Selective Al^{3+} and Fe^{3+} Detection Using Imidazole-Oxadiazole Sensors: Bioimaging Evidence from Zebrafish

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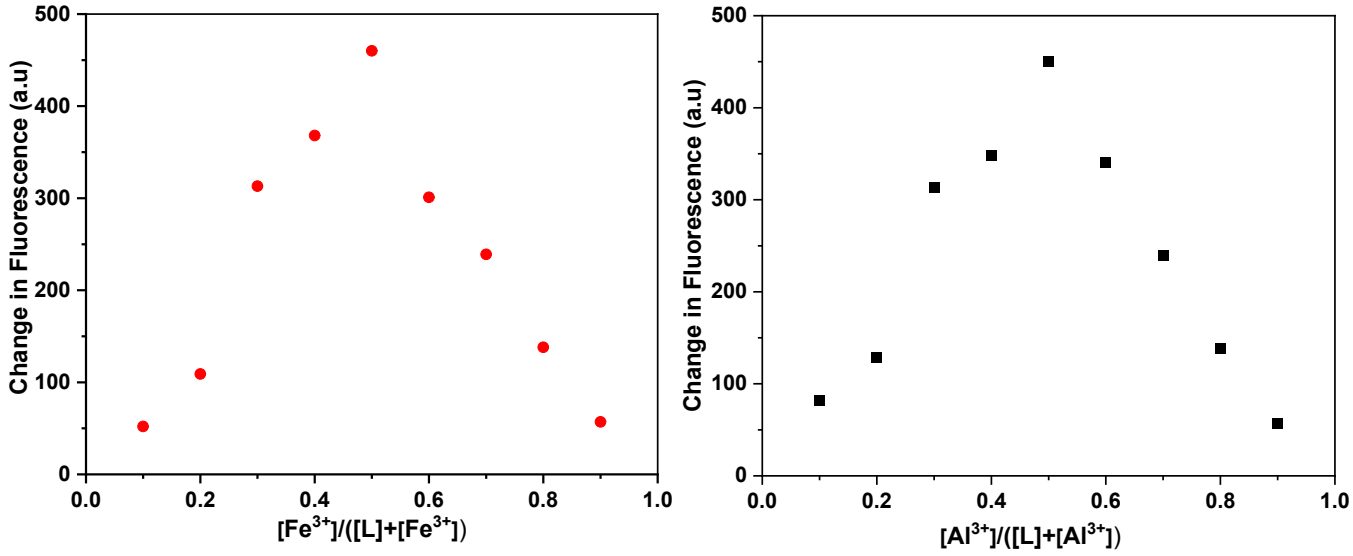


Fig. S1. Job's plot of **TIMF** with metal ions

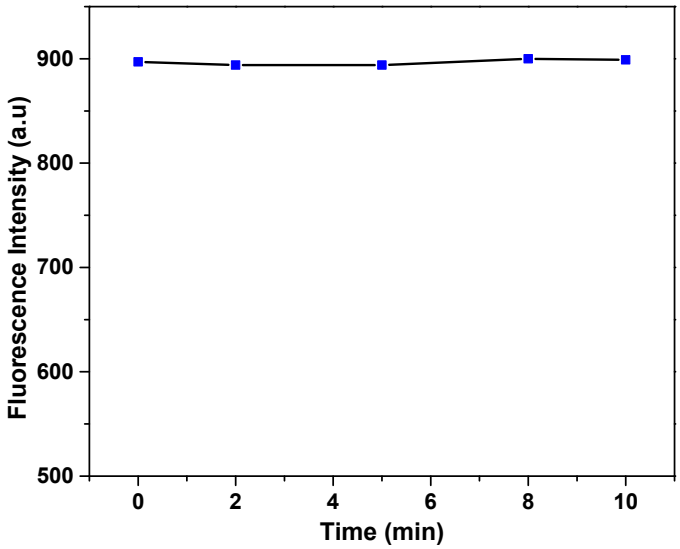


Fig. S2. Time dependent fluorescence studies of **TIMF** in absence of metal ions.

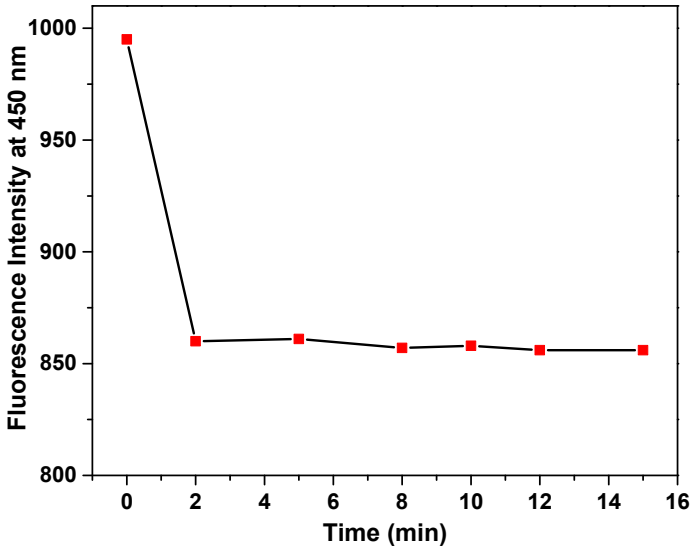


Fig. S3. Time dependent fluorescence studies of **TIMF** in presence of metal ions.

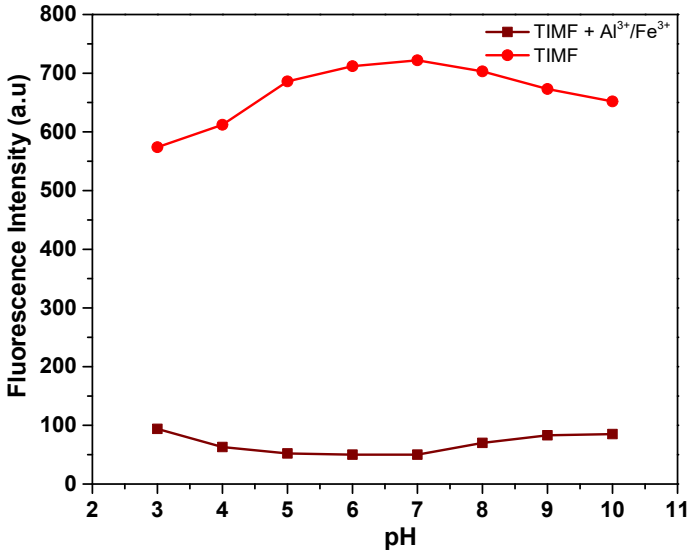


Fig. S4. Fluorescence studies of **TIMF** in absence and presence of Al^{3+}/Fe^{3+} ions at different pH.

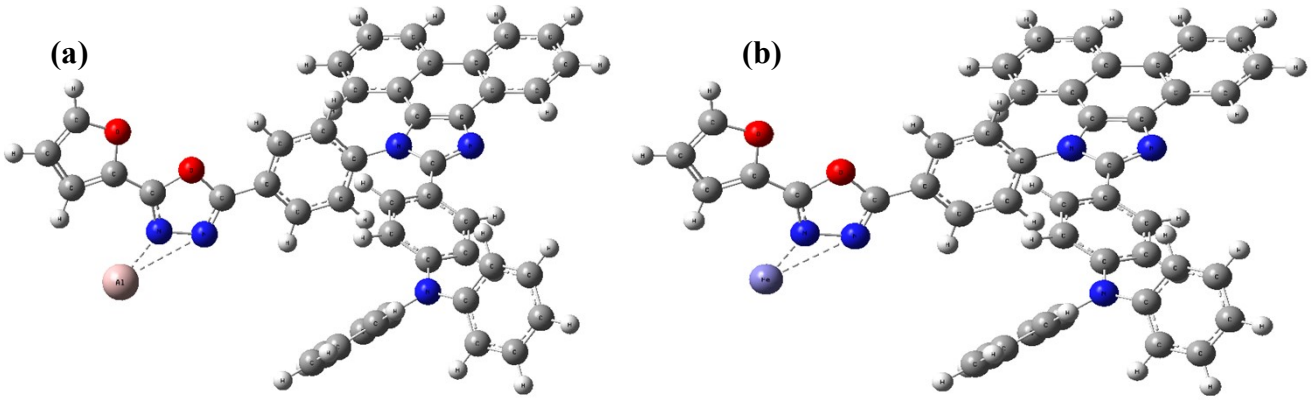


Fig. S5. Binding mode of **TIMF** with Al^{3+} (a) and Fe^{3+} (b).

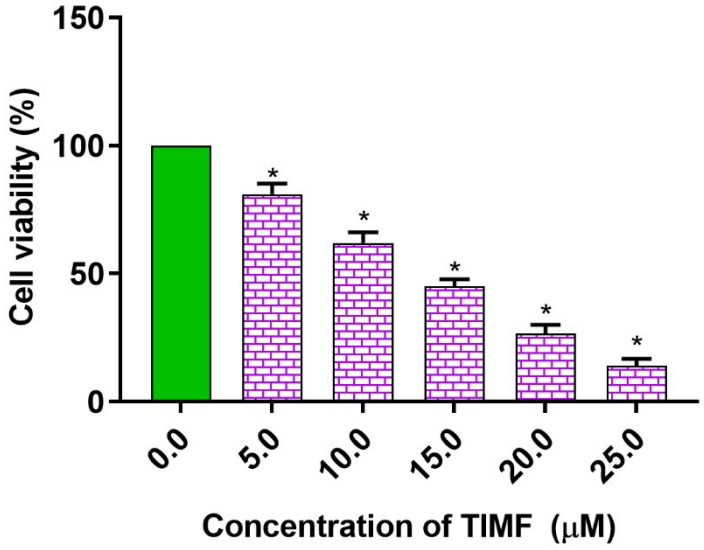


Fig. S6. Dose-dependent effect of ligands on the viability of MDA-MB-231 cells determined by MTT assay. Three times (n = 3) of the analysis were performed, and the results were shown as mean ± standard deviation. The statistical significance of the control and test samples was determined using Dunnett's test, and a *p*-value of 0.05 or lower was considered significant. The asterisk (#) indicates that there is no significant variance between the test sample and the control sample within the respective study. While the asterisk (*) denotes the significance of the test sample relative to the control sample within the respective study.

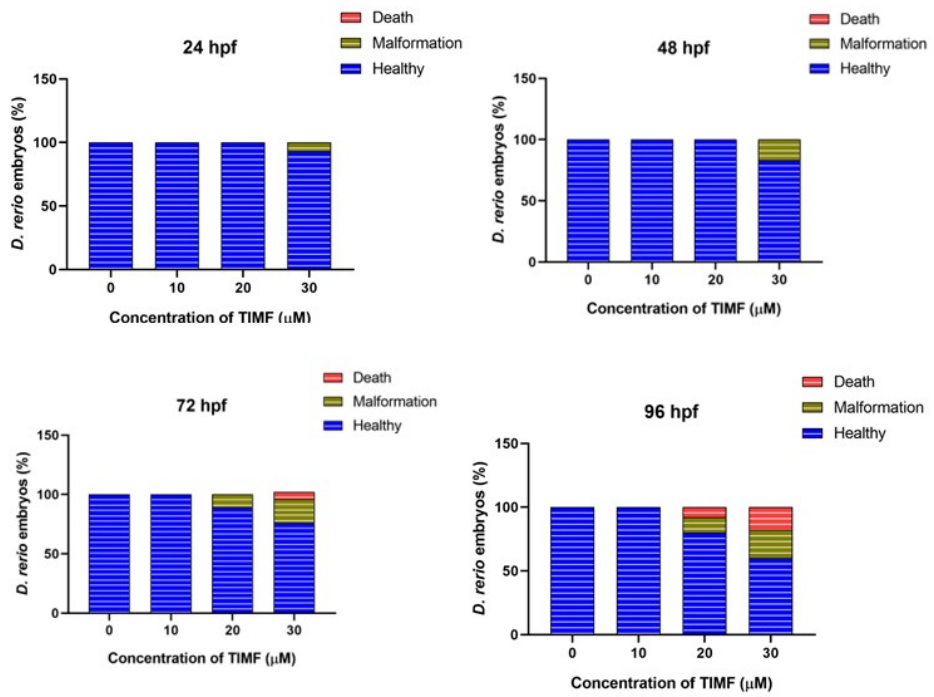
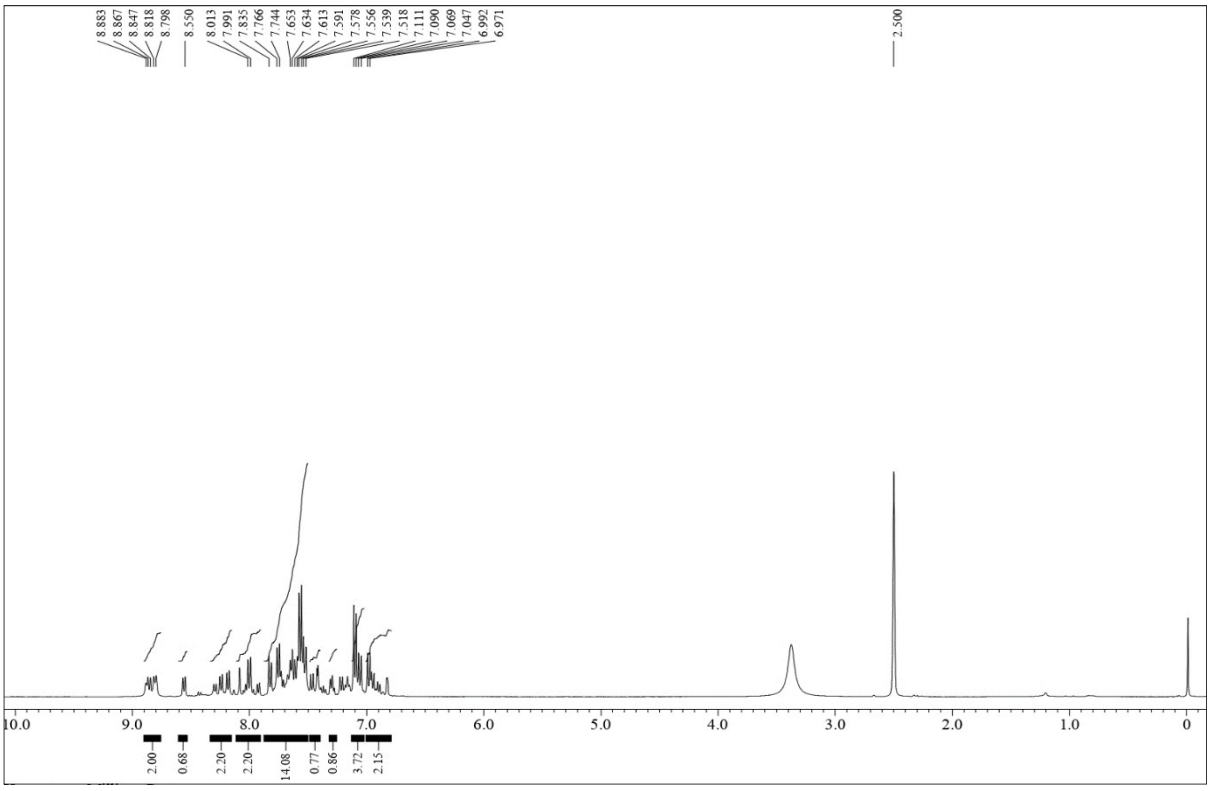
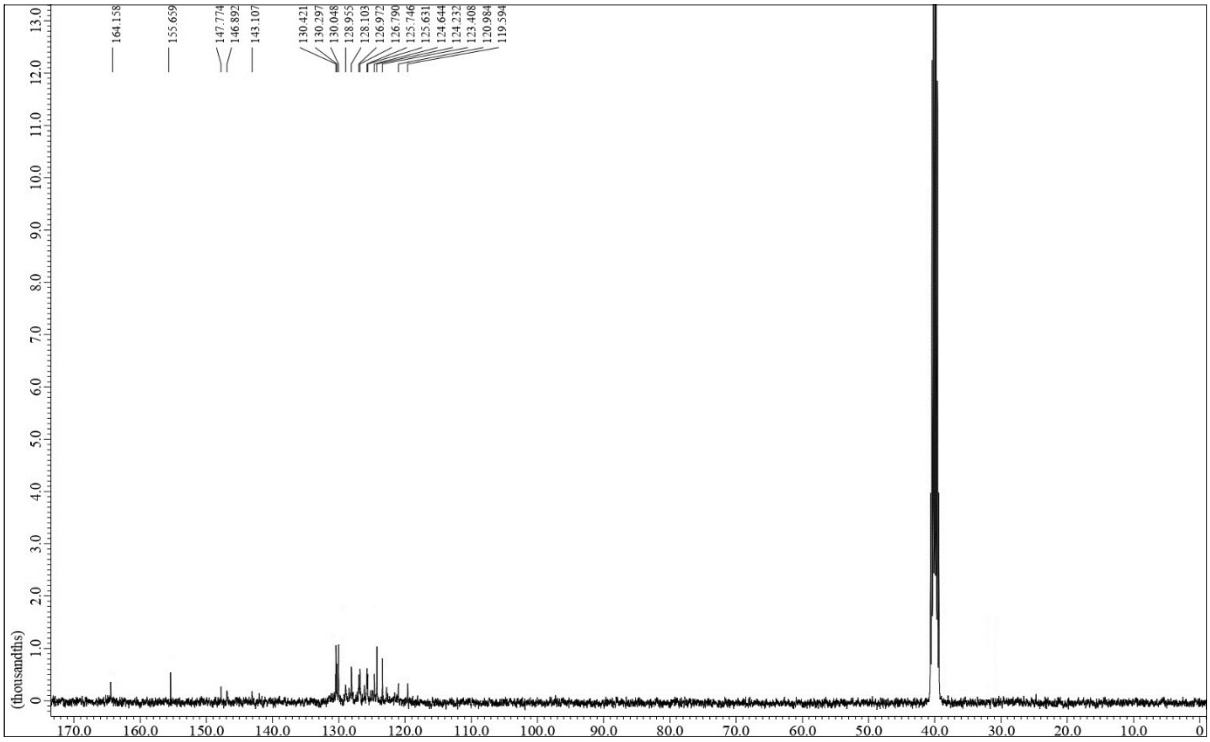


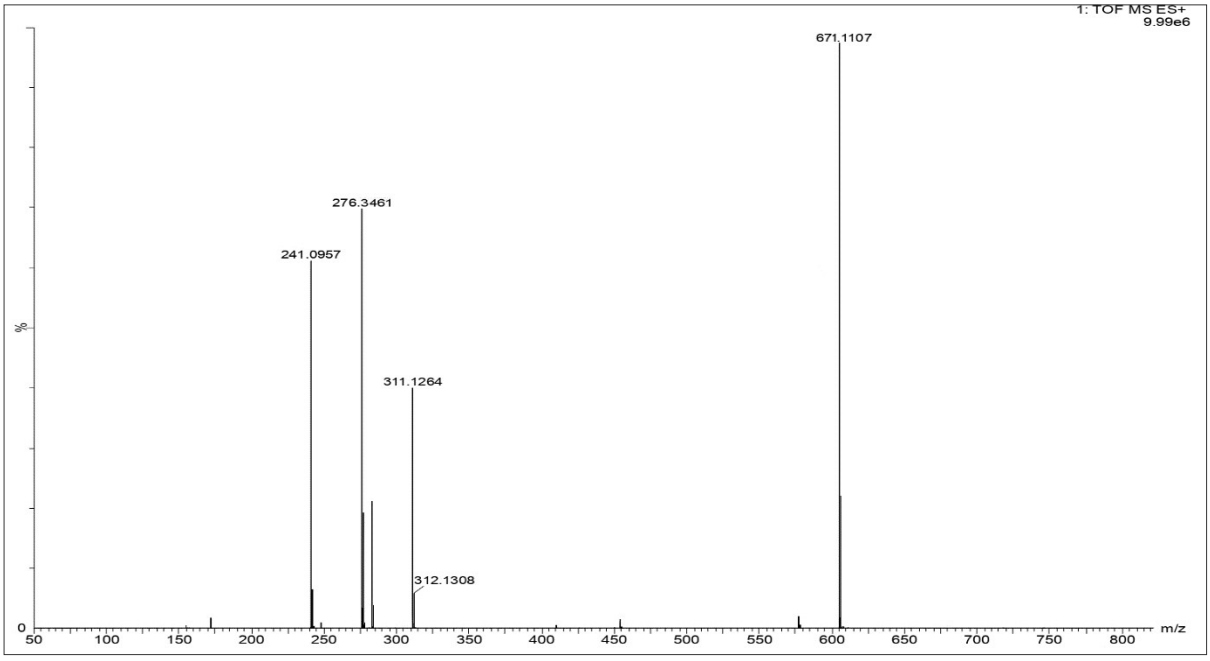
Fig. S7. Dose-dependent toxic effect (developmental defects and death) of **TIMF** on *D. rerio* embryos for 24, 48, 72, and 96 hpf.



Spectrum No. 1 ^{1}H NMR spectrum of **TIMF** (DMSO- $d_{6,}$ 400 MHz)



Spectrum No. 2 ^{13}C NMR spectrum of **TIMF** (DMSO- $d_{6,}$ 100 MHz)



Spectrum No. 3 Mass spectrum of **TIMTB**

Experimental Section

Material and methods

Spectroscopic grade solvents used in the present study were procured from Sigma Aldrich and S. D. Fine Chem Ltd., India. Spectroscopic studies on the fluorophores were carried out maintaining the concentration of fluorescent probes at 10^{-6}M. Absorption and fluorescence spectra of the fluorophores were recorded using Carry-300 UV-Vis spectrophotometer and Hitachi F-7000 fluorescence spectrophotometers, respectively. All the solvatochromic data were analysed using Origin 8.5 software. Theoretical computations were carried out using Gaussian 09W program.Penicillin, streptomycin, dimethyl sulfoxide (DMSO), molecular biology grade water, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), trypsin, Dulbecco's phosphate-buffered saline (DPBS), fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were acquired from Sigma-Aldrich, India. The plastic ware was obtained from Tarsons products, India.

In-vitro and *in-vivo* toxicity and bioimaging analysis

Different ligand concentrations were tested for *in-vitro* and *in-vivo* toxicity in MDA-MB-231 cells (human breast adenocarcinoma) and *Danio Rerio* embryos, respectively. MTT assay and cell morphological analysis were used to assess the toxicity in MDA-MB cells. Deformity and mortality were considered indicators of toxicity in *D. rerio* embryos. At the safest ligand concentration, bioimaging of Al^{3+} was successfully performed in MDA-MB and *D. rerio* embryos.

In-vitro toxicity and bioimaging in MDA-MB-231 cells

The national centre for cell science (NCCS) in India's repository cell line center supplied the MDA-MB-231 cells. The cells were cultured in DMEM media with 10% FBS at 37 °C, 5% CO_2, and 95% air in a humidified incubator. The cells were grown in 25 cm^2 flasks

with alternate days of media replenishment. Confluent cells that were 80 to 90% were used in the toxicology and bioimaging experiments.

The effect of ligands on cell viability was revealed by MTT assay and the study was conducted in accordance with Kalagatur et al.,.[1] A total of 1.8×10^3 cells were plated in a 96-well cell culture plate, allowed to adhere for overnight, and then subjected to 24 h of treatment with various doses of ligands distinctly (up to 20.0 μM).Afterward, 100 μL of MTT reagent (5.0 mg/mL in DPBS) was replaced with culture medium and incubated for 4 h. Following, the MTT solution was replaced with DMSO for 30 min to liquefy the formazan crystals and the multiplate reader was used to measure the optical density of formazan solution at 570 nm. The control cells weren't exposed to ligand. The cell viability of ligand-treated cells was calculated as a percentage of control cells (100%).

The effect of ligands on cells' morphology was observed by a bright-field inverted microscope. Cells were plated in 24-well cell culture plates at a density of about 1.5×10^4 cells per well, allowed to settle overnight, and then exposed to ligands distinctly at various concentrations (up to 20 μM) for 24 h in DMEM devoid of FBS. Cells not treated with ligands were regarded as the control group. Cell morphology was examined using a bright-field inverted microscope after a 24 h incubation period.

Following the toxicity assessment of the ligands, bioimaging of Al^{3+} in MDA-MB-231 cells was done at the safest ligand concentration. Briefly, cells were seeded at a density of approximately 1.5×10^4 per well on 24-well cell culture plates and allowed to settle overnight.The ligands were distinctly exposed to cells at the safest level in DPBS devoid of FBS for 3 h. Following, cells were washed twice with DPBS and treated to different concentrations of Al^{3+} for 30 min. Succeeding, fluorescent images were captured under the DAPI filter using an inverted fluorescence microscope (EVOS FLC, Thermo Fisher Scientific,

USA). The fluorescence intensity was recorded at 220 nm using a microplate reader (Synergy H1, BioTek, USA).

2.2.2 *In-vivo* toxicity and bioimaging in the *Danio rerio* embryo

Briefly, wild-type adult *D. rerio* were acquired from the aquarium market in Coimbatore, Tamil Nadu, India and maintenance and acclimatization of *D. rerio* were carried out in accordance with OECD guidelines, (2013). According to the OECD 236 recommendations, fish breeding (2 males:1 female), egg collection, and embryo toxicity tests were performed.[2] From 4 to 96 h post-fertilization (hpf), the embryos (n = 50 per group) were individually treated to varied doses of ligands(up to 20 µMin embryo medium) in a Petri dish. The control embryos weren't exposed to ligand. At various times, namely 24, 48, 72, and 96 hpf after ligand exposure, mortality and developmental defects of embryos were observed under an inverted microscope (EVOS FLC, Thermo Fisher Scientific, USA). Dead embryos are those that disintegrate or coagulate throughout development and don't have a heartbeat. Pericardial edema, non-tail separation, yolk sac edema, hyperemia, lack of somite development, translucent eyes, and spinal curvature are considered deformation symptoms during embryogenesis.

Succeeding the toxicity assessment of ligands, bioimaging of Al^{3+} was successfully carried out in *D. rerio* embryos at the safest concentration of ligand. Briefly, ligand was distinctly treated to 96 hpf embryos at the safest concentration for 3 h in the embryonic medium. Following, embryos were washed for twice with embryonic medium and treated with different concentrations of Al^{3+} for 30 min. Again, embryos were washed twice with embryonic medium, and images were captured under a DAPI filter using an inverted fluorescence microscope (EVOS FLS, Thermo Fisher Scientific, USA), and fluorescence intensitywas recorded at 250 nm using a microplate reader (Synergy H1, BioTek, USA).

Ethical statement

Ethical review and approval was not required for the animal study because all the experiments in zebrafish were performed followed by the guidelines agreed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (cpcsea.nic.in/WriteReadData/userfiles/file/SOP_CPCSEA_inner_page.pdf). Hence, this is not mandatory regarding the ethical issues, as it is not mentioned in the CPCSEA guidelines yet.

Reference

(1) N.K. Kalagatur, E.F. Abd_Allah, S. Poda, K. Kadirvelu, A. Hashem, V. Mudili, C. Siddaiah, Quercetin Mitigates the Deoxynivalenol Mycotoxin Induced Apoptosis in SH-SY5Y Cells by Modulating the Oxidative Stress Mediators, Saudi J. Biol. Sci. 28(1) (2021) 465–477.