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Self Immolative Dioxetane Based Chemiluminescent Probe for H₂O₂ Detection

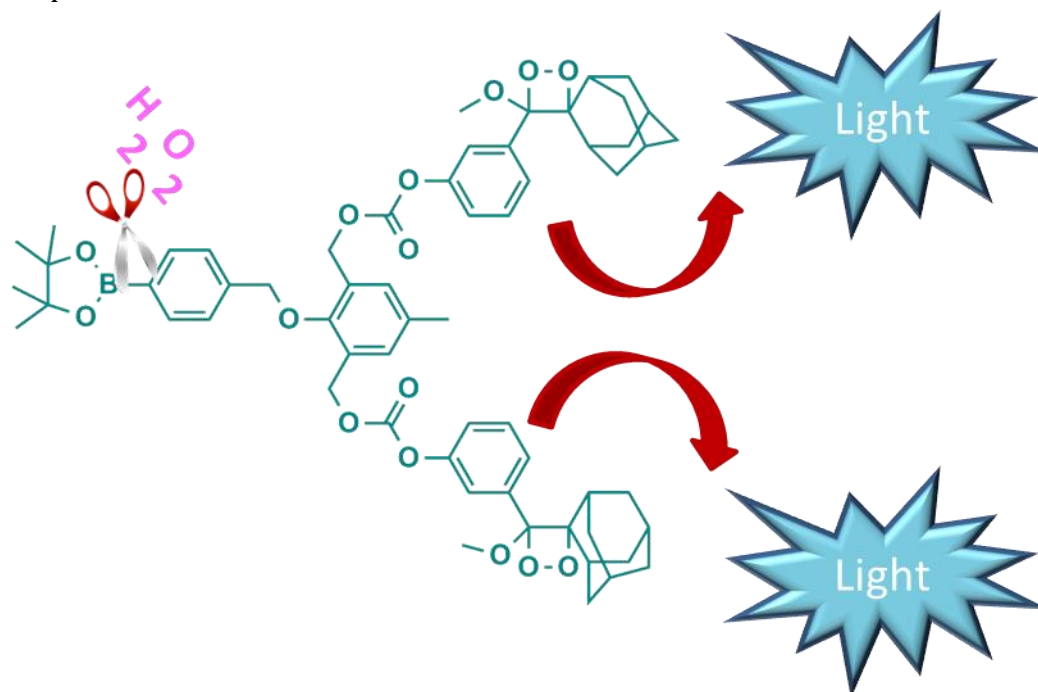
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Graphical abstract

**Highlights**

An amplified chemiluminogenic response is obtained via self-immolation of chemiluminogenic modules when electronically triggered dioxetane cleavage is initiated by H_2O_2 mediated deprotection of boronate ester group. Through the self-immolation, H_2O_2 can be detected selectively at low micromolar concentrations in aqueous solutions.

Abstract

Chemiluminescent detection of H_2O_2 has been achieved by using self immolative dioxetane based probe which enables the signal amplification via disassembly of two chemiluminogenic modules at the same time in response to single analyte. Upon treatment of the probe with H_2O_2 , boronate ester was deprotected subsequently to trigger the decomposition of 1,2-dioxetane ring via CIEEL mechanism which results in light emission as a selective sign of H_2O_2 .

Key Words: Hydrogen peroxide, self-immolation, 1,2-dioxetanes, chemosensors, chemiluminescence.

1. Introduction

As a relatively mild reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) is recognized as a messenger molecule in various signaling processes therewithal it is also included in many biological processes like biosynthesis, immune response and cell signaling [1-3]. Additionally, in many enzymatic reactions, H_2O_2 is also produced as a

byproduct. On the other hand, like other reactive oxygen species, excessive production of H_2O_2 can cause oxidative stress which brings about the development of many diseases such as Alzheimer's disease, Parkinson's disease, cardiovascular disease and Huntington's disease [4-6]. In other words, in terms of beneficial signaling processes and harmful oxidative stress properties, there is a delicate balance for the amounts of ROS. This balance is disrupted in favor of oxidative stress with aging [7, 8]. So, the determination of these species will allow us to identify the production, accumulation, trafficking of ROS and also related diseases. Therefore, development of analytical techniques for selective and sensitive detection of H_2O_2 is urgently needed in a high-throughput fashion. In the literature, there are many proposals for the detection of H_2O_2 by using chromogenic and fluorogenic probes [9-13]. As a new insights into the optical sensing systems, chemiluminescence (CL) based ones would be promising due to their superior advantages such as operational simplicity, cost effectiveness, rapid and high sensitivity of the target, being free from interferences caused by light scattering and reduced background noise due to the absence of photonic excitation [14-16]. In CL based systems, light emission is obtained as a result of specific chemical reaction which is unique to the analyte of interest. Due to the emission of light upon interaction with the analyte of interest, CL is also accepted as cold light. As a result, chemiluminescence based sensor is designed to transform chemical information into a useful signal as a light emission. Although chemiluminescence sensors have shorter life times compared to fluorescence sensors, they frequently have lower background emission that makes the CL as a powerful analytical technique offering high sensitivity, wide linear range and simple instrumentation [17,18]. Although, there are a few reports for the chemiluminescence based detection of H_2O_2 [19-20], until now, to the best of our knowledge, no reports based on the chemiluminescence based detection of H_2O_2 by using 1,2-dioxetane based self-immolative systems have been published.

As a chemiluminogenic unit, our choice was a stable 1,2-dioxetanes which is a four-membered cyclic peroxide usually implicated as the reactive intermediates in bioluminescence as well as oxalate esters, luminol and acridinium esters [21-23]. Use of 1,2-dioxetane derivatives as chemiluminogenic unit is a very promising alternative strategy since it offers direct chemiluminogenic response which can be triggered by the cleavage of a chemical bond under mild reaction conditions specific to the analyte of interest [24-26]. The decomposition of 1,2-dioxetanes that produces chemiluminescence is probably one of the most interesting type of chemical reactions and therefore it has been focus of many chemical and biological research [27,28]. The decomposition of 1,2-dioxetanes is initiated with the removal of triggering moiety which results in two main products, one of which is formed in the excited state undergoing an electron transfer according to CIEEL (Chemically Initiated Electron Exchange Luminescence) mechanism and eventually relaxes radiatively with a peak emission at 466 nm which depends on the structure of the 1,2-dioxetanes. The chemically initiated decomposition mechanism of 1,2-dioxetanes is generally triggered with a catalyst such as a base or a metal ion. Until now, designed chemiluminogenic agents rely on the decomposition of single substituted 1,2-dioxetanes upon interaction with the single analyte [17,29-33]. However, self-immolation enables the fragmentation of large molecules leading to the formation of multiple excited state anions upon interaction with the single analyte which could lead to valuable signal amplification in chemosensors targeting reactive analytes when used judiciously [34-36].

Herein, we design and synthesize 1,2-dioxetane based self immolative chemiluminogenic probe for H₂O₂. We wanted to incorporate a self immolative linker to trigger two chemiluminescence processes at the same time, in response to single H₂O₂ mediated deprotection event. Self-immolation of the probe was initiated with the deprotection of the boronate ester group leading to light emission. Until now, to the best of our knowledge, no reports for chemiluminescent detection of H₂O₂ by using a self immolative 1,2-dioxetane based sensors have been published. This finding may serve as a platform for future H₂O₂ sensor applications. This study provides a new insight into applications of chemiluminescence based sensors.

2. Materials and Methods

2.1 Materials

Spectrophotometric grade solvents were used for spectroscopy experiments. Flash column chromatography (FCC) was performed by using a flash grade silica gel (Merck Silica Gel 60 (40–63 μ m)). Reactions were monitored by thin layer chromatography (TLC) using precoated silica gel plates (Merck Silica Gel PF-254), visualized by UV-Vis light. All organic extracts were dehydrated over anhydrous Na₂SO₄ and concentrated by using rotary evaporator before being subjected to FCC. All other chemicals and solvents were supplied from commercial sources and used as received.

2.2 Instruments

¹H NMR and ¹³C NMR spectra were recorded on Bruker Spectrospin Avance DPX 400 spectrometer using CDCl₃ as the solvent. Chemical shifts values are reported in ppm from tetramethylsilane as internal standard. Spin multiplicities are reported as the following: s (singlet), d (doublet), m (multiplet). HRMS data were acquired on an Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS. Chemiluminescence measurements were done on a Varian Eclipse spectrofluorometer. A pH meter (Oakton, manufactured by Eutech instruments) was used to determine the pH.

2.3 Synthesis of Compounds

2.3.1 Synthesis of Compound (8)

Compound **7** (0.191 g, 0.555 mmol) and compound **4** (0.150 g, 0.555 mmol) were dissolved in acetone. K₂CO₃ (0.105 g, 0.761 mmol) and catalytic amount of 18-crown-6 was added to reaction mixture which was refluxed for 6 hours. The progress of the reaction was monitored by TLC. When starting material was consumed, mixture was diluted with diethyl ether and washed with saturated NH₄Cl solution. After washing with brine, combined organic phases were dried over anhydrous Na₂SO₄. After removal of the solvent, the residue was purified by silica gel flash column chromatography using EtOAc/ Hexane (1:5, v/v) as the eluent. Compound **8** was obtained as colorless waxy oil (0.212 g, 78%). ¹H NMR (400 MHz, CDCl₃) δ 7.85 (d, *J*= 7.7 Hz, 2H), 7.47 (d, *J*=7.7 Hz, 2H), 7.27 (t, *J*=7.7 Hz, 1H), 6.90-6.95 (m, 3H), 5.12 (s, 2H), 3.30 (s, 2H), 3.27 (s, 1H), 2.63 (s, 1H), 1.74-1.99 (s, 1H), 1.37 (s, 12H). ¹³C NMR (100 MHz, CDCl₃) δ 158.5, 143.3, 140.2, 136.8, 135.0, 131.6, 135.0, 131.6, 128.9, 126.5, 122.2, 115.6, 114.1, 83.8, 69.8, 57.7, 39.2, 39.0, 37.2, 32.2, 30.2, 28.3, 24.8 ppm. MS (TOF- ESI): *m/z*: Calcd for C₃₁H₃₉BO₄: 486.30505 [M+H]⁺, Found: 486.30941 [M+H]⁺, Δ = -8.97 ppm.

2.3.2 Synthesis of Probe 1

Compound **8** (0.070 g, 0.144 mmol) was dissolved in DCM. Methylene blue (5 mg) was added to the reaction mixture which was irradiated while oxygen gas was passing through it. The progress of the reaction was monitored by TLC. When TLC showed no starting material, the mixture was concentrated under vacuo and the residue was subjected to the silica gel flash column chromatography by using DCM as the eluent. Probe **1** was obtained as white solid (0.061 g, 81%). ¹H NMR (400 MHz, CDCl₃) δ 7.83 (d, *J* = 7.9 Hz, 2H), 7.45 (d, *J* = 7.9 Hz, 2H), 7.13-7.39 (m, *br*, 3H), 7.02-7.05 (m, 1H), 5.18 (s, 2H), 3.23 (s, 3H), 3.02 (s, 1H), 2.09 (s, 1H), 1.42-1.89 (m, 12H), 1.36 (s, 12H). ¹³C NMR (100 MHz, CDCl₃) δ 139.9, 136.1, 135.0, 129.2, 126.4, 116.4, 112.0, 95.4, 83.8, 69.9, 49.9, 36.4, 34.6, 33.0, 32.8, 32.3, 31.6, 26.0, 25.8, 24.8 ppm.

2.3.3 Synthesis of Compound (9)

2,6-Bis(hydroxymethyl)-*p*-cresol (1.0 g, 5.95 mmol) was dissolved in DMF and cooled to 0 °C by using ice-bath. Imidazole (0.809 g, 11.9 mmol) was added to reaction mixture at 0 °C. After 30 min, *tert*-butyldimethylsilyl chloride (1.820 g, 11.9 mmol) was added to reaction mixture which was left to stir at room temperature for 12 hours. When starting material was consumed, mixture was diluted with diethyl ether and washed with saturated NH₄Cl solution. After washing with brine, combined organic phases were dried over anhydrous Na₂SO₄. After removal of the solvent, the residue was purified by silica gel flash column chromatography using EtOAc/ Hexane (1:5, v/v) as the eluent. Compound **9** was obtained as colorless waxy oil (2.1 g, 89%). ¹H NMR (400 MHz, CDCl₃) δ 8.04 (s, 1H), 6.93 (s, 1H), 4.85 (s, 4H), 0.97 (s, 12H), 0.15 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 150.9, 128.2, 126.2, 125.7, 63.0, 64.8, 25.8, 20.6, 18.3, -5.4 ppm. MS (TOF- ESI): *m/z*: Calcd for C₂₁H₄₀O₃Si₂: 419.24082 [M+Na]⁺, Found: 419.24523 [M+Na]⁺, Δ = -10.52 ppm.

2.3.4 Synthesis of Compound (10)

Compound **9** (0.50 g, 1.26 mmol) and compound **7** (0.433 g, 1.26 mmol) were dissolved in acetone. K₂CO₃ (0.209 g, 2.52 mmol) and catalytic amount of 18-crown-6 was added to reaction mixture which was refluxed for 6 hours. The progress of the reaction was monitored by TLC. When starting material was consumed, mixture was diluted with diethyl ether and washed with saturated NH₄Cl solution. After washing with brine, combined organic phases were dried over anhydrous Na₂SO₄. After removal of the solvent, the residue was purified by silica gel flash column chromatography using EtOAc/ Hexane (1:5, v/v) as the eluent. Compound **10** was obtained as colorless waxy oil (0.718 g, 93%). ¹H NMR (400 MHz, CDCl₃) δ 7.97 (d, *J* = 7.7 Hz, 2H), 7.54 (d, *J* = 7.7 Hz, 2H), 7.30 (s, 2H), 5.02 (s, 2H), 4.82 (s, 4H), 2.45 (s, 3H), 1.45 (s, 12H), 1.04 (s, 12H), 0.19 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 151.2, 140.8, 135.1, 133.8, 133.6, 128.0, 127.0, 83.8, 76.2, 63.1, 60.4, 26.0, 24.9, 21.2, 18.4, -5.1 ppm. MS (TOF- ESI): *m/z*: Calcd for C₃₄H₅₇BO₅Si₂: 634.37661 [M+Na]⁺, Found: 635.37999 [M+Na]⁺, Δ = -11.3 ppm.

2.3.5 Synthesis of Compound (11)

Compound **10** (0.287 g, 0.468 mmol) was dissolved in methanol and catalytic amount of *p*-toluenesulfonic acid was added to reaction mixture which was stirred at room temperature for 2 hours. The progress of the reaction was monitored by TLC. When

starting material was consumed, mixture was diluted with DCM and washed with saturated NaHCO_3 solution. After washing with brine, combined organic phases were dried over anhydrous Na_2SO_4 . After removal of the solvent, the residue was purified by silica gel flash column chromatography using DCM as the eluent. Compound **11** was obtained as colorless waxy oil (0.176 g, 98%). ^1H NMR (400 MHz, CDCl_3) δ 7.83 (d, J = 7.8 Hz, 2H), 7.39 (d, J =7.8 Hz, 2H), 7.30 (s, 2H), 7.12 (s, 2H), 4.85 (s, 2H), 4.60 (s, 4H), 3.12 (s, br, 2H), 2.28 (s, 3H), 1.37 (s, 12H). ^{13}C NMR (100 MHz, CDCl_3) δ 152.2, 140.0, 135.1, 134.2, 133.8, 129.3, 127.1, 83.9, 76.6, 60.4, 24.8, 20.8 ppm. MS (TOF- ESI): m/z : Calcd for $\text{C}_{22}\text{H}_{29}\text{BO}_5$: 406.20366 $[\text{M}+\text{Na}]^+$, Found: 406.20562 $[\text{M}+\text{Na}]^+$, Δ = -4.83 ppm.

2.3.6 Synthesis of Compound (12)

Compound **11** (0.40 g, 1.04 mmol) was dissolved in dry THF and mixture was cooled to 0 °C by using ice-bath. Diisopropyl amine (1.21 mL, 8.0 mmol) and pyridine (0.05 mL) were added to reaction mixture under Ar. *p*-nitrochloroformate (0.897 g, 4.16 mmol) dissolved in dry THF was added dropwise to reaction mixture under Ar at 0 °C. The progress of the reaction was monitored by TLC. When starting material was consumed, mixture was diluted with DCM and washed with saturated NaH_4Cl solution. After washing with brine, combined organic phases were dried over anhydrous Na_2SO_4 . After removal of the solvent, the residue was purified by silica gel flash column chromatography using DCM/Hexane (3:1, v/v) as the eluent. Compound **12** was obtained as colorless waxy oil (0.580 g, 78%). ^1H NMR (400 MHz, CDCl_3) δ 8.26 (d, J = 9.1 Hz, 4H), 7.86 (d, J =7.8 Hz, 2H), 7.50 (d, J =7.8 Hz, 2H), 7.36 (s, 2H), 7.32 (d, J =9.1 Hz, 4H), 5.36 (s, 4H), 5.08 (s, 2H), 2.40 (s, 3H), 1.39 (s, 12H). ^{13}C NMR (100 MHz, CDCl_3) δ 155.4, 154.5, 152.3, 145.4, 139.6, 135.1, 134.8, 132.7, 128.1, 126.7, 126.1, 125.2, 121.7, 115.6, 84.0, 66.2, 24.8, 20.7 ppm. MS (TOF- ESI): m/z : Calcd for $\text{C}_{36}\text{H}_{35}\text{BN}_2\text{O}_{13}$: 736.21607 $[\text{M}+\text{Na}]^+$, Found: 736.21755 $[\text{M}+\text{Na}]^+$, Δ = -1.94 ppm.

2.3.7 Synthesis of Compound (13)

Compound **12** (0.03 g, 0.420 mmol) was dissolved in dry DCM. DMAP (0.123 g, 1.0 mmol) was added to reaction mixture under Ar. Compound **4** (0.272 g, 1.0 mmol) dissolved in dry DCM was added dropwise to the reaction mixture which was stirred at room temperature overnight. The progress of the reaction was monitored by TLC. When the starting material was consumed, the mixture was concentrated under vacuo and the residue was subjected to the silica gel flash column chromatography using EtOAc/Hexane (1:10, v/v) as the eluent. Compound **13** was obtained as white solid (0.258 g, 63%). ^1H NMR (400 MHz, CDCl_3) δ 7.87 (d, J = 8.0 Hz, 2H), 7.51 (d, J =7.9 Hz, 2H), 7.34-7.38 (m, 4H), 7.22 (m, 2H), 7.36 (s, 2H), 7.15 (m, 2H), 7.08-7.11 (m, 2H), 5.34 (s, 4H), 5.06 (s, 2H), 3.31 (s, 3H), 3.26 (s, 2H), 2.67 (s, 2H), 2.39 (s, 3H), 1.80-1.99 (m, 28H), 1.37 (s, 12H). ^{13}C NMR (100 MHz, CDCl_3) δ 154.1, 153.5, 150.9, 142.5, 139.7, 137.1, 135.1, 132.7, 132.2, 128.9, 128.5, 127.1, 126.9, 121.7, 119.9, 83.8, 57.9, 39.2, 39.1, 37.1, 32.1, 30.2, 28.2, 24.8, 20.8 ppm. MS (TOF- ESI): m/z : Calcd for $\text{C}_{60}\text{H}_{69}\text{BO}_{11}$: 1062.45505 $[\text{M}+\text{Na}]^+$, Found: 1062.45455 $[\text{M}+\text{Na}]^+$, Δ = 10.12 ppm.

2.3.8 Synthesis of Probe 2

Synthesis of probe **2** was achieved by using same procedure as in probe **1** starting with compound **14** (0.128 g, 0.13 mmol). Probe **2** was obtained as white solid (0.122 g, 90%). ^1H NMR (400 MHz, CDCl_3) δ 8.14 (d, J = 9.0 Hz, 1H), 7.86 (d, J = 8.2 Hz, 2H), 7.51-7.45 (m,

6H), 7.36 (s, 2H), 7.22 (d, J = 8.2 Hz, 2H), 6.91 (d, J = 9.0 Hz, 1H), 5.33 (s, 4H), 5.05 (s, 2H), 3.23 (s, 3H), 3.05 (s, 2H), 2.39 (s, 3H), 2.15 (s, 2H), 1.90-1.47 (m, 28H), 1.37 (s, 12H). ^{13}C NMR (100 MHz, CDCl_3) δ 154.1, 153.4, 151.1, 139.6, 135.1, 134.6, 132.3, 129.3, 128.4, 127.0, 126.1, 122.1, 115.6, 111.5, 95.5, 83.9, 65.7, 50.6, 39.3, 36.3, 34.7, 33.1, 32.8, 32.2, 31.6, 31.5, 25.9, 25.8, 24.8, 20.8 ppm.

3. Results and Discussions

Our strategy relies on the selective H_2O_2 mediated deprotection of boronate esters which leads to the formation of *m*-oxybenzoate anion as a result of the decomposition of the probes **1** and **2**. So, we designed and synthesized two different probes **1** and **2** for comparison. In the case of probe **1** which is the reference probe, single analyte leads to formation of unamplified chemiluminogenic response whereas in the case of probe **2** which is the amplifier probe, disassembly of the self immolative molecule upon interaction with single H_2O_2 leads to complete fragmentation and thus in turn, signal amplification is achieved.

3.1. Synthesis of probes

Compounds 1-4 and 5-7 for the synthesis of probe **1** and **2** were prepared according to the literature [37, 38]. To that end, the synthesis of triggering moiety was started with the protection of 4-formyl phenyl boronic acid. Formyl group was reduced to obtain compound **6** which is further converted into iodinated form **7**. Meanwhile, 3-hydroxybenzaldehyde was reacted with benzoyl chloride and resulting phenyl ester **1** was converted into dimethyl acetal derivative **2** subsequent to the conversion of this into phosphonate derivative **3** via Arbuzov reaction. Compound **3** was reacted with 2-adamantanone to complete the synthesis of chemiluminescence precursor, compound **4** which is further reacted with compound **7** to complete the synthesis of probe **1**. The self immolative linker was prepared according to Shabat and co-workers [39, 40]. Triggering moiety was introduced to self immolative core group which was obtained by the silyl protection of 2,6-bis(hydroxymethyl)-*p*-cresol. Construction of the target compounds continued with the incorporation of carbonate groups into the silyl deprotected compound **11**. Chemiluminogenic units were assembled by the reaction of activated linker with the 3-hydroxyphenyl moiety of the reporter compound **4**. In the final step, the electron-rich enol ethers were efficiently photooxygenated to yield 1,2-dioxetane derivatives as probe **1** and probe **2** (Scheme 1).

3.2 Chemiluminescence measurements

In order to determine the H_2O_2 sensing capabilities of probe **1** and **2**, a solution of H_2O_2 was added in portions (50 μM – 2.5 mM) and after each addition, the CL spectra were recorded. As expected, both probe **1** and **2** showed increase in CL response (CL on-mode) after each addition depending on the decomposition of boronate group and formation of the phenolate ion. Very bright blue chemiluminescence is obtained upon addition of H_2O_2 and larger concentrations lead to more intense luminescence (Fig. 1).

Data provide clear evidence that probe **2** amplifies the chemiluminogenic signal quarter times more according to probe **1** and senses the lower amount of H_2O_2 (Fig.2). The detection limits were calculated as $75\ \mu\text{M}$ for probe **2** ($\sigma = 0.007887$, $m = 314.36$). For probe **1**, the detection limit was determined as $240\ \mu\text{M}$ ($\sigma = 0.020766$, $m = 258.55$) in DMSO and $0.67\ \text{mM}$ in DMSO/ NaHCO_3 ($140\ \text{mM}$, 90/10, pH 8.3). So, probe **2** had more light emission due to the presence of the two 1,2-dioxetane groups. In other words, the more 1,2-dioxetane modules means larger production of excited state intermediate, which means enhanced CL intensity.

We also studied the optimal buffer ratios for CL response of probe **2** at different percentages of buffer (NaHCO_3 , $140\ \text{mM}$, 90/10, pH 8.3) in DMSO (Fig.3). The CL intensities decreased with increasing buffer percentages due to the possibility of protonation of phenoxide ion which prevents charge transfer from phenoxide ion to 1,2-dioxetane group. Spectroscopic characterization of probe **1** was given in supplementary material.

3.3 Selectivity over other related species

Further, the selectivity of probe **2** for other reactive oxygen species was also investigated. To that end, selectivity of probe **2** toward various reactive oxygen species like tert-butyl hydroperoxide (TBHP), hypochlorite (OCl^-), superoxide (O_2^-), hydroxyl radical ($\cdot\text{OH}$), tert-butoxy radical ($\cdot\text{O}^t\text{Bu}$) are determined according to the literature [41] and displayed in Fig. 4. Thus, treatment of the probe **2** with a number of potential competitor ROS does not resulted in any luminescence. CL data provide clear evidence that amplifier **2** offer very good selectivity for H_2O_2 . No other species was able to decompose the boronate groups; as a result no changes in the CL spectra were observed (Fig.4). Digital photographs of the solutions show the selectivity of chemiluminescent amplifier probe **2** under ambient light (Fig.5). H_2O_2 in DMSO-buffer mixture elicits clear response with luminescence intensity reflecting H_2O_2 concentration.

3.4 Proposed decomposition mechanism

All experiments are in complete agreement with the following mechanisms described in Scheme 2. We have proposed that chemiluminescent disassembly of the amplifier probe was preceded via hydroboration-oxidation reaction between H_2O_2 and boronate ester leading to formation of phenoxide ion and thus, releases the activated core molecule **A**. Subsequent dissociation of the intermediate compound **A** leads to the formation of activated form of 1,2-dioxetane **B** due to the pK_a of the medium. Phenoxide ion of 1,2-dioxetane **B** transfers electron to O-O of dioxetane to initiate its decomposition for the generation of excited *m*-oxybenzoate anion **C** while it relaxes back to ground state, resulting in the emission of photon as shown in Scheme 2.

4. Conclusion

In summary, we demonstrated that multiple chemiluminogenic groups can be triggered by using self-immolative linkers. This approach offers a chemical avenue for

enhancing the signal produced in response to a given analyte. We have described the synthesis and CL properties of self immolative probe **2** for the selective detection of H₂O₂. Upon treatment of probe with H₂O₂, deprotection of boranate subsequently trigger the decomposition of 1,2-dioxetane ring via intramolecular charge transfer due to the negatively charged phenolate groups. This decomposition results in formation of CL as a signal of H₂O₂. Probe **2** has fulfilled the H₂O₂ detection in a highly selective manner. We believe that this study is likely to provide new insights into the development of chemiluminescence based H₂O₂ sensors and bright chemiluminescence of the probe **2** or structurally related derivatives could provide a promising alternative.

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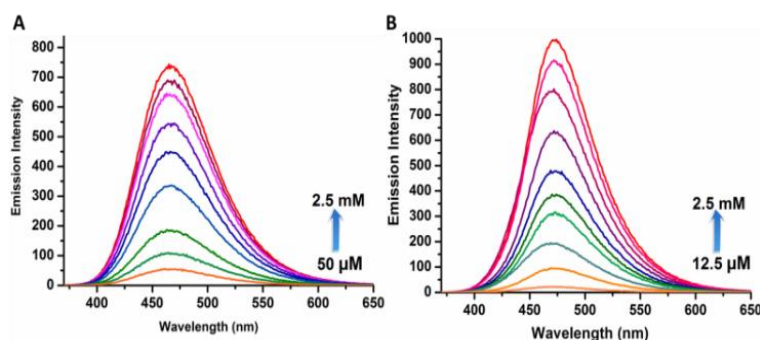


Figure 1. Chemiluminescence response of probes **1** (A) and **2** (B) in the presence of increasing H_2O_2 concentrations wherein 1 to 50 equiv. in (A) and 0,25 to 50 equiv. in (B). Probe concentration is 50 μM . Spectra were acquired in DMSO/ NaHCO_3 (140 mM, 90/10, pH 8.3) for both probes and all data were obtained after incubation with H_2O_2 at 25°C for 30 sec.

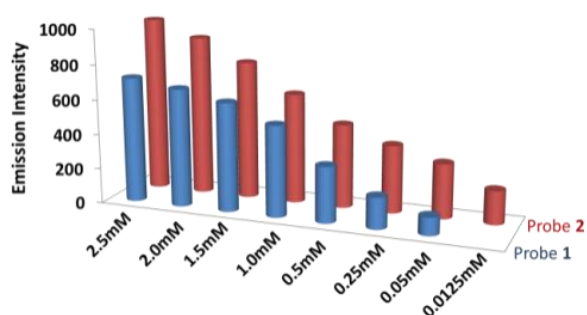


Figure 2. Comparison of chemiluminescence response of probe **1** and **2** in the presence of increasing H_2O_2 concentrations wherein 1 to 50 equiv. in probe **1** and 0,25 to 50 equiv. in probe **2**. Probe concentration is 50 μM . Spectra were acquired in DMSO/ NaHCO_3 (140mM, 90/10, pH 8.3) for both probes and all data were obtained after incubation with H_2O_2 at 25°C for 30 sec.

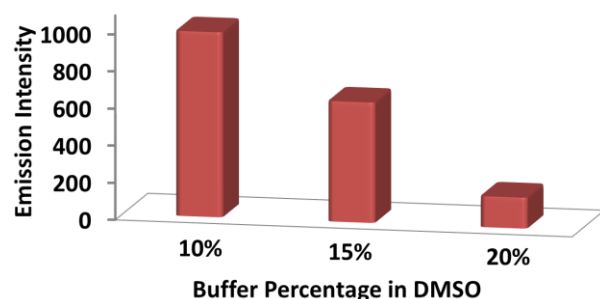


Figure 3. Chemiluminescence response of probe **2** at different percentages of buffer (NaHCO_3 , 140 mM, 90/10, pH 8.3) in DMSO. Probe concentration is 50 μM . All data were obtained after incubation with H_2O_2 at 25°C for 30 sec.

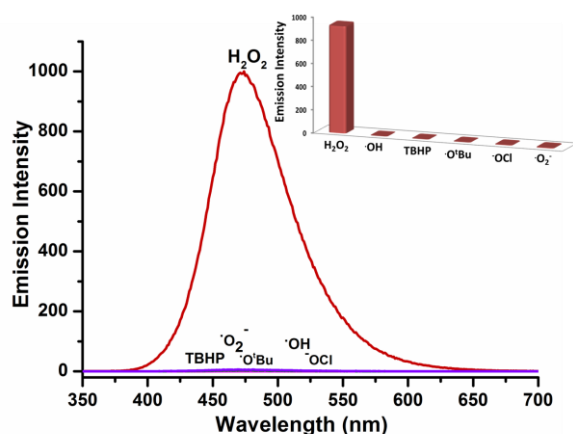


Figure 4. Chemiluminescence responses of 50 μM probe **2** to various reactive oxygen species (ROS). Spectra were acquired in DMSO/ NaHCO_3 (140 mM, 90/10, pH 8.3) for both probes and all data were obtained after incubation with the appropriate ROS at 25°C for 30 sec. Data shown are for 10 mM for O_2^- ; 250 μM for all other ROS. Tert-butyl hydroperoxide (TBHP), and hypochlorite (OCl^-) were delivered from 30%, 70%, and 5% aqueous solutions, respectively. Superoxide (O_2^-) was added as solid KO_2 . Hydroxyl radical ($\cdot\text{OH}$) and tert-butoxy radical ($\cdot\text{O}^t\text{Bu}$) were generated by reaction of 2.5 mM Fe^{2+} with 250 μM H_2O_2 or 250 μM TBHP, respectively.

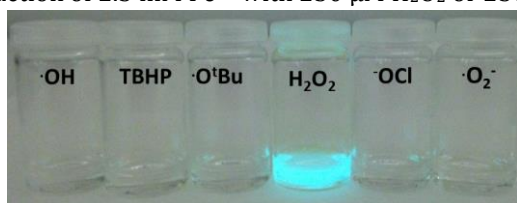
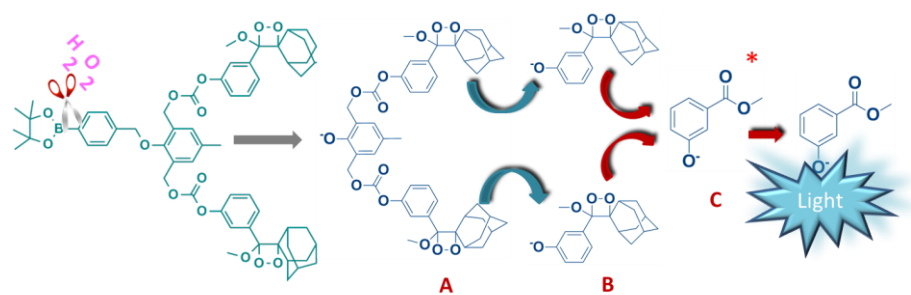


Figure 5. The digital photograph showing to the selective chemiluminescent response of probe **2** under ambient light.

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Scheme 2. Proposed chemiluminescent decomposition process catalysed by H_2O_2 for probe 2.