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Lophine derivatives as activators in peroxoaxalate chemiluminescence


Lophine and four of its derivatives were used as activators (ACTs) of the chemiluminescent peroxoaxalate (PO) reaction of bis(2,4,6-trichlorophenyl)oxalate with $H_2O_2$, catalysed by imidazole. Kinetic emission assays have shown that with the tested compounds the reaction mechanism, regarding the formation of the High Energy Intermediate (HEI) of the PO reaction, occurs as previously seen for commonly used ACTs. A bimolecular interaction of the compounds with the HEI leads to chemiexcitation through the Chemically Initiated Electron Exchange Luminescence (CIEEL) mechanism, as confirmed by a linear free-energy correlation between the relative catalytic rate constants and the oxidation potentials of the compounds. The yields of excited state formation and light emission, in the range of $10^{-2}$ to $10^{-3}$ E mol$^{-1}$, are comparable to the ones seen with commonly used ACTs. A Hammett plot with $\rho = -0.90$ indicates the build up of a partial positive charge on the transition step of the catalytic process, consistent with the formation of a radical cation of the ACT, being an additional validation of the CIEEL mechanism in this system.

Introduction

Chemiluminescence (CL) is the emission of light as one of the products of a chemical transformation. One of the first CL reactions to be described was the autooxidation of lophine (1), reported by Radziszewski in 1877, who observed a yellow emission from its reaction with oxygen in the presence of a strong base. The reaction mechanism (Scheme 1) involves the oxidation of lophine by oxygen, leading to a hydroperoxide, which then forms a 1,2-dioxetane intermediate by cyclization. Finally, the decomposition of this unstable cyclic peroxide leads to the electronic excited state of an amidine, which decays to the fundamental state emitting light.

Due to the CL and fluorescent properties of lophine and its derivatives, much has been done in using them in analytical systems combining HPLC and FIA techniques, for the determination of trace amounts of inorganic and organic compounds. Nowadays, the attention received by lophine and its derivatives is given specially due to their use as fluorescent labels for amines, phenols and carboxylic acids. Nonetheless, new features have been developed with focus on its CL properties, for example, applying lophine as an enhancer for the classical luminol CL reaction.

To expand the use of lophine derivatives, we have applied compounds 1–5 in other CL system, the peroxoaxalate reaction (PO, Scheme 2); these substituted lophines were used as activators (ACTs) of this CL transformation, therefore, the mechanism of excited states generation is completely different from the one involving a 1,2-dioxetane, as described in Scheme 1. As a matter of fact, in the PO system an electron transfer (ET) takes place from the ACT to the high-energy intermediate (HEI), the later being generated on a previous reaction between an oxalic ester and $H_2O_2$, catalysed by base (see below). Since an ET is proven to occur in the PO system, the use of lophine derivatives as activators allows one to probe the influence of different substituents with electron-donating and withdrawing properties on a common structural motif, i.e. the 2,4,5-triphenylimidazole system.
The peroxoate (PO) reaction

The reaction between bis(2,4,6-trichlorophenyl)oxalate (TCPO) and \( \text{H}_2\text{O}_2 \) catalysed by imidazole (IMI-H) was studied in depth by Stevani et al.\(^{10}\) Several polycondensed aromatic hydrocarbons (PAH), such as rubrene, perylene and anthracene derivatives were used as ACTs, due to their high fluorescence quantum yields (\( \Phi_{\text{FL}} \)) and low oxidation potentials (\( \text{E}^{\circ} \)).\(^6\) The mechanism for such transformation can be summarized as follows (Scheme 2):\(^7,10\) imidazole act as a nucleophilic catalyst leading to the formation of an \( 1,1'\)-oxalylimidazolic intermediate (step 1); \( \text{H}_2\text{O}_2 \) performs a nucleophilic attack on such intermediate, forming a peroxyacid (step 2); this peroxyacid undergoes a cyclization, leading to the PO reaction HEI, the \( 1,2\)-dioxetanedione (step 3);\(^7\) in the last step, the chemiexcitation process occurs through a bimolecular interaction between the peroxodic HEI and the ACT (step 4); steps 1 to 3 can all occur with IMI-H acting as a basic catalyst.

\[
\text{HEI} + \text{ACT} \xrightarrow{k_{\text{cat}}} \left[ \text{HEI}^{-} \text{ACT}^{+} \right] \xrightarrow{\text{CO}_2} \text{ACT}^{*} \xrightarrow{\text{hv}} \text{ACT}^{-} + \text{ACT}^{+} + \text{CO}_2
\]

Scheme 3 HEI consumption pathways; (top) unimolecular decomposition without light generation; (bottom) bimolecular ACT-catalysed decomposition, through the light emitting-CIEEL mechanism.

Since the use of lophine and its derivatives as ACTs of the peroxoate reaction is proposed here, it is recommended that CL kinetic assays are performed to address: (i) if the overall mechanism for IAE formation is the same as with commonly used ACTs, and (ii) confirm the initial ET occurring at the chemiexcitation step, as predicted by the CIEEL sequence.

Experimental

General materials and procedures

Ethyl acetate (EtOAc, Sigma-Aldrich, ACS reagent, \( \geq 99.5\% \)), imidazole (IMI-H, Sigma, ACS reagent, \( \geq 99\% \) by titration, crystalline), bis(2,4,6-trichlorophenyl)oxalate (TCPO, Sigma, BioReagent, suitable for chemiluminescence, \( \geq 99.0\% \)), hexane (Synth, analytical grade), benzaldehyde (Sigma-Aldrich, \( \geq 99\% \)), 4-hydroxybenzaldehyde (Aldrich, 98\%), 4-anisaldehyde (Aldrich, 98\%), 4-bromobenzaldehyde (Sigma-Aldrich, 99\%), 4-nitrobenzaldehyde (Aldrich, 98\%), benzil (Aldrich, 98\%), ammonium acetate (Sigma-Aldrich, reagent grade, \( \geq 98\% \)) and acetic acid (Sigma-Aldrich, Reagent Plus, \( \geq 99\% \)) were used as received. Deionized water was obtained through a Milli-Q Millipore purifying system (conductivity 18.2 M\( \Omega \) cm). Quartz cuvettes (Hellma, QS Suprasil) had a volume of 3.0 mL and an optical path of 10.00 mm, with two polished sides for absorption assays and four sides for fluorescence or chemiluminescence assays. Small sample volumes (\( \mu \text{L} \)) were transferred through Hamilton microsyringes. \( \text{H}_2\text{O}_2 \) stock solutions were prepared diluting a 60\% aqueous solution (Solvay Peróxidos do Brasil Ltda.) in EtOAc, the residual water being removed by the addition of
MgSO$_4$ followed by filtration; the final H$_2$O$_2$ concentration was determined iodometrically.$^{12}$

**Equipment**

UV-Vis absorption spectra were recorded on a Varian Cary 60 with a multicell holder thermostatted at 25 °C by a Varian Cary PCB 1500 system. Fluorescence spectra were recorded on a Varian Cary Eclipse with a single-cell holder thermostatted by a Varian Cary PCB 1500 system; kinetic chemiluminescence precipitate was observed. Afterwards, 50 mL of ice-cold with a 65 µm radius), in dimethylsulfoxide (DMSO) as solvent electrode (Pt) and working electrode (microelectrode Pt disc reference electrode (Ag|AgCl, concentrated KCl), auxiliary electrode emitted by the sample. CHN composition was obtained in a PerkinElmer CHN 2400 analyzer, using benzoic acid as standard. NMR spectra were obtained at 25 °C on a Bruker AIII PerkinElmer CHN 2400 analyzer, using benzoic acid as standard.

**Lophine derivatives 1–5**

The known compounds 1–5 were prepared following a general procedure based on Benisvy et al.$^{13}$ A 100 mL single-neck round-bottomed flask was charged with a mixture of the substituted benzaldehyde (8.8 mmol), benzil (8.6 mmol) and ammonium acetate (32 mmol), in 30 mL of glacial acetic acid. A reflux condenser was set up and the solution was kept under reflux for 2 to 3 hours; in some cases, the formation of a precipitate was observed. Afterwards, 50 mL of ice-cold deionized water were added, forcing the precipitation of the lophine; the crude product was collected by filtration, washed with water (5 × 15 mL) and dried by suction. The resulting solid was dissolved in EtOAc and dried over MgSO$_4$. The solution was filtered and the solvent removed by rotary evaporation, yielding a solid that was purified by recrystallization from EtOAc or EtOAc/hexane.

2,4,5-Triphenyl-1H-imidazole (lophine, 1). 1.85 g of a white colored cotton-like solid (71%). Found: C, 84.5; H, 5.4; N, 9.3; Calc. for C$_{22}$H$_{21}$N$_2$: C, 85.1; H, 5.4; N, 9.5%. δ$_H$ (300 MHz; DMSO-d$_6$) 7.3-7.6 (13 H, m, ArH), 8.1 (2 H, d, J = 7.2 Hz, ArH), 12.7 (1 H, br s, NH). δ$_C$ (75 MHz; DMSO-d$_6$) 125.22, 126.50, 127.21, 128.27, 128.46, 128.71, 130.37, 131.05, 135.28, 137.11, 145.54.

2-(4-Hydroxyphenyl)-4,5-diphenyl-1H-imidazole (2). 1.33 g of slightly yellow crystals (48%). Found: C, 80.4; H, 5.3; N, 8.8; Calc. for C$_{22}$H$_{19}$N$_2$O: C, 80.8; H, 5.2; N, 9.0%. δ$_H$ (500 MHz; DMSO-d$_6$) 5.97 (2 H, dt, J = 8.7 and 2.7 Hz, ArH), 6.3-6.7 (10 H, m, ArH), 7.02 (2 H, dt, J = 8.7 and 2.7 Hz, ArH), 8.83 (1 H, br s, ArOH), 11.52 (1 H, br s, NH). δ$_C$ (125 MHz; DMSO-d$_6$) 18.54, 56.01, 115.37, 121.63, 126.31, 126.81, 127.01, 127.33, 127.49, 128.10, 128.29, 128.58, 131.30, 135.41, 136.57, 146.03, 157.75.

2-(4-Methoxyphenyl)-4,5-diphenyl-1H-imidazole (3). 1.41 g of a white solid (50%). Found: C, 80.9; H, 5.5; N, 8.5; Calc. for C$_{22}$H$_{21}$N$_2$O: C, 80.9; H, 5.6; N, 8.6%. δ$_H$ (300 MHz; DMSO-d$_6$) 3.82 (3 H, s, OMe), 7.05 (2 H, d, J = 9 Hz, ArH), 7.2-7.5 (10 H, m, ArH) 8.03 (2 H, d, J = 9 Hz, ArH), 12.5 (1 H, br s, NH). δ$_C$ (75 MHz; DMSO-d$_6$) 55.28, 114.21, 123.10, 126.53, 126.71, 127.20, 127.77, 128.25, 128.51, 128.76, 131.18, 135.44, 136.70, 145.60, 159.39.

2-(4-Bromophenyl)-4,5-diphenyl-1H-imidazole (4). 1.01 g of a white solid (31%). Found: C, 67.9; H, 4.1; N, 7.4; Br, 21.4; Calc. for C$_{22}$H$_{19}$Br$_2$: C, 67.2; H, 4.0; N, 7.5; Br, 21.3%. δ$_H$ (300 MHz; DMSO-d$_6$) 7.37 (6H, br s, ArH), 7.53 (4 H, d, J = 6.9 Hz, ArH), 7.69 (2 H, d, J = 8.7 Hz, ArH), 8.04 (2 H, d, J = 8.7 Hz, ArH), 12.8 (1 H, br s, NH). δ$_C$ (75 MHz; DMSO-d$_6$) 121.75, 127.59, 128.86, 129.98, 132.12, 144.93.

2-(4-Nitrophenyl)-4,5-diphenyl-1H-imidazole (5). 1.17 g of a deep-yellow solid (39%). Found: C, 72.9; H, 4.4; N, 12.5; Calc. for C$_{22}$H$_{19}$Br$_2$: C, 73.9; H, 4.4; N, 12.3%. δ$_H$ (300 MHz; DMSO-d$_6$) 7.10-7.25 (3 H, m, ArH), 7.30-7.40 (3 H, m, ArH) 7.47 (2 H, dd, J = 8 and 1.5 Hz, ArH), 7.54 (2 H, dd, J = 8 and 1.5 Hz, ArH), 8.2-8.38 (4 H, m, ArH). δ$_C$ (75 MHz; DMSO-d$_6$) 124.05, 125.89, 126.95, 127.56, 128.21, 128.33, 128.72, 128.80, 130.25, 131.00, 134.90, 136.63, 139.01, 143.82, 146.80.

**Chemiluminescence kinetic assays**

All CL kinetic assays were carried out in fluorescence quartz cuvettes. To a cuvette charged with EtOAc, the desired amounts of IMI-H, H$_2$O$_2$ and ACT stock solutions (also prepared in EtOAc) were added, and the mixture was allowed to thermally equilibrate with the cell holder at 25 °C for five minutes. The reaction was started with the injection of the TCPO stock solution (prepared in EtOAc), immediately followed by data acquisition on the spectrofluorimeter. The sum of all the volumes added, from EtOAc to the reagents stock solutions, was kept constant at 3.0 mL. The reagents final concentrations were the following: (i) [TCPO] was kept at 0.1 mmol L$^{-1}$ throughout all the experiments; (ii) when the [IMI-H] was varied (from 0.2 to 20 mmol L$^{-1}$), the [H$_2$O$_2$] and [ACT] were 10.0 and 1.0 mmol L$^{-1}$, respectively; (iii) when the [H$_2$O$_2$] was varied (from 0.25 to 10 mmol L$^{-1}$), the [IMI-H] and [ACT] were both 1.0 mmol L$^{-1}$; (iv) when the [ACT] was varied (from 0.1 to 1.0 or 10.0 mmol L$^{-1}$), the [IMI-H] and [H$_2$O$_2$] were 1.0 and 10.0 mmol L$^{-1}$, respectively. The curves of light intensity vs. time (i.e. CL-intensity time-profiles) were observed for at least three half-lives, and fitted by a double exponential function.
Quantum yields

Fluorescence quantum yields (Φ_{FL}) for the lophine derivatives 1–5 were determined using anthracene (Φ_{FL} = 0.27)\(^{14}\) as a relative standard. The chemiluminescence quantum yields (Φ_{CL} in E mol\(^{-1}\)) of the kinetic assays were determined by correcting Φ, which is in arbitrary units (a.u.), by a calibration factor in E a.u.\(^{-1}\) \(f_{cal, Eq. 1}\)\(^{8}\), \(f_{cal}\) was determined using the luminol standard\(^{15}\) under instrumental conditions identical to the ones used for the kinetic assays. Also, a correction factor for the photomultiplier’s wavelength sensibility \(f_{PMT}\) was taken into account \(f_{PMT}\). Eq. 1\)^{5}\) was observed (Figure 1), with bioluminescent \(k_{1(2)}\) and termolecular \(k_{1(3)}\) rate constants of 2.4 ± 0.1 L mol\(^{-1}\) s\(^{-1}\) and (9.03 ± 0.05) × 10\(^{-2}\) L\(^{-2}\) mol\(^{-2}\) s\(^{-1}\), which are in agreement with the ones found for DPA, of 1.4 ± 0.1 L mol\(^{-1}\) s\(^{-1}\) and (9.78 ± 0.08) × 10\(^{-2}\) L\(^{-2}\) mol\(^{-2}\) s\(^{-1}\). This relationship of \(k_{1}\) with the concentration of imidazole is related to its role as a nucleophilic and basic catalyst (step 1, Scheme 2).\(^{10}\)

\[ k_{1} = k_{1(2)}[\text{IMI-H}] + k_{1(3)}[\text{IMI-H}]^{2} \] (3)

Results and Discussion

The PO mechanism with lophine derivatives

Lophine (1), as well as its derivatives 2–5, are fluorescent molecules with a Φ_{FL} ~ 0.4, except for the bromo-substituted 4; they absorb over 300 nm, with molar extinction coefficients of at least 20000 L mol\(^{-1}\) cm\(^{-1}\), fluorescing above 380 nm (Table 1). Despite of being used in several analytical systems,\(^{3}\) including applying the CL emission of 1 for ions detection,\(^{16}\) lophine and its derivatives have never been used before as ACTs of the PO system.

### Table 1. Photophysical properties of the lophine derivatives 1–5.

<table>
<thead>
<tr>
<th>ACT</th>
<th>(\lambda_{\text{ABS}}) (nm)</th>
<th>(\epsilon) (\left(\text{L mol}^{-1}\text{cm}^{-1}\right))</th>
<th>(\lambda_{\text{FL}}) (nm)</th>
<th>(\Phi_{\text{FL}})</th>
<th>(\Phi_{\text{CL}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>309</td>
<td>21400 ± 60</td>
<td>382</td>
<td>0.45</td>
<td>28694</td>
</tr>
<tr>
<td>2</td>
<td>25720 ± 40</td>
<td>391</td>
<td>0.39</td>
<td>28409</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>304</td>
<td>25400 ± 90</td>
<td>390</td>
<td>0.57</td>
<td>28490</td>
</tr>
<tr>
<td>4</td>
<td>316</td>
<td>33500 ± 20</td>
<td>380</td>
<td>0.08</td>
<td>28409</td>
</tr>
<tr>
<td>5</td>
<td>390</td>
<td>31600 ± 50</td>
<td>544</td>
<td>0.45</td>
<td>21668</td>
</tr>
</tbody>
</table>

\(^{1}\) Absorption and emission maxima wavelengths. \(^{2}\) Determined as the angular coefficient of \(\lambda_{\text{ABS}}\) vs. concentration linear plots (Figure S1), setting the linear coefficient as zero; \(r > 0.9999\) in all cases. \(^{3}\) 0–0 transition obtained at the crossing point of absorption and emission spectra (Figure S2).

Before searching for evidences of an ET in the chemiexcitation process (Scheme 3), we verified if compounds 1–5 would affect the slower, rate-determining steps of the PO reaction (steps 1 and 2, Scheme 2), which are the ones observable kinetically. Our results will be compared to the ones found by da Silva et al. and Stevani et al.,\(^{10}\) which have worked with 9,10-diphenylanthracene (DPA) to rationalize the PO mechanism in EtOAc (Scheme 2).

Cl-intensity time-profiles were recorded for different concentrations of 1–5 while keeping the concentration of the other reagents constant (Figure S3); after fitting with a double exponential equation, values for the CL maximum intensity \(I_{\text{max}}\) and the rise \(k_{1}\) and fall \(k_{2}\) rate constants were determined (Table S1). It has been previously shown\(^{10}\) that the fall rate constant \(k_{2}\) corresponds to step 1, while the rise rate constant \(k_{1}\) corresponds to step 2 (Scheme 2). \(k_{1}\) and \(k_{2}\) did not change with the concentration of 1–5 (Table S1), with overall averages of \((3.7 ± 0.3) \times 10^{-3}\) and \((3 ± 1) \times 10^{-1}\) s\(^{-1}\), respectively; therefore, the kinetically observable steps 1 and 2 (Scheme 2) of the PO reaction are independent of the type and concentration of lophine derivative applied as ACT. Such mean values are in agreement with those found previously for DPA – \(k_{1} = 3.5 \times 10^{-3}\) and \(k_{2} = 1.3 \times 10^{-1}\) s\(^{-1}\).\(^{10}\) It is important to notice that the emitting species of the CL reaction is the fluorescent state of the compound, as seen by the clear overlay of CL and FL spectra (Figure S4).

CL kinetic assays were also performed varying the concentration of IMI-H or H\(_{2}\)O\(_{2}\), while keeping the other reagents’ concentration constant, using \([1] = 1.0\) mmol L\(^{-1}\) as ACT (Tables S2 and S3). A quadratic relationship of \(k_{1}\) with the [IMI-H] (Eq. 3) was observed (Figure 1), with bioluminescent \(k_{1(2)}\) and termolecular \(k_{1(3)}\) rate constants of 2.4 ± 0.1 L mol\(^{-1}\) s\(^{-1}\) and (9.03 ± 0.05) × 10\(^{-2}\) L\(^{-2}\) mol\(^{-2}\) s\(^{-1}\), which are in agreement with the ones found for DPA, of 1.4 ± 0.1 L mol\(^{-1}\) s\(^{-1}\) and (9.78 ± 0.08) × 10\(^{-2}\) L\(^{-2}\) mol\(^{-2}\) s\(^{-1}\).\(^{10}\) This relationship of \(k_{1}\) with the concentration of imidazole is related to its role as a nucleophilic and basic catalyst (step 1, Scheme 2).\(^{10}\)
The second step of the PO sequence (step 2, Scheme 2) involves \( \text{H}_2\text{O}_2 \) acting as a nucleophile, with basic catalysis by IMI-H,\textsuperscript{10} and a first-order dependence of \( k_2 \) on both reagents (Figures 1 and 2). From the slope of linear plots, corrected for the fixed concentration of \([\text{H}_2\text{O}_2] = 10.0 \text{ mmol L}^{-1}\) or [IMI-H] = 1.0 mmol L\(^{-1}\), values were found for the termolecular rate constant \( k_{2(3)} = 7.1 \times 10^4 \) and \( 4.3 \times 10^3 \) L mol\(^{-2}\) s\(^{-3}\), respectively, from the imidazole (Figure 1) and \( \text{H}_2\text{O}_2 \) (Figure 2) \( k_2 \) dependence. Considering both determinations, a mean value of \( k_{2(3)} = (6 \pm 1) \times 10^4 \) L mol\(^{-2}\) s\(^{-1}\) is found, which is in agreement with the \( 2 \times 10^4 \) L mol\(^{-2}\) s\(^{-1}\) one found previously with DPA as ACT.\textsuperscript{10}

\[
k_2 = k_{2(3)}[\text{IMI-H}][\text{H}_2\text{O}_2]
\]  

Figure 2. Dependence of the rate constant \( k_2 \) on \( \text{H}_2\text{O}_2 \) concentration (in EtOAc, at 25 °C, [TCPO] = 0.1 mmol L\(^{-1}\), [IMI-H] = [1] = 1.0 mmol L\(^{-1}\)). The \( k_2 \) values show a linear correlation (Eq. 4): \( k_{2(3)}[\text{IMI-H}] = (4.3 \pm 0.1) \times 10^4 \text{ L mol}^{-1} \text{s}^{-1}; r = 0.99093 \).

Therefore, given that the observed rate constants \( k_1 \) and \( k_2 \) of the CL-intensity time-profiles do not depend on the [ACT] for 1–5, and that the values for the rate constants \( k_{1(2)} \), \( k_{1(4)} \), and \( k_{2(3)} \) are in agreement with formerly reported values,\textsuperscript{10} it can be rationalized that: (i) compounds 1–5 do not take part on the kinetically observable steps of the PO mechanism, and (ii) the same overall mechanism is operating for HEI formation (steps 1 to 3, Scheme 2), when these lophine derivatives are used as ACTs, alike commonly used activators such as DPA.\textsuperscript{8,10}

Control experiments were conducted to verify if the lophine derivatives 1–5 emit light through an oxidation process (Scheme 1) that could occur concomitantly to the PO reaction. No light emission was detected on the absence of TCPO, with the system \([1–5] = [\text{IMI-H}] = 1.0 \text{ mmol L}^{-1} \) and \([\text{H}_2\text{O}_2] = 10.0 \text{ mmol L}^{-1}\) (Figure S3), therefore, indicating that the classical CL oxidation reaction of these lophine derivatives is negligible on the experimental conditions used. Also, it was verified that compounds 1–5 could act as basic catalysts of the PO reaction, since these are all hindered imidazole derivatives. There was no light emission for the system \([1–5] = 1.0 \text{ mmol L}^{-1}, [\text{H}_2\text{O}_2] = 10.0 \text{ mmol L}^{-1} \) and [TCPO] = 0.1 mmol L\(^{-1}\) (Figure S3), contrarily to what happens on the system additionally containing IMI-H. Thus, it’s reasonable to assume that derivatives 1–5 do not act as catalysts of the PO reaction and are not involved on the rate-limiting steps of this CL system.

The chemiexcitation mechanism with lophine derivatives

The CL-intensity time profiles’ fitted parameters \( I_{\text{max}}, k_1 \) and \( k_2 \) (Table S1) were used to simulate and extrapolate the intensity to zero counts, and to calculate \( \Phi \) – the integrated areas under the light emission curves. \( Q \) was used to determine the CL quantum yields (\( \Phi_{\text{CL}} \), Table S1), which in turn were converted to the singlet excited states formation quantum yields (\( \Phi_{S} \), Table S1) (see Experimental section). \( \Phi_{S} \) is always related to a particular experimental condition (temperature, solvent, concentration of reagents, etc); its relationship to the ACT concentration (Eq. 5) can be deduced directly from a simplified kinetic scheme.\textsuperscript{8,17}

\[
\frac{I}{\Phi_{S}} = \frac{I}{\Phi_{S}^{\infty}} + \left( \frac{k_{D}}{\Phi_{S}^{\infty}k_{\text{CAT}}} \right) \frac{1}{[\text{ACT}]}
\]  

Besides of being dependent on the unimolecular decomposition rate constant of the HEI (\( k_{D} \)) and on the bimolecular ACT-catalysed reaction rate constant (\( k_{\text{CAT}} \), \( \Phi_{S} \) is also related to \( \Phi_{S}^{\infty} \), which is the singlet excited state formation quantum yield at infinite ACT concentration ([ACT] = ∞). This hypothetical experimental condition is one where every HEI molecule that is formed will be intercepted by one ACT molecule.\textsuperscript{8} Therefore, \( \Phi_{S}^{\infty} \) can be seen as the maximum possible excitation yield of the CL system with that particular ACT, being essentially independent of the rate by which the activator interacts with the HEI. For each derivative 1–5, \( \Phi_{S}^{\infty} \) was obtained through the linear intercept of a double reciprocal plot \( I/\Phi_{S} \) vs. \( 1/[\text{ACT}] \) (Eq. 6, Figure 3 and Table 2).

Figure 3. Double reciprocal plots (\( I/\Phi_{S} \) vs. \( 1/[\text{ACT}] \)) for the lophine derivatives 1–5, used as activators on the imidazole-catalysed reaction of TCPO with \( \text{H}_2\text{O}_2 \).
From Table 2, values for $\Phi_{\text{CL}}$ on the same hypothetical condition of $[\text{ACT}] = \infty$ ($\Phi_{\text{CL}}^{\infty}$) were determined (Table 2). The slopes of the double reciprocal plots (Figure 3, see Eq. 6) were used to determine the ratio $k_{\text{CAT}}/k_D$ (Table 2), which is a relationship between the ACT-catalysed and unimolecular decomposition rate constants of the HEI. Once that the charmiexcitation process (step 4 in Scheme 2) is too fast when compared to the previous steps of the PO reaction (steps 1 to 3, Scheme 2), a direct measurement of $k_{\text{CAT}}$ and $k_D$ is not possible from our kinetic measurements – this can be done only on very specific experimental conditions (see Cisca et al.). Nonetheless, the $k_D$ rate constant is independent of the nature and concentration of the ACT, being related only to the HEI unimolecular decomposition process (Scheme 3). Therefore, changes on the $k_{\text{CAT}}/k_D$ ratio are actually associated to variations on the $k_{\text{CAT}}$ rate constant, due to the bimolecular interaction of the ACT with the HEI (Scheme 3).

The obtained data (Table 2) can be compared to the results found by Stevani et al., who have worked with the same PO system and on identical experimental conditions. They have used PAH derivatives as activators, such as rubrene (RUB), DPA and anthracene (ANT), used as activators. These data were determined in identical experimental conditions ([TCPO] = 0.1 mmol L$^{-1}$, [IMI-H] = 1.0 mmol L$^{-1}$ and [H$_2$O$_2$] = 10.0 mmol L$^{-1}$, in EtOAc at 25 °C).

A free-energy correlation between $k_{\text{CAT}}/k_D$ and $E^{\infty}$ (Figure 4) gives a value for $\alpha = 0.130 \pm 0.003$, i.e. 0.13, indicating the occurrence of an ET process most likely via the CIEEL mechanism (Scheme 3). This relatively low value indicates an early transition state (CIEEL) mechanism. The HEI-peroxodic O–O bond cleavage processes, as these two events are supposed to occur concomitantly (Scheme 4). The $\alpha = 0.13$ value found here for the PO reaction with lophine derivatives 1–5 is in agreement with the ones reported for several CIEEL systems, in the range 0.1–0.3, which includes variations of the PO system and the activated decomposition of isolated four-membered strained cyclic peroxides, called 1,2-dioxetanones.
To the best of our knowledge, the imidazole-catalysed reaction of TCPO with H₂O₂ using RUB as activator is the non-enzymatic system bearing the highest reported emission quantum yield (ΦₑCL = 0.67 E mol⁻¹, Table 2).⁸,¹⁸ The average emission yield ΦₑCL = 1.08 × 10⁻² E mol⁻¹ for compounds 1–3 is, respectively, two and one orders of magnitude lower than ΦₑCL for RUB and DPA, being comparable to the yield reported for ANT (Table 2). Such overall low ΦₑCL values for 1–3, when compared to the PAH-activated ones, are due to the low ΦₑCL values (Table 2) of these systems, given that these derivatives have ΦₑCL values close to 0.4 (Table 1). It has been reported before¹⁰ that imidazole can quench the CL emission of the PO reaction, lowering the emission yields (Table S2). Despite of compounds 1–5 being imidazole derivatives, no evidences were found that could indicate such quenching action, additionally to their role as ACTs, when in high concentrations up to 10.0 mmol L⁻¹. In this concentration, the observed Φₑ and ΦₑCL values (Table S4) are close to the ΦₑCL and Φₑ values, which, as stated above, can be perceived as the maximum possible excitation and emission yields of the CL system. If a quenching process was supposed to occur in such condition, low values for Φₑ and ΦₑCL would have been observed.

Table 3. Singlet energy (Eₛ) of the lophine derivatives 1–5 and energy balance for the electron back-transfer process (EBT, Scheme 3).

<table>
<thead>
<tr>
<th>ACT</th>
<th>Eₛ (kJ mol⁻¹)</th>
<th>ΔGₑEBT (kJ mol⁻¹)</th>
<th>ΔGₑCL (kJ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>340</td>
<td>−333</td>
<td>+7</td>
</tr>
<tr>
<td>3</td>
<td>341</td>
<td>−343</td>
<td>−2</td>
</tr>
<tr>
<td>1</td>
<td>343</td>
<td>−356</td>
<td>−13</td>
</tr>
<tr>
<td>4</td>
<td>340</td>
<td>−359</td>
<td>−19</td>
</tr>
<tr>
<td>5</td>
<td>259</td>
<td>−369</td>
<td>−110</td>
</tr>
</tbody>
</table>

† Obtained from the energy gap between the S₀ and S₁ fundamental vibrational states (ν₀,ν₁ Table 1).  † ΔGₑEBT = −F(EₑACT − EₑCL), where both EₑACT (from Table 2) and EₑCL = −2.44 V are vs. Standard Hydrogen Electrode; F is the Faraday constant.  †† ΔGₑCL = EₑCL + Eₛ.

The ten-fold lower ΦₑCL = 2.29 × 10⁻⁴ E mol⁻¹ observed for derivative 4 (Table 2) is clearly related to its low fluorescence emission quantum yield (ΦₑFL = 0.08, Table 1), a probable offshoot of the heavy atom effect.²² Contrarily, the ΦₑCL and Φₑ values obtained with derivative 5 are much higher than the ones for 1–4, being comparable to the yields formerly reported for DPA (Table 2). Such a higher chemiexcitation yield for 5 can be addressed to the larger accessibility of its electronic excited state, as indicated by the very exergonic ΔGₑEBT = −110 kJ mol⁻¹ (Table 3), which is the free energy associated with the electron back-transfer process that leads to ACT molecules on the excited state (Scheme 3). As discussed elsewhere,⁸ ΦₑCL is strongly related to the energy balance involved in the formation of the excited state (i.e., ΔGₑEBT), which, in turn, depends on the energy gap between S₀ and S₁ states (i.e., Eₛ), and on the free energy associated with the annihilation of the CO₂⁺ and ACT⁺ radical ions leading to species on the fundamental state (i.e., ΔGₑCL). Thus, the overall low ΦₑCL = 3 × 10⁻⁵ E mol⁻¹ chemiexcitation quantum yield for lophine derivatives 1–4 is a consequence of the low accessibility of their electronically excited states, since ΔGₑEBT is much less exergonic for these derivatives (Table 3).

Hammett plot for the PO reaction with lophine derivatives

Information regarding the build up of charge in the transition state of the chemiexcitation step of CL reactions can be obtained through Hammett plots.²³ However, this is not the case for the PO reaction with commonly used ACTs, such as RUB, DPA and ANT, which vary drastically in structure. Since the lophine derivatives 1–5 are different only on a substituent attached to the aryl-imidazolic system, this enables a linear free-energy correlation of the rate constants kₑCAT/kₑCATH – obtained through the kₑCAT/kₑH values (Table 2) – with the Hammett substituent constants (σᵣ). Such plot (Figure 5) shows a good linear dependence (r > 0.97) with ρ = −0.90 ± 0.03. The negative sign of the parameter ρ indicates the formation of a
partial positive charge in the transition state of the rate-determining step – namely, the electron transfer from the ACT to the HEI on the chemiexcitation pathway, with formation of ACT∗, as predicted by the CIEEL sequence (Schemes 3 and 4). Even \( k_{\text{CT}} \) being composed by \( K_{\text{CT}} \) and \( k_{\text{ET}} \) (Eq. 7), it can be stated that the influence of the substituent is mainly on the ET process, since a \( p \) value close to unity indicates that this bimolecular reaction is as sensitive to the substituent as benzoic acid. It can be assumed that the electronic effect of the substituents on the imidazo moiety, where the electron density is probably higher in these lophine derivatives, is basically electron-withdrawing inductive (–I). Thus, there is no direct evidence for the resonance between the imidazolic and the acidity of benzoic acid derivatives. Rationale applied in Hammett’s model system, concerning direct evidence for the resonance between the imidazolic and the acidity of benzoic acid derivatives.

Conclusions

This work has addressed the use of lophine derivatives 1–5 as ACTs of the chemiluminescent PO reaction. Kinetic evidences have shown that such compounds do not act on the slow steps of the reaction, regarding the formation of the HEI. Alike commonly used ACTs, lophines 1–5 act only on the chemiexcitation step, which occurs through the CIEEL mechanism. Average low excited state formation and emission quantum yields (\( \approx 10^{-3} \) E mol\(^{-1}\)) were observed, except for derivative 5, which showed yields ten fold higher. The use of linear free-energy correlations provided evidences not only for an electron transfer, but also for the formation of a partial positive charge on the ACT during the chemiexcitation step, as expected for the CIEEL sequence (Scheme 3).

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Notes and references

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"Lophine derivatives as activators in peroxyoxalate chemiluminescence"

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Lophine and four of its derivatives were applied, for the first time, as activators of the chemiluminescent peroxyoxalate reaction.