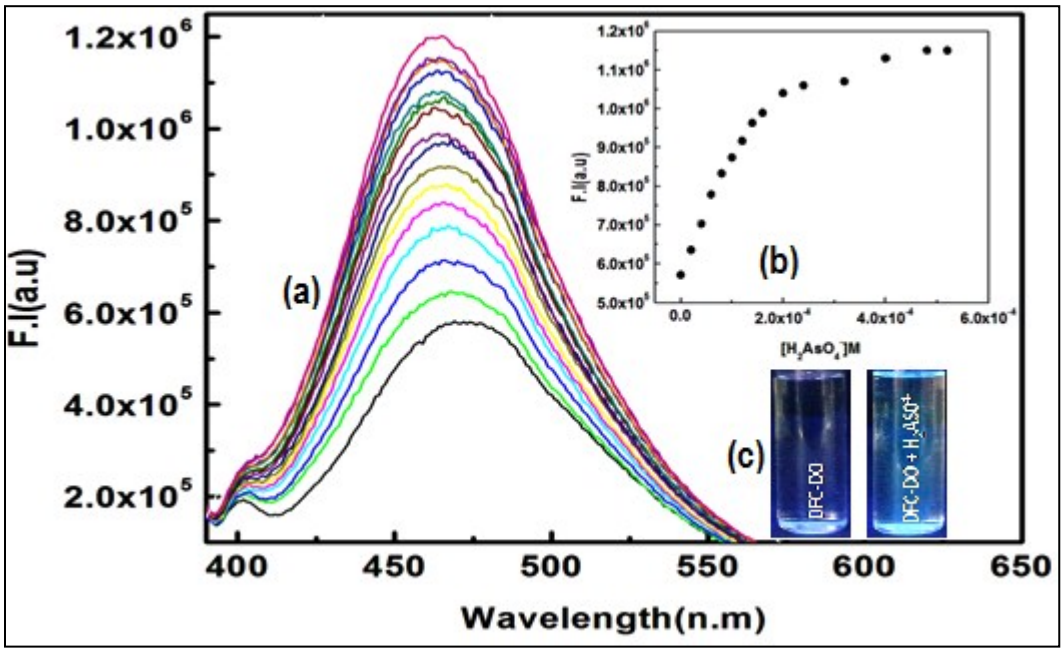


Fig.S3. Change in fluorescence spectra of DFC-DO (20 μM) upon addition of $H_2AsO_4^-$ in HEPES buffer at pH 7.24 in H_2O at 25 °C, [$H_2AsO_4^-$] = 0–1200 μM. (b) Plot of F.I. (at 460 nm) vs. [$H_2AsO_4^-$]; (c) UV-exposed emission image of and DFC-DO–$H_2AsO_4^-$.

Calculation for LOD value

To determine the detection limit, fluorescence titration of **DFC-DO** with $AsO_2^-$and $H_2AsO_4^-$was carried out by adding aliquots of micromolar concentration of $AsO_2^-$and $H_2AsO_4^-$.

However, the detection limits (LOD) of AsO_2^- and $H_2AsO_4^-$ have been calculated by 3σ method.

$$LOD = 3 \times S_d/S$$

Where, S_d is the standard deviation of the intercept of the blank (DFC-DO) obtained from a plot of FI vs. [DFC-DO], and S is the slope obtained from linear part of the plot of FI vs. [AsO_2^-] and [$H_2AsO_4^-$] respectively.

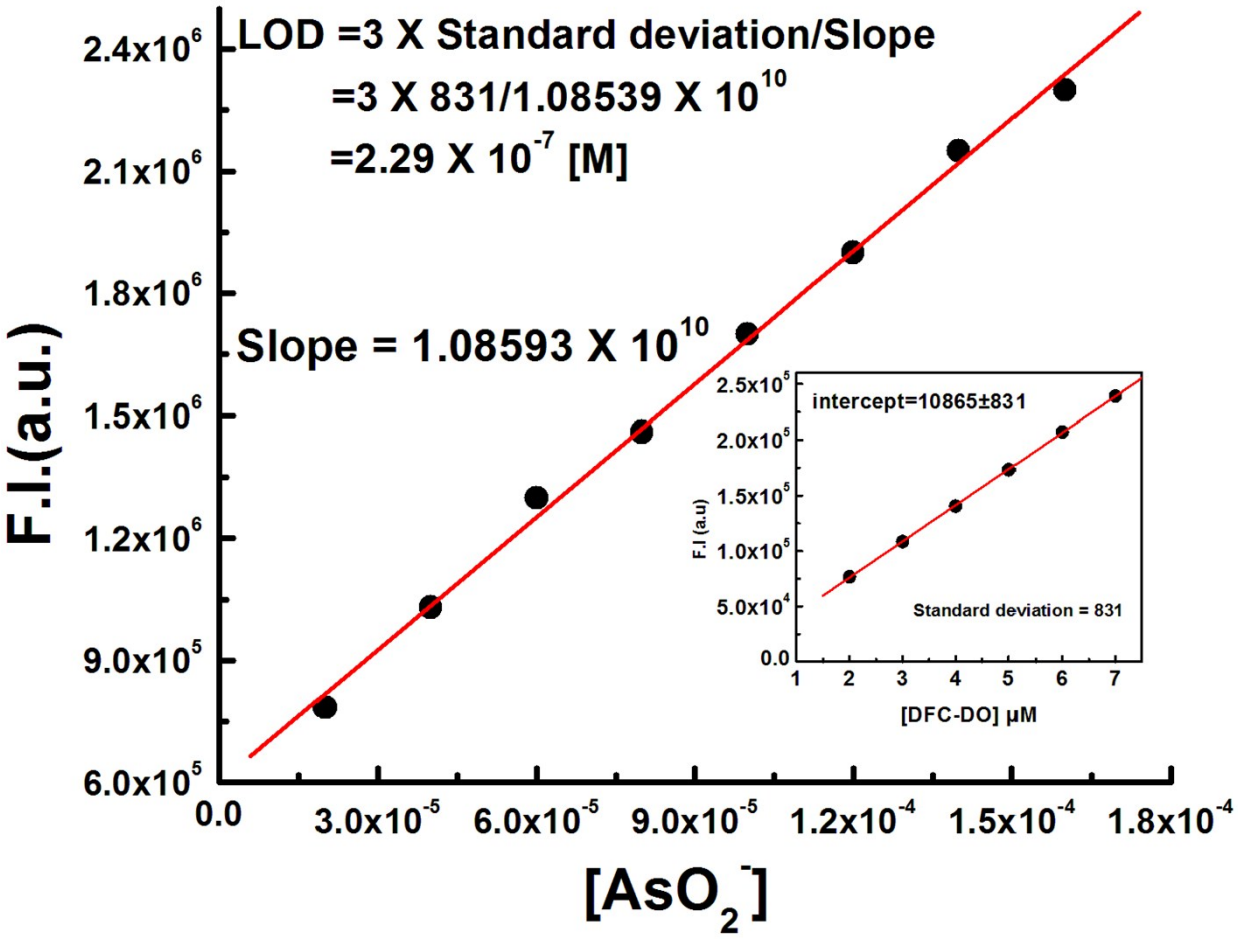


Fig.S4(a). Determination of LOD of AsO_2^-

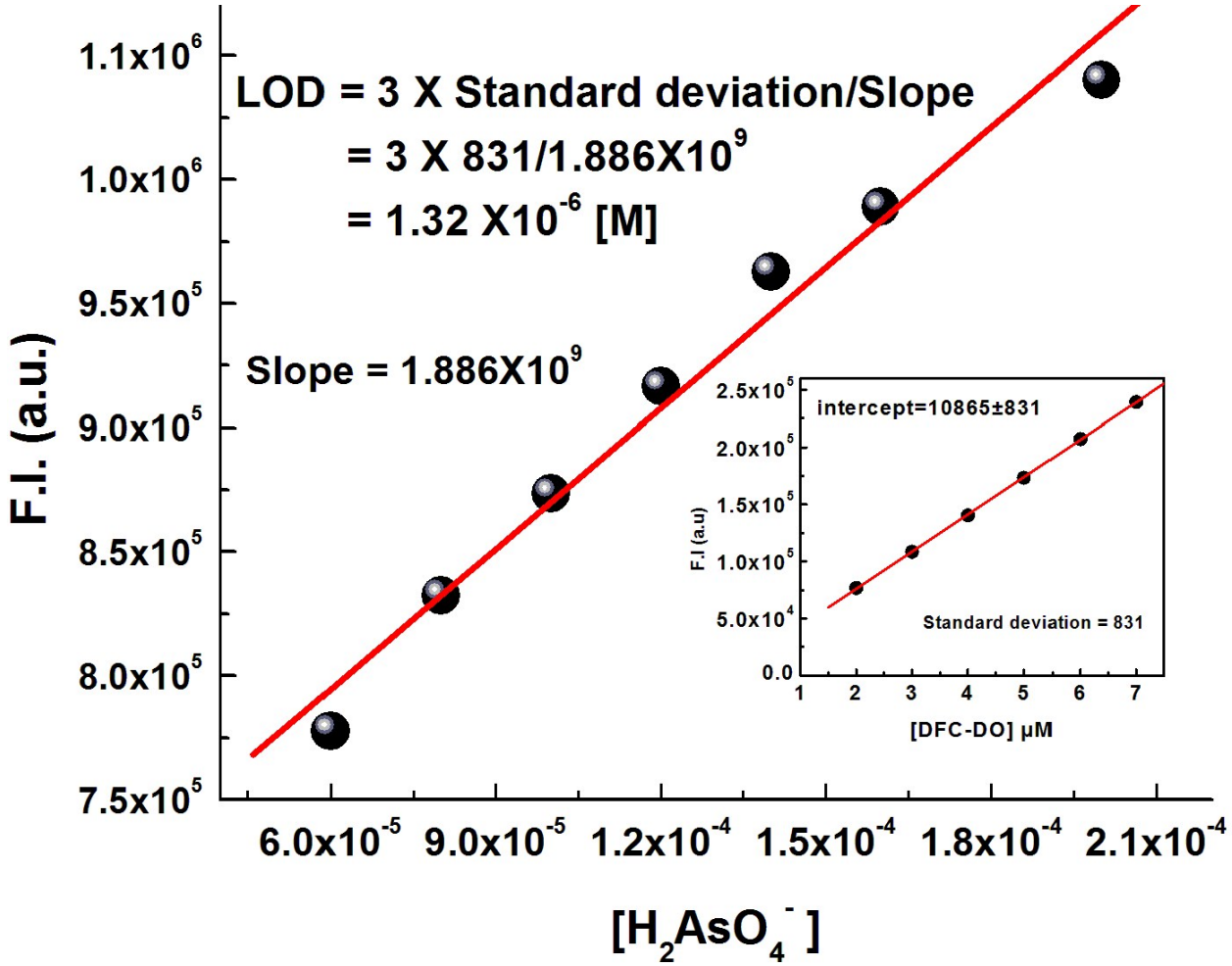


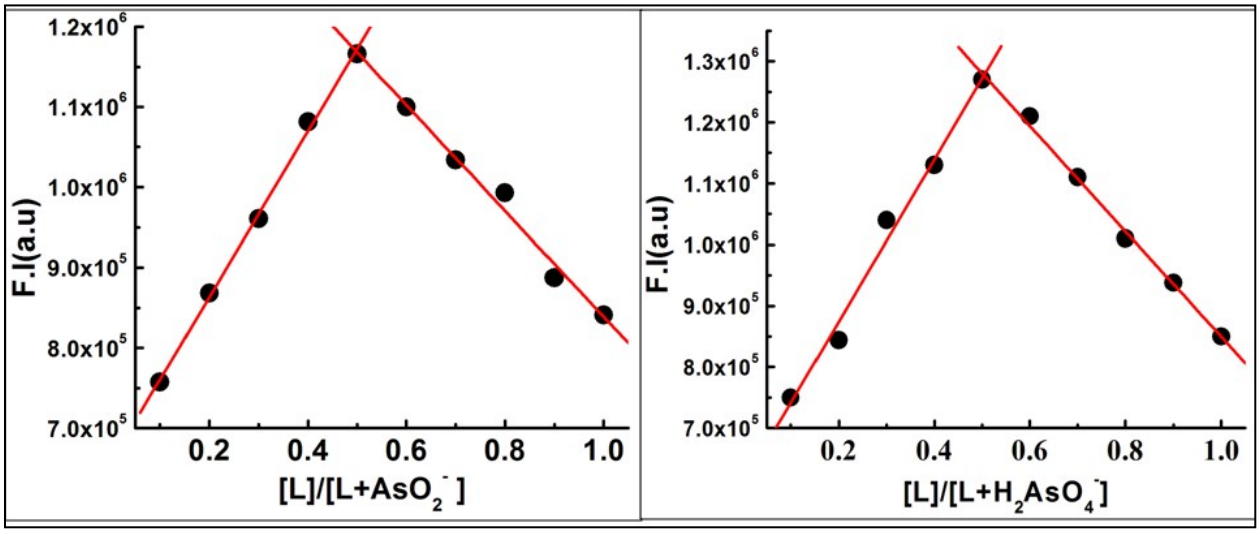
Fig.S4(b). Determination of LOD of $H_2AsO_4^-$

The LOD value obtained for $[AsO_2^-]$ is 0.23 µM and for $[H_2AsO_4^-]$ becomes 1.32µM.

JOB's Plot

This method is based on the measurement of F.I. of a series of solutions in which molar concentrations of two reactants vary but their sum remains constant. The fluorescence intensity of each solution was measured at a suitable wavelength and plotted against the mole fraction of one reactant. A maximum in fluorescence intensity appeared at the mole ratio corresponding to the combining ratio of the reactants.

The composition of the complex was determined by JOB's method and found to be (1:1) with respect to ligand for both AsO_2^- and $H_2AsO_4^-$. Fig.S5(a) and Fig.S5(b).respectively.



(a) (b)

Fig.S5. JOB's plot for(a) AsO_2^- and (b) $H_2AsO_4^-$.

Mass Spectroscopy :

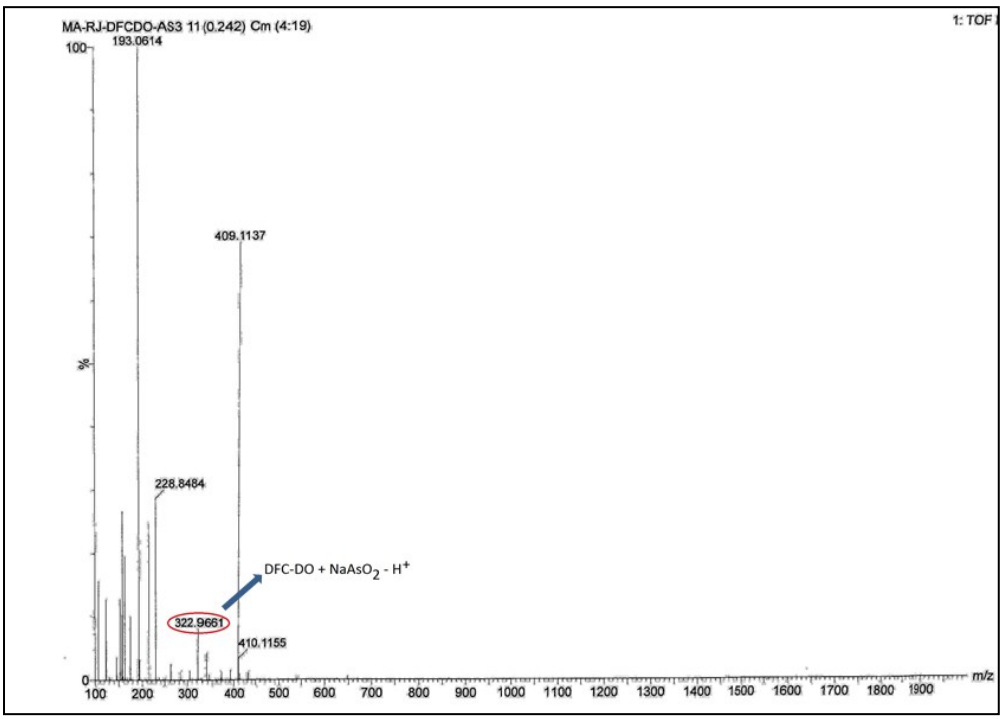


Fig.S6(a) Mass spectra of DEC-DO + $NaAsO_2$ - H^+

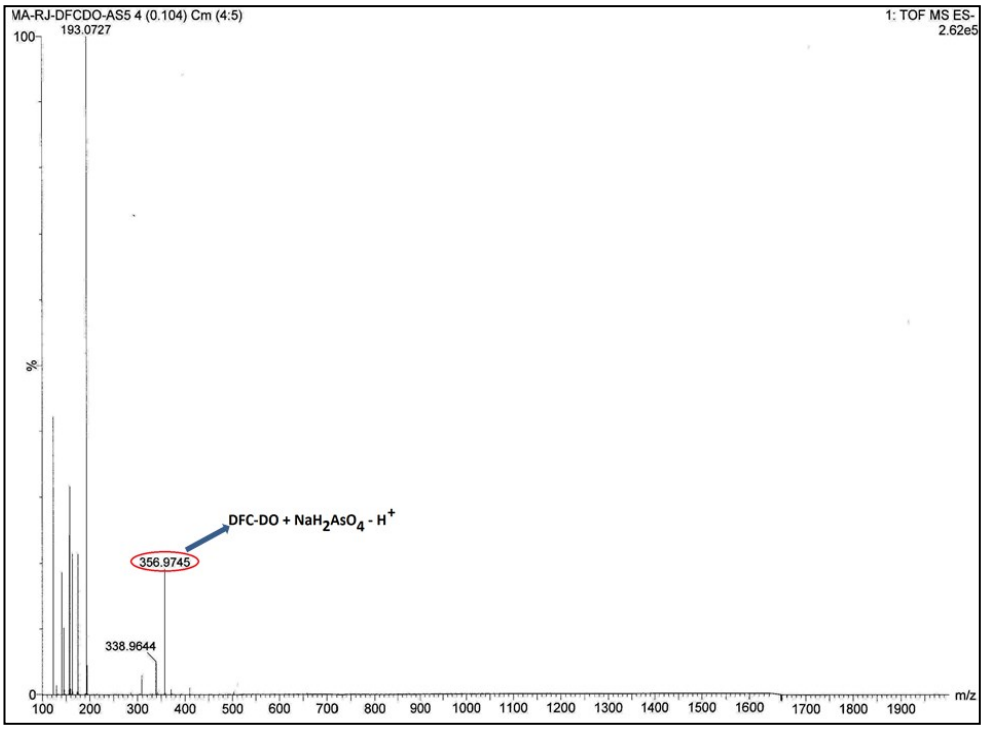


Fig.S6(b) Mass spectra of DEC-DO + NaH_2AsO_4 - H^+

Selectivity Study

The selective sensing of analyte is an important criterion for a successful sensor. In order to check the selectivity of the probe (DFC-DO) towards AsO_2^- and $H_2AsO_4^-$ detection we carried fluorescence experiments with 20 μM DFC-DO and 600equivalentof different anions. It was interesting to note that the detection of AsO_2^- and $H_2AsO_4^-$ was not perturbed by biologically abundant Na^+, K^+, Ca^{2+} etc metal ions. Several transition metal ions, namely Cr^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and heavy metal ions like Cd^{2+}, Pb^{2+}, and Hg^{2+} also caused no interference (**Fig. S7(a)** and **Fig. S7(b))**. Also the other anions do not interfere in the detection of AsO_2^- and $H_2AsO_4^-$.

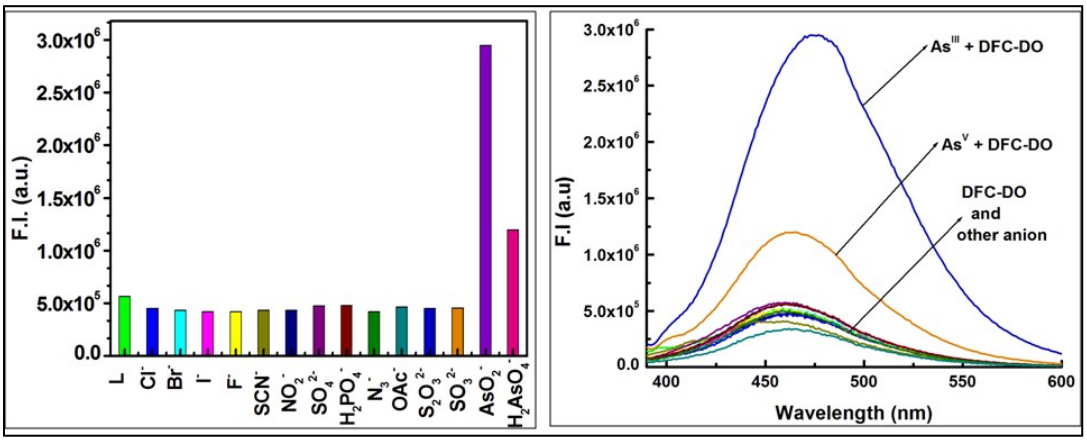


Fig.S7(a). Selectivity of DFC-DO (20 μM) for AsO_2^- and $H_2AsO_4^-$. Fluorescence response of DFC-DO following the addition of 1200 μM of the different anions of interest in aqueous HEPES buffer solution (pH 7.24): where anions =Cl^-, Br^-, I- F-, SCN-, NO_2^-, SO_4^{2-}, $H_2PO_4^-$, N_3^-, CH_3COO^-, $S_2O_3^{2-}$, SO_3^{2-}, AsO_2^-, $H_2AsO_4^-$, λ_{ex} = 340 nm.

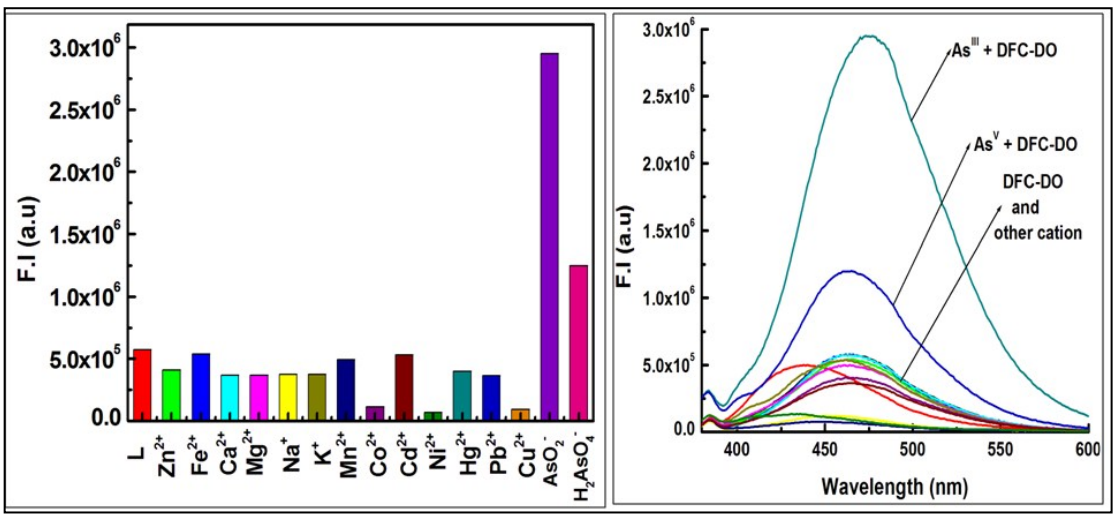


Fig.S7(b) Selectivity of DFC-DO (20 μM) for AsO_2^- and $H_2AsO_4^-$fluorescence response of DFC-DO in pure water buffered with 10 mM HEPES at pH 7.24 following the addition of 1200 μM of the cations of interest. Where cations = Zn^{2+}, Fe^{2+}, Ca^{2+}, Mg^{2+}, Na^+, K^+, Mn^{2+}, Co^{2+}, Ni^{2+}, Hg^{2+}, Pb^{2+}, Cu^{2+}, AsO_2^-, $H_2AsO_4^-$, λ_{ex} = 340 nm.

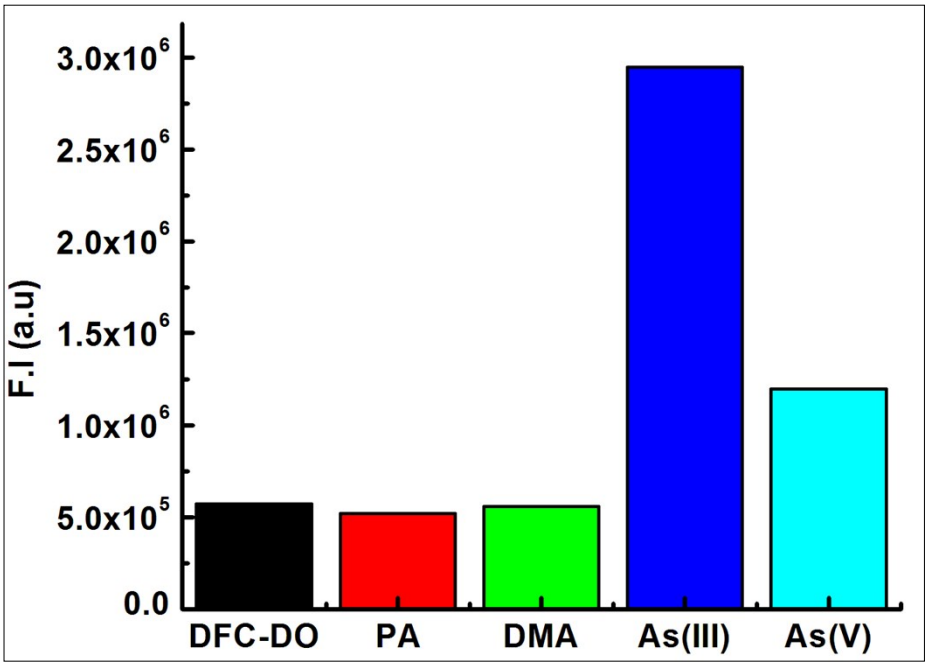


Fig.S7(c). Fluorescence response of DFC-DO(20 μM) in pure water buffered with 10 mM HEPES at pH 7.24 with different arsenic species, Phenylarsonic acid (PA), Dimethylarsenic acid (DMA), $NaAs^{III}O_2$ and $NaH_2As^{V}O_4$.λ_{ex} = 340 nm

pH Study:

For biological applications, the appropriate *p*H condition for a successful probe is utmost importance. The probe **DFC-DO** fluoresces rather very weakly between pH 2 and 8 while DFC-DO–$H_2AsO_4^-$ and DFC-DO–AsO_2^- complexes fluoresce extensively between pH 6 and 8 in 10mM HEPES buffer, clearly indicating that this pH range is suitable for fluorescence studies for the recognition of $H_2AsO_4^-$ and AsO_2^-.**Fig. S8.**

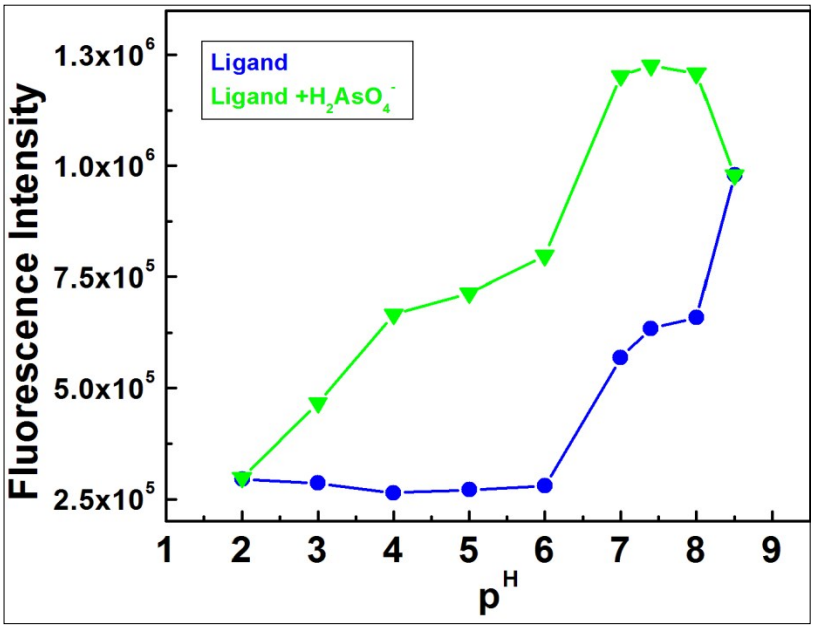


Fig.S8(a).pH dependence of fluorescence responses of DFC-DO and DFC-DO + $H_2AsO_4^-$ inaqueous HEPES buffer solution (pH 7.24).

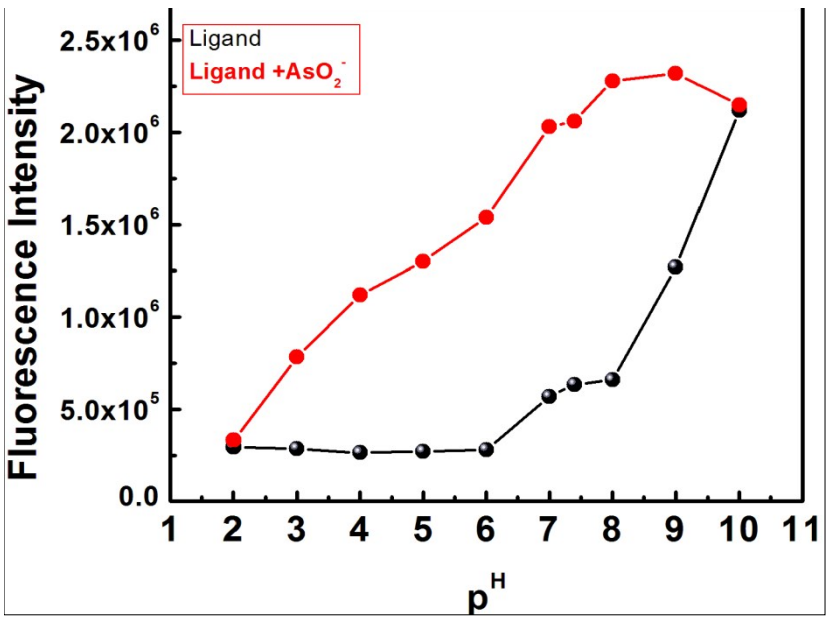


Fig.S8(b).pH dependence of fluorescence responses of DFC-DO and DFC-DO + AsO_2^- inpure water HEPES buffered solution (pH 7.24).

^{1}H-NMR Titration :

The co-ordination modes were further supported by ^{1}H-NMR studies (**Fig.S9**) which clearly showed the protons of the free ligand (oxime –OH)11.46 ppm, (phenolic -OH) 10.57 ppm shifted to a up field (ppm) and broadened, in presence of slight excess of $AsO_2^-$and $H_2AsO_4^-$ respectively. Azomethine proton remains unchanged due to non-participating behavior. However, ^{1}H-NMR peaks corresponding to phenolic -OH and oxime –OH vanishes on adding excess ions, suggesting their involvement in bonding towards AsO_2^- and $H_2AsO_4^-$.

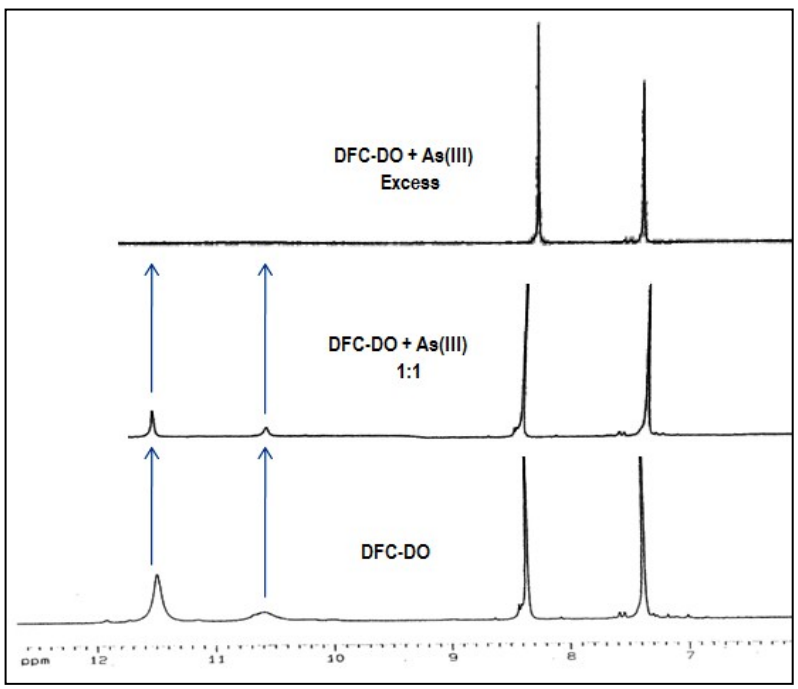


Fig.S9(a).^{1}H-NMR for DFC-DO and AsO_2^-.

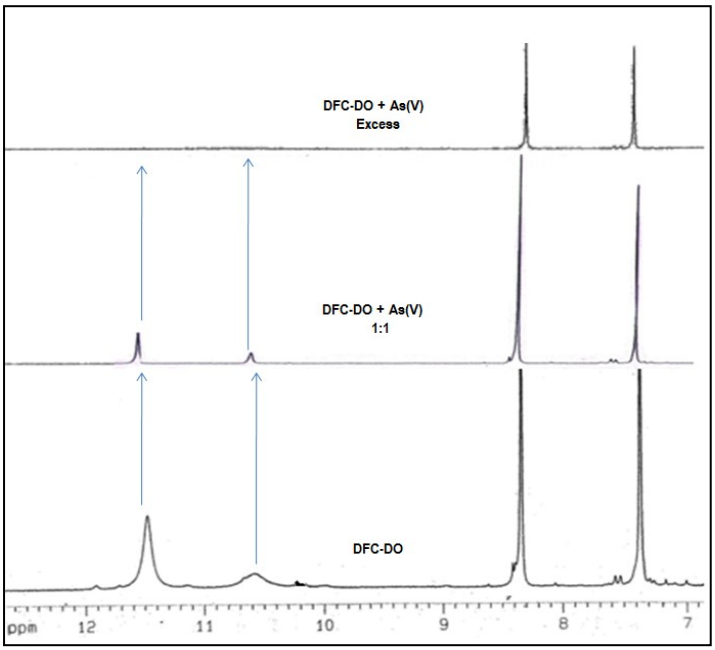


Fig.S9(b). ^{1}H-NMR for DFC-DO and $H_2AsO_4^-$.

Calculation of Quantum Yield:

Fluorescence quantum yields (Φ) were estimated by integrating the area under the fluorescence curves using the equation,

$$\Phi_{sample} = (OD_{std} \times A_{sample})/(OD_{sample} \times A_{std}) \times \Phi_{std}$$

Where, A_{sample} and A_{std} are the area under the fluorescence spectral curves and OD_{sample},OD_{std} are the optical densities of the sample and standard, respectively at the excitation wavelength.

Quninesulphate has been used as the standard with Φ_{std} = 0.54 in water formeasuring the quantum yields of **DFC-DO** and **DFC-DO–AsO_2^-** and **DFC-DO–$H_2AsO_4^-$** systems.

Cell culture

Human hepatocellular liver carcinoma cells (HepG2) cell line (NCCS, Pune, India), were grown in DMEM supplemented with 10% FBS and antibiotics (penicillin-100 µg/ml; streptomycin-50 µg/ml). Cells were cultured at 37ºC in 95% air, 5% CO2 incubator.

Cell Cytotoxicity Assay

To determine % cell viability of ligand (**DFC-DO**),MTT assay was performed on HepG2 cells (1×10^5 cells/well) which were cultured in a 96-well plate at 37°C.These were exposed to varying concentrations of L, 0.5, 1, 5, 10, 20, 40, 60, 80 and 100 µM respectively for 12h. 10 µl of MTT solution (5 mg/ml1X) in phosphate-buffered saline (PBS)) was added to each well of a 96-well culture plate and again incubated continuously at 37^0C for a period of 4 h. All media were removed from wells and 100µl of DMSO was added to each well and absorbance was measured at 550nm (EMax Precision MicroPlate Reader, Molecular Devices, USA). All experiments were performed in triplicate and the relative cell viability (%) was expressed as a percentage relative to the untreated control cells.

Cell Imaging Study

HepG2 cells were culture and incubated in 35x10 mm culture dish over coverslip for 24 h at 37°C in conditioned media (DMEM, 10% FBS). The HepG2 cells were allowed to incubate separately with 10 µM $H_2AsO_4^-$ (in 2 culture dishes), 10µm AsO_2^- (in 2 culture dishes) and 10 µm **DFC-DO** for 2 h, 4 h and 6 h at 37°C. After incubation for 2 h , 4 h and 6 h, each medium were discarded from $H_2AsO_4^-$ (from 1st culture dish), AsO_2^- (from 1st culture dish) and **DFC-DO** treated culture dish and washed twice with 1X PBS. Now the coverslip containing HepG2 cells were mounted over the slide. Bright field and fluorescence images of HepG2 cells were taken by fluorescence microscope (Leica DM3000, Germany) with an objective lens of 40X magnification. Simultaneously, on the another culture dishes which were preincubated with 10µM $H_2AsO_4^-$ (2nd culture dish) and 10µM AsO_4^- (2nd culture dish) at 2h, 4h and 6h at 37°C, washed twice with 1X PBS to remove the medium containing extracellular $H_2AsO_4^-$ and AsO_2^- and, then incubated with 10µM of **DFC-DO** for 30 min at 37^0C, then further washed with 1X PBS for two times to remove the extraneous **DFC-DO**. HepG2 cells were mounted over the slide and bright field and fluorescence images were taken at 40X

magnification. The cell imaging studies were carried out three times (Fig.5 (Experimental set-I), Fig. S11 (Experimental set-II and III).

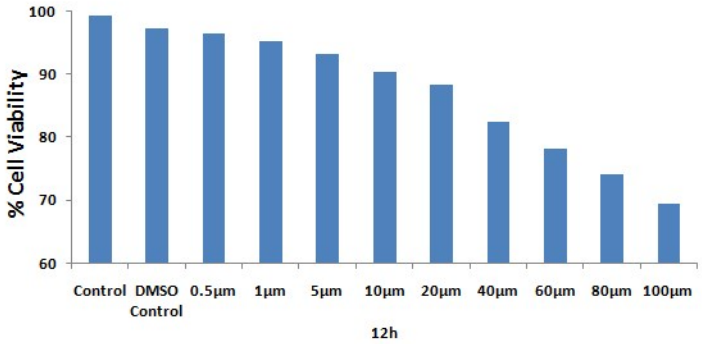
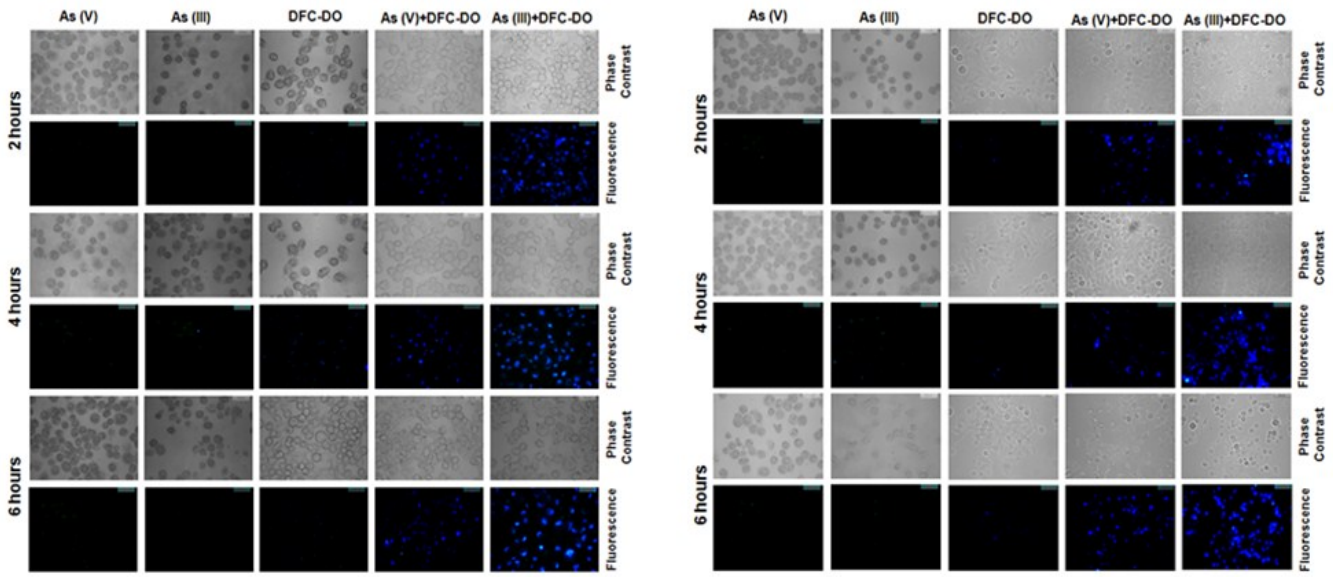


Fig.S10. MTT assay of ligand DFC-DO



Experimental Set II **Experimental Set III**

Fig.S11. The phase contrast and fluorescence images (40X) of HepG2 cells were taken after incubation with 10 µM $H_2AsO_4^-$, 10 µM AsO_2^- and 10 µM DFC-DO for 2 h, 4 h and 6 h at 37ºC followed by washing with 1X PBS for two times to remove the extraneous $H_2AsO_4^-$, AsO_2^- and DFC-DO species (Column 1, 2 and 3). Concurrently, HepG2 cells were pre-incubated with 10 µM $H_2AsO_4^-$, 10 µM AsO_2^- for 2 h, 4 h and 6 h at 37º C followed by washing with 1X PBS for two times to remove the extraneous $H_2AsO_4^-$ and AsO_2^- specie and, then incubated with 10µM **DFC-DO** for 30 minutes at 37ºC, then further washed with 1X PBS for two times to remove the extraneous **DFC-DO** and observed under microscope (column 4 and 5) as mentioned previously. We have performed three parallel sets (Set I{Fig.5}, Set II and Set III).

Determination of $H_2AsO_4^-$ in water at different places

Table S1. Determination of $H_2AsO_4^-$ in water at different places.

PLACE	$H_2AsO_4^-$ added(μM)	$H_2AsO_4^-$ found (μM) [a]	Recovery (%)	RSD(%)[b,1]
Baruipur (tube well water) 24 Parganas (S)	20	20.15	99.9	2.2
Barasat (tube well water) 24 Parganas (N)	30	30.154	99.5	1.2
Sonarpur (tube well water) 24 Parganas (S)	40	40.474	100.1	1.9
Damdam (tube well water) 24 Parganas (N)	60	60.554	100.2	1.96

[a] mean of five determination; [b]Relative standard deviation from five determinations.

DFT calculations

Ground state electronic structure calculations in gas phase of both the ligand and H-bonded adducts have been carried out using DFT[2] method associated with the conductor-like polarizable continuum model (CPCM).[3-5] Becke's hybrid function[6] with the Lee-Yang-Parr (LYP) correlation function[7] was used throughout the study. The geometry of the ligand and complex was fully optimized without any symmetry constraints.

All the calculations were performed with the Gaussian 09W software package.[8]For geometry optimization of both the ligand and the complexes ($AsO_4^{2-}$and AsO_2^-) in ground state we used 6-31G as basis set under B_3LYP for all the atoms (As, C, H, N and O).

References

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