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PRODRUGS FOR TARGETING HYPOXIC TISSUES : REGIOSPECIFIC ELIMINATION OF ASPIRIN FROM REDUCED INDOLEQUINONES

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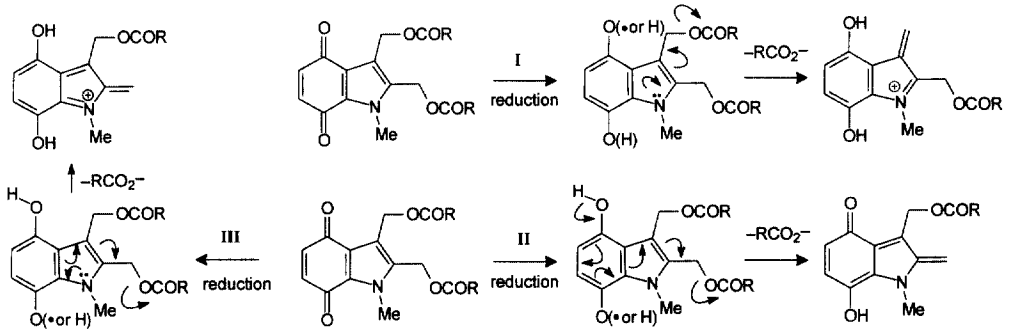
Abstract: A series of regioisomeric derivatives of a 1–methylindole–4,7–dione were synthesised, substituted with a 2–acetoxybenzoate leaving group linked through the (indol–2–yl)methyl or (indol–3–yl)methyl (or propenyl) positions. Reductive elimination of the leaving group occurred from the (indol–3–yl)methyl derivatives but not the 2–substituted regioisomers, indicating that only the C–3 position may be utilised in bioreductively–activated drug delivery, which was demonstrated with an aspirin prodrug.

The targeted delivery of bioreductively–activated cytotoxic agents to the hypoxic cells of solid tumours has been an area of intense investigation.[1] Reduction often involves free radicals as intermediates, which react rapidly with oxygen to form superoxide radicals, inhibiting drug reduction.[2] Alternatively, under low oxygen tensions, these radical intermediates may react with biomolecules or be further reduced to toxic species. The elevation of cellular oxidative stress accompanying oxygen inhibition of reduction is generally less damaging than drug reduction to toxic products; therefore, such drugs offer selective toxicity to hypoxic cells. As part of our studies on such compounds, we have recently synthesised and evaluated a series of indolequinones bearing a variety of leaving groups at the (indol–3–yl)methyl position.[3]



Scheme 1. Possible pathways for reductive elimination of RCO_2^- (e.g. aspirin).

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Reductive elimination of carboxylate and phenolic (indol–3–yl)methyl substituents to produce the iminium derivative (Scheme 1, Pathway I) was inhibited by oxygen and the utilisation of indolequinones as the basis for a hypoxia–selective, bioreductively–activated delivery system was recognised. It was unknown whether analogous elimination from C–2 may occur, *via* quinone methide type intermediates (Scheme 1, Pathway II). This has been previously shown with naphthoquinones bearing halide leaving groups in the corresponding position.[4] Alternatively, an orthoquinodimethane type intermediate could be envisaged (Pathway III). A series of indolequinones with 2–acetoxybenzoic acid (aspirin) conjugated in the 2– and/or 3–position were thus synthesised in order to establish whether the reductive elimination of this leaving group could occur *via* both pathways, or is regiospecific. Aspirin was selected as a model leaving group of therapeutic interest because it and other steroidal and non–steroidal anti–inflammatory drugs (NSAIDs) are often used to control rheumatoid joint inflammation, which is known to be associated with the presence of hypoxia.[5–7] Aspirin prodrugs based on reductively–activated indolequinone conjugates might have benefit since delivery of the active agent to hypoxic tissues should be enhanced relative to other tissues.

The precursor and parent indolequinone diol **1**,[8] 2– and 3–hydroxymethyl and 2– or 3–hydroxypropenyl compounds[9,10] were treated with acetyl salicyloyl chloride in the presence of pyridine to give the desired C–2 and/or C–3 substituted carboxylate derivatives (Table 1, structures **2** – **6**) in good yields. Structure and purity determinations for all the new compounds were based on TLC, HPLC, LCMS and NMR data.[11]

Indolequinone solutions (typically 50 μM in 2–propanol/water (50%, v/v) containing 4 mM phosphate buffer at pH 7.4) were saturated with N_2O in gas–tight vials before irradiation with a ^{60}Co source. Under these experimental conditions the primary radicals from the radiolysis of 2–propanol/water are rapidly converted to the reductant, the 2–propanol radical [$E((CH_3)_2C^{\cdot}OH/(CH_3)_2CO, H^+) = -1.8V$] which easily reduces the indolequinones [$E(Q/Q^{\cdot-}) \sim -0.3$ to -0.4 V] [3,10] to generate the semiquinone radical ($Q^{\cdot-}$). This can disproportionate to the hydroquinone (QH_2), the system mimicking drug activation by cellular reductases:

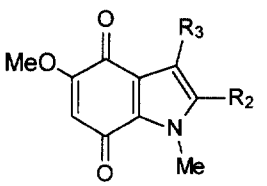
$$Q + (CH_3)_2C^{\cdot}OH \rightarrow Q^{\cdot-} + (CH_3)_2CO + H^+$$

$$Q^{\cdot-} + Q^{\cdot-} + 2H^+ \rightleftarrows QH_2 + Q$$

A dose rate of 6 – 6.5 Gy min^{-1} was used, as determined by Fricke dosimetry[12] and an absorbed dose of 1 Gy = 0.67 μM $(CH_3)_2C^{\cdot}OH$ radicals. Total doses of ~ 20 Gy ensured less than 50 % conversion of drug to products. Radiation–chemical yields (μmol J^{-1}) were then calculated from the linear plots of indolequinone reduced and aspirin produced versus the absorbed dose (Gy). Product analysis following γ–radiolysis was

performed by gradient HPLC separation on a base–deactivated reverse–phase column with detection at 280 nm and concentrations were determined from peak areas. In certain cases where authentic samples were not available, products were identified by LCMS fragmentation patterns.[13]

Table 1. Percentage efficiencies of reductive elimination from indolequinone prodrugs.



Q	R_2	R_3	$-G(Q)^a$ /μmol J^{-1}	$G(Aspirin)^a$ /μmol J^{-1}	efficiency %
1	$CH=CHCH_2OH$	CH_2OH	0.1^b	–	–
2	Me	$CH_2OCO(o\text{–}OAc(C_6H_4))$	1.63	1.40	86
3	H	$CH=CHCH_2OCO(o\text{–}OAc(C_6H_4))$	0.86	0.64	74
4	$CH=CHCH_2OCO(o\text{–}OAc(C_6H_4))$	H	0^c	0^c	0^c
5	$CH_2OCO(o\text{–}OAc(C_6H_4))$	H	0^c	0^c	0^c
6	$CH=CHCH_2OCO(o\text{–}OAc(C_6H_4))$	$CH_2OCO(o\text{–}OAc(C_6H_4))^d$	1.09	0.89	82

[a]2–Propanol/water (50%, v/v); $G((CH_3)_2C^{\cdot}OH = 0.67$ μmol J^{-1}. [b]Reduction of **1** did not generate a significant quantity of the corresponding isopropyl ether. [c] Reduction of Q would be followed by re–oxidation of QH_2 to Q during HPLC, i.e. no apparent loss of Q. [d]Preferred leaving group eliminated.

The leaving group efficiency (%) from derivatives **2** – **6** determined from the radiation chemical yields of the parent indolequinone lost relative to aspirin generated are displayed in Table 1. Derivatives **2** and **3** eliminated aspirin efficiently (> 70%) from the (indol–3–yl)methyl position. Associated products following the reduction of **6** were the alcohol **6a** and the 3–(isopropoxy)methyl derivative **6b** which has previously been shown to be due to solvent trapping of the iminium derivative formed[3] as depicted in

Scheme 1, Pathway I. The structures of **6a** and **6b** can be assigned as shown in Figure 1 since the results for compound **4** demonstrate that an acyl group cannot be eliminated from the 2–position.

The product profile following the reduction of the *bis*–conjugated derivative **6** is shown in Figure 1. Initially, reduction of **6** by $(CH_3)_2C'OH$ (~ 16 μM) generated aspirin with equivalent efficiency to derivatives **2** and **3**, with the concomitant formation of **6a** and **6b**. Both these quinones are generated by autoxidation of their respective hydroquinones following the unavoidable introduction of oxygen during HPLC sampling.

$$QH_2 + O_2 \rightarrow Q + H_2O_2$$

When **6** had been completely reduced to products **6a** and **6b**, further reduction after oxygen removal did not liberate the second aspirin molecule from the (indol–2–yl)methyl position. Furthermore, reductive elimination from both C–2 and C–3 would be expected to generate the diol **1** but this was not detected. Aspirin was efficiently released from **2** (the (indol–3–yl)methyl–conjugate) and **3** (the (indol–3–yl)propenyl–conjugate) but was not observed from the 2–substituted analogues **4** and **5**.

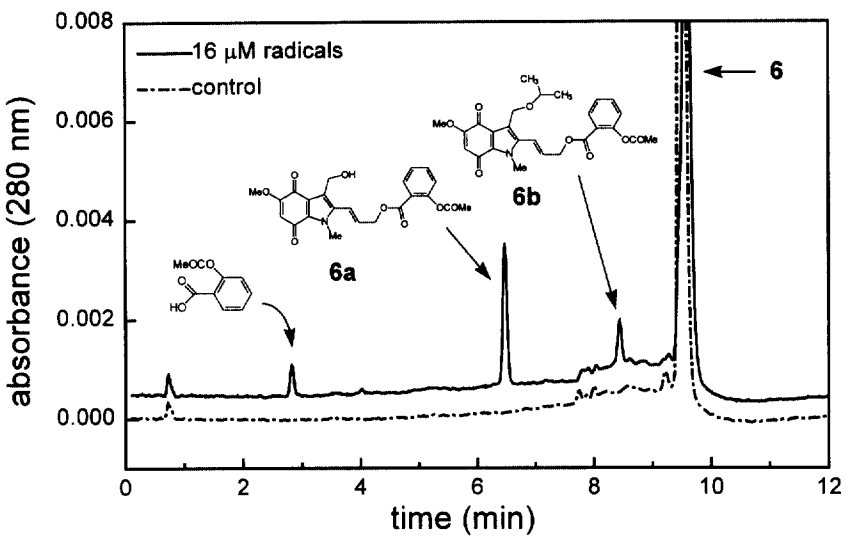


Figure 1. Reductive elimination of aspirin from the (indol–3–yl)methyl position of indolequinone 6.

In conclusion, the only release mechanism of utility for these prodrugs is elimination from the (indol–3–yl)methyl position (presumably facilitated by the nitrogen lone pair), with no evidence for

quinone–methide induced elimination as has been observed for some other quinone systems with halide leaving groups.[4] This is in agreement with some previous studies on related mitosenes, in which nucleophiles were shown to add preferentially to C–10 under reductive conditions, although release of the corresponding acyl groups was not quantified.[14] This preferred position of prodrug attachment results in highly efficient release of a model drug following reduction and clearly merits further investigation. From a chemical–kinetic point of view the selectivity of indolequinones for hypoxia in tumours or arthritic tissue will rely on establishing a balance between the rates of oxidation (by oxygen) of $Q^{\cdot-}$ and/or QH_2 and the rate of competing reductive elimination from the (indol–3–yl)methyl position in $Q^{\cdot-}$ and/or QH_2.[2,15] The application of indolequinones to target NSAIDs or other biologically active agents to rheumatoid or other tissue containing hypoxic regions is being actively pursued.

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11. Analysis of compounds **2 – 6**:

3–(2–Acetoxybenzoyloxy)methyl–1,2–dimethyl–5–methoxyindole–4,7–dione (2) (69%) mp 159–161 °C; δ_H 7.99 (1H, dd, J 8.1, 1.4, Ar–*H*), 7.01–7.53 (3H, m, Ar–*H*), 5.63 (1H, s, *H*–6), 5.47 (2H, s, CH_2), 3.90 (3H, s, OCH_3), 3.81 (3H, s, N–CH_3), 2.31 (3H, s, $OCOCH_3$), 2.27 (3H, s, CH_3); Fnd: C; 63.8, H; 4.8, N; 3.7, Calcd. C; 63.5, H; 4.8, N; 3.5%.

2–(2–Acetoxybenzoyloxy)propen–1–yl–5–methoxy–1–methylindole–4,7–dione (3) (48%) mp 141–143 °C; δ_H 8.09 (1H, d, *J* 6.3, Ar–*H*), 7.59 (1H, t, *J* 6.3, Ar–*H*), 7.34 (1H, t, *J* 7.3, Ar–*H*), 7.14 (1H, d, *J* 7.9, Ar–*H*), 6.81 (1H, s, *H*–3), 6.63 (1H, d, *J* 15.8, C*H*=CHCH$_2$–), 6.41 (1H, d, *J* 18.8, CH=C*H*CH$_2$–), 5.67 (1H, s, *H*–6), 4.96 (2H, d, *J* 4.9, CH_2), 3.98 (3H, s, OCH_3), 3.82 (3H, s, N–CH_3), 2.33 (3H, s, $OCOCH_3$); *m/z* (EI) 409.1158 (M, $C_{22}H_{19}NO_7$ requires 409.1162).

2–(2–Acetoxybenzoyloxy)methyl–5–methoxy–1–methylindole–4,7–dione (4) (41%) mp 136–138 °C; δ_H 8.02 (1H, dd, *J* 7.9, 1.7, Ar–*H*), 7.63 (1H, dt, *J* 7.9, 1.7, Ar–*H*), 7.35 (1H, dt, *J* 7.6, 1.32, Ar–*H*), 7.13 (1H, dd, *J* 8.3, 1.32, Ar–*H*), 6.77 (1H, s, *H*–3), 5.71 (1H, s, *H*–6), 5.30 (2H, s, CH_2), 3.98 (3H, s, OCH_3), 3.84 (3H, s, N–CH_3), 2.19 (3H, s, $OCOCH_3$); *m/z* (EI) 383.1020 (M, $C_{20}H_{17}NO_7$ requires 383.1005).

3–(2–Acetoxybenzoyloxy)propen–1–yl–5–methoxy–1–methylindole–4,7–dione (5) (48%) mp 132–133 °C; δ_H 8.06 (1H, d, *J* 6.3, Ar–*H*), 7.59 (1H, t, *J* 6.3, Ar–*H*), 7.33 (1H, t, *J* 7.3, Ar–*H*), 7.11 (1H, d, *J* 7.9, Ar–*H*), 6.86 (1H, s, *H*–2), 6.65 (1H, d, *J* 15.8, C*H*=CHCH$_2$–), 6.43 (1H, d, *J* 18.8, CH=C*H*CH$_2$–), 5.68 (1H, s, *H*–6), 5.51 (2H, d, *J* 4.9, CH_2), 3.94 (3H, s, OCH_3), 3.83 (3H, s, N–CH_3), 2.32 (3H, s, $OCOCH_3$); *m/z* (EI) 409.1160 (M, $C_{22}H_{19}NO_7$ requires 409.1162).

2–(2–Acetoxybenzoyloxy)propen–1–yl–3–(2–acetoxybenzoyloxy)methyl–5–methoxy–1–methylindole–4,7–dione (6) (37%) mp 141–143 °C; δ_H 8.04 (1H, d, *J* 6.3, Ar–*H*), 7.97 (1H, d, *J* 6.3, Ar–*H*), 7.58 (2H, 2xdt, Ar–*H*), 7.34 (2H, 2xdt, Ar–*H*), 7.13 (2H, 2xdd, Ar–*H*) 6.56 (1H, d, C*H*=CHCH$_2$–), 6.28 (1H, m, CH=C*H*CH$_2$–), 5.70 (1H, s, *H*–6), 5.49 (2H, d, *J* 5.3, ind–CH_2), 4.99 (2H, d, *J* 4.9, CH=CHC*H*$_2$–), 3.97 (3H, s, OCH_3), 3.82 (3H, s, N–CH_3), 2.30 (3H, s, $OCOCH_3$), 2.28 (3H, s, $OCOCH_3$); *m/z* (EI) 601.1579 (M, $C_{30}H_{26}NO_{11}$ requires 601.1584).

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