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Electrochemical behavior of indolone-N-oxides: Relationship to structure and antiplasmodial activity

Karine Reybier a,b,⁎, Thi Hoang Yen Nguyen a,b, Hany Ibrahim a,b, Pierre Perio a,b, Armelle Montrose a,b, Paul-Louis Fabre c,d,1, Françoise Nepveu a,b

a Université de Toulouse, UPS, PHARMA-DEV, UMR 152, 118 route de Narbonne, F-31062 Toulouse cedex 9, France
b IRD, PHARMA-DEV, UMR 152, F-31062 Toulouse cedex 9, France
c Université de Toulouse, UPS, Laboratoire de Génie Chimique, F-31062 Toulouse cedex 9, France
d CNRS, Laboratoire de Génie Chimique, UMR 5503, F-31062 Toulouse cedex 9, France

⁎ Corresponding author at: PHARMA-DEV, UMR 152, Université Paul Sabatier, 118 route de Narbonne, F-31062 Toulouse cedex 9, France. Tel.: +33 5 6225 9804; fax: +33 5 6225 9802.
E-mail address: karine.reybier-vuattoux@univ-tlse3.fr (K. Reybier).
1 The author is BES member.

A B S T R A C T

Indolone-N-oxides exert high parasiticidal activity at the nanomolar level in vitro against Plasmodium falciparum, the parasite responsible for malaria. The bioreductive character of these molecules was investigated using cyclic voltammetry and EPR spectroelectrochemistry to examine the relationship between electrochemical behavior and antimalarial activity and to understand their mechanisms of action. For all the compounds (37 compounds) studied, the voltammograms recorded in acetonitrile showed a well-defined and reversible redox couple followed by a second complicated electron transfer. The first reduction (−0.88 V < E 1/2 < −0.50 V vs. SCE) was attributed to the reduction of the N-oxide function to form a radical nitroxide anion. The second reduction (−1.65 V < E 1/2 < −1.14 V vs. SCE) was assigned to the reduction of the ketone function. By coupling electrochemistry with EPR spectroscopy, the EPR spectra confirmed the formation of the nitroxide anion radical. Moreover, the experiments demonstrated that a slow protonation occurs at the carbon of the nitrone function and not at the NO function. A relationship between electrochemical behavior and indolone-N-oxide structure can be established for compounds with \( R_1 = -\text{OCH}_3 \), \( R_2 = H \), and electron-withdrawing substituents on the phenyl group at \( R_3 \). The results help in the design of new molecules with more potent in vivo antimalarial activity.

1. Introduction

The N-oxide moiety is a reducible function that leads to biologically active compounds when introduced into conjugated systems such as heterocyclic and pseudo-quinoid compounds. These derivatives are thought to exert their biological action by the generation of reactive oxygen species through a redox cycling process that causes oxidative stress. This may explain how these compounds act as anti-infective agents towards a large number of microorganisms. This is the case of quinoxaline 1,4-di-N-oxide derivatives acting as anti-infectious [1–4], or mono-N-oxides such as benzofuroxan, nitrofurane and nitroimidazole, derivatives with antiprotozoal activities [5–7], and of indolone-N-oxides with various antimicrobial activities [8–10]. The precise mechanism by which these drugs act in different biological models has not yet been fully elucidated [11]. Recently we reported the antimalarial activity of a series of indolone-N-oxides (Scheme 1). The biological evaluation and structure–activity relationship (SAR) studies proved the efficient antimalarial activity of these derivatives in vitro and in vivo against Plasmodium falciparum (Pf) [12,13]. Proteomic studies that we have reported suggest that these indolone-N-oxides cause a profound destabilization of the membrane of the Pf infected red blood cells (RBC) through a mechanism apparently triggered by the activation of a redox signaling pathway, which could be initiated by a chemical redox reaction in the RBC [14]. These results gave

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doi:10.1016/j.bioelechem.2012.04.001

Scheme 1. Structure of the indolone-N-oxide series.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>LogP&lt;sub&gt;oct&lt;/sub&gt; (VCCLAB)</th>
<th>I&lt;sub&gt;C50&lt;/sub&gt;/nM FcB1 strain&lt;sup&gt;b&lt;/sup&gt;</th>
<th>E&lt;sub&gt;i&lt;/sub&gt;/mV&lt;sup&gt;c&lt;/sup&gt; (vs. SCE)</th>
<th>R&lt;sub&gt;p&lt;/sub&gt;</th>
<th>ΔEp/mV&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>O—CH&lt;sub&gt;2&lt;/sub&gt;—O</td>
<td>4'-Chlorophenyl</td>
<td>2.07</td>
<td>75 ± 63</td>
<td>−0.705</td>
<td>0.27(1)</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>H H</td>
<td>4'-Phenoxyphenyl</td>
<td>3.51</td>
<td>264 ± 60</td>
<td>−0.749</td>
<td>0.60(0.86)</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>H H</td>
<td>4'-Methoxyphenyl</td>
<td>2.01</td>
<td>195 ± 20</td>
<td>−0.744</td>
<td>0.89(1)</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>H H</td>
<td>4'-6'-Methoxy-naphthalen-2-yl</td>
<td>3.03</td>
<td>560 ± 15</td>
<td>−0.748</td>
<td>0.70(0.86)</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>O—CH&lt;sub&gt;2&lt;/sub&gt;—O</td>
<td>4'-Phenoxyphenyl</td>
<td>3.00</td>
<td>165 ± 40</td>
<td>−0.788</td>
<td>0.50(0.84)</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>H H</td>
<td>3', 4'-Dichlorophenyl</td>
<td>3.14</td>
<td>155 ± 20</td>
<td>−0.635</td>
<td>0.92(1)</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Chlorophenyl</td>
<td>2.58</td>
<td>210 ± 10</td>
<td>−0.727</td>
<td>1(1)</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>3'-Trifluoromethyl-4'-chlorophenyl</td>
<td>3.32</td>
<td>50 ± 39</td>
<td>−0.611</td>
<td>0.37(0.72)</td>
<td>75</td>
<td></td>
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<td>12</td>
<td>H OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4'-Chlorophenyl</td>
<td>2.57</td>
<td>148 ± 31</td>
<td>−0.729</td>
<td>0.85(0.95)</td>
<td>70</td>
<td></td>
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<tr>
<td>13</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>3', 4'-Dichlorophenyl</td>
<td>3.18</td>
<td>100 ± 7</td>
<td>−0.634</td>
<td>0.89(1)</td>
<td>68</td>
<td></td>
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<td>17</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>Phenyl</td>
<td>2.00</td>
<td>184 ± 53</td>
<td>−0.760</td>
<td>0.30(0.50)</td>
<td>100</td>
<td></td>
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<tr>
<td>18</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Tolyl</td>
<td>2.25</td>
<td>52 ± 48</td>
<td>−0.794</td>
<td>1(1)</td>
<td>74</td>
<td></td>
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<td>19</td>
<td>H CF&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4'-Chlorophenyl</td>
<td>3.26</td>
<td>186 ± 15</td>
<td>−1.588</td>
<td>0.35</td>
<td>176</td>
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<td>20</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Methoxy-naphthalen-2-yl</td>
<td>3.03</td>
<td>135 ± 45</td>
<td>−1.226</td>
<td>0.30</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Chlorophenyl-4-yl</td>
<td>4.06</td>
<td>630 ± 248</td>
<td>−1.726</td>
<td>1(1)</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Methoxyphenyl</td>
<td>2.04</td>
<td>40 ± 39</td>
<td>−1.782</td>
<td>0.44</td>
<td>166</td>
<td></td>
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<tr>
<td>24</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Phenoxyphenyl</td>
<td>3.51</td>
<td>193 ± 55</td>
<td>−1.509</td>
<td>0.85</td>
<td>172</td>
<td></td>
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<tr>
<td>25</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Trifluoromethoxyphenyl</td>
<td>2.90</td>
<td>20 ± 6</td>
<td>−1.590</td>
<td>0.80</td>
<td>172</td>
<td></td>
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<tr>
<td>26</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Dimethylaminophenyl</td>
<td>2.30</td>
<td>&lt;3</td>
<td>−1.582</td>
<td>0.60(0.80)</td>
<td>82</td>
<td></td>
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<tr>
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<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Isopropoxyphenyl</td>
<td>2.81</td>
<td>17 ± 2</td>
<td>−1.816</td>
<td>0.31</td>
<td>252</td>
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<tr>
<td>29</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Methoxy-3'-tolyl</td>
<td>2.25</td>
<td>44 ± 4</td>
<td>−1.836</td>
<td>0.75(1)</td>
<td>94</td>
<td></td>
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<tr>
<td>30</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Aminophenyl</td>
<td>1.58</td>
<td>24 ± 19</td>
<td>−1.876</td>
<td>0.83(1)</td>
<td>68</td>
<td></td>
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<td>35</td>
<td>H OCTF&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4'-Methoxyphenyl</td>
<td>2.88</td>
<td>133 ± 50</td>
<td>−1.681</td>
<td>0.95(1)</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Trifluoromethylphenyl</td>
<td>2.74</td>
<td>43 ± 23</td>
<td>−1.643</td>
<td>0.92(0.96)</td>
<td>68</td>
<td></td>
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<tr>
<td>39</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>3'-Chlorophenyl</td>
<td>2.60</td>
<td>21 ± 1</td>
<td>−1.387</td>
<td>0.45</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>3'-Methyl-4'-tolyl</td>
<td>2.48</td>
<td>37 ± 28</td>
<td>−1.574</td>
<td>0.44</td>
<td>226</td>
<td></td>
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<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>4'-Tert-butylphenyl</td>
<td>3.63</td>
<td>204 ± 14</td>
<td>−0.791</td>
<td>1(1)</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>OCH&lt;sub&gt;3&lt;/sub&gt; H</td>
<td>2'-Thiopen-3-yl</td>
<td>1.93</td>
<td>270 ± 16</td>
<td>−0.785</td>
<td>0.91(1)</td>
<td>70</td>
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<tr>
<td>48</td>
<td>H NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4'-Methoxyphenyl</td>
<td>1.97</td>
<td>345 ± 81</td>
<td>−0.477</td>
<td>0.81(1)</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>H H</td>
<td>i-Butyl</td>
<td>1.61</td>
<td>&gt;4.500&lt;sup&gt;f&lt;/sup&gt;</td>
<td>−0.842</td>
<td>0.86(1)</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>H H</td>
<td>n-Propyl</td>
<td>1.41</td>
<td>12,160 ± 500</td>
<td>−0.838</td>
<td>0.77(0.90)</td>
<td>80</td>
<td></td>
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<tr>
<td>56</td>
<td>H H</td>
<td>4'-Nitrophenyl</td>
<td>1.96</td>
<td>56 ± 4</td>
<td>−0.518</td>
<td>0.90(1)</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>H OPh</td>
<td>4'-Phenoxyphenyl</td>
<td>4.79</td>
<td>665 ± 135</td>
<td>−0.780</td>
<td>0.35(0.67)</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>H H</td>
<td>4'-Ethylphenyl</td>
<td>2.81</td>
<td>2,750 ± 150</td>
<td>−0.726</td>
<td>0.67(1)</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>H H</td>
<td>4'-Trifluoromethylphenyl</td>
<td>2.71</td>
<td>1,170 ± 80</td>
<td>−0.652</td>
<td>1(1)</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>H (CO)CH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>4'-Methoxyphenyl</td>
<td>584 ± 27</td>
<td>−0.603</td>
<td>0.79(1)</td>
<td>72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>ΔE<sub>p</sub> = E<sub>i</sub>/mV<sup>c</sup> (vs. SCE) − E<sub>i</sub>/mV<sup>d</sup> (vs. ferrocyanide/ferrocyanide)<sup>d</sup>.

<sup>b</sup>IC<sub>C50</sub>/nM FcB1 strain<sup>b</sup> = [IC<sub>C50</sub>/nM] (FcB1 strain<sup>b</sup>)

<sup>c</sup>E<sub>i</sub>/mV<sup>c</sup> (vs. SCE) = E<sub>i</sub>/mV<sup>c</sup> (FcB1 strain<sup>b</sup>)

<sup>d</sup>R<sub>p</sub> = R<sub>p</sub> (FcB1 strain<sup>b</sup>)

<sup>e</sup>ΔEp/mV<sup>d</sup> = ΔE<sub>p</sub>/mV<sup>d</sup> (FcB1 strain<sup>b</sup>)

<sup>f</sup>R<sub>p</sub> = R<sub>p</sub> (FcB1 strain<sup>b</sup>)
rise to pharmacomodulation studies to develop new hits and leads. The conjugated system between the nitrone group and the ketonic function of the indolone moiety was found to be essential for optimal in vitro antimalarial activity.

In parallel, we explored some ADMET properties (Absorption, Distribution, Metabolism, Excretion and Toxicity) with the binding affinity of these compounds to human serum albumin [15] and cell based metabolism studies. In this context, we showed that indolone-N-oxides undergo a bioreductive transformation in red blood cells [16]. In previous studies we evaluated the spin trapping properties of these compounds and demonstrated their ability to trap hetero- and carbon-centered radicals [17]. Major differences in antiplasmodial activities as well as in spin trapping properties studied by EPR spectroscopy were observed between aryl and alkyl substituted indolones at R². It can be noted that indolone-N-oxides are built around three reducible functions, the N-oxide moiety, the highly electrophilic carbon in the nitrone moiety and the ketone function. At this point we considered it necessary to study the redox properties of indolone-N-oxides to establish the relationship between the electrochemical behavior and the biological activity and to continue with the study of the structures required to obtain more potent in vivo antimalarial activity.

We report here the redox behavior of 37 indolone-N-oxides using cyclic and stationary voltammetry, and EPR spectroelectrochemical measurements. The relationships between the substituents, redox potentials, lipophilicity and antimalarial activities were examined.

2. Experimental

2.1. Reagents

Acetonitrile (ACN, Fisher) and tetrabutylammonium perchlorate (TBAP, Fluka) were obtained commercially at the highest purity available and used without further purification. The methods for the synthesis of indolone-N-oxides have been previously reported [12]. The two new compounds (67, 68) were obtained from Idealp-Pharma (Villeurbanne, France).

2.2. Voltammetric measurements

Electrochemical experiments were carried out at 25 °C in acetonitrile containing TBAP 0.1 mol L⁻¹ using a Voltalab 80 PC2 402 (Radiometer) with a conventional cell with three electrodes: reference electrode, a double junction calomel electrode (SCE); counter electrode, a platinum electrode sheet (5 × 5 mm); working electrode, a platinum disk (0.5 mm diameter) for cyclic voltammetry and a rotating disk electrode (EDL, Tacussel) for stationary conditions. All solutions were deoxygenated by argon bubbling through the solution for 15 min and maintaining a blanket of the inert gas over the solution during the experiment. Voltamperograms were recorded for a compound concentration equal to 10⁻³ mol L⁻¹ (ferrocene or indolone-N-oxides) and a potential scan rate ranging from 0.02 V/s up to 25 V/s depending on experiments. The electrochemical data shown in Table 1 were recorded at a potential scan speed of 0.1 V/s.

2.3. EPR spectroelectrochemical analysis

For spectroelectrochemical measurements, the EPR spectrometer was coupled to a potentiotstat-galvanostat (EC&G Princeton Applied Research-Model 362). A PHN 81 (Tacussel) voltameter was used to control the applied potential. A flat quartz cell adapted to electrochemical measurements (Bruker, Wissembourg, France) was used for analysis. The electrochemical reduction was performed using a three-electrode set-up: the working and counter-electrode were platinum and the reference electrode was a silver wire. The applied potential was chosen to be on the diffusion plateau of the first reduction wave obtained under stationary conditions: Eₐₜ₈ italiani = Eₙₐₘ₈₋₂ V. The electrolysis potential was applied for 5 min to the solution containing the compound in acetonitrile/TBAP and the EPR spectrum was immediately recorded as a function of time. EPR spectra were obtained at X-band at room temperature on a Bruker EMX-8/27 (9.86 GHz) equipped with a high-sensitivity cavity (4119/HS 0205) and a gaussmeter (Bruker, Wissembourg, France). WINEPR and SIMFONIA software (Bruker, Wissembourg, France) were used for EPR data processing and spectrum computer simulation. Typical scanning parameters were scan rate, 1.2 G/s; scan number, 1; modulation amplitude, 1 G; modulation frequency, 100 kHz; microwave power, 20 mW; sweep width, 100 G; sweep time, 83.88 s; time constant, 40.96 ms; center field, 3 480 G; receiver gain 5 × 10⁴.

2.4. Biological properties

Log P and antimalarial in vivo activities of the compounds studied have been previously reported [12] and are given in Table 1 (same numbering as in Ref. [12]). The IC₅₀ value is defined as the concentration inhibiting the growth of the parasite Pf FcB1 reference strain by 50% when cultured in red blood cells.

3. Results and discussion

3.1. Electrochemical behavior

In the electroactivity domain of CH₂CN/TBAP, the cyclic voltammograms of the compounds showed oxidation and reduction processes at a platinum electrode. As this paper deals with the reductive properties of the indolone-N-oxides, only the cathodic behavior is described. Table 1 reports the electrochemical data obtained at a potential scan speed of 0.1 V/s: E₁/₂ = (Eₕᵣ₇ᵣ₈ + E₁ᵣ₋ᵣ₇ᵣ₈)/2; Rlp = |Eₕᵣ₇ᵣ₈/E₁ᵣ₋ᵣ₇ᵣ₈| and ΔEₚ = |Eₕᵣ₇ᵣ₈ − E₁ᵣ₋ᵣ₇ᵣ₈|.

Fig. 1 shows typical voltammograms obtained for the series. The voltammogram is characterized by two electron transfers. The first electron transfer (−0.882 (26) < E₁/₂ < −0.477 (48) vs. SCE) is perfectly reversible (Rlp around 1) independently of the scan rate when the potential scan is restricted to this system. As expected the
The irreversibility of this reduction is indicative of high chemical reactivity of the intermediate, the radical-anion C––O⁻.

The ketone reduction potential depends on the delocalization [18] whereas the nature of the products formed depends on the medium: pinacol, through a monoelectron transfer, or alcohol through a bielectron transfer [18,19] that requires protonation. Pinacol originates from the dimerization and then protonation of the radical-anion. The reversibility of the second electrochemical system depends on the stability of the radical-anion C––O⁻: the peak ratio $R_{p}$ is lower than unity (Table 1) but when the potential scan rate was increased (around 5 V/s) a quasi-reversible system was observed (data not shown). In dry acetonitrile, the one electron reduction yields the C––O⁻ radical-anion, which gives pinacol after dimerization.

Voltammograms were recorded with increasing quantities of water (Fig. 2). Addition of small quantities of water has a slight effect on the first reduction process whereas it considerably affects the second one. The potential of the first redox process (N-oxide reduction) is slightly displaced towards less negative values when increasing the percentage of water (around 120 mV on the figure). Moreover, the system remains reversible, in particular when the potential scan is restricted to this system. The anodic displacement of the reduction potential can be explained by the protonation of the nitroxide radical-anion according to the potential-pH diagram theory, the water being the proton source. This protonation is confirmed by the EPR spectroelectrochemical analysis (cf. 3.2).

On the contrary, the potential of the second electron transfer (ketone reduction) is strongly displaced towards less negative values when the percentage of water is increased (around 500 mV). The forward peak current also increases showing that the system moves from a one electron transfer to a bielectronic transfer, leading to the production of alcohol [19,20].

For compounds 48, 56, and 67 that have a third reducible function in their structure, the signals are consistent with those reported in the literature. The acetonitrone moiety in 67 is reduced around −2 V [20–22]. In the case of the NO₂ derivatives, their reductions are monoelectronic [23,24] in aprotic solvents. The potential of NO₂ reduction for 48 is then attributed at −0.932 V and for 56 at −0.987 V. The C=O reduction potentials are in the same order as the reported values of other compounds (Table 1). However, as part of our study, only the data corresponding to the first reduction step were correlated with anti-malarial properties.

3.2. EPR spectroelectrochemical analysis

To confirm the radical nature of the species formed after the first one-electron step reduction and to study its stability, electrochemistry was coupled with electron paramagnetic resonance (EPR) spectroscopy. An illustration of the EPR spectrum obtained and its evolution over time is shown in Fig. 3 for compound 1 after 5 min of electrolysis in acetonitrile containing TBAP 0.1 mol L⁻¹ (potential on the plateau of the diffusion wave due to the first electrochemical system: $E_{\text{applied}} = E_{1/2} = 0.2$ V). The spectrum recorded 4 min after the end of electrolysis (Fig. 3a) consists in a triplet characterized, as demonstrated by simulation, by the hyperfine splitting constants

![Fig. 1. Electrochemical properties of indolone-N-oxides. Cyclic voltammograms of 1 and 49 (1 mmol L⁻¹) at a platinum electrode in ACN-TBAP (0.1 mol L⁻¹), potential scan rate 0.1 V/s.](image)

![Scheme 2. Reduction of indole N-oxide.](image)

- Values are in agreement with the quasi-reversible definition of an electron transfer. The peak currents are linearly related to the square root of the potential scan rate as for a diffusion controlled process.
- The peak currents are linearly related to the square root of the potential scan rate 0.1 V/s.
- The second electron transfer is then attributed to the ketone function.
This nitroxide radical-anion detected is relatively stable (about 10 min). This stability is consistent with an aprotic medium and the highly conjugated character of the indolone-N-oxide structure allowing the delocalization of the unpaired electron. The high stability of the spin adducts derived from indolone-N-oxydes has already been demonstrated in previous studies dealing with spin trapping properties of these molecules [17]. This triplet evolves with time to a signal (Fig. 3c) with hyperfine splitting constants $a_N = 9.5$ G and $a_H^\beta = 2.3$ G indicating that the carbon of the nitroxide anionic form has been protonated (Scheme 3). A protonation on the nitroxide function would give a hydroxylamine form which is EPR silent. Before the protonation at carbon 2, the unpaired electron is strongly delocalized on the whole structure which is nearly planar and it couples with the nuclear spins of the nitrogen and proton at C4 and C7 (for compound 1). X-ray crystallography of a similar compound (2-phenylisatogen) shows that the isatogen ring and the phenyl ring are almost fully co-planar [25,26]. After protonation at C2, the planarity of the molecule is decreased thus reducing the electronic delocalization and resulting in a higher electronic density at the NO bond. The unpaired electron then couples with the nuclear spins of the nitrogen and

\[ a_N = 6.27 \text{ G}, \quad a_H^\gamma = 1 \text{ G} \text{ (protons on carbons 4 and 7 for compound 1)}. \]

Fig. 2. Electrochemical properties of indolone-N-oxydes. Cyclic voltammograms of 49 (1 mmol L\(^{-1}\)) at a platinum electrode in ACN-TBAP (0.1 mol L\(^{-1}\)) with increasing amount of water, potential scan rate 0.1 V/s.

Fig. 3. EPR spectra of the reduced form of indolone-N-oxide: EPR spectra and the corresponding simulated spectra recorded for compound 1 (1 mmol L\(^{-1}\)) a) 4 min, b) 12 min and c) 23 min after the end of the 5 min electrolysis at $-0.9$ V vs. SCE in ACN containing TBAP (0.1 mol L\(^{-1}\)).
proton at C2. From this structural change resulting from the protonation at C2, the higher electronic density on the NO bond leads to a higher hyperfine splitting constant, \(a_N\), shifting from 6.27 G to 9.5 G. These observations are in agreement with Scheme 3. For compound 1, a mixture of the two species (nitroxide radical-anion and its protonated form) is observed over about 8 min. As demonstrated by the simulation, the spectrum recorded 12 min after the end of electrolysis is the superimposition, in the same proportion, of the spectra of the two species (Fig. 3b). The coupling between the electrochemistry and EPR spectroscopy leads to a better understanding of each step of the reduction/protonation process compared with that reported for some isatogens [27].

3.3. Relationship between reduction potentials and substituents on indolone-N-oxides

All the compounds studied gave at least two electrochemical systems: the first one presented a half-wave potential ranging from \(-0.882\) to \(-0.477\) V vs. SCE while for the second one the limits are in the domain \([-1.650, -1.264]\) V vs. SCE. These values are highly dependent on the three substituents, \(R_1\), \(R_2\) and \(R_3\). Studies reported for other N-oxide derivatives, such as phosphorylated nitrones [28], phenyl-butyl nitrite derivatives [29] or quinoxaline-N, N′-dioxides [11], showed such a relationship. In our series, the presence of electron-donating groups induces an important shift of the first reduction wave. For example compounds 49 or 50, \((R^3 = \text{alkyl})\) have the highest reduction potential value. With the introduction of electron-withdrawing substituents (Cl, CF\(_3\), O—CF\(_3\), NO\(_2\)) on the phenyl ring \((R^3 = \text{aryl})\) (compounds 1, 8, 9, 11, 12, 13, 19, 25, 37, 39, 56 and 61) or on the indolone moiety at \(R^2\) (compounds 19, 35, 48), the products are more readily reduced. For example, the substitution of the alkyl chain \((R^1)\) (compounds 49 or 50) by a 3,4-dichlorophenyl (compound 8) or by a 4-trifluoromethylphenyl (compound 61) group resulted in a positive shift equal to 23 mV for the first cathodic wave. This shift was only 9 mV when \(R^2\) was a 4-ethylphenyl group (compound 60). By comparing the redox potentials of compounds 9, 37 and 25 or 56 and 61 the withdrawing effect of substituents appears clear: \(\text{NO}_2 > \text{CF}_3 > \text{Cl}\). Similarly, by comparing compounds 37 and 25, one can note that the replacement of CF\(_3\) by O-CF\(_3\) induces, as expected, a negative shift in the reduction potential. A smaller shift is recorded with the introduction of an electron-donating group \((-\text{O—R or Ph—O—R})\) as \(R^1\) or \(R^3\) (compounds 2, 4, 5, 7, 18, 20, 22, 24, 27, 29 and 57). By comparing compounds 9 and 12 it appears that the position of the methoxy group \((R^1\) or \(R^2\)) did not affect the reduction potential.

These effects are consistent with the facilitation of reduction by lowering the electron density at C2 which is confirmed by the Hammett plot (Fig. 4), considering the \(\sigma_p\) values for substituents in the 4-position of the phenyl ring [27,30]. As predicted by Hammett, the number and position of electron-withdrawing groups influence the reduction potential.

Note that the most cathodic potentials were obtained in the case of amine substituents (compounds 26 and 30). The addition of a
methoxy group at R\(^1\) does not modify the reduction potential as seen by comparing compounds 2 with 24 and 37 with 61.

3.4. Relationship between the electrochemical behavior and antiplasmodial activity

The indolone-N-oxides gave rise to two reduction steps in ACN/TBAP. The first reduction of the N-oxide moiety is reversible and involves the formation of a stable nitrooxide radical-anion as confirmed by EPR spectroscopy. This relatively stable radical could be the starting point for the redox events deleterious to the parasite. In this case, the ease of the first reduction of the N-oxide moiety would play an important role in the antiplasmodial activity. Indeed, further experiments carried out on the series have demonstrated that the antiplasmodial properties of these indolone-N-oxides were controlled by a bioreductive transformation in red blood cells [16].

Many parameters including solubility and log P, absorption, diffusion and metabolism, as well as the cellular targets affect the biological activities of compounds. It is clear that a global analysis of each parameter versus the biological activity studied may not produce a clear relationship. The effects of these parameters may be additive or subtractive, rendering any correlation difficult to observe on a large series. No simple relationship was found between the reduction potential and the antiplasmodial activity. For example, one can note that compounds with aliphatic substitutions (49, 50 and 60) are hardly reduced and poorly active against Plasmodium. On the contrary, compounds having amine groups (26, 30 and 68) also have very cathodic reduction potentials whereas they are among the most biologically active compounds of the series. The amine groups at R\(^1\) on these compounds give cationic protonated forms at the pH of the cell model tested (parasitized red blood cells), which are determinant for the absorption mechanism of the cell. In the case of these compounds (26, 30 and 68), the absorption surpasses the electron withdrawing effects of substituents compared with other compounds. Interestingly, we previously showed that antiplasmodial activities were more strongly affected by the substitutions at R\(^1\) than by substitutions at the phenyl group of the indolone moiety (R\(^2\) and R\(^3\)). Consequently, the relationship between activity and the ease of reduction have been studied for molecules with R\(^3\) = ―OCH\(_3\) and R\(^3\) = H, compounds distinguished by electron-withdrawing or donating substituents on the phenyl group at R\(^3\). The corresponding graphs are shown in Fig. 5. In the case of electron-withdrawing substituents (Fig. 5a), the antiplasmodial activity increases when the compounds are more readily reduced. These results are consistent with an antiplasmodial property controlled by a bioreductive transformation in red blood cells [16] and in agreement with mechanisms of action that we recently reported [14].

On the other hand, for electron-donating substituents (Fig. 5b), a large variation in activity is observed, whereas the potential is almost constant due to their weak influence. In this second case, variation in lipophilicity could explain such disparate IC\(_{50}\) values. Indeed, in this group, the less potent compounds 20, 24 and 42 are characterized by the highest partition coefficient (log P > 3), reaching 3.63 for 42.

4. Conclusion

The thirty seven indolone-N-oxides studied in aprotic solvents, showed two reduction steps located around −0.68 ± 0.2 V and −1.45 ± 0.2 V vs. SCE. The first reduction step was reversible for all the compounds studied under the conditions of the study and ascribed to the reduction of the C=N—O double bond, whereas the second irreversible reduction step was ascribed to the carbonyl reduction. EPR spectroscopy analysis confirmed that this first reduction gives rise to the formation of a relatively stable radical, which could be the starting point for the redox events deleterious to the parasite. A relationship between electrochemical behavior and indolone-N-oxide structure can be established for compounds having electron-withdrawing substituents. The insertion of an electron-withdrawing group on the N-oxide ring results in a less negative reduction potential making the bio-reduction easier and thus the compound more active against Plasmodium. These results will orientate the selection of substituents for the design of new molecules with better pharmacological activities.

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