Rates of reductive elimination of substituted nitrophenols from the (indol-3-yl)methyl position of indolequinones

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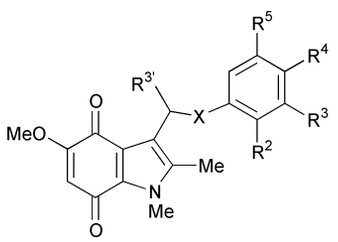
A series of indolequinones bearing substituted nitrophenols on the (indol-3-yl)methyl position was synthesised. The nitrophenol leaving groups were appropriately substituted to give a wide range (4 units) in phenolic pK_a value. The rate of reductive elimination of phenoxide anions from the (indol-3-yl)methyl position of semiquinone radicals was dependent upon this pK_a, with a decrease in 3.8 pK units shortening the half-life from 28 to 1.5 ms. Only 2,4-dinitrophenol (pK_a = 3.9) was eliminated from an unsubstituted (indol-3-yl)methyl position at a rate that would compete with reoxidation of the radical by oxygen. A nitrothiophenol leaving group was eliminated comparatively slowly and only from the hydroquinone. These studies demonstrate the dependence upon leaving group pK_a of the rate of reductive elimination from the (indol-3-yl)methyl position of indolequinones.

Introduction

There has been much interest in the exploitation of indolequinones as bioreductively activated cytotoxins designed to target the hypoxic sub-population of cells present in many solid tumours.[1–7] In addition to the 'direct' activation to cytotoxic species, of increasing importance is the alternative bioactivation route where reduction of an indolequinone initiates fragmentation of a linking bond and the release of a bioactive agent, the biological activity of which is masked in the prodrug form.[8,9] We have demonstrated that indolequinones are capable of efficiently eliminating a range of leaving groups, coupled through various linkers, from the (indol-3-yl)methyl position following one-electron reduction to the semiquinone radical anion ($Q^{\bullet -}$) or two-electron reduction to the hydroquinone (QH_2).[10] From a physico-chemical viewpoint, a key factor in the design of these prodrugs is the ability to control rates of reductive fragmentation relative to the reactivities of intermediate $Q^{\bullet -}/QH_2$ species with oxygen.[11,12] The effects of indolequinone substitution patterns on rates of elimination of the drug model 4-nitrophenol have been studied recently.[13] By incorporating radical-stabilising substituents (*e.g.* methyl, thienyl) at the indolyl carbinyl position it was possible to significantly enhance the rate of reductive fragmentation directly from the $Q^{\bullet -}$ radical. The use of 4-nitrophenol as a model leaving group facilitated structural optimisation of the indolequinone moiety but the importance of the leaving group itself in controlling rates of fragmentation has yet to be elucidated.

For this study we have synthesised a series of substituted nitrophenols coupled to an indolequinone through a phenolic ether bond (Fig. 1, **1**–**8**) and the thiophenol analogue **9**, and determined the rates of reductive fragmentation by pulse radiolysis.[11] The indolequinones were reduced in a controlled and quantifiable manner using radiolytically-produced reducing radicals which mimic reduction by (one- and two-electron reducing) activating enzymes such as NADPH P450 reductase[6] and NQO1 (DT-diaphorase)[14,15] in biological systems.



Compound	$R^{3'}$	R^2	R^3	R^4	R^5	X
1	H	NO_2	H	NO_2	H	O
2	H	OMe	H	H	NO_2	O
3	H	OMe	H	NO_2	H	O
4	H	CHO	H	NO_2	H	O
5	H	H	CHO	NO_2	H	O
6	H	F	H	NO_2	H	O
7	Me	F	H	NO_2	H	O
8	H	H	H	NO_2	H	O
9	H	H	H	NO_2	H	S

Fig. 1 Structures of indolequinones **1**–**9** bearing substituted nitrophenols (or nitrothiophenol) in the (indol-3-yl)methyl position.

Results

Synthesis

The 3-aryloxymethylindolequinones **1**–**8** were prepared from the corresponding 3-hydroxymethylquinones **10** and **11** as outlined in Scheme 1. Thus, in the case of the 2,4-dinitrophenol derivative **1**, reaction of the alcohol with 2,4-dinitrofluorobenzene in the presence of silver(I) oxide gave the desired aryloxy compound **1** in modest yield. The other aryloxy derivatives **2**–**8** were prepared from the 3-hydroxymethyl compound and substituted phenol using the Mitsunobu reaction. The thioether **9** was prepared similarly. The 3-isopropoxymethylindolequinone **12** was prepared from **10** as previously described.[13]

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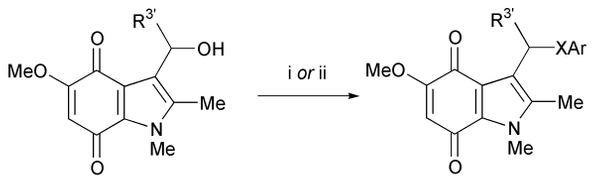

10 $R^{3'}$ = H
11 $R^{3'}$ = Me

Scheme 1 *Reagents and conditions*: [for X = O, Ar = 2,4-$(NO_2)_2C_6H_3$] i, 2,4-dinitrofluorobenzene, Ag_2O, THF; [all other Ar] ii, ArOH (or ArSH), $EtO_2CN{=}NCO_2Et$, Ph_3P, THF.

Free radical chemistry

Reductive fragmentation at the (indol-3-yl)methyl position. In agreement with previous studies,[7,10,11,13] we observed that the reaction of the propan-2-ol radical ($(CH_3)_2C^{\bullet}OH$) with all the indolequinones **1**–**11** in aqueous solution at pH > 6 yields transients with absorption spectra characterised by two maxima, one in the UV (λ_{max} *ca.* 350 nm), and a considerably weaker one in the visible (λ_{max} *ca.* 600 nm). These transients have been identified as the semiquinone radicals ($Q^{\bullet -}$),[7,10,11,13] which are fully formed (k_1 ~10^9 dm^3 mol^{-1} s^{-1}) by ~200 μs after the electron pulse according to reaction (1).

$$Q + (CH_3)_2C^{\bullet}OH \rightarrow Q^{\bullet -} + (CH_3)_2CO + H^+ \qquad (1)$$

From the pH dependence of the absorption at 350 nm, a pK_a ($QH^{\bullet}/Q^{\bullet -}$) = 5.5 ± 0.1 for **10** was obtained and was typical for the semiquinone radicals of all the indolequinones in the present study. Traditionally the hydroxymethyl compounds exhibit poor leaving group ability and as expected the semiquinone radicals decayed by pure second-order kinetics *via* the disproportionation reaction (2).

$$Q^{\bullet -} + Q^{\bullet -} + 2H^+ \rightleftharpoons QH_2 + Q \qquad (2)$$

In contrast, the decay of the semiquinone radical of **3** was associated with an increase in absorption between 350–500 nm ascribed to the 2-methoxy-4-nitrophenoxide anion. In propan-2-ol–water (50%, v/v) 2-methoxy-4-nitrophenol has a pK_a (LG–OH $\rightleftharpoons$ LG–O$^-$ + H$^+$) = 7.56 ± 0.1 (LG = leaving group) and when deprotonated exhibits a ground-state absorption maximum at ~420 nm. As previously observed for **8** under similar experimental conditions (where 4-nitrophenol has a slightly higher pK_a = 7.71 ± 0.03), the reductive elimination of 2-methoxy-4-nitrophenol from **3** is biphasic (see Fig. 2a): the first phase of release complete in ~100 ms and a second slower phase which is complete 10 s after reduction. This biphasic release of the 2-methoxy-4-nitrophenoxide anion reflects complex kinetics associated with reductive elimination initially from the $Q^{\bullet -}$ radical *via* reaction (3) and then from the hydroquinone *via* reaction (4). The latter is formed when reaction (2) begins to compete with reaction (3).

$$(Q{-}LG)^{\bullet -} \rightarrow Q^{\bullet +} + LG \qquad (3)$$

$$(QH_2{-}LG) \rightarrow QH_2^+ + LG^- \qquad (4)$$

The rates of reductive elimination of 2-methoxy-4-nitrophenoxide anion from both the $Q^{\bullet -}$ radical and QH_2 were derived using a data-fitting model in FACSIMILE. A model comprising reactions (2)–(4) was used to give 'best' fits to kinetic traces (see Fig. 2 for an example of a fitted kinetic trace) obtained by pulse radiolysis and gave $k_3 = 25.5 \pm 0.1$ s^{-1} and $k_4 = 4.1 \pm 0.1$ s^{-1} for **3**. Table 1 contains the rate constants for the reductive elimination of substituted nitrophenols from the (indol-3-yl)methyl position of indolequinones **1**–**8**. Steady-state γ-radiolysis confirmed that in all cases reduction of the parent indolequinone generated stoichiometric amounts of the corresponding leaving group, with the reactive species formed from the indolequinone, presumably an iminium ion, being intercepted by water or propan-2-ol solvents to give the

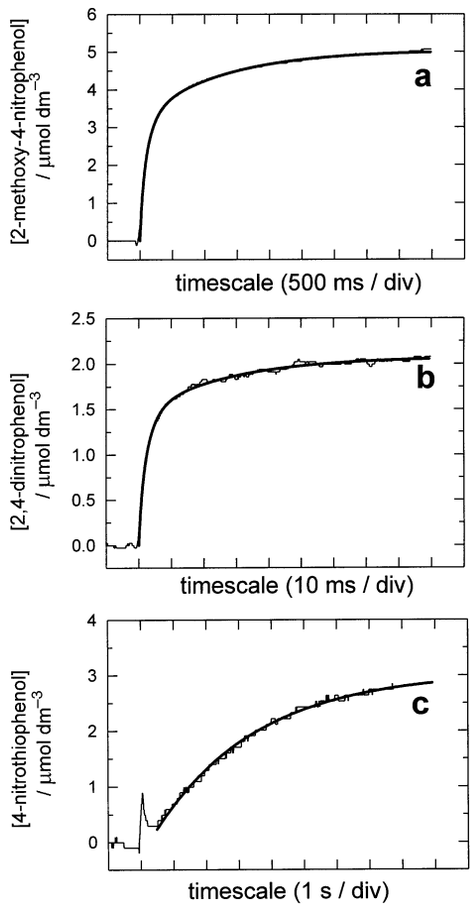

Fig. 2 Changes in the concentration of substituted nitrophenols as measured by pulse radiolysis of indolequinones (50 μmol dm^{-3}) in an N_2O-saturated propan-2-ol–water mixture (50%, v/v) at pH 9. Panels (a–c) show the reductive elimination of substituted nitrophenols from indolequinones **3** (10.8 Gy ~7.2 μmol dm^{-3} $(CH_3)_2C^{\bullet}OH$ radicals recorded at 434 nm), **1** (3.1 Gy ~2.1 μmol dm^{-3} $(CH_3)_2C^{\bullet}OH$ radicals recorded at 400 nm), and **9** (10 Gy ~6.7 μmol dm^{-3} $(CH_3)_2C^{\bullet}OH$ radicals recorded at 410 nm).

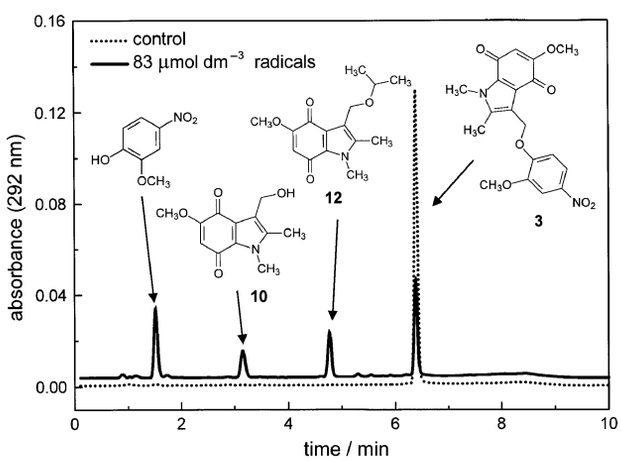

Fig. 3 HPLC chromatogram showing typical product profiles obtained by reductive elimination from the (indol-3-yl)methyl position of indolequinone **3**. Conditions: γ-radiolysis (124 Gy) of **3** (40 μM) in an N_2O-saturated propan-2-ol–water mixture (50%, v/v) containing phosphate buffer (4 mmol dm^{-3}) at pH 7.4.

Table 1 Rate constants for the reductive elimination of substituted nitrophenols and nitrothiophenol leaving groups (LGs) from both semiquinone radical and hydroquinone species, ground-state pK_a of the LGs, and radiation chemical yields for loss of parent indolequinone (Q) and release of LGs

Q	k_3(Q–LG$^{\bullet-}$ ⟶ Q$^{\bullet+}$ + LG)/s^{-1}	k_4(QH$_2$–LG ⟶ Q$^+$ + LG$^-$)/s^{-1}	pK_a(LG–OH ⇌ LG–O$^-$ + H$^+$)[a]	$G(-Q)$[b]/ μmol J^{-1}	G(LG$^-$)[b]/ μmol J^{-1}
1	465 ± 7	—	3.87 ± 0.02	0.71 ± 0.01	0.58 ± 0.01
2	—	~0.3	9.20 ± 0.10	1.57 ± 0.01	1.43 ± 0.01
3	25.5 ± 0.1	4.1 ± 0.1	7.56 ± 0.02	1.21 ± 0.01	1.22 ± 0.01
4	49.9 ± 0.2	—	5.42 ± 0.07	~0.65	~0.61
5	31.2 ± 0.1	2.2 ± 0.1	6.82 ± 0.06	0.70 ± 0.01	0.71 ± 0.01
6	41.3 ± 0.2	—	6.33 ± 0.05	1.10 ± 0.01	1.11 ± 0.01
7	520 ± 5	—	6.33 ± 0.05	2.12 ± 0.01	2.10 ± 0.01
8	25.2 ± 0.1	1.7 ± 0.1	7.71 ± 0.03	1.60 ± 0.01	1.50 ± 0.01
9	—	~0.3	4.31 ± 0.07	0.32 ± 0.01	0.27 ± 0.01

[a] Excluding indolequinone **9** which contains a thioether linking bond. [b] Propan-2-ol–water (50%, v/v); $G(CH_3)_2C^{\bullet}OH$ = 0.67 μmol J^{-1}; dose rate ~3.9 Gy min^{-1}.

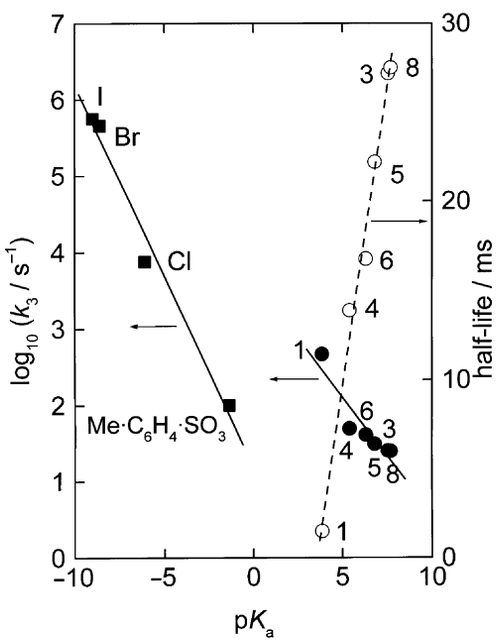

Fig. 4 Linear correlation between the half-lives (open circles) and $\log_{10}$ k_3 (solid circles) for the reductive elimination of the substituted nitrophenols from the semiquinone radical *vs.* pK_a of the phenolic hydroxy group. For comparative purposes the plot also contains a $\log_{10}$ k *vs.* pK_a plot (solid squares) for the reductive elimination of leaving groups from 4-nitrobenzyl halides and toluene-*p*-sulfonate (data taken from ref. 17).

alcohol **10** and the isopropyl ether **12** (Fig. 3). For example, the radiation chemical yield for the loss of **3** G(–Q–LG) = 1.02 ± 0.1 μmol J^{-1} equals that for the generation of the leaving group (LG = 2-methoxy-4-nitrophenol) G(LG) = 1.02 ± 0.1 μmol J^{-1}. There is a strong correlation between the pK_a of the phenol moiety and the rate of reductive elimination from the Q$^{\bullet-}$ radical. For example, 2-methoxy-5-nitrophenol has a high pK_a (LG–OH ⇌ LG–O$^-$ + H$^+$) = 9.2 ± 0.1 and as a consequence elimination of this leaving group following reduction of indolequinone **2** is only observed from the hydroquinone (k_4 ~0.3 s^{-1}). In contrast, 2,4-dinitrophenol, with a much lower pK_a (LG–OH ⇌ LG–O$^-$ + H$^+$) = 3.87 ± 0.02, is rapidly released following the reduction of indolequinone **1**. The formation of the 2,4-dinitrophenoxide anion (see Fig. 2b) follows first-order kinetics and matches the decay of the corresponding Q–LG$^{\bullet-}$ radical and is consistent with reaction (3) being the predominant decay pathway (k_3 ~465 s^{-1}). Even at high doses per pulse (*ca.* 30 Gy) the radiation chemical yields G(Q–LG)$^{\bullet-}$ = G(2,4-dinitrophenoxide anion) are stoichiometric. Poor solubility of the parent indolequinone **1** precluded the use of higher doses per pulse and since reaction (3) out-competed reaction (2) it was not possible to determine the rate of reductive elimination from the hydroquinone. Fig. 4 shows the linear correlation between the half-life for the reductive elimination of the substituted nitrophenols from the Q$^{\bullet-}$ radicals ($t_{1/2} = 0.7/k_3$) in milliseconds, *versus* the pK_a of the phenolic group. Lowering the pK_a of the leaving group by ~4 pH units shortened the half-life for reductive elimination by ($t_{1/2} = (0.7/k_3)$) ~26 ms. Also shown in Fig. 4 is a test of a linear relationship between log k_3 and pK_a and a comparison with elimination rates of the leaving groups shown in the radical anions of 4-nitrobenzyl halides and toluene-*p*-sulfonates (see Discussion).[16,17]

Our previous studies have shown that incorporation of a methyl or heterocyclic substituent at the (indol-3-yl)methyl position significantly enhanced the rate of reductive elimination from the Q$^{\bullet-}$ radical.[13] The rate of reductive fragmentation of **6** is k_3 = 41 s^{-1} and substitution with methyl at the R$^{3\prime}$ position to give **7** increases the rate 10-fold to k_3 = 520 s^{-1}.

Reductive fragmentation of a thioether linker. Fig. 2c shows a typical kinetic trace showing the reductive elimination of nitrothiophenol from **9**. Despite the fact that the nitrothiophenol has a relatively low pK_a (LG–SH ⇌ LG–S$^-$ + H$^+$) = 3.87 ± 0.02, no evidence was obtained for reductive elimination directly from the Q–LG$^{\bullet-}$ radical. Instead, the Q–LG$^{\bullet-}$ radical decays by second-order kinetics to the hydroquinone which then fragments to release the nitrothiophenol (k_4 ~0.3 s^{-1}). Steady-state γ-radiolysis confirmed that the radiation chemical yield for the loss of **9** G(–Q–LG) = 0.37 ± 0.01 μmol J^{-1} approximately equals that for the generation of the leaving group (LG = nitrothiophenol) G(LG) = 0.28 ± 0.1 μmol J^{-1}. However, both yields were about one-half that expected ($G(CH_3)_2C^{\bullet}OH$ = 0.67 μmol J^{-1}), indicating that reductive elimination occurs primarily from the hydroquinone. This slow elimination from **9** contrasts with the fast elimination from **1** (for leaving groups of comparable pK_a) and highlights the dramatic differences between thioether and phenolic ether 'linkers' in terms of reductive elimination from the (indol-3-yl)methyl position of indolequinones.

Semiquinone and hydroquinone reactivities with oxygen. In order to put the rates of reductive fragmentation of the indolequinones **1**–**9** into context, both semiquinone radical and hydroquinone reactivities towards oxygen were also determined. The one-electron reduction potentials [E(Q/Q$^{\bullet-}$)] for the indolequinone alcohols **10** and **11** are −376 and −302 mV respectively.[10,13] This is reflected in the corresponding rates of electron transfer from the Q$^{\bullet-}$ radical to oxygen in reaction (5),

$$Q^{\bullet-} + O_2 \rightarrow Q + O_2^{\bullet-} \qquad (5)$$

which are $k_5 = 5.2 \times 10^8$ dm^3 mol^{-1} s^{-1} and $k_5 = 1.7 \times 10^8$ dm^3 mol^{-1} s^{-1} for **10** and **11** respectively.

The indolequinone alcohols exhibit much poorer leaving group behaviour than their corresponding substituted nitrophenol conjugates **1**–**9**, and were therefore utilised for the study of hydroquinone autoxidation without the added complication of reductive elimination of leaving groups.

Discussion

The rates of reductive elimination from the (indol-3-yl)methyl position of indolequinones **1**–**8** are dependent upon the pK_a of the hydroxy group of the substituted nitrophenol. The span of the line in Fig. 4 (6.8 ± 0.4 pH^{-1}) indicates that a decrease in pK_a by four units shortens the half-life for reductive elimination from the $Q^{\bullet -}$ radical by *ca.* 27 ms. A similar effect has previously been achieved by substitution with a methyl or heterocyclic moiety at the (indol-3-yl)methyl position in **8**. This substitution also shortens the half-life for reductive elimination by *ca.* 26 ms.

A number of studies have established relationships between rates of cleavage of radical anions and free energy changes.[18] Over a limited range an approximately linear relationship between log *k* and free energy may be found, and in the case of cleavage of radical anions of α-aryloxyacetophenones in DMF, a linear relationship was established between log *k* and the pK_a values of the phenols corresponding to the leaving groups, with a slope of the regression line equal to -0.53.[19] In Fig. 4 the corresponding plot covers a limited range and the linear fit is less satisfactory, but the slope equals -0.31 ± 0.06. Also shown in Fig. 4 is an analysis of the rates of elimination of leaving groups from 4-nitrobenzyl halides and toluene-*p*-sulfonate, where the slope of the plot of log *k vs.* pK_a equals -0.50 ± 0.05. If this relationship for 4-nitrobenzyl radical anions extends to other leaving groups such as tertiary amines, *i.e.* alkylating mustard pro-drugs that are nitrobenzyl derivatives linked to substituted trialkylamines or dimethylanilines [$O_2NC_6H_4CH_2$-N^+R_3],[20,21] then it is no surprise that the radical anions disproportionate before release of the amine leaving group can be observed.[22] The corresponding aliphatic or aromatic tertiary amines have pK_a values around 6.4 or 5 respectively. The relationship in Fig. 4, if applicable, would predict half-lives for elimination of these amines in excess of 10 s (of the order of 70 s for elimination of $MeN(CH_2CH_2Cl)_2$ from the radical anion of the 4-nitrobenzyl prodrug).

Substitution by methyl on the linker (*e.g.* **7** *vs.* **6**) increases the rate of elimination of the leaving group by a factor of ~12. This is of the same order as that found in cleavage of radical anions of 4-nitrobenzyl halides substituted on the exocyclic carbon with methyl (~11–24).[16,17]

Clearly both lowering the pK_a of the leaving group and incorporation of suitable radical-stabilising substituents at the indolyl carbinyl position can have a dramatic effect on rates of elimination. This is exemplified by indolequinone **7**, the semiquinone of which has the shortest half-life ($t_{1/2} = (0.7/k_3)$ ~1.3 ms) of the indolequinones studied to date. However pK_a is clearly not the only factor since the correlation appears to apply only to the elimination of phenoxides since a thioether linker, as exemplified by indolequinone **9**, fragments only slowly from the hydroquinone despite nitrothiophenol having a low pK_a (~4.3) which would normally favour the elimination of substituted nitrophenols. There have been a number of studies that compare the relative leaving ability of RS^- and RO^- groups in unimolecular and bimolecular processes.[23] For leaving groups of the *same* pK_a, it has been shown that RO^- (or ArO^-) can be a better leaving group than RS^- (or ArS^-) by over 3 orders of magnitude, the difference reflecting the intrinsic nature of the heteroatom.[23c]

The semiquinone radical derived from **10** reacts with oxygen with a first order rate constant of $k_5 = 5.2 \times 10^8$ dm^3 mol^{-1} s^{-1}. This value indicates a half-life ($0.7/k_5[O_2]$) of ~0.3 ms at a tumour relevant oxygen concentration ($[O_2]$ ~5 μmol dm^{-3}). At the same concentration of oxygen the half-life for the elimination of the leaving group from the semiquinone radical of **11** is $t_{1/2} = 0.8$ ms. Reaction (5) represents the primary competing reaction to the reductive elimination reaction (3) and the balance between these reactions is likely to control release of a bioactive agent as leaving group in hypoxic tumour cells. In this work we have studied elimination of substituted nitrophenols as representative models for bioactive agents that might be released following fragmentation of a phenolic ether linker. It is predicted that the half-lives for the reductive elimination from indolequinones **3**–**6**, **8** which fall in the range $t_{1/2} = 28$–14 ms, would be too long to compete with the very short half-lives of their semiquinone radicals on reaction with oxygen ($t_{1/2} < 1$ ms), since even at a low oxygen concentration of 5 μmol dm^{-3}, elimination of the leaving group would be efficiently inhibited. The half-life for **1** is $t_{1/2}$ ~1.5 ms which begins to approach a level where reductive fragmentation can begin to compete with $Q^{\bullet -}$ reactivity with oxygen. Many biologically active compounds with phenolic hydroxy groups available for coupling to the (indol-3-yl)methyl position of indolequinones are unlikely to have as sufficiently low a pK_a as 2,4-dinitrophenol (pK_a ~3.87). Consequently, a significant lowering of the pK_a of the leaving group or active drug (without compromising biological activity) to facilitate faster rates of reductive fragmentation is one possible strategy for targeting delivery to hypoxic tissues. However, a more productive strategy, as exemplified by indolequinone **7**, would involve manipulation of leaving group pK_a coupled with the required substituent at the indole carbinyl position.

In conclusion, this study demonstrates the dependence upon leaving group pK_a of the rate of reductive elimination from the (indol-3-yl)methyl position of indolequinones. Therefore, in addition to α-substitution of these positions, manipulation of the pK_a of potential agents to be reductively-eliminated may be a strategy for enhancing hypoxic targeting if this can be achieved without compromising the activity of the drug to be delivered.

Experimental

General procedures and materials

NMR spectra: *J* values are given in Hz. Elemental analyses were determined at the University of Exeter and all compounds characterized by HRMS were chromatographically homogeneous. Solutions in organic solvents were dried by standard procedures, and dimethylformamide, toluene and tetrahydrofuran were anhydrous commercial grades. Silica gel for flash column chromatography was Merck Kieselgel 60 H grade (230–400 mesh) or Matrex silica 60. Propan-2-ol was obtained from Sigma-Aldrich Chemical Company Ltd (Gillingham, Dorset, UK), and nitrous oxide was obtained from the British Oxygen Company (Gillingham, Kent, UK).

Pulse radiolysis

The redox properties of the indolequinones and the kinetic characteristics of their semiquinone radicals ($Q^{\bullet -}$) were investigated by pulse radiolysis. Semiquinone radicals were generated following reduction of the parent indolequinone by the propan-2-ol radical [$(CH_3)_2C^{\bullet}OH$]. Kinetic spectrophotometry with sub-microsecond time resolution was used to monitor the reactions involving the $Q^{\bullet -}$ radical and the reductive elimination of substituted nitrophenols. Experiments were performed using a 6 MeV linear accelerator as described previously.[10] The absorbed radiation dose per electron pulse (typically 1–30 Gy) was determined by the thiocyanate dosimeter.[24]

For measurements of the reductive elimination of substituted nitrophenols from indolequinones over longer time-scales up to 10 s, a solid-state light source was developed to minimize possible sample photobleaching. The source uses a number of

narrow-band (15–30 nm) light-emitting diodes (LEDs) which cover the range ~430–900 nm. Thus 12 LEDs were positioned in front of an optical fiber and positioning was achieved using a rotating wheel servo system. The output end of the fiber was at the focus of an aspheric lens, producing a highly collimated beam to illuminate the sample cell. This novel system was utilized in combination with a tungsten lamp and photodiode detector to determine the rates of substituted nitrophenol release. For indolequinones where the disproportionation of semiquinone radicals could compete with the release of substituted nitrophenols a simulated data fitting model (FACSIMILE for Windows version 2.00, AEA Technology) provided estimates of rate constants from experimental data.

Steady-state γ-radiolysis

HPLC analysis was carried out using indolequinone solutions (40 μmol dm^{-3}) which were saturated with N_2O gas in gas-tight syringes before irradiation in a ^{60}Co source. An absorbed dose of 1 Gy equals 0.67 μmol dm^{-3} $(CH_3)_2C^{\bullet}OH$ radicals in the radiolysis of an N_2O-saturated propan-2-ol–water (50%, v/v) solution as determined by ferricyanide reduction. A dose rate of 3.9 Gy min^{-1} was used, as determined by Fricke dosimetry.[25]

High-performance liquid chromatography

Product analysis following γ-radiolysis of indolequinone solutions was performed by gradient separation on a 100 mm × 3.2 mm base-deactivated reverse-phase column (Hichrom RPB, Hichrom, Reading, UK) at a flow rate of 1 cm^3 min^{-1}. The eluents were A: 5 mmol dm^{-3} KH_2PO_4, 5 mmol dm^{-3} H_3PO_4 and B: 75% acetonitrile, 25% water with a gradient of 35–95% for 5–8 min. Detection was at 292 nm using a Waters 616 pump, 717 detector, 996 photodiode array detector and Millennium data acquisition (Watford, UK).

Synthesis

The synthesis of the following indolequinones has previously been described in the literature: 5-methoxy-1,2-dimethyl-3-(4-nitrophenoxymethyl)indole-4,7-dione **8**,[10] 3-hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione **10**,[10] 3-(1-hydroxyethyl)-5-methoxy-1,2-dimethylindole-4,7-dione **11**,[13] and 3-(isopropoxy)methyl-5-methoxy-1,2-dimethylindole-4,7-dione **12**.[10]

5-Methoxy-1,2-dimethyl-3-(2,4-dinitrophenoxymethyl)indole-4,7-dione 1. A solution of 3-hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione **10** (0.04 g, 0.17 mmol), 2,4-dinitrofluorobenzene (0.063 g, 0.34 mmol) and silver(I) oxide (0.079 g, 0.34 mmol) in THF (15 cm^3) was stirred at room temperature for 5 days. The crude mixture was filtered through Celite, and the filtrate concentrated and purified by column chromatography (20% ethyl acetate–80% light petroleum elution), to yield the title compound (0.015 g, 22%) as an orange crystalline solid, mp 203–205 °C (from ethyl acetate–light petroleum) (Found: C, 52.5; H, 3.5; N, 9.9. $C_{18}H_{15}N_3O_8 \cdot 0.5H_2O$ requires C, 52.9; H, 3.9; N, 10.2%); ν_{max} (KBr)/cm^{-1} 3114, 3047, 2925, 2848, 1686, 1643, 1604; λ_{max} (DMF)/nm 448 (log ε 3.21), 292 (4.38); δ_H (300 MHz; $CDCl_3$) 8.68 (1H, d, *J* 2.8, ArH), 8.38 (1H, dd, *J* 9.3, *J* 2.8, ArH), 7.57 (1H, d, *J* 9.3, ArH), 5.66, 5.65 (3H, 2s, CH_2, 6-H), 3.90 (3H, s, OMe), 3.83 (3H, s, NMe), 2.36 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 178.7, 178.5, 159.5, 156.1, 140.2, 139.2, 129.0 (CH), 128.9, 121.6 (CH), 120.9, 115.9 (CH), 114.3, 106.9 (CH), 62.7 (CH_2), 56.6 (OMe), 32.5 (NMe), 9.9 (Me); *m*/*z* (CI, relative intensity) 419 ($[M + NH_4]^+$, 100%).

General method for Mitsunobu reaction. Diethyl azodicarboxylate (4 equiv.) was added to a solution of the 3-hydroxymethylindolequinone **10** or the 3-(1-hydroxyethyl)-indolequinone **11** (0.2 mmol), triphenylphosphine (3 equiv.) and the nitrophenol (3 equiv.) in THF (15 cm^3). The solution was stirred for 1 h. The solvent was removed *in vacuo* and the residue dissolved in ethyl acetate and washed with sodium hydroxide (1 mol dm^{-3}), hydrochloric acid (1 mol dm^{-3}), water, dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography.

5-Methoxy-3-(2-methoxy-5-nitrophenoxymethyl)-1,2-dimethylindole-4,7-dione 2. Chromatography (80% dichloromethane–20% ethyl acetate elution), to yield the title compound (31%) as an orange crystalline solid, mp 253–255 °C (from dichloromethane–ethyl acetate) (Found: C, 57.6; H, 4.5; N, 6.9. $C_{19}H_{18}N_2O_7 \cdot 0.5H_2O$ requires C, 57.7; H, 4.8; N, 7.1%); ν_{max} (KBr)/cm^{-1} 3099, 2925, 2848, 1669, 1631, 1601; λ_{max} (DMF)/nm 456 (log ε 3.30), 340 (4.08), 292 (4.40); δ_H (300 MHz; $CDCl_3$) 7.91 (2H, m, ArH), 6.89 (1H, dd, *J* 7.3, *J* 2.2, ArH), 5.62 (1H, s, 6-H), 5.36 (2H, s, CH_2), 3.92 (3H, s, OMe), 3.90 (3H, s, OMe), 3.81 (3H, s, NMe), 2.32 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 178.8, 177.9, 159.7, 155.2, 147.9, 141.4, 138.1, 129.0, 121.6, 118.1 (CH), 115.8, 110.1 (CH), 109.2 (CH), 106.7 (CH), 61.9 (CH_2), 56.44 (OMe), 56.37 (OMe), 32.4 (NMe), 9.8 (Me); *m*/*z* (CI, relative intensity) 404 ($[M + NH_4]^+$, 100%).

5-Methoxy-3-(2-methoxy-4-nitrophenoxymethyl)-1,2-dimethylindole-4,7-dione 3. Chromatography (80% dichloromethane–20% ethyl acetate), to yield the title compound (75%) as an orange crystalline solid, mp 237–239 °C (from dichloromethane–ethyl acetate) (Found: C, 58.1; H, 4.6; N, 6.9. $C_{19}H_{18}N_2O_7 \cdot 0.3H_2O$ requires C, 58.2; H, 4.8; N, 7.2%); ν_{max} (KBr)/cm^{-1} 3088, 2945, 2914, 1669, 1633, 1602; λ_{max} (MeOH)/nm 456 (log ε 2.89), 336 (3.70), 292 (4.02); δ_H (300 MHz; $CDCl_3$) 7.86 (1H, dd, *J* 8.9, *J* 2.6, ArH), 7.71 (1H, d, *J* 2.6, ArH), 7.12 (1H, d, *J* 8.9, ArH), 5.62 (1H, s, 6-H), 5.44 (2H, s, CH_2), 3.90 (3H, s, OMe), 3.87 (3H, s, OMe), 3.81 (3H, s, NMe), 2.32 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 178.7, 178.3, 159.5, 153.5, 149.3, 141.5, 138.4, 128.8, 121.3, 117.8 (CH), 115.8, 112.0 (CH), 106.8 (CH), 106.6 (CH), 61.7 (CH_2), 56.5 (OMe), 56.3 (OMe), 32.4 (NMe), 9.9 (Me); *m*/*z* (EI, relative intensity) 386 (MH^+, 96%), 369 (62), 324 (47).

3-(2-Formyl-4-nitrophenoxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione 4. Chromatography (5% ethyl acetate–dichloromethane), to yield the title compound (46%) as a yellow–orange crystalline solid, mp 225–227 °C (from ether–hexane) (Found: C, 55.7; H, 4.1; N, 6.4. $C_{19}H_{16}N_2O_7 \cdot 1.4H_2O$ requires C, 55.7; H, 4.6; N, 6.8%) (Found: MH^+ 385.1042. $C_{19}H_{16}N_2O_7 + H$ requires 385.1035); ν_{max} (KBr)/cm^{-1} 2960, 2842, 1690, 1642, 1597; λ_{max} (MeOH)/nm 456 (log ε 3.02), 320 (4.07), 292 (4.32); δ_H (300 MHz; $CDCl_3$) 10.4 (1H, s, CHO), 8.67 (1H, d, *J* 2.9, ArH), 8.41 (1H, dd, *J* 9.2, *J* 2.9, ArH), 7.39 (1H, d, *J* 9.2, ArH), 5.66 (1H, s, H-6), 5.56 (2H, s, CH_2), 3.92 (3H, s, OMe), 3.83 (3H, s, NMe), 2.34 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 187.5, 178.6, 178.4, 166.7, 164.7, 159.6, 141.7, 138.2, 130.6 (CH), 124.9, 124.6 (CH), 121.2, 114.8, 114.1 (CH), 106.9 (CH), 61.9 (CH_2), 56.5 (OMe), 32.5 (NMe), 9.9 (Me); *m*/*z* (CI, relative intensity) 385 (MH^+, 100%), 357 (46), 340 (30), 292 (33).

3-(3-Formyl-4-nitrophenoxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione 5. Chromatography (5% ethyl acetate–dichloromethane), to yield the title compound (18%) as an orange crystalline solid, mp 214–216 °C (from ethyl acetate–hexane) (Found: C, 58.7; H, 4.0; N, 7.0. $C_{19}H_{16}N_2O_7 \cdot 0.25H_2O$ requires C, 58.7; H, 4.3; N, 7.2%); ν_{max} (KBr)/cm^{-1} 3068, 2924, 2848, 1735, 1693, 1671, 1643, 1595; λ_{max} (DMF)/nm 448 (log ε 3.22), 332 (4.00), 288 (4.32); δ_H (300 MHz; $CDCl_3$) 10.50 (1H, s, CHO), 8.14 (1H, d, *J* 9.1, ArH), 7.40 (1H, d, *J* 2.8, ArH), 7.25 (1H, dd, *J* 9.1, *J* 2.8, ArH), 5.64 (1H, s, 6-H), 5.41 (2H, s, CH_2), 3.91 (3H, s, OMe), 3.82 (3H, s, NMe), 2.31 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 188.5, 178.7, 178.2, 163.1, 159.6, 142.2, 138.1,

134.3, 129.1, 127.3 (CH), 121.3, 118.7 (CH), 115.1, 115.0 (CH), 106.8 (CH), 61.5 (CH_2), 56.5 (OMe), 32.4 (NMe), 9.8 (Me); *m*/*z* (CI, relative intensity) 402 ($[M + NH_4]^+$, 5%), 222 (30), 220 (20), 192 (55), 138 (100).

3-(2-Fluoro-4-nitrophenoxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione 6. Chromatography (5% ethyl acetate–dichloromethane), to yield the title compound (68%) as an orange crystalline solid, mp 219–221 °C (from ethyl acetate–hexane) (Found: C, 57.4; H, 3.8; N, 7.4. $C_{18}H_{15}FN_2O_6$ requires C, 57.7; H, 4.0; N, 7.5%); ν_{max} (KBr)/cm^{-1} 3073, 2945, 2837, 1675, 1642, 1598; λ_{max} (DMF)/nm 448 (log ε 3.19), 316 (4.10), 288 (4.25); δ_H (300 MHz; $CDCl_3$) 8.02 (1H, m, ArH), 7.94 (1H, dd, *J* 10.6, *J* 2.7, ArH), 7.27 (1H, m, ArH), 5.64 (1H, s, 6-H), 5.48 (2H, s, CH_2), 3.89 (3H, s, OMe), 3.82 (3H, s, NMe), 2.33 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 178.6, 178.4, 159.6, 152.2 (d, J_{CF} 10), 151.5 (d, J_{CF} 249), 141.0 (d, J_{CF} 7), 138.4, 129.0, 121.2, 121.0 (d, J_{CF} 4), 115.2, 114.2 (d, J_{CF} 2), 112.4 (d, J_{CF} 23), 106.8 (CH), 62.0 (CH_2), 56.5 (OMe), 32.4 (NMe), 9.8 (Me); *m*/*z* (CI, relative intensity) 392 ($[M + NH_4]^+$, 3%), 235 (18), 220 ($M^+ - C_6H_3FNO_2$, 100).

3-[1-(2-Fluoro-4-nitrophenoxy)ethyl]-5-methoxy-1,2-dimethylindole-4,7-dione 7. Chromatography (5% ethyl acetate–dichloromethane), to yield the title compound (84%) as an orange crystalline solid, mp 175–177 °C (from ethyl acetate–hexane) (Found: C, 58.7; H, 4.2; N, 7.1. $C_{19}H_{17}FN_2O_6$ requires C, 58.7; H, 4.4; N, 7.2%); ν_{max} (KBr)/cm^{-1} 3109, 3006, 2945, 1671, 1619, 1593; λ_{max} (MeOH)/nm 460 (log ε 3.11), 292 (4.16); δ_H (300 MHz; $CDCl_3$) 7.92 (2H, m, ArH), 7.00 (1H, apparent t, ArH), 6.45 (1H, q, *J* 6.5, C*H*(Me)OAr), 5.65 (1H, s, 6-H), 3.85 (3H, s, OMe), 3.82 (3H, s, NMe), 2.33 (3H, s, Me), 1.73 (3H, d, *J* 6.5, Me); δ_C (100 MHz; $CDCl_3$) 178.7, 178.4, 159.5, 152.7, 151.4 (d, J_{CF} 249), 151.3 (d, J_{CF} 10), 140.6 (d, J_{CF} 8), 135.8, 128.5, 121.6, 120.9 (d, J_{CF} 4), 120.0, 114.4 (d, J_{CF} 2), 112.2 (d, J_{CF} 23), 106.9 (CH), 70.2 (CH), 56.5 (OMe), 32.1 (NMe), 21.9 (Me), 10.4 (Me); *m*/*z* (CI, relative intensity) 406 ($[M + NH_4]^+$, 28%), 389 (MH^+, 73), 317 (70), 290 (60).

5-Methoxy-1,2-dimethyl-3-(4-nitrophenylthiomethyl)indole-4,7-dione 9. Chromatography (5% ethyl acetate–dichloromethane elution) to yield the title compound (16%) as an orange crystalline solid, mp 215–217 °C (from dichloromethane–ethyl acetate) (Found: C, 57.5; H, 4.0; N, 7.3. $C_{18}H_{16}N_2O_5S \cdot 0.2H_2O$ requires C, 57.5; H, 4.4; N, 7.4%); ν_{max} (KBr)/cm^{-1} 2851, 1665, 1639, 1601; λ_{max} (MeOH)/nm 460 (log ε 3.09), 340 (3.94), 292 (4.05); δ_H (300 MHz; $CDCl_3$) 8.10 (2H, *J* 7.0, ArH), 7.46 (2H, *J* 7.0, ArH), 5.62 (1H, s, 6-H), 4.49 (2H, s, CH_2), 3.89 (3H, s, OMe), 3.81 (3H, s, NMe), 2.25 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 178.5, 178.0, 159.6, 147.0, 145.4, 136.8, 128.8, 127.7 (CH), 123.8 (CH), 121.0, 116.0, 106.8 (CH), 56.5 (OMe), 32.5 (NMe), 27.0 (CH_2), 9.6 (Me); *m*/*z* (CI, relative intensity) 390 ($[M + NH_4]^+$, 83%), 373 (MH^+, 100).

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References

1 W. N. Speckamp and E. A. Oostveen, International Patent: WO 87/06227, 1987.
2 E. A. Oostveen and W. N. Speckamp, *Tetrahedron*, 1987, **43**, 255.
3 M. Maliepaard, N. J. de Mol, L. H. M. Janssen, J. C. Hoogvliet, W. van der Neut, W. Verboom and D. N. Reinhoudt, *J. Med. Chem.*, 1993, **36**, 2091.
4 G. E. Adams and I. J. Stratford, *Int. J. Radiat. Oncol. Biol. Phys.*, 1994, **29**, 231.
5 A. S. Cotterill, C. J. Moody, R. J. Mortimer, C. L. Norton, N. O'Sullivan, M. A. Stephens, N. R. Stradiotto, E. Swann and I. J. Stratford, *J. Med. Chem.*, 1994, **37**, 3834.
6 M. P. Saunders, M. Jaffar, A. V. Patterson, J. Nolan, M. A. Naylor, R. M. Phillips, A. L. Harris and I. J. Stratford, *Biochem. Pharmacol.*, 2000, **59**, 993.
7 M. A. Naylor, M. Jaffar, M. A. Stephens, S. Butler, K. B. Patel, S. A. Everett, G. E. Adams and I. J. Stratford, *J. Med. Chem.*, 1997, **40**, 2335.
8 C. J. Moody and E. Swann, *Farmaco*, 1997, **52**, 271.
9 M. Jaffar, M. A. Naylor, N. Robertson and I. J. Stratford, *Anti-Cancer Drug Des.*, 1998, **13**, 593.
10 M. A. Naylor, E. Swann, S. A. Everett, M. Jaffar, J. Nolan, N. Robertson, S. D. Lockyer, K. B. Patel, M. F. Dennis, M. R. L. Stratford, P. Wardman, G. E. Adams, C. J. Moody and I. J. Stratford, *J. Med. Chem.*, 1998, **41**, 2720.
11 S. A. Everett, M. A. Naylor, J. Nolan, K. B. Patel and P. Wardman, *Anti-Cancer Drug Des.*, 1998, **13**, 635.
12 P. Wardman, *Curr. Med. Chem.*, 2001, **8**, 739.
13 S. A. Everett, M. A. Naylor, P. Barraja, E. Swann, K. B. Patel, M. R. L. Stratford, A. R. Hudnott, B. Vojnovic, R. J. Locke, P. Wardman and C. J. Moody, *J. Chem. Soc., Perkin Trans. 2*, 2001, 843.
14 H. D. Beall, S. Winski, E. Swann, A. R. Hudnott, A. S. Cotterill, N. O'Sullivan, S. J. Green, R. Bien, D. Siegel, D. Ross and C. J. Moody, *J. Med. Chem.*, 1998, **41**, 4755.
15 R. M. Phillips, M. A. Naylor, M. Jaffar, S. W. Doughty, S. A. Everett, A. G. Breen, G. A. Choudry and I. J. Stratford, *J. Med. Chem.*, 1999, **42**, 4071.
16 M. Meot-Ner, P. Neta, R. K. Norris and K. Wilson, *J. Phys. Chem.*, 1986, **90**, 168.
17 J. P. Bays, S. T. Blumer, S. Baral-Tosh, D. Behar and P. Neta, *J. Am. Chem. Soc.*, 1983, **103**, 320.
18 C. P. Andrieux, J.-M. Savéant, A. Tallec, R. Tardivel and C. Tardy, *J. Am. Chem. Soc.*, 1996, **118**, 9788.
19 M. L. Andersen, N. Mathivanan and D. D. M. Wayner, *J. Am. Chem. Soc.*, 1996, **118**, 4871.
20 W. A. Denny, W. R. Wilson and M. P. Hay, *Br. J. Cancer*, 1996, **74 (Suppl. XXVII)**, S32.
21 W. R. Wilson, M. Tercel, R. F. Anderson and W. A. Denny, *Anti-Cancer Drug Des.*, 1998, **13**, 663.
22 R. F. Anderson, W. A. Denny, W. Li, J. E. Packer, M. Tercel and W. R. Wilson, *J. Phys. Chem. A*, 1997, **101**, 9704.
23 For example, see: (*a*) D. J. Hupe and W. P. Jencks, *J. Am. Chem. Soc.*, 1977, **99**, 451; (*b*) J. L. Jensen and W. P. Jencks, *J. Am. Chem. Soc.*, 1979, **101**, 1476; (*c*) K. T. Douglas and M. Alborz, *J. Chem. Soc., Chem. Commun.*, 1981, 551; (*d*) E. Castro and C. Ureta, *J. Org. Chem.*, 1989, **54**, 2153; E. Castro and C. Ureta, *J. Chem. Soc., Perkin Trans. 2*, 1991, 63.
24 B. H. J. Bielski, *Radiat. Phys. Chem.*, 1993, **41**, 527.
25 K. Sehested, O. L. Rasmussen and H. Fricke, *J. Phys. Chem.*, 1968, **72**, 626.