Rhodamine-Appended Benzophenone Probe for Trace Quantity Detection of Pd²⁺ in Living Cells

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ABSTRACT: Designing a fluorogenic probe for the determination of Pd²⁺ is a challenging analytical task. Pd²⁺ is a potentially toxic and harmful substance even at a very low level of contamination in the end product. Herein, a promising spirolactam-functionalized chemosensor, rhodamine-appended benzophenone (HBR), is designed and characterized by spectroscopic (¹H NMR, ¹³C NMR, ESI-MS, and FT-IR) data along with the single-crystal X-ray diffraction technique. It acts as a highly sensitive and selective fluorogenic chemosensor for Pd²⁺ ions over other environmentally relevant cations in aqueous ethanol (1:1, v/v) at pH 7.4. The limit of detection (LOD) is 34 nM that is far below the WHO recommended Pd uptake (47 μM). The plausible mechanism involves the specific binding of HBR with Pd²⁺ and the formation of 1:1 stoichiometry of the complex, which has been supported by ESI-MS, FT-IR data, Job plot, and association constant data (Benesi-Hildebrand plot). The computation study has been attempted to explain the ring cleavage fluorescence enhancement scheme of HBR upon binding with Pd²⁺. Furthermore, this “turn-on” probe has successfully applied to image the Pd²⁺ ion in cultured MDA-MB-231 cells.

INTRODUCTION

In the past few years, rhodamine-based probes have attracted tremendous interest recently due to their excellent photophysical properties such as impressive quantum yields, high extinction coefficients, great photostability, etc. where the signal transduction mechanism involves the transformation of the lactonized form (fluorescence-off mode) to the delactonized form (fluorescence-on mode) by binding to some specific metal ions especially to Pd²⁺ ions.¹ In this aspect, optical detection techniques (colorimetric and fluorometric) with appropriate probes are more effective for sensing of Pd²⁺ ions due to their sensitivity, simplicity, and nondestructive nature. Several rhodamine-based receptors were reported in literature for sensing of mercury,²,³ copper,⁴ zinc,⁵ cadmium,⁶ chromium,⁷ iron,⁸ aluminum,⁹ and also for palladium.¹⁰,¹¹ However, researchers are interested to develop a convenient and efficient method to detect Pd²⁺ ions in the presence of other cations.¹² Hence, the specific and rapid detection of the potentially toxic heavy cations (such as lead, cadmium, mercury, palladium, etc.) is important due to their lethal impact on the ecosystem and human health. These ions enter into our body via contaminated foods, drinks, beverages, medicines, etc.¹²,¹³ In this regard, the Pd²⁺ ion is considered to be a highly dangerous substance because of its binding with −SH groups of different enzymes like kinase, carbonic anhydrase, aldolase, creatine, succinate dehydrogenase, alkaline phosphatase, and prolyl hydroxylase to dysfunction the enzymatic process.¹⁴ Palladium has also severe effects on DNA, proteins, and other macromolecules, such as vitamin B₆, etc.¹⁵ The WHO permissible limit of palladium is 5–10 ppm (5–10 ppm equals to 46.98–93.97 μM), and the maximum dietary intake value is ∼1.5–15 μg/day/person (∼1.5–15 μg equals to 14–140 nM).¹⁶,¹⁷ Therefore, it is very urgent to develop an efficient, technically simple, cheap, and trustworthy technique for quantitative estimation of the trace quantity of Pd²⁺ ions both in the living cells and environment. Other methods for the detection of Pd²⁺ ions including XRF (X-ray fluorescence), ICP-AES (inductively coupled plasma atomic emission spectroscopy), FAAS (flame atomic absorption spectrometry), AAS (atomic absorption spectrometry), ICP-MS (inductively coupled plasma mass spectrometry), and SPME-HPLC (solid-phase microextraction coupled with high-performance liquid chromatography)¹⁸,¹⁹ often necessitate costly, sophisticated instrumentation, and time consuming.
sample preparation steps. In this aspect, optical detection techniques (colorimetric and fluorometric) with appropriate probes are more effective for sensing of Pd^{2+} ions. In this work, we have synthesized an ethylenediamine-bridged 2-hydroxy benzophenone-rhodamine-B dyad (HBR), which acts as a fluorogenic sensor and capable of detecting Pd^{2+} ions with the naked eye. The response is very fast and the detection limit is reasonably very low (34 nM). The compound HBR has been verified by spectroscopic data (1H NMR, 13C NMR, mass, and FT-IR spectroscopies) and X-ray single-crystal data. The practical application of the probe HBR has been examined in the MDA-MB-231 cell for the detection of exogenous Pd^{2+} ions by fluorescence cell imaging processes. Sensing ability has been studied by both absorption and fluorescence techniques.

## RESULTS AND DISCUSSION

### Formulation of Probe HBR

The chemosensor (HBR) has been synthesized from ethylenediamine derivatives of rhodamine B and 2-hydroxy benzophenone (Figure S1, Scheme 1) and characterized by spectroscopic (FT-IR, 1H NMR, 13C NMR, and ESI-MS) data and by the single-crystal X-ray diffraction technique (Figures S2, S3, S5, and S7). A suitable single crystal is obtained by slow evaporation of methanol solution of HBR for a week. HBR crystallizes in the monoclinic crystal system with the P2_{1}/c space group and Z = 4. The structure of HBR (Figure 1) indicates the formation of the unique cyclic lactam ring (fluorescence-off mode) from the condensation of acid chloride and ethylene diamine molecules. Two planes of the cyclic lactam ring in the HBR probe are attached in an appropriately vertical position (δΔ = 2.13°).

Other aromatic rings are more or less planar with either the pyran ring (maximum deviation 4.75°) or the pyrrol-2-one ring (deviation 0.85°). Thus, the structural distortion is more or less close to the reported rhodamine derivatives. In the molecular assembly, the H-bonding performs the pivotal role during the generation of the supramolecular assembly (Figure 2b). Here, both intermolecular and intramolecular types of hydrogen bonding are present. The intramolecular hydrogen bonding is in between O−H (O3−H3) and the N (N6) atom (H−N, 1.84 Å; angle O−H−N, 144°), which is classical in nature. Similarly, there is a nonclassical hydrogen bonding in between the C−H (C68−H68A) moiety and the O (O4) atom (H−O, 2.52 Å; angle C−H−O, 102°). The intermolecular hydrogen bonding is (C46−H46−O2, C59−H59C−O6) in the range of 2.58−3.44 Å. In the structural architecture, there are some allowable C−H···π (2.76−2.96 Å) and π···π (3.817(5) to 3.820(6) Å) interactions, which improve the stability of aggregated motifs (Figure 2c). As far as we could possibly know, only a few crystal structures of the rhodamine spirolactam moiety have been reported.

### Absorption Spectroscopic Studies

The sensor, HBR, is colorless and reveals almost no absorbance beyond 400 nm, which is mainly due to its spirolactam structure. A specific signal of the tertary C atom at ~64.8 ppm in the 13C NMR spectrum and the structure of single crystal for HBR also characterized its spirolactam form. However, upon the gradual addition of Pd^{2+} ions (0−25 μM) in aqueous ethanolic solution (v/v, 1:1) of HBR at pH 7.4, a significant enhancement in absorbance at 572 nm is observed (Figure 3). This is accompanied by an obvious color change from colorless to pink and is signifying the binding of Pd^{2+}ions. Moreover, the same absorption experiment has been performed with other common metal ions (K+, Na+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Mn^{2+}, Al^{3+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Tb^{3+}, Eu^{3+}, Gd^{3+}, Sm^{3+}, Cr^{3+}, Ag+, Hg^{2+}, Cu^{2+}, Sn^{2+}, Rb^{+}, Pt^{2+}, and Rh^{3+}), and no perceptible spectral change is observed (Figure 4). Further, these metal ions are found to be noninterfering to the response of Pd^{2+} ions. Therefore, the color change of HBR with the addition of Pd^{2+} ions can be visualized clearly by the naked eye. This is an exciting feature by which we can easily detect Pd^{2+}ions without using high-value sophisticated instrumental methods.

### Fluorescence Property and Binding Behavior

The fluorescence measurements are performed at the maximum excitation wavelength of 572 nm and also to reveal the nature of binding ability of the probe HBR. Fluorescence titrations of chemosensors with Pd^{2+} ions have been carried out using 2 mL of 20 μM of HBR in aqueous ethanolic solution (v/v, 1:1) at pH 7.4 (HEPES buffer). As shown in Figure 5, the emission...
The intensity of the solution has significantly changed at 595 nm with the gradual addition of Pd$^{2+}$ ions (0−25 μM), along with a visual emission color change from colorless to pink under a UV lamp. The significant increase in the emission intensity upon the addition of Pd$^{2+}$ ions reports the binding of the ion with the probe and initiates the “off−on” switching mechanism. This may be due to the structural change of the sensor, HBR, from nonluminescent spirocyclic forms to the luminescent noncyclic forms as shown in Scheme 2. The luminescence change of HBR to specific binding of Pd$^{2+}$ ions is analyzed from 0 to 1.2 equivalents (Figure 5). In addition, the sensing behavior of HBR has been investigated by adding several metal ions (K$^+$, Na$^+$, Mg$^{2+}$, Ca$^{2+}$, Ba$^{2+}$, Mn$^{2+}$, Al$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Tb$^{3+}$, Eu$^{3+}$, Gd$^{3+}$, Sm$^{3+}$, Cr$^{3+}$, Ag$^+$, Hg$^{2+}$, Cu$^{2+}$, Sn$^{4+}$, Ru$^{3+}$, Pt$^{4+}$, and Rh$^{3+}$) to the EtOH/H$_2$O (v/v, 1:1) solution of HBR at pH 7.4 (10 mM, HEPES buffer). As displayed in Figure 6, only Pd$^{2+}$ induces a strong fluorescent enhancement at 595 nm, whereas other metal ions do not show any noticeable spectral change. Additionally, competitive experiments are carried out by adding several metal ions to the aqueous ethanolic solution of the sensor HBR in the presence of Pd$^{2+}$ ions.
of Pd\textsuperscript{2+} ions (Figure S14), and the results have shown that the Pd\textsuperscript{2+} ion has induced fluorescence enhancement and is not affected by the competitive ions. The observation is clearly demonstrating the excellent selectivity of HBR for sensing Pd\textsuperscript{2+} at biological conditions (pH = 7.4).

**Effect of pH.** The sensor, HBR, alone does not show any fluorescence spectra at pH 7.4, which indicates the non-luminescent lactam form. Fluorescence spectra were recorded for HBR (20 μM) at different pH solutions, and the result of such experiment demonstrated that the change from spirocyclic to ring opening of the rhodamine moiety in HBR alone could happen only for pH ≤ 5.0 (Figure 7) due to the strong protonation. However, the addition of Pd\textsuperscript{2+} led to the fluorescence diminishment over a comparatively wide pH range (8.0 – 12.0), which may be attributed to the formation of Pd(OH)\textsubscript{2}. This affirms that the fluorescence response at ~595 nm is only due to the selectivity of the sensor HBR to Pd\textsuperscript{2+} ions in approximately physiological pH ranges.

The reversibility is a significant feature of any probes to be used as a chemosensor for practical application. The reversibility is tested by adding an aqueous solution of KSCN (c = 2 × 10\textsuperscript{-4} M) to the pink colored Pd\textsuperscript{2+}-complex (c = 2 × 10\textsuperscript{-5} M) solution. The solution color turned from pink to colorless, and fluorescence is quenched gradually in the presence of excess KSCN as shown in Figure 8 (Scheme 2), which may be due to the formation of Pd(SCN)\textsubscript{2} and HBR.

**In Vitro Detection of Pd\textsuperscript{2+} Ions.** All the above chemical and spectroscopic properties of HBR show that HBR specifically binds with Pd\textsuperscript{2+} ions in the presence of other metal ions. The exposure of different heavy metal ions has an immense effect to induce several devastating diseases in the human body. Among the heavy metals, Pd\textsuperscript{2+} has a major role in the occurrence and progression of cardiovascular disorders as well as cancer. Therefore, the selective detection of Pd\textsuperscript{2+} in the cell through a highly sensitive analytical method is a very demanding and challenging area of research. Toward this objective, an experiment has been modeled to examine the presence of Pd\textsuperscript{2+} in living cells using the HBR and MDA-MB-231 cell lines. The toxicity of HBR in MDA-MB-231 cell lines is checked in different concentrations up to 100 μM through the MTT assay (Figure 9). After 24 h treatment of HBR, the cell viability is decreased with the increase in drug concentrations. This result suggests that this compound shows slight toxicity in this cancer cell line, which might be considered as an anticancer compound.

The ability of HBR for selective binding of Pd\textsuperscript{2+} ions in the living cell model is tested using MDA-MB-231 cell lines.
Above, the spectroscopic study indicated that after binding with Pd$^{2+}$, HBR showed emission in the visible range. Therefore, we used the microscopic technique to visualize the Pd$^{2+}$ by adding HBR. The cells were treated with Pd$^{2+}$ ions (5 μM) along with HBR. After 4 h treatment, the cells were fixed and captured the images of cellular morphology under a microscope in the red channel. The images showed the red fluorescence in the cell body, which further supports the metal chelating property of HBR (Figure 10). Therefore, it may be concluded that HBR can be used to identify the presence of Pd$^{2+}$ in cells. Both the spectroscopic study and cellular images showed that HBR specifically bonded Pd$^{2+}$ ions and might be used to capture Pd$^{2+}$ ions.

**Density Functional Theory Calculation.** The crystallographic parameters of HBR are used to optimize the structure and proposed complex (Figures S12 and S13), which are compared with analogous coordination environment. Also, the identity of structure has been confirmed by vibrational frequency calculation of selected functions and on relating with experimental spectra. The DFT-optimized geometry of the complex with the Pd$^{2+}$ ion is a distorted square planar where HBR coordinates to Pd$^{2+}$ through O, N, N, and O centers. The calculated Pd−O (benzophenone), Pd−N (imine), Pd−N (rhodamine) and Pd−O (rhodamine) distances are 2.00, 1.98, 1.96, and 2.47 Å, respectively, and they are comparable with the analogous reported structure.22,24 Owing to the coordination, the distances C−O (spirolactam) are elongated from 1.25 to 1.31 Å, and C=N (imine) is extended from 1.29 to 1.32 Å (Tables S2 and S3). Upon specific chelation of Pd$^{2+}$ ions to HBR, the HOMO and LUMO energy gaps have been decreased as compared to that of free HBR. The HOMO−LUMO energy gap in HBR is 3.73 eV (332 nm), while that in the complex [BR$^-$−Pd$^{2+}$]$^+$, it is 2.38 eV (521 nm). These are in close agreement to the observed longest wavelength absorption band of HBR and [BR$^-$−Pd$^{2+}$]$^+$, respectively (Figure 11). The fractional contribution of different components of orbitals is depicted, and qualitative molecular orbital concepts are also obtained.

**Proposed Binding Mode of HBR with Pd$^{2+}$.** The changes in absorption spectra took place up to the 1:1 ([Pd$^{2+}$]/[HBR]) ratio, which shows the formation of the complex of [BR$^-$−Pd$^{2+}$]$^+$. This [Pd$^{2+}$]/[HBR] ratio is confirmed by the Job plot analysis (Figure S11). The ESI-MS data show m/z_{obs} = 769.2051, where the m/z_{calc} for [BR$^-$−Pd$^{2+}$]$^+$ is 769.2364 (Figure S6). The observed peak has provided the additional support for the 1:1 complex formation of [BR$^-$−Pd$^{2+}$]$^+$. The association constant (K_{assoc}) has been found to be 1.99 × 10^{4} M$^{-1}$ from the Benesi–Hildebrand plot obtained by using data available from systematic emission spectral titrations (Figure S10). The binding of HBR to Pd$^{2+}$ through the oxygen of −OH via deprotonation, the oxygen of C=O in γ-lactam, nitrogens of C=N (Schiff base), and C=N via spirolactam ring opening was suggested, which is consistent with FT-IR spectra (Figures S7 and S8) because a broad peak at 3412 cm$^{-1}$ for −OH (HBR) disappeared, shifting of stretching frequencies of “C=O” from 1690 to 1680.5 cm$^{-1}$, C=N (Schiff base) remains unchanged and C=N from 1514 to 1585 cm$^{-1}$ respectively in the complex, [BR$^-$−Pd$^{2+}$]$^+$. The plausible sensing mechanism has also been supported by the 1H NMR spectrum of the complex, [BR$^-$−Pd$^{2+}$]$^+$. The disappearance of the phenolic −OH peak (δ = 14.80 ppm, s) and downfield shifts of a, b, c, and d protons of HBR clearly indicate that Pd$^{2+}$ induces the formation of a delocalized xanthene moiety of rhodamine B (Figures S2 and S4).
CONCLUSIONS

A rhodamine spirolactam-based dyad, 2-hydroxy benzophenone-rhodamine (HBR), has been synthesized, which served as an effective "turn-on" fluorescent and a colorimetric probe for Pd²⁺ ions in mixed ethanol-aqueous media over 26 other tested metal ions with a very low detection limit of 34 nM. Addition of Pd²⁺ ions to the sensor has enhanced the emission intensity by 53-fold through the ring opening of the spirolactam form of the rhodamine moiety. The recognition events have been effectively used to the biomaging of Pd²⁺ ions in the intracellular level of breast cancer cell lines in the physiological medium.

EXPERIMENTAL SECTION

Materials and Methods. Unless otherwise noted, all the solvents and inorganic salts were purchased from Spectrochem. Rhodamine B, 2-hydroxy benzophenone, and ethylenediamine were purchased from Sigma-Aldrich. All reactions were monitored by thin layer chromatography (TLC) plates. Column chromatography was done by using silica gel (60–120 mesh). All metal salts were used as either their nitrate or chloride salts. Aqueous solutions for spectroscopic measurements were prepared using Milli-Q water (Millipore). ¹H and ¹³C NMR spectra were taken on a 400 MHz spectrometer [Bruker(AC)] in CDCl₃ where the internal reference was tetramethylsilane (TMS). ESI mass spectra were collected from a water HRMS model XEVO-G2QTOF#YCA3S1 spectrometer. Fluorescence and UV–vis spectrum measurements were performed on a Perkin Elmer spectrophotometer model LSS5 and a Perkin Elmer Lambda 25 spectrophotometer, respectively. FT-IR spectra (KBr disk, 4000–400 cm⁻¹) were acquired on a Perkin Elmer LX-1FTIR spectrophotometer. The fluorescence quantum yield was calculated using fluorescein as a reference with a known quantum yield (ϕᵣ = 0.52 in 0.1 M NaOH).²⁵

The quantum yield was calculated by using the following equation:

\[
\frac{\phi_s}{\phi_R} = \frac{A_s}{A_R} \times \left( \frac{(Abs)_R}{(Abs)_S} \right) \times \left( \frac{n_s^2}{n_R^2} \right)
\]

where \(n_s\) is the fluorescence quantum yield of the samples, \(\phi_R\) is the fluorescence quantum yield of the reference, \(A_s\) is the respective areas under emission spectra of the sample, \(A_R\) is the respective areas under emission spectra of the reference, (Abs)ₗ is the absorbance of the sample at the excitation wavelength, (Abs)ₗ is the absorbance of the reference at the excitation wavelength, \(n_s^2\) is the refractive index of the solvent, and \(n_R^2\) is the refractive index of the solvent used for the reference.

Synthesis of 2-Hydroxy Benzophenone-Rhodamine (HBR). The sensor (HBR) was achieved according to Figure S1 and Scheme 1. The ethylenediamine derivative of rhodamine B (1) was synthesized by refluxing rhodamine B with POCl₃ followed by ethylenediamine. A mixture of the compound 1 (1.0 equiv) and 2-hydroxy benzophenone (1.0 equiv) was refluxed for 4 h in dry ethanol (10 mL) under inert atmosphere. After cooling to ambient temperature, the solvent was evaporated and the obtained residue was purified by silica column chromatography using ethyl acetate/petroleum ether (1:5, v/v) as an eluent to afford a yellow solid of the compound HBR in 85% yield. mp: 158 °C, IR. Bands: 3412, 2974, 2938, 1690, 1612, 1514, 1228 cm⁻¹. ESI-MS: m/z calc'd for C₄₁H₃₄N₈O₃ [M + H]⁺ (m/z): 665.3413; found, 665.4254.¹H NMR (400 MHz, DMSO-d₆), δ (ppm): 18.40 (s, 1H, −OH), 7.75 (d, J = 5.3 Hz, 1H, Ar), 7.51–7.42 (m, 2H, Ar), 7.42–7.33 (m, 3H, Ar), 7.21 (t, J = 8.6 Hz, 1H, Ar), 6.98–6.92 (m, 1H, Ar), 6.90–6.85 (m, 2H, Ar), 6.83–6.78 (m, 1H, Ar), 6.62–6.54 (m, 1H, Ar), 6.49 (dd, J = 8.0, 1.7 Hz, 1H, Ar), 6.28 (d, J = 2.5 Hz, 2H, Ar), 6.20 (d, 2H, Ar), 6.14 (dd, J = 9.0, 2.5 Hz, 2H, Ar), 3.25 (q, 2H = 7.0 Hz, 8H, 4CH₂).²⁸ (t, J = 6.5 Hz, 2H, =NCH₂), 2.46 (t, J = 6.5 Hz, 2H, ([−(C═O)]N−CH₃)).¹³C NMR δ in ppm (CDCl₃ solvent): 174.8, 168.4, 163.2, 153.8, 153.1, 148.7, 133.9, 132.4, 132.3, 131.3, 130.9, 128.8, 128.7, 128.5, 128.0, 127.0, 123.7, 122.9, 119.8, 118.0, 117.1, 108.0, 105.4, 97.7, 64.8, 49.2, 44.4, 40.6, 12.7.

Preparation of Solutions. Stock solutions of HBR (20 × 10⁻⁶ M) for electronic absorption and fluorescence measurements were prepared in aqueous ethanolic solution (v/v, 1:1) at pH 7.4 buffered with HEPES. Stock solutions of the several metal ions (K⁺, Na⁺, Mg²⁺, Ca²⁺, Ba²⁺, Mn²⁺, Al³⁺, Co³⁺, Ni²⁺, Pb²⁺, Zn²⁺, Cd²⁺, Fe³⁺, Fe²⁺, Tb³⁺, Eu³⁺, Gd³⁺, Sm³⁺, Eu⁴⁺, Ag⁺, Hg²⁺, Cu²⁺, Sn⁴⁺, Ru⁴⁺, Pd²⁺, Pt²⁺, and Rh⁵⁺) were dissolved with HEPES. Stock solutions of HBR (20 × 10⁻⁶ M) for electronic absorption and fluorescence measurements were prepared in aqueous ethanolic solution (v/v, 1:1) at pH 7.4 buffered with HEPES. Stock solutions of the several metal ions (K⁺, Na⁺, Mg²⁺, Ca²⁺, Ba²⁺, Mn²⁺, Al³⁺, Co³⁺, Ni²⁺, Pb²⁺, Zn²⁺, Cd²⁺, Fe³⁺, Fe²⁺, Tb³⁺, Eu³⁺, Gd³⁺, Sm³⁺, Eu⁴⁺, Ag⁺, Hg²⁺, Cu²⁺, Sn⁴⁺, Ru⁴⁺, Pd²⁺, Pt²⁺, and Rh⁵⁺) were dissolved with HEPES. Stock solutions of HBR (20 × 10⁻⁶ M) for electronic absorption and fluorescence measurements were prepared in aqueous ethanolic solution (v/v, 1:1) at pH 7.4 buffered with HEPES. Stock solutions of the several metal ions (K⁺, Na⁺, Mg²⁺, Ca²⁺, Ba²⁺, Mn²⁺, Al³⁺, Co³⁺, Ni²⁺, Pb²⁺, Zn²⁺, Cd²⁺, Fe³⁺, Fe²⁺, Tb³⁺, Eu³⁺, Gd³⁺, Sm³⁺, Eu⁴⁺, Ag⁺, Hg²⁺, Cu²⁺, Sn⁴⁺, Ru⁴⁺, Pd²⁺, Pt²⁺, and Rh⁵⁺) were dissolved with HEPES. Stock solutions of HBR (20 × 10⁻⁶ M) for electronic absorption and fluorescence measurements were prepared in aqueous ethanolic solution (v/v, 1:1) at pH 7.4 buffered with HEPES.

X-Ray Measurement. The crystal structure of the organic compound was determined by a single-crystal X-ray diffraction method. A prismatic yellow-colored single crystal having an appropriate dimension (0.16 × 0.06 × 0.01 mm) was picked up with nylon loops and mounted for data collection via a Bruker SMART APEX II diffractometer, having graphite-monochromated Mo Kα radiation (λ, 0.71073 Å). Unit cell parameters and experimental details during the crystal data collection and structure refinement are briefly reported in Table S1. The unit cell parameters and crystal-orientation matrices were determined by least-square refinement of all reflections within the hkl range -11 < h < 14, -15 < k < 15, -49 < l < 49. The intensity data of single crystal were enhanced for Lorentz and polarization effects.²⁶ Crystal data of the organic compound were collected applying the condition I > 2σ(I). The collected data of the above compound were integrated using SAINT program, and the absorption correction was made with SADABS. Full-matrix least-square refinements on F² of the data were carried out using SHELXL-97²⁷ with anisotropic displacement parameters for all non-hydrogen atoms. Hydrogen atoms present in the organic molecule were placed in their geometrically idealized positions to constrain their parent atoms. The refinement shows high alert levels, which are not monitored by crystal solution software. We have tried further to get better quality crystals but failed. The crystallinity of the compound is not good; however, we have chosen the best single crystal among them.

Computational Method. All calculations of HBR and proposed complex were performed at the B3LYP²⁸ level using the Gaussian 09 software²⁹ package. The X-ray coordinates of HBR were used to generate an optimize structure. The lan12d
basis set was assigned for the elements. All the ground state \( (S_0) \) stationary points were fully optimized at the B3LYP/lanl2dz and the excited states at the TD-B3LYP/lanl2dz method.\(^{30}\) Frequency verification vertical electronic excitations based on B3LYP-optimized geometries were calculated using the time-dependent density functional theory (TDDFT) formalism\(^{32}\) in methanol using the conductor-like polarizable continuum model (CPCM).\(^{32}\) GaussSum\(^{33}\) was used to estimate the fractional contributions of various groups to each molecular orbital.

**Cell Viability Assay.** Cell viability assay was performed in MDA-MB-231 cell lines. MDA-MB cell lines were procured from NCCS Pune, India. The DMEM media with 10% fetal bovine serum was used to culture cells. Cells were seeded in 96-well plate (density 10,000 cells per well). After 24 h of plating of the cells, cells were treated with HBR in different concentrations. Again, the cells were incubated for another 24 h. Then, cells were washed and the MTT experiment was performed. Results were represented in the percent of viability, which was calculated from the absorbance value of 96-well plates. Percentage viability was calculated following the below formula.

\[
\frac{[A_{550}(\text{treated cell}) - A_{550}(\text{background})]}{A_{550}(\text{untreated cell}) - A_{550}(\text{background})} \times 100
\]

**Cellular Uptake of HBR in Presence of Pd\(^{2+}\) Ions by Microscopic Imaging.** For this experiment, MDA-MB-231 was also used. Cells were seeded in 6-well plates for 24 h. Then, cells were treated with HBR (5 mM) in the DMEM media for 4 h. Then, Pd\(^{2+}\) ions (5 \( \mu \)M) were added and incubated for another 4 h. A four percent formaldehyde concentration was prepared in PBS buffer and used to fix the cell for 30 min. Further, after removing of formaldehyde solution, PBS buffer was used to wash the cells. Finally, cell morphology was checked through a microscope in 40x objective. Images were taken in an Olympus microscope having an AndoriXon 3897 EMCCD camera.

### ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01860.

X-ray crystal details for HBR (CIF)

NMR, ESI-MS, FT-IR, and X-Ray crystallographic data of HBR along with additional required spectra (PDF)

### Accession Codes

CCDC number 1920481 contains the crystallographic data of HBR. The copies of these data may be picked up free of cost from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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**Notes**

The authors declare no competing financial interest.

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