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BIOREDUCTIVELY–ACTIVATED PRODRUGS FOR TARGETING HYPOXIC TISSUES: ELIMINATION OF ASPIRIN FROM 2–NITROIMIDAZOLE DERIVATIVES

S. A. Everett, M. A. Naylor, K. B. Patel, M. R. L. Stratford and P. Wardman*

Gray Laboratory Cancer Research Trust, Mount Vernon Hospital, Northwood, Middlesex, HA6 2JR, U.K.

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Abstract: 2–Nitroimidazoles were synthesised substituted with aspirin or salicylic acid, as leaving groups linked through the (imidazol–5–yl)methyl position. Activation of aqueous solutions by $CO_2^{\bullet-}$ (a model one-electron reductant) resulted in release of aspirin or salicylate, probably via the 2-hydroxyaminoimidazole. The analogous 2-nitroimidazole with bromide as leaving group eliminated bromide in < 1 ms via the radical-anion.

Hypoxia or hypoxia/reperfusion injury is a feature of many solid tumours, arthritic joints, ischaemic episodes, inflammatory bowel disease and other common conditions. Prodrugs which might release an active agent selectively in hypoxic tissues could therefore have use in diverse diseases. Tumour hypoxia can be an important prognostic indicator for survival following radiotherapy.[1,2] Drugs which are reductively and selectively activated in hypoxic regions of tumours [3-5] have included nitroarenes which fragment on reduction to release an 'effector'.[6] We and others have recently reported the analogous elimination of aspirin from reduced indolequinones,[7] and the factors which influence release of 'leaving groups' from indolequinones.[8,9]

Nitroheterocycles, especially nitroimidazoles,[10] are extensively used in medicine, and reductive elimination of leaving groups has been studied involving 4- and 5-nitroimidazoles, 5-nitrofurans, and a 5-nitropyrrole (as well as from nitrobenzenes).[6,11-16] However, to our knowledge little has been reported about reductive elimination from 2-nitroimidazoles in spite of this nitroheterocyclic moiety being by far the most extensively studied in the context of tumour therapy and diagnosis of hypoxia, reflecting the favourable, predictable and controllable redox properties of 2-nitroimidazoles.[17,18] 2-Nitroimidazoles are of special interest in targeting hypoxic tumour cells since reductive metabolism of this moiety is being used as the basis for diagnosis of tumour hypoxia, with many studies involving both histological and non-invasive techniques.[19-26] Hence there is an extensive literature, including clinical data, concerning the extent of reductive activation of 2-nitroimidazoles in tumour cells and its dependence on oxygen tension. We demonstrate here the potential for delivery of therapeutics to hypoxic tissues using 2-nitroimidazole as the basis for the reductively-activated 'trigger'. Aspirin and salicylate were selected as model leaving groups partly because of therapeutic interest in controlling rheumatoid joint inflammation, known to be associated with hypoxia;[27] bromide as an alternative leaving group permitted comparison with nitrobenzyl halides.[28]

* Fax + 44 1923 835 210; e-mail: wardman@graylab.ac.uk

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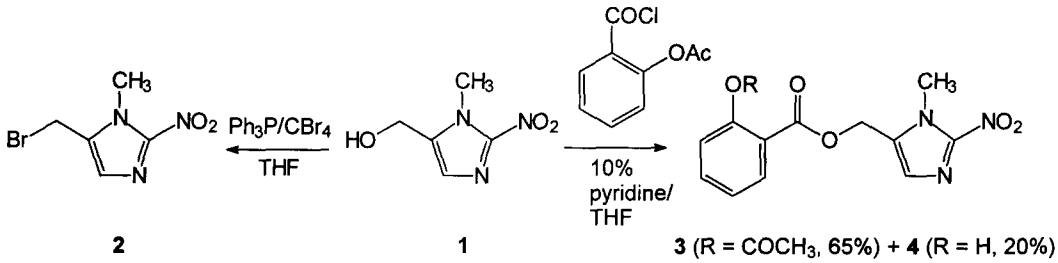
1-Methyl-2-nitroimidazole-5-carboxaldehyde was reduced ($NaBH_4$/MeOH) to the 5-hydroxymethyl derivative (Scheme 1, **1**);[29] treatment with acetyl salicyloyl chloride in the presence of pyridine gave the salicylate prodrugs. Treatment of the alcohol with triphenylphosphine/CBr_4 gave the required bromomethyl analogue. Structure and purity determinations for the new compounds were based on TLC, HPLC, LCMS, CHN and NMR data.



Scheme 1. Synthetic routes to model compound **2** and prodrugs **3** and **4**.

One-electron reductants are important in the activation of 2-nitroimidazoles,[10,30,31] and we used as a controllable reductant the one-electron donor, $CO_2^{\bullet-}$ (generated radiolytically). Aqueous solutions contained nitroimidazoles (50–100 μM), HCOONa (0.1 M), phosphate buffer (4 mM, pH 7.4), and N_2O (~25 mM). The primary radicals from water radiolysis are scavenged by HCO_2^-/N_2O to yield $CO_2^{\bullet-}$ which then reduces the nitroimidazoles.[32] γ-Radiolysis (dose rate ~ 0.1 Gy s^{-1}) generates nitro radical-anions with a yield of ~0.68 μM Gy^{-1}, and pulse radiolysis produces the radicals in similar yield in ~1 μs, monitored by kinetic spectrophotometry.[33] Product analysis following γ-radiolysis was performed by gradient HPLC separation on a base-deactivated reverse-phase column with absorbance detection between 200-320 nm; concentrations were determined from peak areas. Where authentic samples were not available, products were qualitatively identified by LCMS fragmentation patterns. Radiation-chemical yields were calculated from linear plots of nitroimidazole reduced or salicylate produced versus the absorbed dose.

Figure 1 shows typical product profiles obtained on the reduction of **2** and **3**, and Table 1 lists the yields for the reduction of the nitroimidazoles and elimination of Br^- or salicylate. Bromide was released from **2** with a yield corresponding to 1 Br^- : 1 $CO_2^{\bullet-}$, also generating a dimer (identified by its molecular ion in LCMS). This could be formed *via* combination of two of the carbon–centred radicals shown in Scheme 2, although delocalization in the radical may result in other, ring-coupled structures. However, **3** or **4** eliminated aspirin or salicylate respectively with a stoichiometry of about 1 aspirin : 4 $CO_2^{\bullet-}$ without forming a dimer, and in both cases no products with intact imidazole moieties were detected by LCMS, presumably due to the formation of glyoxal, guanidines or other fragmentation products of the heterocyclic core which are seen after reduction of 2-nitroimidazoles.[34,35] The stoichiometry of reduction at pH 7.4 and pH 9.1 was identical (2-nitroimidazole radicals are longer-lived at higher pH).[32]

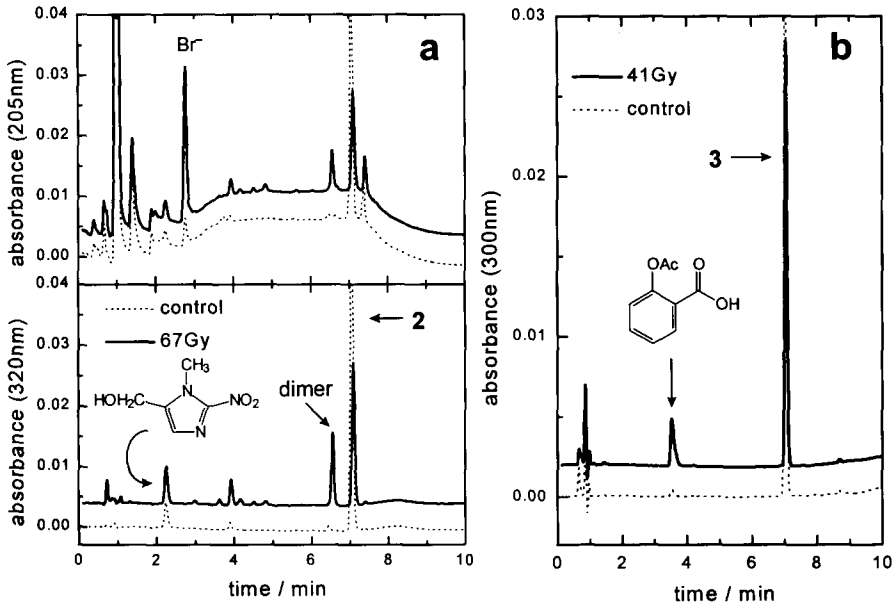
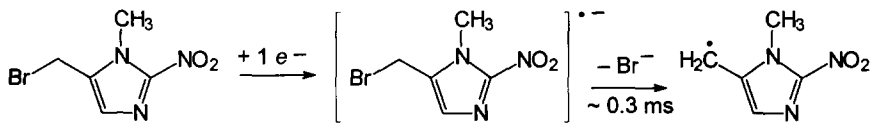


Figure 1. Product profiles obtained on reduction of **2** (a) and **3** (b).

Table 1. Radiation-chemical yields for reductive elimination of leaving groups (LG) at pH 7.4.

compound	loss of parent/μmol J^{-1}	yield of LG [a] /μmol J^{-1}
2	0.68 ± 0.02 [b]	0.62 ± 0.02 [c]
3	0.19 ± 0.01	0.20 ± 0.02
4	0.21 ± 0.02	0.21 ± 0.02

[a] LG = Br^- (**2**), aspirin (**3**), salicylate (**4**). [b] Equals the yield of radicals produced. [c] Corrected for hydrolysis of **2** during experiment (< 5%).

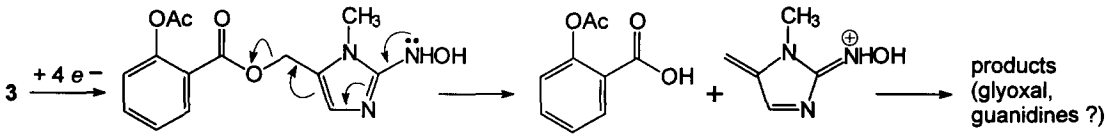


Scheme 2. Reductive elimination of bromide from the radical-anion of **2**.

The lifetimes of the nitro radical-anions were monitored at 440 nm by pulse radiolysis. Radical-anions of **2**, **3** and **4** decayed exponentially at low concentrations, as found for other 2-nitroimidazoles.[32] At pH 7.4 the half-life of the decay of the absorption of the radical from **2** was ~0.3 ms, much shorter than that of simple 2-nitroimidazoles without leaving groups (e.g. misonidazole or etanidazole, ~0.1 s at this pH).[32] The stoichiometry of bromide release indicates that reductive elimination of Br^- occurs directly from the radical-anion (Scheme 2), and we ascribe the decay on the sub-millisecond timescale to this elimination. However, the

absorptions of the radicals of **3** and **4** decayed considerably slower than that of **2** (half-lives ~30 ms for **3**, pH 9.1; ~70 ms or ~250 ms for **4** at pH 7.4 or 9.1 respectively). The stoichiometries of aspirin/salicylate release from **3** and **4** respectively indicate that reductive elimination does not occur from the radical-anions; with both compounds the observed first–order decay kinetics probably do not correspond to elimination from the (imidazol–5–yl)methyl position, but rather an alternative pathway which appears to be common to 2–nitroimidazoles in general and precedes formation of higher reduction products via radical disproportionation.[32] The efficiencies of reduction of **3** and **4** correspond fairly well to 4-electron reduction, i.e. formation of 2-hydroxyaminoimidazoles, behaviour typical of other 2-nitroimidazoles,[36] although elimination yields slightly higher than one-quarter of the 'radical yield' of ~0.68 μmol J^{-1} may indicate some minor fragmentation and/or reaction of the resulting radical with HCO_2^-.[14]

These results, combined with the product analysis, demonstrate that the reductive elimination of aspirin from **3** (and salicylate from **4**) probably occurs largely from the hydroxyamine (Scheme 3) rather than directly from the radical-anion exemplified by **2** (Scheme 2).



Scheme 3. Proposed mechanism for the reductive elimination of aspirin from **3**.

In conclusion, we have demonstrated the reductive elimination of bromide, aspirin or salicylate from the (imidazol–5–yl)methyl position of 2–nitroimidazoles; the mechanism depends on the leaving group. The salicylate models studied here suffer from the problem of an ester linkage susceptible to cleavage by non-specific esterases, but alternative linker groups are possible. Bromide release from **2** occurs on the sub-millisecond timescale, but still ~100-fold slower than the corresponding elimination from 4-nitrobenzyl bromide.[28] We have discussed elsewhere the kinetics of the competition between release of leaving group and electron-transfer from the undissociated radical-anion to oxygen, which is one basis for selective toxicity towards hypoxic cells based on 'futile' redox cycling.[10,18,37,38] In considering the merits of e.g. quinones or nitroarenes as bioreductive 'triggers', an important difference between radical-anions of nitroimidazoles or quinones of similar reduction potential is that electron-transfer to oxygen is some ~100-fold slower for the former.[37] This means that elimination of the leaving group in the radical-anion need not be as fast with nitroimidazoles as with quinones if the reaction of the radical-anion with oxygen is to be balanced with leaving group elimination, at the low oxygen tensions characteristic of tumours;[39] however, oxygen could inhibit nitroreduction by mechanisms other than redox cycling.[12]

The present results demonstrate the potential to exploit the characteristics of 2-nitroimidazoles as a basis for drug delivery to hypoxic tissues. The reducible moiety has advantageous redox properties for enzyme-

catalyzed reduction, readily manipulated in a predictable manner; it has enhanced aqueous solubility characteristics compared to some quinones, readily enhanced by substitution at the imidazol-1-yl position; there are many studies of mechanisms of hypoxic and aerobic toxicity *in vitro*,[40,41] and, especially, of tolerance in humans of administration of high and repeated doses of simple 2-nitroimidazoles.[42-44]

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References and notes: 5–Bromomethyl–1–methyl–2–nitroimidazole **2**, ^{1}H–NMR ($CDCl_3$) δ 7.14 (1H, s, Im–H), 4.47 (2H, s, CH_2Br), 4.02 (3H, s, Im–NMe). MS (EI) *m/z* (relative intensity) 219/221 (M^+, 5 %), 140 (100%), 110 (5 %). (1–Methyl–2–nitroimidazol–5–yl)methyl 2–acetoxybenzoate **3**, mp 154–155°C; ^{1}H–NMR ($CDCl_3$) δ 7.0–8.06 (4H, m, Ar–H), 7.27 (1H, s, Im–H), 5.31 (2H, s, CH_2O), 4.02 (3H, s, Im–NMe), 2.2 (3H, s, COMe). Calcd for $C_{14}H_{13}N_3O_6$: C; 52.67, H; 4.10, N; 3.15. Found: C; 52.89, H; 4.09, N; 13.16%. (1–Methyl–2–nitroimidazol–5–yl)methyl 2–hydroxybenzoate **4**, mp 150–152°C (dec.); ^{1}H–NMR ($CDCl_3$) δ 7.02–7.83 (4H, m, Ar–H), 7.24 (1H, s, Im–H), 5.37 (2H, s, CH_2O), 4.07 (3H, s, Im–NMe).

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