Cyclic voltammetric determination of free radical species from nitroimidazopyran: a new antituberculosis agent

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Abstract

PA-824 (2-nitro-6-(4-trifluoromethoxy-benzyloxy)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine) is being tested as antituberculosis drug. Little is known on the action mechanism of PA-824; however the reduction of the nitro group seems to be a key step in the metabolic activation, as is observed for the well-known bactericidal metronidazole. Consequently, this paper is focused on the cyclic voltammetric behavior of PA-824 with the aim of revealing the formation and stability of the corresponding nitro radical anion and its comparison with the metronidazole behavior.

Both compounds PA-824 and metronidazole reveal, in aprotic medium (DMSO + 0.1 tetrabutylammonium hexafluorophosphate), a similar reduction pattern showing a well-resolved couple due to nitro reduction to form the corresponding nitro radical anion. The electrode reaction obeys an EC_2 mechanism with a dimerization reaction as the chemical step in aprotic medium. Using cyclic voltammetry theory for a dimerization reaction we have calculated the second-order decay constants, $k_{2,\mathrm{dim}}$, and the half-life time, $t_{1/2}$, for the nitro radical anions formed from PA-824 and metronidazole. We have obtained $k_{2,\mathrm{dim}}$ values of 2.22×10^2 and 2.58×10^4 $M^{-1}s^{-1}$ for metronidazole and PA-824, respectively. Our voltammetric results show that the PA-824 nitro radical anion requires more energy for formation (about 200 mV) and it is approximately 100 times less stable than the metronidazole radical anion.

Keywords: Nitro radical anion; Cyclic voltammetry; Metronidazole

1. Introduction

Tuberculosis is the greatest single infection, which causes mortality world wide, killing approximately two million people annually [1]. Furthermore, the synergy between tuberculosis and AIDS [2] and the surge of multidrug-resistant pathogens have reaffirmed tuberculosis as a primary public health problem. Consequently the search for new antitubercular drugs is a permanent challenge. Recently, Stover et al. [3] reported a series of compounds containing a nitroimidazopyran moiety that possesses antitubercular activity. One of these derivatives PA-824 (2-nitro-6-(4-trifluoromethoxy-benzyloxy)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine) (Fig. 1) exhibited potent bactericidal activity against both replicating and static *Mycobacterium tuberculosis* and promising oral activity in animal infection models. Currently, little is known on the mechanism of action of PA-824 towards tuberculosis. Initial evidence [3] indicates that the metabolic activation of PA-824 by *M. tuberculosis* may involve a nitro-reduction step analogous to that required for metronidazole activation [4]; consequently, the reduction of the nitro group in the PA-824 molecule could also be a key step in the metabolic activation.

In view of the similarity between the electrochemical reactions at the electrode|solution interface, and the enzymatic redox reactions, knowledge of the electrochemical mechanism of reduction of this compound would be very useful in providing additional information on the mechanism of action. Consequently, we have focused our current research on investigating the redox behavior of PA-824 in comparison to the well-known redox behavior of metronidazole.

The electrochemistry of metronidazole has been reported at different electrodes such as mercury [5–9],

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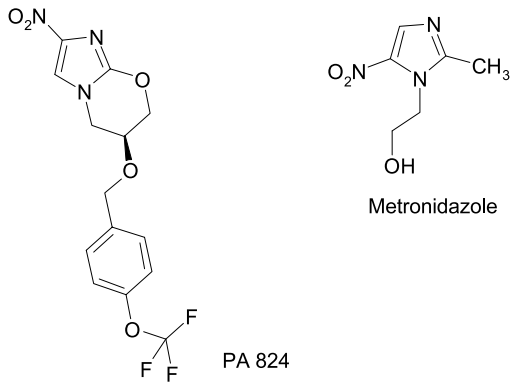

Fig. 1. Chemical structures of PA-824 and metronidazole.

glassy carbon, DNA-modified glassy carbon and a mercury thin film electrode [10,11]. According to these studies, the reduction of metronidazole in acid medium is pH-dependent and four electrons are involved in the complete reduction to the hydroxylamine derivative. In aprotic medium the reduction of metronidazole occurs with a first reversible 1-electron couple due to the nitro/nitro radical anion redox pair and a second irreversible peak due to the 3-electron and 4-proton further reduction of the nitro radical anion to the hydroxylamine derivative. Furthermore the published results revealed the voltammetric feasibility of studying the formation and stabilization of the nitro radical anion from metronidazole [10,11].

On the other hand PA-824 has received less attention: only one voltammetric study, mainly focused on its analytical determination, has been published recently [12]. Considering that participation of enzymatically-generated free radicals could be relevant to the antitubercular mechanism of action, the elucidation of the voltammetric characteristics of the nitro radical anion would be worth conducting. In the present contribution, a cyclic voltammetric approach, in order to study the formation and stability of the corresponding nitro radical anion from PA-824 and its comparison with metronidazole, is carried out.

2. Experimental

2.1. Materials

PA-824 was obtained from PathoGenesis Corporation, Seattle, Washington, USA. Metronidazol was obtained from Laboratorio Chile, Santiago, Chile. All reagents employed were of analytical grade.

1 or 5 mM of each drug solution in two different media were used for the voltammetric studies. For a mixed medium, a mixture of 60/40: DMF + citrate buffer was used with 0.3 M KCl as the supporting electrolyte. The pH was adjusted with small aliquots of concentrated NaOH or HCl, respectively.

Di methyl sulphoxide (DMSO) and 0.1 M tetrabutyl ammonium hexafluorophosphate (TBAF), as solvent and supporting electrolyte, respectively, were used in aprotic media.

2.2. Apparatus

Electrochemical experiments were performed with a totally automated BAS CV-100 voltammetric analyzer. All experiments were carried out at a constant temperature of 25 ± 0.1 °C using a 10 ml thermostatic cell. A static mercury drop electrode (SMDE mode in a controlled growth mercury electrode stand from BAS), with a drop area of 0.42 mm^2 and a 3 mm diameter glassy carbon electrode (BAS) were used as working electrodes with a platinum wire as the counter electrode. All potentials were measured against Ag|AgCl|3M Cl^-.

Simulated CV curves were obtained using the DIGISIM® 2.1 CV simulator for Windows software (BAS, USA). The software was run using a Gateway 2000 PC.

2.3. Methods

The pH measurements were corrected according to the following equation [13]:

$$pH^* - B = \log U_H^0$$

where pH^* equals $-\log a_H$ in the mixed solvent, B is the pH meter reading and the term $\log U_H^0$ is the correction factor for the glass electrode, which was calculated from different mixtures of DMF and aqueous solvent, according to a previously reported procedure [14].

In the kinetic analysis carried out by cyclic voltammetry, the return-to-forward peak current ratio I_{pa}/I_{pc} for the reversible first-electron transfer (the $ArNO_2$/$ArNO_2^{\cdot-}$ couple) was measured from each cyclic voltammogram, varying the scan rate from 0.1 to 10 V/s according to the procedure described by Nicholson [15].

Using the theoretical approach of Olmstead et al. [16,17], the I_{pa}/I_{pc} values measured experimentally at each scan rate were inserted into a working curve to determine the parameter ω, which incorporates the effects of rate constant, drug concentration and scan rate. A plot of ω versus τ resulted in a linear relationship described by the equation

$$\omega = k_2 c_0 \tau,$$

where k_2 is the second-order rate constant for the chemical reaction of $ArNO_2^{\cdot-}$, c_0 is the nitrocompound concentration and $\tau = (E_\lambda - E_{1/2})/v$. Consequently we can obtain the second-order rate constant for the decomposition of the nitro radical anion from the slope of the straight line ω versus τ. The assumption that the decomposition of $ArNO_2^{\cdot-}$ follows second-order kinetics

is supported by the linear relation between the kinetic parameter ω and the time constant τ.

3. Results and discussion

In mixed medium (DMF + citrate: 60/40, 0.3 M KCl) the electroreduction of PA-824 at different pHs was studied. From the cyclic voltammograms at pHs 3, 6, 9 and 12 (Fig. 2) it was possible to observe differences in the PA-824 electroreduction according to the pH solution, indicating a change in the overall redox mechanism. At pH lower than 7, there are two main cathodic signals, the first one (signal I) appearing around −500 and −800 mV, pH 3 and 6, respectively, and a second signal (II) beyond −1500 mV.

According to a previous electrochemical study performed with PA-824 [12] it was well established that at acidic pH and potentials lower than −1000 mV, the electrochemical reduction of PA-824 involves a 4-electron and 4-proton transfer to the nitro group to form the corresponding hydroxylamine derivative:

$$\text{R-NO}_2 + 4\text{e}^- + 4\text{H}^+ \rightarrow \text{R-NHOH} + \text{H}_2\text{O} \quad \text{(peak I)} \qquad (1)$$

On the other hand the second signal (II) can be ascribed to the reduction of the diazole moiety, as was previously described for other nitro imidazolic compounds [18]. Up to pH 7 the intensity of peak I decays, concomitant to the appearance of other peaks, one negative peak (III) appearing at a potential approximately 500 mV more negative than peak I and another anodic signal (IV) around −500 mV. Doing shortened sweeps, involving only peak I, at the same pH (Fig. 3),

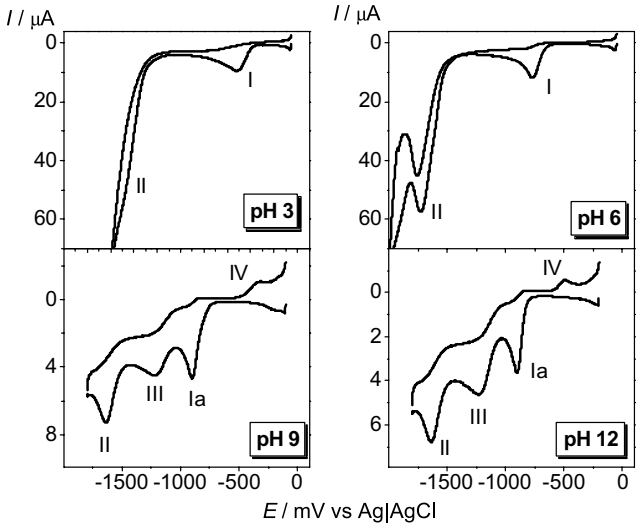

Fig. 2. Cyclic voltammograms of 1 mM PA-824 solution at different pHs in mixed media (60/40: DMF + citrate buffer, 0.3 M KCl). Sweep rate 1 V/s.

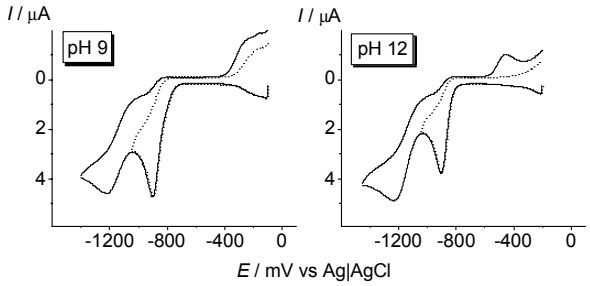

Fig. 3. Cyclic voltammograms of 1 mM PA-824 solution in mixed media (60/40: DMF + citrate buffer, 0.3 M KCl) pH 9 and 12 at different potential amplitudes. Sweep rate 1 V/s.

the anodic signal did not appear, indicating that peak IV is totally dependent on the product formed in the negative sweep corresponding to peak III. According to previous information about the electroreduction of other related nitroheterocyclic compounds [19,20], the two cathodic peaks observed in alkaline pH can be ascribed to the splitting of the main peak I, according to:

$$\text{R-NO}_2 + \text{e}^- \rightleftarrows \text{R-NO}_2^{\cdot -} \quad \text{(peak Ia)} \qquad (2)$$

$$\text{R-NO}_2^{\cdot -} + 3\text{e}^- + 4\text{H}^+ \rightarrow \text{R-NHOH} + \text{H}_2\text{O} \quad \text{(peak III)} \qquad (3)$$

The anodic peak IV corresponds to the electrooxidation of the hydroxylamine derivative formed in the negative sweep, according to:

$$\text{R-NHOH} \rightleftarrows \text{R-NO} + 2\text{e}^- + 2\text{H}^+ \quad \text{(peak IV)} \qquad (4)$$

In spite of the nitro radical anion formation, peak Ia could be detected in alkaline medium; apparently the half-life of the radical species was not high enough to obtain a reversible electrochemical couple, as was obtained for a series of other nitrofurans [19,20], nitroimidazoles [21,22] and nitrobenzene [23,24] derivatives under the same electrochemical conditions. Consequently, a comparison of the half-life of the radical species between PA-824 and metronidazole in this aqueous alkaline medium was not possible.

In order to obtain suitable conditions wherein the nitro radical anion from PA-824 would be more stable, a totally aprotic medium formed by 100% DMSO with TBAF as supporting electrolyte was used. Under these conditions a very well resolved reversible cyclic voltammogram without any interfering signals was obtained (Fig. 4). From the study of the dependence of this couple on the sweep rate (0.1 and 10 V/s) a linear increase of the peak current with the square root of the sweep rate was observed. Moreover the difference between the cathodic and anodic peak potentials, ΔE_p, has a mean value of 64.7 ± 4.5 mV and the $\log I_p$ versus log (sweep rate) plot shows a slope of 0.475. All of the above results indicate that the electron transfer follows a reversible diffusion controlled one-electron

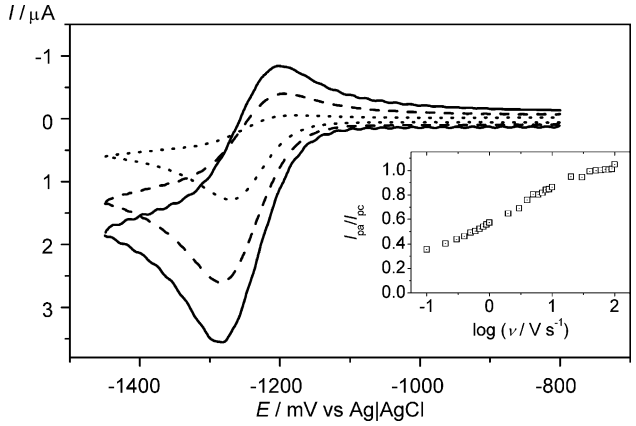

Fig. 4. Cyclic voltammograms of the isolated couple of 1 mM PA-824 in aprotic media (100% DMSO + 0.1 M TBAF) at different sweep rates. Inset: dependence of the current ratio on sweep rate for the same experiment.

process corresponding to the $ArNO_2/ArNO_2^{\cdot-}$ couple of PA-824.

On the other hand, the current ratio, I_{pa}/I_{pc}, increases with the increase of sweep rate up to 1 (Inset, Fig. 4), showing the typical variation of an ECi mechanism [25], i.e., with values lower than 1 at low sweep rates and values ~1 at higher sweep rates. Furthermore, the effect of PA-824 concentration on the current ratio was evaluated, an inverse correlation being found between them, with lower I_{pa}/I_{pc} values at higher PA-824 concentration values (data not shown). This fact implies a second-order chemical reaction for the ECi mechanism.

According to current thinking, an electrochemically formed nitro radical anion can undergo two different decay paths, disproportionation or dimerization. Consequently, in the sixties, Olmstead et al. [16,17] developed theoretical approaches to evaluate the corresponding decay constants, $k_{2,disp}$ or $k_{2,dim}$. Applying the theoretical curves to our experimental data, we found that a straight line is obtained in both cases (Fig. 5); consequently, we were unable to decide whether disproportionation or dimerization is the chemical step with this approach. Moreover from the slope, values of 8.40×10^4 and 2.58×10^4 M^{-1} s^{-1} were obtained for $k_{2,disp}$ and $k_{2,dim}$, respectively.

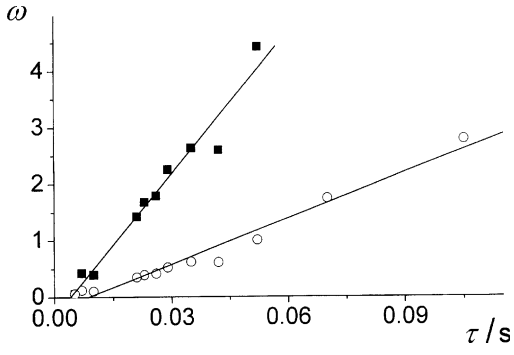

Fig. 5. Plot of the kinetic parameter, ω, with the time constant, τ, for PA-824 according the theoretical approach of Olmstead et al. [16,17] for disproportionation (■) and dimerization (○) mechanisms.

We have used digital simulation software to decide between and dimerization as the chemical decay of the nitro radical anion. The overall mechanism used for simulation was as follows:

$$A + e^- \rightleftarrows B \quad (E^0, \alpha, k^0)$$

and then, a disproportionation decay reaction:

$$2B \rightarrow A + C \quad (k_{2,disp})$$

or a dimerization decay reaction:

$$2B \rightarrow D \quad (k_{2,dim})$$

wherein A and B are the nitro and nitro radical anion derivatives, respectively.

For the simulation procedure we have selected the following parameters:

Electrochemical step:	$E^0/V = -1.27$, $\alpha = 0.5$, $k_s = 1 \times 10^4$ cm s^{-1}
Dimerization step:	$k_{2,dim} = 2.58 \times 10^4$ M^{-1} s^{-1}
Disproportionation step:	$k_{2,disp} = 8.4 \times 10^4$ M^{-1} s^{-1}

In Fig. 6, the experimental (solid lines) and simulated (dotted lines) cyclic voltammograms for both mechanisms at different sweep rates are shown. From this figure it is clear that the dimerization mechanism fits much better than the disproportionation mechanism. Therefore we conclude that dimerization is the more

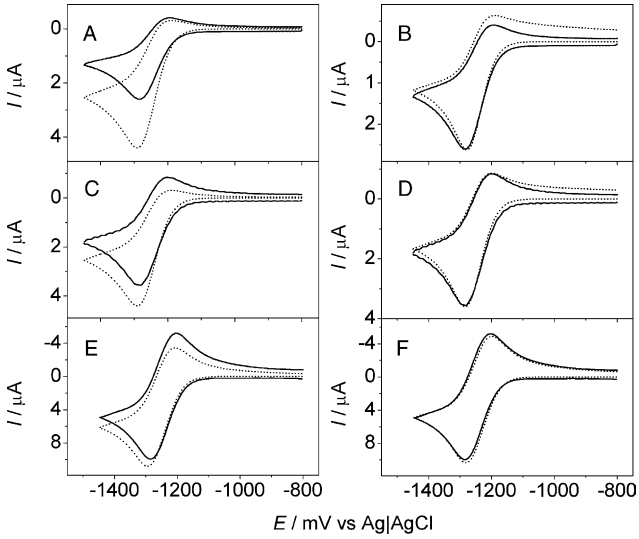

Fig. 6. Comparison of experimental (solid line) and simulated (dotted line) cyclic voltammograms of 1 mM PA 824 solution in aprotic medium considering a disproportionation chemical reaction (A, C and E) and dimerization chemical reaction (B, D and F). Sweep rates: (A) and (B) 0.5 V/s; (C) and (D) 1 V/s and (E) and (F) 5 V/s.

Table 1
Peak potentials, dimerization decay constants values ($k_{2,dim}$) and half-lives ($t_{1/2}$) at 1 mM concentration for the corresponding Ar–NO_2/Ar–$NO_2^{\cdot-}$ couples obtained from PA-824 and metronidazole

	PA-824		Metronidazol	
	HMDE	GCE	HMDE	GCE
10^{-2} $k_{2,dim}$/M^{-1} s^{-1}	258 ± 61	63.1 ± 5.6	2.22 ± 0.38	1.52 ± 0.24
$t_{1/2}$/s	0.04	0.16	4.50	6.58
E_p/mV	−1284	−1275	−1090	−1082

Values were obtained in aprotic media on mercury (HMDE) and glassy carbon (GCE) electrodes.

likely chemical path to explain the deactivation of the nitro radical anion from PA-824 in DMSO.

A glassy carbon electrode was also used to study the electroreduction of PA-824. In this case, the same EC_{2i} electrodic mechanism with a dimerization reaction as the chemical step was obtained (data not shown). In Table 1 the dimerization second-order decay constants and the half-life time are summarized showing that the nitro radical anion is more stable when the electrode material is glassy carbon, i.e., the dimerization reaction is favored at a mercury electrode.

On the other hand, as the literature reports that PA-824 exerts its antitubercular mechanism of action in a similar way to metronidazole [3], a good challenge would be a comparison between the capabilities of nitro radical anion formation and the radical stabilization from both type of compounds. Consequently, we have applied the same electrochemical procedure to characterize the nitro radical anion from metronidazole and compared it with PA-824.

The cyclic voltammograms and the corresponding I_{pa}/I_{pc} behavior with the sweep rate for the nitro reduction of metronidazol in DMSO are shown in Fig. 7.

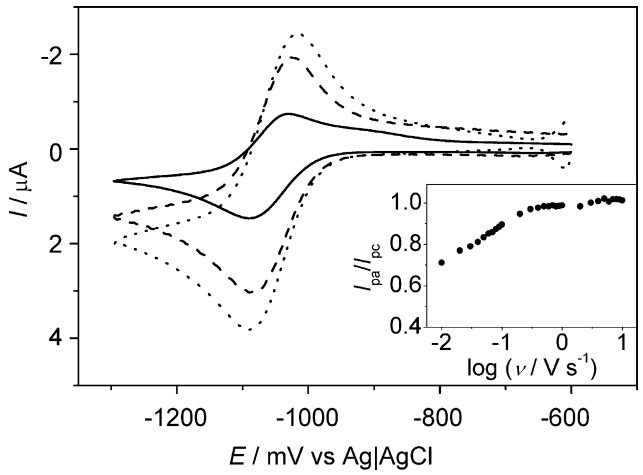

Fig. 7. Cyclic voltammograms of the isolated couple of 1 mM Metronidazole in aprotic media (100% DMSO + 0.1 M TBAH) at different sweep rates 0.5 V/s (solid line) 0.5 V/s (dashed line) and 1 V/s (short dashed line). Inset: dependence of the current ratio on sweep rate for the same experiment.

From this figure it is clear that the electrode mechanism involved ECi behavior, since the I_{pa}/I_{pc} values increased to unity at higher sweep rates. In a similar way as in the case of PA-824, the nitro radical anion from metronidazole obeyed second-order kinetics for the chemical step and it also fitted better to a dimerization reaction. According to the theory of Olmstead et al. [17] we have obtained $k_{2,dim}$ values of $2.22 \pm 0.38 \times 10^2$ and $1.52 \pm 0.24 \times 10^2$ M^{-1} s^{-1} for metronidazole in DMSO at mercury and a GCE, respectively. Wardman [26] and Henry et al. [27], using pulse radiolysis measurements, have previously reported k_2 values of 4.2×10^4 (pH 7.4) and 7.9×10^4 M^{-1} s^{-1} (pH 7) for metronidazole in aqueous media. Tocher and Edwards [28], using electrochemical measurements, have reported a k_2 value of 8.4×10^4 M^{-1} s^{-1} which was obtained as an extrapolation in 100 % aqueous media. More recently, La Scalea et al. [9] reported a k_2 value of 6.6×10^3 M^{-1} s^{-1} in a mixed media containing 50% DMF in citrate buffer (pH 7.4). It is clear from these results that the rate constants decreased as the aprotic solvent percentage increased. This fact explains the lower k_2 value obtained for metronidazole under the totally aprotic conditions of our experiments.

For an adequate comparison between PA-824 and metronidazole we have used the same aprotic media. In Table 1 the $k_{2,dim}$ values obtained with mercury and glassy carbon electrodes for both the PA-824 and metronidazole radical decay constants are summarized. The results in Table 1 reveal two main aspects. Firstly, based on the nitro reduction peak potentials, we can affirm that the formation of PA-824 nitro radical anion is energetically less favored than that of metronidazole. Secondly, based on the $k_{2,dim}$ values obtained, we can affirm that metronidazole generates more stable radical species than PA-824. This affirmation is clearly reflected in the $t_{1/2}$ values obtained that are of the order of seconds in the case of metronidazole and of the order of tenths or hundredths of seconds in the case of PA-824. In spite of the fact that PA-824 produces a nitro radical anion with major energetic requirements and that the radicals produced are less stable those from metronidazole; our results clearly show that PA-824 is capable of forming stable free radicals in a similar way as metronidazole.

4. Concluding remarks

Our cyclic voltammetric results show that PA-824, a new antituberculosis agent, is electrochemically reduced via formation of a relatively stable nitro radical anion in a similar way as that of the electrochemical reduction of the well-known bactericidal drug metronidazole. However PA-824 radical requires more energy for formation (about 200 mV) and it is approximately 100 times less stable than metronidazole radical anion.

In view of the fact that metronidazole is a well-known drug for which the mechanism of action involves free radical interactions, a possible similar mechanism involving nitro radical anion interactions should be considered for PA-824.

Acknowledgements

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