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Electrochemical, *in vitro* and cell culture analysis of integrated redox catalysts: implications for cancer therapy

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Integrated catalysts as redox sensitisers towards cancer.

Various malignant carcinomas, such as the human kidney, lung and prostate, are characterised by the presence of oxidative stressors. These cells also have a significantly reduced level of the essential antioxidant enzymes glutathione peroxidase (GPx) and catalase.[1,2] We have recently proposed to utilise this abnormal redox state to selectively target carcinomas by supplementation with redox catalysts.[3] Organoselenides and organotellurides efficiently mimic the activity of the selenoenzyme GPx (Fig. 1). In contrast to GPx, these mimics lack substrate specificity and can utilise a structurally diverse range of cellular thiols in their redox cycle, a toxic process in cells with low glutathione levels. This approach therefore has the potential to selectively kill oxidatively stressed cancer cells with a mutated antioxidant defence while being non-toxic to surrounding cells with a normal redox balance.

Theoretically, catalytic drugs have many advantages over conventional, stoichiometric chemotherapy: high selectivity for cancer cells, low drug concentration for therapeutic effect and a reliance on internal stressors, removing the requirement for external stressors such as radiation. Our initial work has demonstrated that this approach is effective in an *in vitro* cell culture tumour model.[3] A high catalyst concentration (μM) was, however, required to exert a significant cytotoxic effect, emphasising the urgent need to develop more efficient catalysts.

As an alternative to investigating the effect of simple structural alterations we have adopted an "integrated" approach, to increase efficiency by incorporating an additional catalytic centre into the molecule. A quinone redox system was chosen because of its known activity in bioreductive anticancer agents.[4] Like the chalcogen centre, quinone agents utilise several mechanisms to exert a cytotoxic effect[5] and of especial interest in the development of integrated catalysts is their ability to redox cycle with triplet oxygen, forming superoxide and peroxide in the process. This is especially advantageous as the peroxide formed as a result of redox cycling of the quinone moiety has the potential to activate the chalcogen moiety of the catalyst. Although bioreductive agents act *via* a different mechanism (the prodrug is activated by cellular reductase enzymes whose expression varies widely between cell lines),[6] the sensitivity towards the status of the cellular redox environment makes quinone redox systems interesting to both approaches.

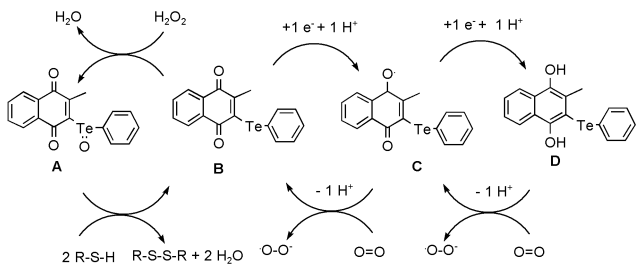

Fig. 1 Redox-cycling of integrated catalysts. Oxidation of integrated catalyst **B** at the chalcogen centre leads to the telluroxide **A** that can be reduced by thiol proteins regenerating the catalyst, **B**. Alternatively, one electron reduction of **B** by NADH, catalysed by quinone metabolising enzymes, leads to the semiquinone radical **C**. This can undergo further reduction to the dihydroquinone **D**. Oxidation of either **C** or **D** by molecular oxygen results in the generation of superoxide and regeneration of **B**.

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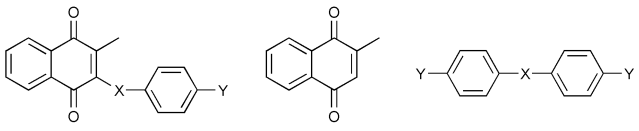
We report here the synthesis, electrochemical, *in vitro* and cell culture analysis of structurally related, integrated catalysts. We show that the chalcogen centre provides GPx activity, increasing the rate of thiol oxidation in the presence of oxidative stressors. The quinone component is also redox active and possibly gives rise to superoxide generation.

Compounds **1–4** were synthesised *via* bromination of **5** followed by nucleophilic displacement of the bromine substituent by the chalcogen anion following established procedures.[7,8] Cyclic voltammograms were recorded using a glassy carbon electrode at scan rates of 100 to 500 mV s^{-1} from a potential of −600 mV to +1400 mV *vs.* the standard Ag/AgCl electrode (SSE) (Figs. 2, 3) as described previously.[9]



Comp	X	Y	Quinone Epa_1/mV	Quinone Epc_1/mV	Chalcogen Epa_2/mV	MT Zn Release ± 5% (no peroxide)	MT Zn Release ± 5% (with peroxide)	CR r x 10^{-9} Ms^{-1}
1	S	OCH_3	-70	-302	+1348	3	12	8.6
2	Se	OCH_3	-57	-328	+1128	3	12	5.4
3	Te	H	-115	-341	+823	7	28	8.7
4	Te	OCH_3	-116	-341	+760	5	28	2.7
5	-	-	-166	-277	-	2	9	29.2

Fig. 2 Electrochemical and *in vitro* characterisation of integrated catalysts. Potentials are given *vs.* SSE and are standardised against the ferrocene reference couple[18] measured under the same conditions (scan rate 200 mV s^{-1}). Other experimental details are given in the text.

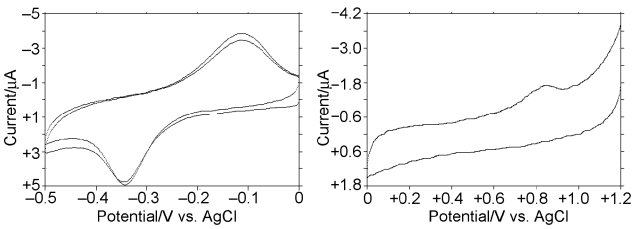


Fig. 3 Cyclic voltammogram of an integrated catalyst containing two redox active sites. **3** (100 μM) was scanned at 200 mV s^{-1} with a glassy carbon electrode and SSE in potassium phosphate buffer (50 mM containing 30% MeOH, pH 7.4). a) Quinone redox couple; b) telluride oxidation peak.

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1–4 exhibited a combination of two distinct redox behaviours: one quinone redox couple (Epa$_1$ and Epc$_1$) at around −200 mV *vs*. SSE and an irreversible oxidation peak (Epa$_2$) between +760 mV (**4**) to +1350 (**1**) characteristic of chalcogen oxidation.[9] The quinone couple exhibited quasi-reversible characteristics[10] with a peak current ratio ($|I$pa$_1/I$pc$_1|$) ≈ 0.7, and transfer coefficients for $\alpha_c n_\alpha$ between 0.9 (**3**) to 1.0 (**4**) and for $\alpha_a n_\alpha$ between 0.6 (**3**) to 0.7 (**4**). Taking α as 0.5 this would imply n_α values of 1.8 for the reduction and 1.4 for the oxidation peak. Since the quinone redox centre was oxidised well before the chalcogen centre, electron withdrawing effects of the quinone led to more positive oxidation potentials for the chalcogen atoms in **2** and **4** when compared to their previously studied symmetrical selenium **6** (Epa = +753 mV) and tellurium **7** (Epa = +368 mV) analogues.[9] Overall, the electrochemical studies confirmed the presence of two fully active, yet interdependent redox centres in **1–4**.

The resulting biochemical activities were evaluated in two bioassays indicative of chalcogen and quinone redox activity *in vitro*. The metallothionein (MT) zinc release assay measures the ability of agents to catalyse the reaction of *tert*-butyl hydroperoxide (*t*-BuOOH) with the biologically abundant zinc/sulfur protein MT.[11–13] Compounds were evaluated according to the total extent of zinc release after 60 min.[12] Maximum zinc release was measured using ebselen as standard and activities are expressed as a percentage of this value.

Figure 2 shows that zinc release from MT in the presence of *t*-BuOOH was significantly enhanced by **1–4**, confirming a peroxidase-like activity. **1** and **2** had a similar, yet low activity while both the tellurium analogues caused a significantly higher zinc release, confirming a previously reported correlation between Epa and zinc release.[9] The presence of the quinone moiety in **4** seems to be detrimental to activity as this compound has both a higher Epa (+760 mV) value and lower activity (28%) than its symmetrical analogue **7** (Epa = +368 mV and 53% zinc release).[9]

The activity of the quinone moiety was assessed in a coupled cytochrome *c* reductase (CR) assay measuring the rate (r) of oxidation of NADH.[14] Porcine heart CR (5 μM), NADH (500 μM) and organochalcogens (50 μM) were incubated together in potassium phosphate buffer (10 mM, pH 7.5) for 10 min. On incubation of NADH with quinone **5** over a 60 min time period, 5 equivalents of NADH were consumed. This oxidation of NADH beyond 2 equivalents per quinone is incompatible with a stoichiometric oxidation as only 2 electrons are required to fully reduce the quinone to the dihydroquinone, suggesting that the dihydroquinone moiety is not the final reduction product and that the reaction involves the generation of the superoxide radical anion, a process frequently observed for quinones such as plumbagin under aerobic conditions *in vitro* (Fig. 1).[15] Regardless of whether the mechanism of the initial quinone reduction step involves a one or two electron transfer, quinones are able to initiate the reduction of triplet oxygen to the superoxide radical anion ($O_2^{-\cdot}$).[16] The superoxide anion is a strong base which can abstract a proton from a biological matrix, forming the hydroperoxyl radical ($HO_2\cdot$), a one electron oxidising species (overall $O_2^{-\cdot} + e^- + 2\ H^+$ / HOOH, $E°$ = +890 mV *vs*. NHE).[17] Hydrogen peroxide formed by either the reduction or the disproportionation of the hydroperoxyl radical also acts as an oxidative stressor (HOOH + 2 H^+ + 2e^- / 2 H_2O, $E°$ = +1760 mV *vs*. NHE).[17]

The effect of H_2O_2 on the pro-oxidant activity of an integrated catalyst was further evaluated in a PC12 cell culture model of cancer.[3] A dose response curve for **3**, the most active compound in both the *in vitro* CR and MT assays was established (Fig. 4a). The effects of H_2O_2 (200 μM) together with the increasing concentrations of either **3** or the control quinone **5** on cell survival were also examined (Fig. 4b). At low concentrations (100 nM), **3** was non-toxic to cells not challenged by H_2O_2, but in the presence of this oxidative stressor cell survival dramatically dropped to 45%. While the parent quinone **5** also promoted cell death under oxidative stress conditions, a significantly higher concentration (3.2 μM) was required to obtain the same effect. **3** was also far superior to **8**, the most active GPx mimic studied earlier.[3] Administration of **8** (10 μM) reduced cell survival to 45%, whereas the same effect was observed for **3** at 100 nM concentration, an increase in efficacy of two orders of magnitude.

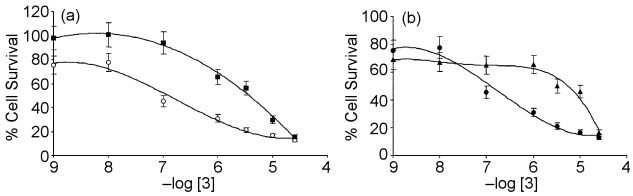

Fig. 4 Effect of **3** and **5** on PC 12 cells. a) **3** in the absence (■) and presence (○) of H_2O_2 (200 μM); b) **3** (●) and **5** (▲) with H_2O_2 (200 μM). Error bars represent the standard deviation (n = 12).

In conclusion, the integrated catalyst **3** was considerably more active in cell culture when compared to the parent quinone **5** and GPx mimic **8**. The *in vitro* results suggest that this increased activity in cell culture is likely to be the result of combining two different redox centres, hence widening the agent's target range, rather than an increase in activity of one individual redox centre. The notion that small quantities of catalysts sensitive to their redox environment can facilitate redox processes utilising species already present in cells could be used in future for therapeutic treatments. Integrated catalysts might be able to effectively, yet selectively, kill certain types of cancer cells whilst being non-toxic towards differentiated cells.

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