

Release of nitrite from the antitubercular nitroimidazole drug PA-824 and analogues upon one-electron reduction in protic, non-aqueous solvent†

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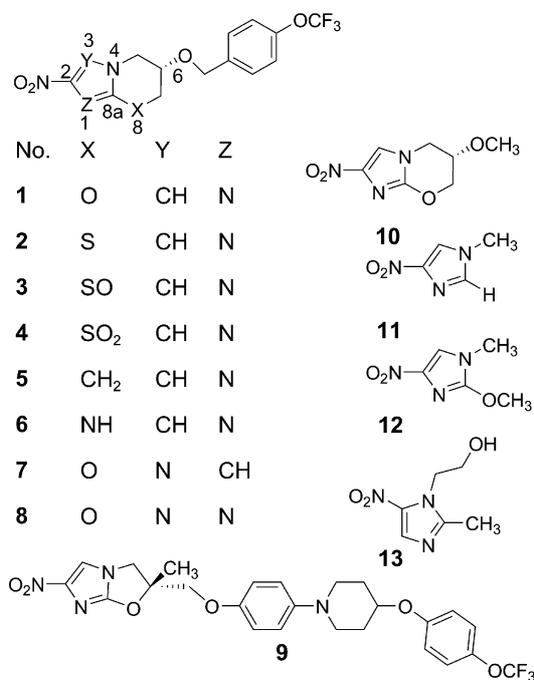
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The one-electron reduction chemistry of the antituberculosis drug PA-824, together with a series of closely related compounds, has been investigated in irradiated anaerobic propan-2-ol solution. The protic solvent, of low dielectric constant, was chosen to mimic the environment of a water-restricting active site of a model protein, which is capable of reducing the compounds. Radiolytic reduction of the compounds containing electron donating substituents in the 2-position of the imidazole ring released nitrite, with compounds that are highly active against *Mycobacterium tuberculosis* exhibiting high yields of nitrite. The release of cytotoxic reactive nitrogen species through a one-electron pathway, by as yet unidentified proteins, may play a role in the activity of this class of compounds against TB. The described radiolytic quantification of nitrite release may have utility as a preliminary screening test for nitroaromatic candidate drugs against the disease.

Introduction

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), kills approximately two million people a year and, in its latent form, infects one third of humanity.¹ The latent form is thought to sustain the pandemic as non replicating Mtb are much more resistant to chemotherapeutics.² Host cells can impose immunity through the bacteriostasis effects of endogenous reactive nitrogen species (RNS),³ reactive oxygen species (ROS) and nutrient deprivation, all of which can drive Mtb into its non replicating state, a state also reached in hypoxia. As most front-line drugs are used to kill replicating Mtb, the non replicating state is resistant. This has necessitated the use of multidrug combinations over extended treatment times and non-compliance in such treatment regimes has given rise to the emergence of drug-resistant tuberculosis.⁴ Hence there is an urgent need to develop new drugs to fight the disease.

The bicyclic nitroimidazoles **1** (PA-824) and **9** (OPC-67683), Scheme 1, are new candidate drugs for treating TB,^{5,6} and are undergoing clinical trials. The drugs are active against both replicating and non-replicating forms of the disease and appear to act in an atypical manner compared to related nitroimidazoles. The 5-nitroimidazole metronidazole (**13**), for example, has modest activity against the disease only in hypoxia⁷ and this activity is thought to arise from reduction of the nitro group to bioactive



Scheme 1

intermediates. Nitroimidazoles and other nitroarenes, generally undergo sequential reduction to initially form the nitro radical anion, which under oxic conditions undergoes redox cycling to form ROS. Further reduction of the radical anion in hypoxia, or direct two-electron (hydride ion) reduction, leads to the formation of nitroso and on to hydroxylamine cytotoxins.

The major metabolites of **9** (by *Mycobacterium bovis*) and **1** (by Mtb), determined under aerobic and hypoxic conditions respectively, are their des-nitro compounds.^{5,8} Analysis of intermediates produced, by both chemical and radiolytic methods, indicate that reduction of the imidazole ring occurs in preference

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to reduction of the nitro group.^{8,9} The F₄₂₀ deazaflavin-dependent protein Rv3547 has been shown to metabolise **1** in hypoxia¹⁰ to form products, which include the des-nitro compound and the release of RNS.⁸ It is proposed that a hydride transfer from the reduced cofactor to the C3-position of the imidazole ring takes place as the initial step.⁸ These authors propose Nef-type chemistry for the release of nitric oxide (NO), a reaction pathway requiring highly acidic conditions which is unknown for nitroheterocycles in solution.¹¹ The protonation of intermediates formed in the active sites of proteins often occurs by proton bridging from neighbouring amino acids and hence a 'biological equivalent' of Nef-type chemistry can be invoked. In this regard, the active site of such proteins can be described as a protic environment, even in the absence of solvent water or with there being only a few molecules of water present in the active site of the protein. The purified F₄₂₀ deazaflavin-dependent nitroreductase metabolises **1** *in vitro* to yield RNS, as detected by the Griess reagent, and these are suggested to be monomeric hyponitrous acid (HNO) and nitrous acid (HNO₂).⁸ Nitrous acid is unstable in solution and readily breaks down to form NO, and the nitrite anion is metabolised in cells by a number of processes, also leading to the formation of NO.¹²

In this study we examine a series of nitroimidazoles related to PA-824 for possible release of RNS in a protic solvent (95% propan-2-ol, 5% water) as a model for a water-restricted active site of proteins which carry out one-electron reduction of substrates, by using the α -hydroxyprop-2-yl radical ((CH₃)₂C•OH) as the reductant. This solvent has previously been used to examine the conversion from outer sphere electron transfer in water to inner sphere electron transfer on changing the solvent, in the one-electron reduction of 4-nitrobenzonitrile.¹³ Our findings are compared with the cytotoxicity of the compounds against Mtb under both hypoxic and aerobic conditions to give some insight into an alternative/additional one-electron reduction pathway of how these drugs might be reduced to yield RNS.

Results and discussion

Pulse radiolysis studies

Time-resolved spectral changes following the pulse radiolysis of **10** in deaerated 95% propan-2-ol–5% water solutions containing **10** (0.5 mM), are presented in Fig. 1. The initial, low-intensity, absorption spectrum of the α -hydroxyprop-2-yl radical ($\lambda < 360$ nm) gave rise to the spectrum built-up over 100 μ s, which subsequently decayed to the absorption spectrum measured 8 ms after the pulse. Both these spectra, measured at 100 μ s (corrected for its partial decay during formation) and at 8 ms after the pulse, exhibit bleaching of the parent compound, **10**, in the 330–370 nm region. The rate of build-up in absorption of the band at 450 nm increased with the concentration of **10**, Fig. 2A, giving a calculated rate constant of $9.1 \pm 0.3 \times 10^7$ M⁻¹ s⁻¹. The solvated electron, produced on the ionization of the solvent and observed at 600 nm, decayed quickly on reaction with **10** (0.5 mM to 2.0 mM) on the microsecond timescale, with a rate constant of 5×10^9 M⁻¹ s⁻¹ (data not shown) but did not give rise to an intermediate which absorbs in the region $\lambda > 270$ nm. The transient spectrum (Fig. 1) measured at 100 μ s after the pulse has some similarities to that previously measured in water, such as bleaching of the ground-

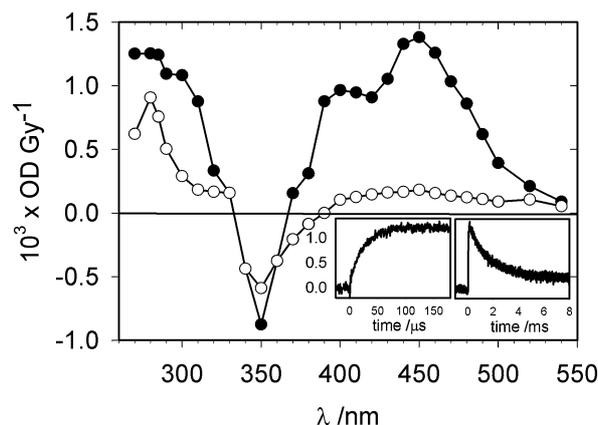


Fig. 1 Time resolved spectra observed following pulse radiolysis (ca. 5 Gy in 200 ns) of **10** (0.5 mM) in deaerated propan-2-ol–water (95 : 5% volume) measured 100 μ s (●), 8 ms (○) after the electron pulse. Insets: time dependent change of transient absorption at 450 nm.

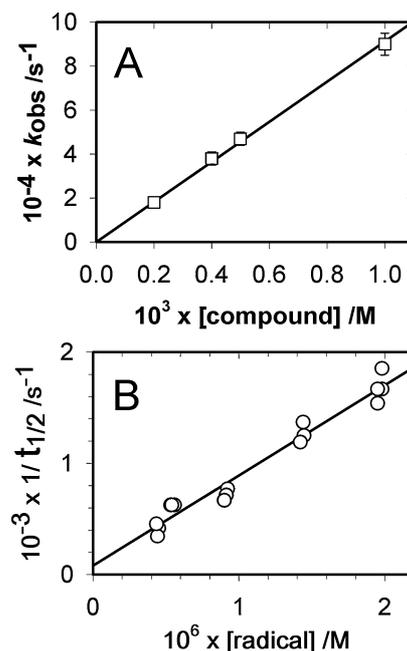
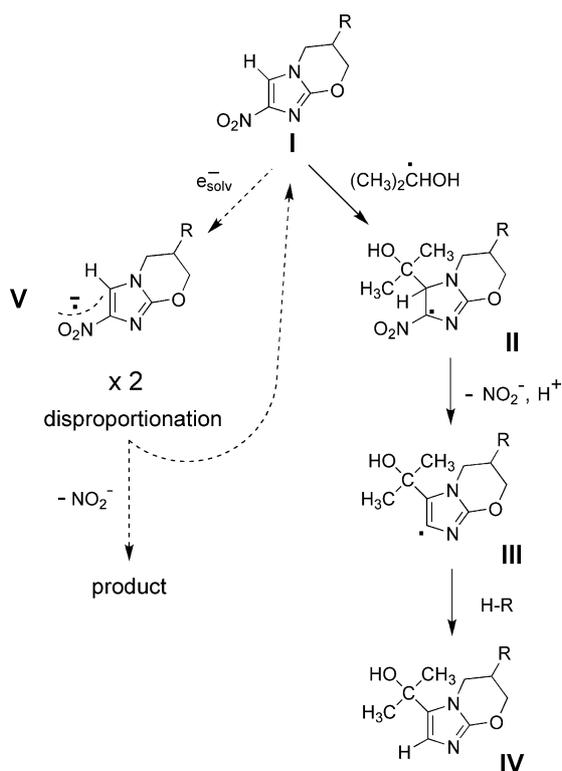


Fig. 2 Kinetic data obtained on the pulse radiolysis of **10** in deaerated propan-2-ol–water (95 : 5% vol): (A) Dependence of the rate of formation of the intermediate absorbing at 450 nm on the concentration of **10**; (B) Dependence of the reciprocal of the first half-life of the decay of radicals absorbing at 450 nm on the initial radical concentration formed with increasing radiation dose.

state absorbance in the 320–380 nm, however, the absorption band in the 400–500 nm region, relative to the bleaching region, is much more intense.

The transient species measured at 100 μ s was observed to decay by a mixed-order kinetic process and was analysed (at 450 nm) as previously described¹⁴ to ascertain both the first-order rate constant of 55 ± 70 s⁻¹ and second-order rate constants of $5.6 \pm 0.2 \times 10^8$ M⁻¹ s⁻¹; Fig. 2B. The first-order rate constant is possibly that for the elimination of nitrite (as nitrous acid) from **II**, Scheme 2, in competition (under pulse radiolysis conditions) with a second-order decay process. The elimination of nitrous acid in this way would lead to the transient formation of aryl radical,



III, which could abstract H-atom from the solvent to give the observed product, IV (see below).

Steady-state radiolysis, production of nitrite

All compounds were subjected to steady-state radiolysis in deaerated propan-2-ol solution and the irradiated solutions analysed for nitrite using the Griess reagent.

Dose response plots were constructed for increasing radiation doses, Fig. 3, from which the yield of nitrite produced, (G -value in $\mu\text{mol J}^{-1}$), was determined for each compound. A wide range in G -values, from 0 up to $0.15 \mu\text{mol J}^{-1}$, were determined for analogues of PA-824 with $E(1) \leq -500 \text{ mV}$. This is most likely, in electronic terms, related to the structural necessity of possessing an electron-donating substituent in the 2-position, as the model

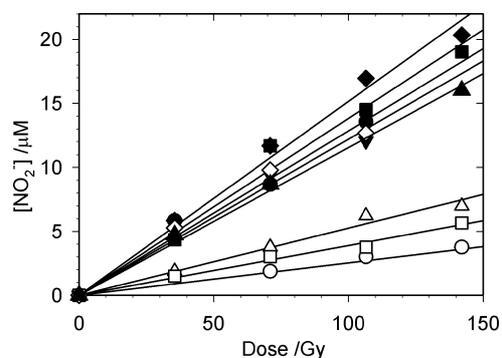


Fig. 3 Representative plots of the yield of nitrite upon irradiation of compounds ($50 \mu\text{M}$) in deaerated solutions containing (i) propan-2-ol; 1, \blacktriangledown ; 3, \triangle ; 6, \square ; 7, \blacklozenge ; 10, \diamond ; 11, \bullet ; 13, \blacksquare ; 14, \circ and (ii) propan-2-ol-water (95 : 5% volume), 1, \blacktriangle .

compound **12** (2-OCH₃) also releases a high concentration of nitrite upon reduction. The maximum G -value corresponds to the *ca.* 75% G -value of total reducing equivalent in irradiated propan-2-ol. The yield of nitrite was found to be unchanged when irradiations were carried out in propan-2-ol-water mixtures (95 : 5% vol).

Steady-state radiolysis, UV-vis spectra and mass analysis of product

The reduction of PA-824 (**1**) upon its radiolysis in deaerated propan-2-ol was monitored using UV-vis spectrophotometry in a closed system containing an integral spectrophotometer cell. Isosbestic points were maintained up until the last four spectra, Fig. 4, enabling the G -value for the loss of **1** to be determined. This was done by converting the absorption at 319 nm into concentration and using the initial slope of a plot of the loss of concentration against radiation dose (inset of Fig. 4). The obtained G -value of $0.15 \mu\text{mol J}^{-1}$ corresponds to *ca.* 75% of the estimated concentration of reducing equivalents produced in irradiated propan-2-ol (Experimental section). The more soluble methyl ether analogue of PA-824, **10**, was used to obtain sufficient product for chemical analysis. This compound was similarly irradiated in deaerated propan-2-ol (5.2 mg in 25 mL) and the decrease in its absorption band centred at 340 nm was followed with increasing radiation dose.

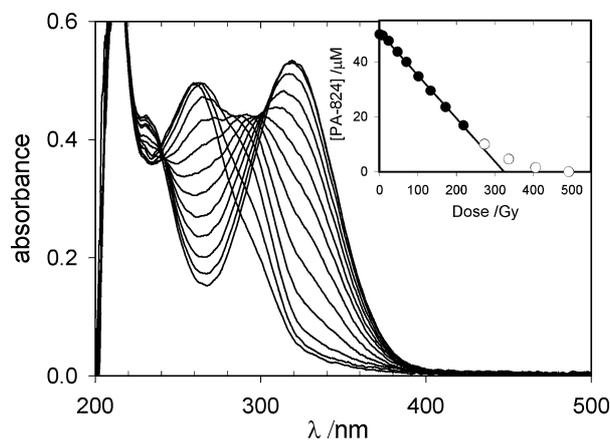


Fig. 4 Changes in UV-vis absorption spectra, observed during the γ -irradiation (absorbed doses 0–500 Gy) of N_2 -saturated propan-2-ol solutions of **1** ($50 \mu\text{M}$). Inset : Plot of absorbance of **1** monitored at λ_{max} 319 nm as a function of absorbed dose.

The radiation was stopped at the point where this absorption band was completely lost (*ca.* 7.3 kGy) and the irradiated sample was then subjected to analysis. Thin layer chromatography indicated clean formation of a single product, as detected under UV light, which was more polar than the starting **10**. This product was purified by column chromatography on silica, to give the desnitro compound **IV** ($\text{R} = \text{OCH}_3$) as a yellow oil, which accounted for approximately three-quarters of the mass of the starting material. The rest of the material was presumably either broken down into non-aromatic compound(s) by the high dose of radiation, or could not be isolated by the chromatographic procedure. High resolution mass spectrometry of the isolated product gave a molecular weight of 212.116, consistent with a

Table 1 Yield of nitrite formed upon radiolytic reduction of compounds in deaerated propan-2-ol; formation of radical adduct; and comparison with the Minimum Inhibitory Concentration/ μM , determined under aerobic (MABA, MIC) or anaerobic (LORA, MAC) conditions

Compound	$E(1)$ pH 7/ mV	$G(\text{NO}_2^-)/\mu\text{mol J}^{-1}$	+13 ion m/z^c g mol^{-1}	MABA ^{a,b} aerobic	LORA ^{a,b} anaerobic	MIC ^a aerobic	MAC ^a anaerobic
1, PA-824	-534 ± 7^e	0.12 ± 0.03	Yes	1.1	4.4	0.80^d	12^d
2	-534 ± 6^b	0.05 ± 0.01	Yes	1.1	15	1.2^d	25^d
3	-476 ± 6^b	0	No	> 128	92	> 100^i	50–100 ⁱ
4	-488 ± 6^b	0	No	> 128	89	> 100^i	25^i
5	$-570 \pm 6^{b,g}$	0.04 ± 0.01	Yes	126	> 128	25^i	250^i
6	$-568 \pm 6^{b,h}$	0.15 ± 0.02	Yes	> 128	> 128	0.80^d	130^d
7	-500 ± 6	0	No	> 128	> 128		
8	-338 ± 6	0	No	> 128	120		
9, OPC-67683	-526 ± 6^f	0.14 ± 0.03	Yes	0.03^f	2.54^f		
10	-527 ± 6^e	0.13 ± 0.02	Yes	105^f	> 128^f		
11	-557 ± 8^f	0	No	—	—		
12	-561 ± 7^f	0.14 ± 0.02	Yes	121^f	> 128^f		
13, metronidazole	-516 ± 7^f	0.025 ± 0.005	Yes	$\gg 64^f$	$\sim 64^f$	> 300^j	62.5^j

^a MIC, minimum inhibitory concentrations towards *Mycobacterium tuberculosis*, MABA, microplate-based assay; LORA, low-oxygen-recovery assay; MIC, minimum aerobic inhibitory concentration; MAC, minimum anaerobic concentration. ^b Data from reference 18; MIC for the lowest concentration effecting an inhibition of >90%. ^c Mass spectrometry evidence for the formation of des-nitro-propan-2-ol adduct in anoxia. ^d Data from reference 8; MIC for the concentration that will inhibit 99% of growth of inoculums. ^e Data from reference 9. ^f This work. ^g Data for 6-OH analogue (without side-chain). ^h Data for 6-OCH₃ analogue (without side-chain). ⁱ Data from reference 30. ^j Data from reference 16.

formula of C₁₀H₁₆N₂O₃. This corresponds to loss of the nitro group from **10**, and the addition of one propan-2-ol molecule. The incorporation of solvent was confirmed by the ¹³C NMR spectrum, which contained resonances for 10 carbon atoms, three more than the starting material. In the ¹H and ¹³C NMR spectra, the chemical shifts of the oxazine ring proton and carbon atoms were little shifted from those of the starting material, suggesting that this ring remained unaltered. In the ¹H NMR spectrum of **IV** (R = OCH₃), a one-proton singlet at δ 6.25 ppm indicated that a single hydrogen atom remained on the imidazole ring, with its upfield value compared with that in the starting material being consistent with loss of the nitro group. Two new, three-proton singlets at δ 1.44 and 1.41 ppm result from the methyl groups of the incorporated propan-2-ol. A new D₂O-exchangeable one-proton singlet at δ 4.95 ppm results from the tertiary alcohol group, indicating that the propan-2-ol has been linked *via* a carbon atom, rather than through oxygen. Complete chemical shift assignments for the molecule were made following COSY, HMBC and HSQC NMR experiments, and are consistent with the proposed structure. Assignment of the site of attachment of the propan-2-ol as being at the 5-position of the imidazole ring was made following NOE experiments performed in a ROESY experiment. The resonances assigned to the methyl groups and alcohol of the propan-2-ol side chain showed correlations to those of the oxazine methylene group attached to the imidazole ring, indicating that they are close together in space. In contrast, the singlet at δ 6.25 ppm resulting from the remaining imidazole ring proton showed a correlation only to the methyl groups of the adjacent propan-2-ol side chain.

Similar irradiation of PA-824 (**1**) gave an analogous product **IV** (R = OCH₂C₆H₄-4-OCF₃) in good yield, which was fully characterised by proton and carbon NMR analyses. Irradiation of OPC-67683 (**9**) under these conditions was slightly less clean, but appeared to give an analogous compound as the major product, as evidenced by the appearance of a characteristic singlet at δ 6.23 ppm in the ¹H NMR spectrum of the crude product, resulting from the imidazole ring proton. In addition, all irradiated samples were analysed by low resolution mass spectrometry for loss of

each parent compound and formation of their major radiolytic products. Loss of the nitrite from each compound and formation of their desnitro-propan-2-ol adduct was scored as a change in mass of +13 g mol⁻¹, Table 1.

Conclusions

Nitrite is released as an RNS following one-electron reduction of the nitroimidazole compounds examined in this study in a protic solvent of low dielectric constant. Such conditions may well mimic the environment of the active site of some enzymes, which carry out one-electron reduction of substrates, when access of the solvent is restricted by, for example, conformational changes in the protein structure upon substrate binding. Compound **10**, PA-824 (**1**) and OPC-67683 (**9**) yield *ca.* 75% of stoichiometric amounts of nitrite following their one-electron reduction in anaerobic propan-2-ol. The G -values for the loss of compounds **1** and **9** equal the nitrite yield of *ca.* 0.15 $\mu\text{mol J}^{-1}$ (Table 1). As we directly observe the reaction of the α -hydroxyprop-2-yl radical with the compounds, we can assume that the release of nitrite is associated with this reaction and that the scavenging of the solvated electron, to form the radical anion, does not release nitrite quantitatively. It is possible that the radical anion reforms the parent compounds, at *ca.* 50% yield, through a disproportionation reaction, with this reaction producing a product and nitrite, Scheme 2. This would lead to the loss of parent compound equalling the yield of nitrite release at *ca.* 75% of the total yield of reducing radicals produced upon irradiation of the solvent (0.15 $\mu\text{mol J}^{-1}$ compared to 0.20 $\mu\text{mol J}^{-1}$). The production of nitrite is accompanied, under steady-state radiolysis conditions, by the formation of an adduct between their des-nitro forms and propan-2-ol. This is consistent with the α -hydroxyprop-2-yl radical adding to the 3-position of the imidazole ring, followed by the elimination of nitrous acid to form an aryl-type radical, **III**, which abstracts a H-atom from the solvent to form the product **IV**, Scheme 2. This mechanism of reduction is substantially different from that for nitrobenzenoid compounds in the same solvent, as exemplified by 4-nitrobenzonitrile. In this

case the α -hydroxyprop-2-yl radical adds to the nitro group and quickly eliminates forming acetone and the nitro radical anion,¹³ which we have determined does not lead to formation of nitrite.

High yields of nitrite are seen for the analogue of **1** without the lipophilic sidechain, compound **10**, the 2-methoxy-4-nitroimidazole model compound **12**, and compound **6**, which has the electron donating NH moiety in the 2-position of the 4-nitroimidazole ring. Other analogues of **1**, which also possess electron donating moieties in the 2-position, **2** and **5**, also produced nitrite upon reduction, but at much lower concentrations.

Activity against Mtb does qualitatively correlate with the production of nitrite, for compounds possessing the protein-targeted lipophilic side-chain, Table 1. (The reason for the disparity in the reported activities of **6** is unknown). However, the fact that some of the compounds are active under hypoxia, even when no nitrite is produced, means that other mechanisms operate as well in hypoxia. It has been reported that hydride-transfer from the F₄₂₀ cofactor leads to RNS formation⁸ and this two-electron pathway could be the major route to produce cytotoxic RNS under hypoxia. It is of interest that metronidazole, a 5-nitroimidazole, can release a low yield of nitrite upon one-electron reductions under the conditions of this present study. Conventionally, the anaerobic activity of metronidazole against protozoa and bacteria is thought to arise from the formation, and subsequent reactions, multi-electron reduced intermediates, such as its hydroxylamine derivative.¹⁵ Recently a 5-nitroimidazole with aerobic activity has been reported. This same study also found that some of PA-824-related compounds tested retained anaerobic sensitivity in mutants lacking the F₄₂₀-dependent nitroreductase activity.¹⁶ This observation implies that other pathways are also operable in activating these compounds to cytotoxins which are active against Mtb. The procedures described in this paper may have utility as a preliminary screening test for potential nitroaromatic candidate drugs, which can be activated by a one-electron pathway, in the fight against TB.

Experimental

Synthesis

All reagents used were of analytical grade. Sodium formate, sodium hydroxide, perchloric acid and phosphate buffers were obtained from Merck and potassium thiocyanate from Riedel-de Haen. All other reagents were obtained from Aldrich Chemical Company. All solutions were prepared in water purified by the Millipore "Milli-Q" system. Solution pH values were adjusted using the phosphate salts (5 mM) and either NaOH or HClO₄ when necessary. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal 2300 Melting Point apparatus. NMR spectra were obtained on a Bruker Avance 400 spectrometer. Spectra are referenced to Me₄Si. Chemical shifts and coupling constants were recorded in units of ppm and Hz, respectively. High resolution mass spectra were determined on a VG-70SE mass spectrometer using an ionizing potential of 70 eV at a nominal resolution of 1000. Atmospheric pressure chemical ionisation mass spectra (APCI-MS) were determined for methanol elutions on a ThermoFinnigan Surveyor MSQ spectrometer. Solutions in organic solvents were dried with anhydrous Na₂SO₄.

Column chromatography was carried out on silica gel, (Merck 230–400 mesh). PA-824 (**1**),¹⁷ compounds **2–8**,¹⁸ OPC-67683 (**9**)¹⁹ compounds **10** and **12**,⁹ 1-methyl-4-nitro-1*H*-imidazole (**12**)²⁰ were synthesised as described. Metronidazole (**13**) was purchased from Sigma.

2-(6-Methoxy-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-3-yl)-2-propanol (**IV**)

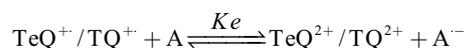
A deaerated solution of the oxazine (**10**) (5.2 mg, 0.025 mmol) in propan-2-ol (25 mL) was irradiated to an absorbed dose of 7.2 kGy. The solution was evaporated to dryness *in vacuo* and the residue was chromatographed on silica. Ethyl acetate eluted fore fractions, then ethyl acetate–methanol (9:1) eluted the product (**IV**, R = OCH₃) as a yellow oil (3.9 mg, 73%). ¹H NMR (DMSO-d₆) δ 6.25 (s, 1H, H-2), 4.95 (s, 1H, OH), 4.48 (dt, J = 13.3, 2.2 Hz, 1H, H-5), 4.40 (dt, J = 11.8, 2.7 Hz, 1H, H-7), 4.21 (br d, J = 11.8 Hz, 1H, H-7), 4.08 (dd, J = 13.3, 3.5 Hz, 1H, H-5), 3.87 (m, 1H, H-6), 3.33 (s, 3H, OCH₃), 1.44, 1.41 (2 s, 6H, CH₃). ¹³C NMR δ 148.6 (C-8a), 132.7 (C-3), 117.4 (C-2), 68.5 (C-6), 66.3 (C-OH), 65.9 (C-7), 55.5 (OCH₃), 45.1 (C-5), 29.7 (CH₃), 29.4 (CH₃). HREIMS found: M = 212.1160. C₁₀H₁₆N₂O₃ requires 212.1161.

Methods

Pulse radiolysis experiments were carried out at room temperature (22 ± 1 °C) using the University of Auckland's 4 MeV linear accelerator, to deliver typical absorbed doses of 2.5–5 Gy in 200 ns. The optical detection system and method of dosimetry have been described previously.²¹ Steady-state radiolysis experiments were performed using a ⁶⁰Co γ -source delivering a dose rate of 7 Gy (J kg⁻¹) min⁻¹. Samples for irradiation were evacuated and purged with O₂-free N₂ gas in glass tubes for three cycles. Nitrite was determined using the Griess reagent.²² Reagents, sulfanilic acid (0.1%), 0.1 mL, HCl (0.2 M), 2 mL, *N*-1-naphthalenediamine dihydrochloride (0.2%), 0.1 mL and water, (1 mL) were added to test samples (1 mL). Colour was allowed to develop for 15 min in the dark and then read at 545 nm (ϵ = 28340 M⁻¹ cm⁻¹) for comparison with standard solutions of sodium nitrite treated in the same way.

One-electron reduction potentials, $E(1)$

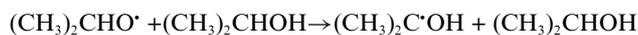
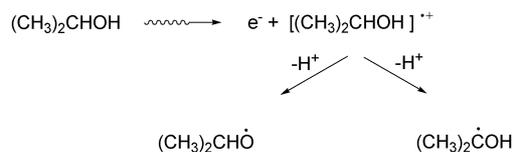
The one-electron reduction potentials of compounds **1**, **9**, **11–13**, $E(A/A^{\cdot-})$, vs. NHE, were determined at pH 7.0 in aqueous solution (5 mM phosphate buffer) by establishing redox equilibria between three mixtures of the one-electron reduced compounds and the reference compounds tetraquat ($E(\text{TeQ}^{2+}/\text{TeQ}^{\cdot+}) = -635 \pm 7$ mV)²³ or triquat ($E(\text{TQ}^{2+}/\text{TQ}^{\cdot+}) = -548 \pm 7$ mV).²⁴ The radiolytically-produced e⁻_{aq} species was used as the primary reductant, with the oxidizing primary radicals ($\cdot\text{OH}$ and H-atom) being scavenged by added 2-methylpropan-2-ol (0.1 M) to form an inert radical. The ΔE values were calculated from the equilibrium constants, K_e (established within 50 μs), using the Nernst equation.



The value for **13** (metronidazole) was found to be lower than that in the literature.²⁵ All these new data, together with data previous published for the analogues of PA-824, are presented in Table 1.

Radiolysis of propan-2-ol

High energy electrons and γ -rays ionize alcohols to form initially an electron and radical cation pair. A small proportion of these primary radicals escape recombination with the electron becoming solvated, *ca.* $0.10 \pm 0.02 \mu\text{mol J}^{-1}$,²⁶⁻²⁸ and the radical cation undergoing ion molecule reactions to form alkoxy and alkyl radicals.²⁹ The alkoxy radicals are oxidizing in nature and because of the relatively lower bond strength of the α -C–H bond of propan-2-ol, a proportion of the radical cation yield gives rise to the α -hydroxyprop-2-yl radical giving an overall yield (*G*-value) of one-electron reductants in irradiated propan-2-ol of *ca.* $0.2 \mu\text{mol J}^{-1}$.



One electron reduction of the nitroheterocyclic compounds dissolved in propan-2-ol then occurs by (i) direct reaction with the solvated electron and (ii) reaction with the α -hydroxyprop-2-yl radical.

Acknowledgements

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References

- World health organization (WHO), *Tuberculosis Fact Sheet* No. 104, Geneva, Switzerland, 2007.
- H. I. Boshoff and C. E. Barry, 3rd, *Nat. Rev. Microbiol.*, 2005, **3**, 70–80.
- J. Chan, K. Tanaka, D. Carroll, J. Flynn and B. R. Bloom, *Infect. Immun.*, 1995, **63**, 736–740.
- M. Laurenzi, A. Ginsberg and M. Spielgelman, *Infect. Disord.: Drug Targets*, 2007, **7**, 105–119.
- M. Matsumoto, H. Hashizume, T. Tomishige, M. Kawasaki, H. Tsubouchi, H. Sasaki, Y. Shimokawa and M. Komatsu, *PLoS Med.*, 2006, **3**, e466.
- C. K. Stover, P. Warren, D. R. VanDevanter, D. R. Sherman, T. M. Arain, M. H. Langhorne, S. W. Anderson, J. A. Towell, Y. Yuan, D. N. McMurray, B. N. Kreiswirth, C. E. Barry and W. R. Baker, *Nature*, 2000, **405**, 962–966.
- L. G. Wayne and H. A. Sramek, *Antimicrob. Agents Chemother.*, 1994, **37**, 2054–2058.
- R. Singh, U. Manjunatha, H. I. M. Boshoff, Y. H. Ha, P. Niyomrattanakit, R. Ledwidge, C. S. Dowd, I. Y. Lee, P. Kim, L. Zhang, S. Kang, T. H. Keller, J. Jiricek and C. E. Barry 3rd, *Science*, 2008, **322**, 1392–1395.
- R. F. Anderson, S. S. Shinde, A. Maroz, M. Boyd, B. D. Palmer and W. A. Denny, *Org. Biomol. Chem.*, 2008, **6**, 1973–1980.
- U. H. Manjunatha, H. Boshoff, C. S. Dowd, L. Zhang, T. J. Albert, J. E. Norton, L. Daniels, T. Dick, S. S. Pang and C. E. Barry, III, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 431–436.
- R. Ballini and M. Petrini, *Tetrahedron*, 2004, **60**, 1017–1047.
- J. O. Lundberg, E. Weitzberg and M. T. Gladwin, *Nat. Rev. Drug Discovery*, 2008, **7**, 156–167.
- V. Jagannadham and S. Steenken, *J. Am. Chem. Soc.*, 1984, **106**, 6542–6551.
- R. F. Anderson, S. S. Shinde, M. P. Hay, S. A. Gamage and W. A. Denny, *J. Am. Chem. Soc.*, 2003, **125**, 748–756.
- R. M. J. Ings, J. A. McFadzean and W. E. Ormerod, *Biochem. Pharmacol.*, 1974, **23**, 1421–1429.
- P. Kim, L. Zhang, U. H. Manjunatha, R. Singh, S. Patel, J. Jiricek, T. H. Keller, H. I. Boshoff, C. E. Barry and C. S. Dowd, *J. Med. Chem.*, 2009, **52**, 1317–1328.
- US Pat.*, 5,668,127.
- A. M. Thompson, A. Blaser, R. F. Anderson, S. S. Shinde, S. G. Franzblau, Z. Ma, W. A. Denny and B. D. Palmer, *J. Med. Chem.*, 2009, **52**, 637–645.
- H. Sasaki, Y. Haraguchi, M. Itotani, H. Kuroda, H. Hashizume, T. Tomishige, M. Kawasaki, M. Matsumoto, M. Komatsu and H. Tsubouchi, *J. Med. Chem.*, 2006, **49**, 7854–7860.
- P. Benjes and R. Grimmett, *Heterocycles*, 1994, **37**, 735–738.
- R. F. Anderson, W. A. Denny, W. Li, J. E. Packer, M. Terrel and W. R. Wilson, *J. Phys. Chem. A*, 1997, **101**, 9704–9709.
- P. Griess, *Ber. Bunsenges. Physik. Chem.*, 1879, **12**, 426–428.
- R. F. Anderson, *Ber. Bunsenges. Physik. Chem.*, 1976, **80**, 969–972.
- E. Stekhan and T. Kuwana, *Ber. Bunsenges. Physik. Chem.*, 1974, **78**, 253–259.
- P. Wardman, *J. Phys. Chem. Ref. Data*, 1989, **18**, 1637–1755.
- J. C. Russell and G. R. Freeman, *J. Phys. Chem.*, 1968, **72**, 808–815.
- M. C. Sauer, S. Arai and L. M. Dorfman, *J. Chem. Phys.*, 1965, **42**, 708–712.
- W. V. Sherman, *J. Phys. Chem.*, 1966, **70**, 667–672.
- F. P. Sargent and E. M. Gardy, *Can. J. Chem.*, 1974, **52**, 3545–3650.
- P. Kim, S. Kang, H. I. Boshoff, J. Jiricek, M. Collins, R. Singh, U. H. Manjunatha, P. Niyomrattanakit, L. Zhang, M. Goodwin, T. Dick, T. H. Keller, C. S. Dowd and C. E. Barry, III, *J. Med. Chem.*, 2009, **52**, 1329–1344.