Internet of Things-Enabled Aggregation-Induced Emission Probe for Cu²⁺ Ions: Comprehensive Investigations and Three-Dimensional Printed Portable Device Design

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ABSTRACT: Herein, we have developed a novel aggregation-induced emission (AIE) probe and three-dimensional (3D) printed portable device for copper (Cu²⁺) sensing in an aqueous medium. A ubiquitous synthetic route has been employed to devise the anthracene-conjugated imidazo[1,5-a]pyridine (TL19) probe as a unique anchor for Cu²⁺ ions. The TL19 is meticulously characterized through pivotal spectroscopic techniques, and the satisfactory results were obtained. The solvatochromic analysis and density functional theory calculations cohesively reveal that the TL19 exhibits the intramolecular charge transfer transition upon photoexcitation. Intriguingly, the TL19 exhibits spherically shaped nanoaggregates and enhanced fluorescence in DMSO/water (10:90) mixtures. This fluorescent nanoaggregate instantaneously responded toward the detection of Cu²⁺ via a deaggregation mechanism. The detection limit is found to be 9 pM in an aqueous medium. Further, the detection of Cu²⁺ in the HeLa cells has also been achieved due to bright green fluorescence, photostability, and biocompatibility nature of TL19 aggregates. On the other hand, an internet of things (IoT)-embedded 3D printed portable device is constructed for the detection of Cu²⁺ ions in real water samples. The Cu²⁺ detection is achieved through an IoT device, and results were acknowledged through an android application in 3.32 s round-trip time. Thus, the IoT-enabled AIE probe could be a prospective device for Cu²⁺ detection in a constrained environment.

INTRODUCTION

Over two decades, Tang’s group reported several interesting findings and has established the concept of aggregation-induced emission (AIE), which provides a tremendous platform for fluorophore research.¹,² In this milieu, several researchers reported the multitudinous AIEgens with tunable emission. The applications of AIEgens are not limited to particular research fields, and they are effectively utilized in diverse areas especially in sensory fields.³⁻¹¹ Notably, AIEgens played a pivotal role as fluorescent chemosensors for various metals, ions, anions, gases, explosives, peroxides, etc.¹²⁻¹⁵ Among the analytes, the sensing of the metal ions has attracted considerable attention in the field of chemosensors. Among the various metal ions, sensing of copper (Cu²⁺) ions has much attracted the researchers owing to its biological importance.¹⁶⁻²⁰ Cu²⁺ is an essential transition metal, which plays a pivotal role in brain functioning, and the blood–brain barrier regulates brain copper homeostasis by eradicating excessive Cu²⁺ ions. The interruption in Cu²⁺ homeostasis results in human genetic diseases such as Menkes (Cu²⁺ deficiency), Wilson’s (Cu²⁺ excess), and Alzheimer’s diseases. Therefore, the monitoring of Cu²⁺ in living cells with higher sensitivity and selectivity has a great deal of interest in biology. In this juncture, the fluorescent chemosensors were primarily developed for the detection of Cu²⁺ in a water medium. However, the paramagnetic nature of Cu²⁺ and aggregation-caused quenching (ACQ) nature²¹⁻²⁷ of organic fluorophores are mainly impacted to achieve high sensitivity of Cu²⁺. Thus, the detection of Cu²⁺ ions principally remains a challenge. To overcome these issues, the aforementioned AIEgens are developed and successfully utilized. The Cu²⁺ detection in water using AIEgens has been moderately reported²⁸⁻³⁰ with a detection limit in the range of 1 nM to 1 μM (Table S1). Therefore, developing a highly sensitive and selective AIEgen for the detection of Cu²⁺ is still a challenge. With this milieu aforementioned in mind, we have synthesized an organic chemosensor based on bidentate 1-(pyridin-2-yl)imidazo[1,5-a]pyridine containing an anthracene arm (Scheme 1).
In addition, this molecule has AIE character and very high sensitivity and selectivity for biologically important Cu^{2+} ions. To the best of our knowledge, this is the first report of an anthracene derivative that undergoes AIE followed by sensing of Cu^{2+} with pM sensitivity through deaggregation of an AIE channel. In the current scenario, internet of things (IoT) is the sophisticated solution to detect the toxic elements in a constrained environment. The IoT devices are capable to do image processing, machine learning, and cloud-based connectivity with a single board computer. Here, we have designed an IoT-embedded 3D printed portable device to sense the Cu^{2+} ions and report the near real-time results to the users' application with image processing and cloud connectivity. The designed system works with low power supply and is able to filter and identify specific colors of the sample. The exact color outputs of the samples were also successfully identified through an OpenCV image processing algorithm.

## RESULTS AND DISCUSSION

The TL19 probe was synthesized according to the synthetic route shown in Scheme 1, and structure characterization data are given in the Supporting Information. ^1^H NMR, ^13^C NMR, and HRMS results of TL19 are shown in Figures S1−S3.

### Photophysical Studies

The typical absorption and emission spectra of TL19 in a DMSO solvent are shown in Figure 1a. Both spectra were bathochromically shifted compared with pristine anthracene, and it could be assigned to intramolecular charge transfer (ICT) from pyridylimidazo[1,5-a]pyridine to anthracene. The solvatochromic analysis was performed to understand the ICT transition operating in TL19. The corresponding spectral profiles and solvatochromic data are depicted in Figure S4 and Table S2, respectively. Results show that by changing polar to nonpolar solvents, the spectral characteristics of TL19 altered significantly. Intriguingly, the absorption spectra of TL19 are bathochromically shifted in a CHCl₃ solvent; this is because of instantaneous stabilization due to rapid reorganization during electronic excitation. On the other hand, the fluorescence spectra of TL19 in polar solvents are bathochromically shifted, and this unanimously indicates that the excited state is stabilized by the polar solvents. In support, the fluorescence lifetime of TL19 is also longer in polar solvents. Moreover, the calculated Stokes shift is higher in polar solvents specifying that intramolecular charge transfer (ICT)-type transition is more pronounced in TL19. Further, using a polarity function (Δf), the plot of Stokes shift versus solvent polarity is constructed; concurrently, the fluorescence lifetime was also considered in this plot (Figure 1b & Figure S5).

The longer fluorescence lifetime and higher Stokes shift in polar solvents unambiguously suggested that TL19 undergoes ICT transition. In order to affirm this, DFT calculations were performed to examine the nature of electronic transitions occurring in TL19. Hence, the calculations were performed in the B3LYP functional 6-311G** basis set (Table S3). On inspecting, the optimized geometry of TL19 indicates that both anthracene and pyridyl groups are lying perpendicular to imidazo[1,5-a]pyridine (Figure 1c). Notably, there are two intermolecular hydrogen-bonding interactions existing between nitrogen atoms of the pyridylimidazo[1,5-a]pyridine...
group and hydrogen atoms of the anthracene unit. Therefore, it could enhance the effective intramolecular charge transfer process. Figure S6 shows the HOMO/LUMO levels of TL19, and from these levels, the ICT transition could be simply rationalized. The electron density of pyridylimidazo[1,5-a]pyridine is localized in HOMO, whereas the electron density of anthracene is localized in the LUMO level upon photoexcitation. Thus, the relocation of electron density from HOMO to LUMO levels illustrates evidence of effective ICT transition in TL19. Further, it is understood from Figure 1b that TL19 exhibits the specific solvent interaction especially with polar solvents. Hence, the photophysical properties of TL19 are investigated in solvent mixtures.

**AIE Studies.** Figure S7 shows the absorption spectra of TL19 measured in various percentages of DMSO/water mixtures (Table S4). There is no significant alteration in the absorption spectral profile up to 70% water. However, the TL19 solution turns turbid at higher water percentages (80 and 90%). Hence, the dramatic changes in the absorption spectral profile were monitored. Notably, the hyperchromic and bathochromic shifts were observed in 8% and 90% water, respectively. Moreover, in both the water percentages, TL19

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Figure 2. (a) DLS, (b) HR-SEM, and (c) HR-TEM images of TL19 aggregates. (d) Stacked geometry of TL19.

Figure 3. (a) UV-illuminated picture of TL19 in various percentages of water. (b) Emission spectra of TL19 measured in various percentages of DMSO/water mixtures. (c) Plot of water percentages vs fluorescence intensity and lifetime. The concentration of TL19 is $1.59 \times 10^{-5}$ M.
reveals the leveling of the absorption tail due to the Mie scattering effect, and this portrays that at higher water percentages, TL19 exhibits the nanosized aggregates.

A dynamic light scattering (DLS) measurement in 90% water shows that the average diameter of the TL19 aggregates is of $\sim$200 nm, indicating the existence of nanosized aggregates (Figure 2a). Further, Figure 2b shows the SEM image in a 1 $\mu$m scale bar, which displays the spherical shape of TL19 aggregates. Further, the HR-TEM image (Figure 2c) also supports the spherical shape of aggregates with the average size of $\sim$18 nm. In order to understand the aggregation mechanism, we have employed the computational methods. Figure 2d shows the optimized geometry of TL19 in a dimer form. Intriguingly, the anthracene units were found to be stacked together, and four intermolecular hydrogen-bonding interactions between nitrogen atoms of the pyridylimidazo[1,5-a]pyridine group and hydrogen atoms of the anthracene unit existed. The presence of hydrogen-bonding interactions stabilizes the stacked geometry of TL19, and hence, aggregation is the favorable channel. Overall results summarize that TL19 in 90% water exhibits the spherically shaped nanoaggregates. Figure 3a reveals the excited state behavior of TL19 aggregates under UV light ($\lambda_{\text{exc}}$ 375 nm) illumination with various percentages of water. To our surprise, the orange emission of TL19 turns green emission at higher water percentage. To expedite this contrary behavior, the fluorescence spectral measurements of TL19 in various percentages were conducted (Figure 3b). Upon varying the water percentages up to 70%, fluorescence of TL19 is deteriorated at regular intervals due to stabilization of the ICT state owing to the increased polarity of the solvent mixtures, which provoked TL19 to be almost nonemissive.

Intriguingly, the observations from steady-state fluorescence results are nicely correlated to time-resolved fluorescence lifetime data (Figure 3c). Surprisingly, the emission intensity started to improve in higher water percentages, and the revived emission peak was centered at 510 nm. Notably, the measured fluorescence quantum yield of TL19 is also improved from 1.8% (in DMSO) to 12% (in DMSO/90% H$_2$O mixture). Thus, the above photophysical results demonstrate the incidence of AIE.

**Copper Sensing Studies.** The AIE nature of TL19 in DMSO/90% H$_2$O mixtures opted for metal ion sensing applications since the pyridylimidazo[1,5-a]pyridine unit is known for metal complexation.$^{31}$ Therefore, the selectivity of TL19 in DMSO/90% H$_2$O solvent mixtures was investigated to identify its specificity toward metal ions by adopting a fluorescence technique. The fluorescence intensity of TL19 was found to alter with various metal ions, and the respective quenching efficiency is calculated and shown in Figure S8. As shown, the drastic fluorescence quenching (>90%) was observed in the presence of Cu$^{2+}$, whereas quenching efficiency became deteriorated for other metal ions used in this work. Concurrently, upon addition of Cu$^{2+}$, the TL19 aggregates become clear solution; this was clearly probed using UV–Visible absorption spectral studies. Figure S9 shows the absorption spectra of TL19 aggregates with various metal ions including Cu$^{2+}$ ions. Intriguingly, other metal ions did not influence the spectral features of TL19 aggregates. However, the Cu$^{2+}$ ion interacts rapidly with TL19 aggregates; as a result, we could see the change in leveling of the tail absorption. Hence, the Cu$^{2+}$ quenching is solely due to a deaggregation mechanism. Notably, the counterions of Cu$^{2+}$ did not intervene in the quenching efficiency. On the other hand, the selectivity of TL19 was investigated with various inorganic and organic acids. The quenching efficiency of TL19 was monitored in the presence of individual acids (100 nM), and the resultant selectivity plot is shown in Figure S10. This figure clearly reveals that the fluorescence intensity of TL19 was not much deteriorated by both organic and inorganic acids. These findings confirm that the TL19 exhibits excellent selectivity toward the sensing of Cu$^{2+}$.

Further, the computational methods have been employed to understand the Cu$^{2+}$ binding site and deaggregation mecha-
nism. Based on the MESP picture of TL19 (Figure 4a), two nucleophilic sites (both imidazole and pyridyl nitrogens) were identified, and CuCl₂ is specifically bound with these two nucleophilic nitrogens (Figure 4b), thereby significant changes in the molecular geometry. The ¹H NMR studies also support that the Cu²⁺ ion binds to the heterocyclic nitrogen donors of TL19 (Figure S11). Notably, after binding of Cu²⁺, both pyridyl and pyridylimidazo[1,5-a]pyridine groups are in planar position, and they are perpendicular to the anthracene unit, while both pyridyl and pyridylimidazo[1,5-a]pyridine groups are mutually perpendicular to the anthracene unit in TL19 alone. This significant change in the molecular geometry upon binding with CuCl₂ leads to play a vital role in the deaggregation of TL19 aggregates.

The sensing ability of TL19 was examined by determining the value of the detection limit (LOD). By employing a fluorescence titration method, the fluorescence spectra of TL19 in DMSO/90% H₂O were recorded as a function of Cu²⁺ as shown in Figure 4c. It was found that the fluorescence intensity of TL19 gradually decreases at an increasing concentration of Cu²⁺ and reaches the end of reaction with a concentration of about 100 nM. The change in fluorescence intensity was plotted as a function of Cu²⁺ as shown in Figure 4d, displaying linearity for a certain concentration range. The LOD was determined to be 9 pM by using the slope value of the linear plot with 3(σ/slope) relation, where σ is the standard deviation of the fluorescence response. Intriguingly, the obtained LOD is very much lower than the limit (31.5 μM) recommended by WHO.³² Thus, the obtained very low LOD signifies the excellent sensitivity nature of TL19 sensors to the detection of Cu²⁺.

Further, to evaluate the on-site sensing efficacy of TL19 aggregates, the real water samples from different sources such as tap water (TW), pond water (PW), sea water (SW), and distilled water (DW) were considered. The collected TW, PW, and SW samples were filtered to remove the fine floating particles, whereas DW was directly used. The TL19 aggregates were prepared in different water samples followed by spiking of Cu²⁺ with different concentrations (0.01, 0.05, and 0.1 nM), and the fluorescence intensity measurements were conducted. Each measurement was conducted in triplicate, and the averaged values are depicted in Table 1. The percentage of recovery was in the range of 97–102 for all real water samples indicating the reliability of the TL19 for Cu²⁺ determination in real water samples.

| sample            | added (nM) | found (nM) | recovery (%) |
|-------------------|------------|------------|--------------|
| tap water         | 0.01       | 0.0097     | 97           |
|                   | 0.05       | 0.0504     | 101          |
|                   | 0.1        | 0.0997     | 100          |
| pond water        | 0.01       | 0.0096     | 96           |
|                   | 0.05       | 0.0512     | 102          |
|                   | 0.1        | 0.0991     | 99           |
| sea water         | 0.01       | 0.0097     | 97           |
|                   | 0.05       | 0.0503     | 101          |
|                   | 0.1        | 0.0997     | 100          |
| distilled water   | 0.01       | 0.0097     | 97           |
|                   | 0.05       | 0.0504     | 101          |
|                   | 0.1        | 0.0995     | 99           |

The ultrasensitive nature of TL19 aggregates toward Cu²⁺ ions is employed in live cells. Before employing the TL19 probe to bioimaging applications, the pH sensitivity must be investigated. Hence, the fluorescence spectra of TL19 were recorded as a function of pH from 1 to 14, and the resultant fluorescence responses with varying pH are shown in Figure S12. The fluorescence intensity was found to be almost unchanged while varying the pH in the range of 5 to 14. On the contrary, the fluorescence intensity of TL19 became weak in the more acidic condition such as pH 1 and 3. Thus, this probe is suitable for bioimaging applications.

The in vitro bioimaging measurement was investigated to examine the sensing capability toward Cu²⁺ in live HeLa cells by using confocal fluorescence microscopy. Before investigation, the photostability of TL19 aggregates was investigated in HeLa cells. The results signify higher photostability of TL19 in HeLa cells (Figure S14a,b) and that it could be a promising candidate for long-term bioimaging. Therefore, TL19 was added with HeLa cells to incubate for 30 min. After incubation, the fluorescence images of TL19-treated cells were recorded at the excitation of 405 nm, which shows strong fluorescence in cells (Figure 5a–c). It was found that the fluorescence signal was observed in the cell membrane alone, indicating that the TL19 favors interaction with the cytoplasm instead of the nucleus. Then, the Cu²⁺ solution (10 nM) was added into the same TL19-treated HeLa cells and incubated for 15 min. In the presence of Cu²⁺, the strong fluorescence nature of TL19 was significantly decreased, which further completely quenched with the addition of a high concentration of Cu²⁺ (100 nM) as shown in Figure 5d–i. These bioimaging results prove the excellent capability of TL19 as a sensor in the detection of Cu²⁺ in live cells.

**Sensing in an IoT Environment.** The practical applicability of the developed fluorescent nanoprobe for the detection of Cu²⁺ ions is investigated using an IoT and cloud-based android application. This application collects the sample...
image with a pixel size of 3280 × 2464 (H × L) from a Raspberry Pi-enabled UV cabinet and uploads the pictures in Google Cloud space. An OpenCV-based RGB color identification python program is set up in a Google cloud environment to identify the sample color and communicates to the android application with the color-identified data. The response time of this application is approximately 3.32 s. The TL19 fluorescent probe is dissolved in tap water and spiked with 100 nM Cu^{2+} ions. The samples were kept in a Raspberry Pi-enabled UV cabinet, and images were collected based on the above application. Figure 6 illustrates the output file from the IoT cloud application with the RGB color output. The intensity of the green component is 195 for TL19 aggregates, which is quenched to 19 upon addition of 100 nM Cu^{2+} ions. Thus, we have validated the TL19 fluorescent nanoprobe for real-time detection of Cu^{2+} ions using an IoT and cloud-based android application.

**SUMMARY**

In summary, an efficient and rapid sensor for Cu^{2+} has been developed by using an AIE strategy. The sensitivity of the fluorescent nanoprobe is 9 pM. The proposed AIEgen is easily launched into cells due to low toxicity, enabling the Cu^{2+} sensing in living cells. Further, the IoT-embedded 3D printed portable device is successfully employed and demonstrated toward the detection of Cu^{2+} ions in real water samples along with the RGB values. The response time of the developed IoT application is 3.32 s, which instigates the rapid detection of Cu^{2+} ions. Thus, this work provides a new avenue for the development of fluorescent nanoprobe in an IoT environment.

**EXPERIMENTAL SECTION**

**Materials.** The starting materials and reagents for this work were procured from commercial sources (Sigma-Aldrich and Alfa Aesar) and used as such. Column chromatography was carried out using a Spectrochem silica gel (60–120 mesh) and analytical grade solvents. Thin-layer chromatography (TLC) was conducted on precoated Merck silica gel F254 plates (0.25 mm). All the reaction steps and manipulations were performed under an argon atmosphere with standard Schlenk techniques. The HPLC grade solvents were used for photophysical studies.

**Methods.** Nuclear magnetic resonance (NMR) spectroscopy was performed on a BRUKER spectrometer operating at 400 MHz for ^1^H and ^1^C, and the respective spectra were collected at ambient probe temperature calibrated to either an internal tetramethylsilane (TMS) standard or residual protio solvent. A high-resolution Q-TOF Premier mass spectrometer was utilized for recording the mass spectrum of TL19. UV–Visible absorption spectra of samples were recorded using a Shimadzu UV-1800 spectrophotometer. The fluorescence spectral measurements were carried out using a Hitachi F-4500 fluorescence spectrometer. For fluorescence studies, very dilute solution with less optical density was used to avoid spectral distortions due to the inner-filter effect and emission reabsorption. Time-resolved fluorescence decays were obtained by the time-correlated single-photon counting (TCSPC) technique. The data analysis was carried out by software provided by IBH (DAS-6), which is based on a deconvolution technique using nonlinear least-squares methods.

Fluorescence quantum yield (ϕ_F) of TL19 was determined by using the following equation (eq 1)

\[ \phi_F = (A_R/A_S)(I_S/I_R)(\eta_S/\eta_R)^2 \phi_R \]  

where the subscripts S and R refer to the sample and the reference, A is the absorbance at an excitation wavelength (366 nm), I is the integrated area of fluorescence, and η is the refractive index. Quinine sulfate (ϕ = 0.5) was used as a standard.

**Computational Methods.** Quantum chemical calculations based on density functional theory (DFT) and time-dependent density functional theory (TD-DFT) were carried out by using Gaussian 09 program. The exactness of the computed results is exclusively based on the choice of the functional and justified from the benchmarking studies. Therefore, we have optimized the TL19 with seven different functionals such as B3LYP, CAM-B3LYP, M06, M06-2X, ω-b97Xd, PBE0, and PBE using the 6-311+G** basis set. Among the functionals, the B3LYP functional was found to ratify with the experimental λmax value of TL19. Hence, geometry optimization of TL19 was carried out with B3LYP in blend with the 6-311+G** basis set and also for computing photophysical properties of TL19. The molecular electrostatic potential (MESP) plot is drawn for TL19 to gain proper insights into the nature of the reactive sites available on the chosen molecules. The self-consistent reaction field-polarizable continuum model (SCRF-PCM) was exploited to assess the DMSO solvent effect in the simulated absorption spectrum.

**Solvatochromic Analysis.** Solvent-dependent spectral shifts are often interpreted in terms of the Lippert equation (eq 2), which describes Stokes’ shift in terms of the change in dipole moment of the TL19 upon excitation and the dependence of energy of the dipole on the dielectric constant and refractive index of the solvent and is given as
detection value of TL19 toward Cu^{2+} was determined from the speed of light, addition of varying concentrations of Cu^{2+}. The lower absorbance of solution was adjusted to a stock solution of each metal ion was prepared in deionized water. For the selectivity experiment, a set of TL19 solution was formed with metal ions. For the quantitative detection of Cu^{2+}, the fluorescence spectra were monitored for all solutions at the excitation wavelength of 405 nm. Subsequently, two different concentrations of Cu^{2+} solutions were added in the same TL19 stock solution and then recording of bioimages.

Copper Sensing Studies. A certain amount of TL19 was dissolved in a DMSO/90% H_{2}O solvent mixture, and the absorbance of solution was adjusted to ~0.1 at 375 nm. The stock solution of each metal ion was prepared in deionized water. For the selectivity experiment, a set of TL19 solution was prepared in which each metal ion solution (0.1 µM) was added individually and mixed well. Then, the fluorescence spectra were monitored for all solutions at the excitation wavelength of 375 nm. The selectivity of TL19 was confirmed toward Cu^{2+} ions based on the obtained fluorescence response with metal ions. For the quantitative detection of Cu^{2+}, the fluorescence spectra of TL19 were recorded by the gradual addition of varying concentrations of Cu^{2+}. The lower detection value of TL19 toward Cu^{2+} was determined from the acquired changes in the fluorescence intensity.

IoT-Embedded 3D Printed Portable Device Design. The proposed IoT-enabled portable UV chamber (Scheme 2)

Scheme 2. Catia Design Diagram of the Proposed IoT-Enabled Portable Device

is 3D printed with an acrylonitrile butadiene styrene (ABS) material. The size of the module is 15 × 9 × 9 cm, and it consists of a compartment to mount the UV light source, a single board computer (Raspberry Pi-ARM V11 architecture 512 MB RAM with a 1GHz processor), and a cuvette. The sample is illuminated with the UV 365 nm light source, and the response is recorded through the Raspberry Pi camera setup (Raspicam-5 Mp, a resolution of 3280 × 2464 pixel, and a focal length of 3.04 mm). The Raspberry Pi is powered with a 5 volt, 2 A h battery bank. The IoT-enabled portable device is operated, and the results are also communicated to the smartphone android application. It also has cloud connectivity to store and forward the data to remote locations.

Photostability, Cytotoxicity, and Bioimaging Studies. For the photostability experiment, the HeLa cells were incubated with TL19 (30 µg/mL) for 30 min. The incubated cells were washed with PBS buffer to remove unreacted TL19. The fluorescence bioimages were recorded at regular times (0, 5, 10, 15, and 20 min) under continuous light irradiation of 405 nm using confocal fluorescence microscopy.

In a typical MTT assay, the diverse concentrations of TL19 (0 to 100 µg/mL) were added into the separate wells consisting of HeLa cells and then incubated for 8, 16, and 24 h. After incubation, the TL19-treated cells were washed thoroughly with PBS buffer, and then, MTT solution (15 µL of 5 mg mL^{-1}) was added and incubated for 4 h. The absorbance of each incubated solution was monitored at 570 nm in a Synergy H1 Hybrid multimode reader (BioTek, USA). The percentage of cell viability was determined based on the absorbance values obtained for different concentrations of TL19 with respect to the control experiment.

For the in vitro bioimaging experiment, the HeLa cells were treated with TL19 (30 µg/mL) for 30 min incubation followed by a wash with PBS buffer. Afterward, the fluorescence bioimages were acquired for TL19-treated HeLa cells at the excitation wavelength of 405 nm. Subsequently, two different concentrations of Cu^{2+} solution were added in the same TL19-treated HeLa cells to observe the change in the fluorescence signal. The cells were incubated for further 15 min in each addition of Cu^{2+} solution and then recording of bioimages.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c05262.

NMR, HRMS characterization data, and spectral data (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

A.K. thanks the SERB Research Scientists scheme (SB/SRS/2018-2019-05/CS, dated 30/01/2019). A.K. also acknowledges the SAIF, IIT Madras for analytical measurements. M.V. is thankful to the Department of Science and Technology, New Delhi, India for the financial support (ref. no. DST-SERB/EMR/2016/007955).

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