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Targeting oxidative stress-related diseases: organochalcogen catalysts as redox sensitizers

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Abstract

Tumor cells proliferate under conditions of oxidative stress. A novel therapeutic approach would be to enhance the cellular effects of the reactive oxygen species formed under these conditions by supplementation with a redox catalyst. This provides a means to target and specifically destroy cancer cells *via* oxidation of redox-sensitive proteins, such as transcription factors, while leaving cells with a normal redox balance largely unaffected. We have previously reported a preliminary observation on the effects of pro-oxidant catalysts that enhance cancer cell death. This paper presents a detailed *in vitro* investigation into the mechanism of action of synthetic glutathione peroxidase mimics on a model Sp1 transcription factor peptide. The structure and redox potential of these mimics correlate with their ability to catalyze the oxidation of this zinc-binding motif by H_2O_2 and these compounds promise therapeutic potential by promoting H_2O_2-induced PC12 cell death.

Keywords: Oxidative stress; Redox catalysts; Chemotherapy; Zinc finger; Cyclic voltammetry

1. Introduction

A multitude of human diseases, including autoimmune, inflammatory, neurodegenerative, viral, and proliferative, are connected to an important biochemical condition known as oxidative stress [1]. Oxidative stress occurs when a redox imbalance exists within the cell that results in the production of high concentrations of toxic species, such as reactive oxygen (ROS), nitrogen (RNS), and sulfur (RSS) species [2–4]. ROS can readily oxidize and therefore inactivate redox-sensitive proteins and also damage membranes and DNA. To prevent the accumulation of such deleterious species, the cell harbors several defense systems that form non-toxic species from such stressor molecules [2]. In certain animal and human cancer cells, however, the redox balance is tilted in favor of oxidation due to low antioxidant enzyme levels [5].

A possible approach to cancer therapy is to utilize this abnormal redox balance to modify the activity of essential proteins and so selectively target cancer cells [6]. A promising candidate is the zinc finger structural motif, which is present in as many as 1% of mammalian proteins. These motifs comprise one or more cysteine ligands and are frequently found in transcription factors where they are a prerequisite for their DNA-binding and transcriptional functions [7]. Since the thiol group of cysteine must be in the reduced state to bind zinc, oxidation of the thiol group leads to the expulsion of zinc, collapse of the zinc finger secondary structure, and inability of the transcription factor to bind to DNA.

The zinc finger motif is contained in the transcription factors AP1, Sp1 Egr1, NFκB, p53, and many other regulatory proteins, such as replication protein A (RPA). Together these zinc finger-containing proteins regulate DNA replication and elongation as well as nucleotide

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Abbreviations: AAS, atomic absorption spectrometry; CV, cyclic voltammetry; DME, dropping mercury electrode; DTDP, 2,2′-dithiodipyridine; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); E_{pa}, anodic oxidation potential; E_{pc}, cathodic reduction potential; GCE, glassy carbon electrode; GPx, glutathione peroxidase; GSSG, glutathione disulfide; HUVEC, human umbilical vein endothelial cells; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; ROS, reactive oxygen species; ZnF, zinc finger peptide fragment.

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excision and repair [8,9]. As such they influence both the rate of cellular proliferation and expression of phenotype. Sp1 belongs to a large family of transcription factors, which have three zinc finger motifs in the C-terminal region. It is an 81 amino acid protein that binds to one of the most common regulatory elements found in promoters, enhancers, and control regions of housekeeping and tissue specific genes called the GC box (GGGGCGGGG). Expression of Sp1 is ubiquitous and implicated in the regulation of numerous genes, including those involved in cell cycle regulation and glycolysis, and well as some non-housekeeping genes, such as metallothionein (MT) [7,10]. On the basis that Sp1 has demonstrated involvement in growth regulation and shown interaction with tumor supressors, it is also likely to be implicated in tumor development [11]. Increase in the level of Sp1 mRNA and Sp1 DNA-binding activity has been reported in epithelial tumors, thus contributing to skin tumor progression [12]. Sp1 has also been implicated in the induction of $cPLA_2$ in lung cancer [13]. Apart from being the most fully characterized transcription factor, it has also been demonstrated that Sp1 is redox-sensitive *in vitro* and *in vivo* and that its oxidative inactivation leads to transcriptional repression of endogenous genes [8,14].

Modification of the cysteine residues in zinc motifs, which alters their redox state and ability to coordinate metal ions, has been considered an attractive target in the design of antiviral and antitumor drugs. This would in turn prevent DNA binding, transcription, protein synthesis, and proliferation, all of which will eventually lead to tumor cell and viral death. Thiol modification can be achieved either by oxidation or by exploiting its reaction with electrophiles [15,16]. It has already been demonstrated that electrophilic compounds react and oxidize retroviral nucleocaspid proteins releasing zinc in the process [17]. The electrochemical analysis of these compounds can be used to predict their activity against retroviral zinc fingers hence assisting in their rational design [17]. It has also been established that agents that destroy the zinc finger structure by chelating zinc (such as picolinic acid) have the therapeutic potential in the control of viral disease and cancer [18]. The inherent problems with administering such agents are the large concentrations required to exert a therapeutic effect and the lack of specificity for the cancer phenotype.

As an alternative strategy, it should be possible to utilize the condition of oxidative stress within certain cancer cells, where levels of ROS are high and GSH levels are low, to catalyze the oxidative destruction of the zinc finger secondary structure. This would, in theory, specifically target cancer cells while leaving cells with normal redox balance largely unaffected. So far little progress has been made in the direction of such catalytic drugs. Studies have shown that the response to oxidative stress alone can activate or inactivate many redox-sensitive factors [19]. As a consequence, the administration of a catalytic agent to increase this basal rate of protein inactivation could, even at small doses, have dramatic effects on transcription factor activity.

A good candidate for this study are mimics of the selenoenzyme glutathione peroxidase (GPx). GPx oxidizes the thiol group of the commonly occurring cellular peptide GSH to the disulfide (GSSG), in the process consuming an oxidant, such as hydrogen peroxide or peroxynitrite. Molecular mimics of this enzyme catalyze the same reaction but frequently lack the substrate specificity of the native enzyme, oxidizing a range of different thiol moieties in the presence of oxidative stressors. Mechanistically, these catalysts undergo oxidation of the chalcogen atom by the ROS, followed by regeneration of the catalyst by reduction with thiols, forming disulfides in the process.

We have recently reported the ability of such peroxidation catalysts to actively destroy the structure of transcription factors and also promote cancer cell death only under oxidative stress conditions [6]. In this paper, we present a preliminary investigation to characterize the oxidative effects of these catalysts on a protein fragment and in cell culture. We demonstrate a correlation between the structures of these compounds, their redox potential, and their catalytic activity in oxidation of a 2-Cys–2-His zinc finger peptide of Sp1. We have previously established a model for the oxidative environment of a tumor by incubating PC12 cells with hydrogen peroxide [6]. Here, we further characterize the ability of these redox catalysts to selectively kill cells under these stress conditions. To evaluate any deleterious effects of the catalysts on cell survival in the *absence* of oxidative stressors, we also show that the catalysts are non-toxic to both, unstressed PC12 cells and normal, non-tumorogenic human umbilical vein endothelial cells (HUVECs).

2. Materials and methods

2.1. Materials

Synthetic GPx mimics were prepared as previously described [20]. The zinc finger peptide fragment (ZnF) of transcription factor Sp1 (ZnF) KFACPECPKRFMRSDHLSKHIKTHQNKK [21] was purchased from the Peptide Synthesis Unit IBMS, University of Southampton (purified to >95% as determined by HPLC). $ZnSO_4$ (99.999%), buffers, thiol reagents, methanol, hydrogen peroxide, and catalase (bovine liver) were purchased from Sigma-Aldrich. Tissue culture grade PBS, fetal bovine serum, L-glutamine, trypsin, and gentamicin were purchased from Gibco. RMPI 1640 (RO883) medium, horse serum, nutrient mixture F-12 HAM medium, fibronectin, hydrogen peroxide, water, endothelial cell growth supplement, and heparin were all purchased from Sigma-Aldrich. The 1000 mg/L zinc standard ''SpectrosoL'' and 69% nitric acid for atomic absorption spectrometry (AAS) were purchased from BDH. All commercial chemicals were

analytical grade and needed no further purification. Solutions and buffers were prepared in MilliQ water and nitrogen flushed prior to use. Rat adrenal PC12 cell lines were obtained from ECACC. HUVECs were isolated from human umbilical cords of normal pregnancies delivered at The Royal Devon and Exeter Hospital with Local Research Ethics Committee approval.

Atomic absorption spectrometry was performed on a Perkin Elmer Analyst 100. UV-Vis spectra were recorded on a CARY 50Bio spectrophotometer (Varian). Cyclic voltammetry (CV) was performed on a 100B/W workstation (BAS). Cells were incubated in a Sanyo CO_2 incubator and cell viability measured using a Dynex Technologies MRX microplate reader. Experiments were repeated in triplicate and data are expressed as the mean ± SD.

2.2. Methods

2.2.1. Preparation and characterization of Zn_1–ZnF

Zn_1–ZnF was prepared from ZnF and $ZnSO_4$ according to an established procedure [22,23]. The concentration of peptide was based on thiol (2:1 SH/ZnF) and amino (7:1 NH_2/ZnF) content. Total thiol content was determined by measurement of DTDP absorbance ($\varepsilon_{343} = 6600\ M^{-1}\ cm^{-1}$) after reaction with the thiol groups of the ZnF. A quantitative ninhydrin assay was used to measure the number of lysine residues ($\varepsilon_{570} = 23{,}200\ M^{-1}\ cm^{-1}$) [24]. Atomic absorption spectroscopy was carried out in order to confirm the number of zinc ions bound per ZnF.

2.2.2. In vitro oxidation of Zn_1–ZnF

Zn_1–ZnF (5 μM) was incubated at 37° with varying concentrations of catalyst (nanomolar range) and H_2O_2 (250 μM) in Tris–HCl (20 mM, pH 7.4) for 10 min. The reaction was aborted by incubating with catalase (1 nM) at room temperature for a further 2 min before adding 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (100 μM) and taking absorbance measurements after a further 5 min incubation period at room temperature to allow the color to develop. DTNB (Ellman's reagent) allows the presence of the thiol group of cysteine to be monitored spectrophotometrically at 412 nm ($\varepsilon_{412} = 13{,}600\ M^{-1}\ cm^{-1}$) due to the release of the 5-thio-2-nitrobenzoate ion (TNB^-). Methanol was used as a control.

2.2.3. Cyclic voltammetry

Cyclic voltammograms of organochalcogens (50–100 μM) were recorded in potassium phosphate buffer (50 mM, pH 7.4) containing 30% methanol (due to the limited solubility of the organochalcogens in aqueous media) using a standard Ag/AgCl reference electrode (SSE) and a glassy carbon (GCE) working electrode. Counter and working electrodes were thoroughly cleaned and polished (Al_2O_3) after each scan. The CV of the ZnF (20 μM) was recorded at a scan rate of 500 mV/s, using a dropping mercury (DME) working electrode on which a new drop (with a clean surface) was generated for each scan.

2.2.4. Cell culture

Rat adrenal PC12 cells were cultured in suspension in RPMI 1640 supplemented with glutamine (2 mM), horse serum (10%), heat-inactivated fetal bovine serum (5%), and gentamicin (250 units/mL) at 5% CO_2 and 37° [25]. Cells were fed three times a week and sub-cultured every 7 days. For experimentation, undifferentiated cells were plated at 100,000 cells per well into 96-well tissue culture plates.

HUVECs were isolated from human umbilical cords within 24 hr of birth from normal pregnancies according to an established procedure [26]. Cells were cultured in Complete HAM's [Nutrient mixture F-12 HAM medium, gentamicin (50 μg/mL), L-glutamine (0.68 mM), and fetal bovine serum (20%)] in uncoated flasks (37°; 5% CO_2, 95% air). After 24 hr cells were treated with trypsin for 3 min and then passaged into fibronectin (20 μg/mL)-coated flasks. Cells were then cultured in Complete HAM's containing Endothelial Cell Growth Supplement (20 μg/mL) and heparin (90 μg/mL) until a confluent monolayer was formed. For experimentation, the cells were treated with trypsin, counted, and plated at 10,000 cells per well into a fibronectin-coated 96-well plate. Cells were incubated for a period of 3 days to form a confluent monolayer.

2.2.5. Measurement of cell survival by the MTT assay

Cell viability was measured using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay in which the reduction of MTT to formazan crystals is catalyzed by mitochondrial dehydrogenase [27]. MTT (0.5 mg/mL, in PBS) was added to the 96-well plate (final concentration, 0.1 mg/mL) and incubated for 3 hr. The reduced MTT crystals were solubilized in 30% DMSO and the colorimetric evaluation of reduced MTT was made at 540 nm.

2.2.6. Evaluation of organochalcogens as redox sensitizers towards cells

The PC12 cells were plated and then pre-incubated for 1 hr with varying concentrations (1 nM–25 μM) of catalyst **5** (selected as the most active compound from above *in vitro* studies) at 37° and 5% CO_2, followed by the addition of H_2O_2 (200 μM) and overnight incubation. Cell viability was then determined using the MTT assay. In a separate experiment, cells were pre-incubated with **5** (10 μM) followed by addition of varying H_2O_2 concentrations (50–500 μM). A H_2O_2 dose–response curve (no compound) was also established as a control (data not shown). **4**–**7** and the commercially available organoselenide ebselen (2-phenyl-1,2-benzisoselenazol-3(2*H*)-one) were all tested at a set concentration (10 μM) in the presence of H_2O_2 (200 μM). All compounds were initially solubilized

in methanol and diluted in medium to the appropriate concentration with 2% methanol to maintain solubility. Methanol and also catalyst alone (no H_2O_2) were incubated as established controls. Cell viability was expressed as a percentage of normal growth (in absence of compounds and H_2O_2). Individual treatments consisted of 6 wells per experiment of which experiments were replicated at least four times and presented as an average (mean ± SD). As a test of toxicity on non-tumorogenic cells, HUVECs were also treated with ebselen and **4**–**7** (10 μM) and cell survival was determined using the MTT assay.

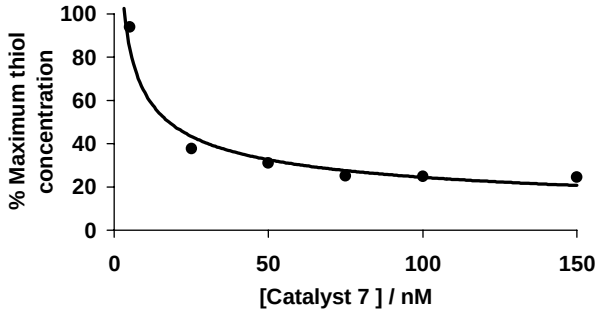


Fig. 2. Oxidation of Sp1 zinc finger by catalyst **7** measured in the DTNB assay. The progression of Zn_1–ZnF oxidation was monitored at 412 nm (generation of TNB^-). Zn_1–ZnF (5 μM) was incubated with H_2O_2 (250 μM) and increasing concentrations of catalyst for 10 min at 37° in 20 mM Tris–HCl buffer (pH 7.4).

3. Results

3.1. Electrochemical analysis of Zn_1–ZnF

Cyclic voltammetry of the zinc finger peptide using the DME gave an oxidation peak in the region of −487 mV and a reduction peak in the region of −560 mV (Fig. 1). The redox potential $E^{\circ\prime}$ for the thiol/disulfide redox couple of zinc finger peptide is therefore calculated as −524 mV vs. SSE.

3.2. Effect of peroxidation catalysts on the oxidation of Zn_1–ZnF by H_2O_2

The third zinc finger of transcription factor Sp1 of molecular mass of 3396 Da was used in all *in vitro* oxidation studies. Only 7% of the zinc finger peptide (10 μM) was oxidized by hydrogen peroxide (250 μM), in the absence of a redox catalyst during the incubation period (10 min). All the catalysts were found to accelerate the oxidizing power of the stressor H_2O_2 (Fig. 2) [6]. Table 1 shows the potent pro-oxidant function of these GPx mimics expressed as an IC_{50} value, which lies in the nanomolar range for all the compounds. The IC_{50} value for the standard ebselen (110 nM) [28] was first determined to ensure accuracy of the DTNB assay. **5** was the most active catalyst with an IC_{50} value of 16 nM. Control experiments included the catalyst solvent methanol, H_2O_2 alone, and catalyst in the absence of H_2O_2, all of which had negligible effects on measurements.

A time course analysis (Fig. 3) of two of the compounds (**6** and **7**) with IC_{50} values of 35 and 18 nM, respectively, shows a linear relationship between catalytic turnover and

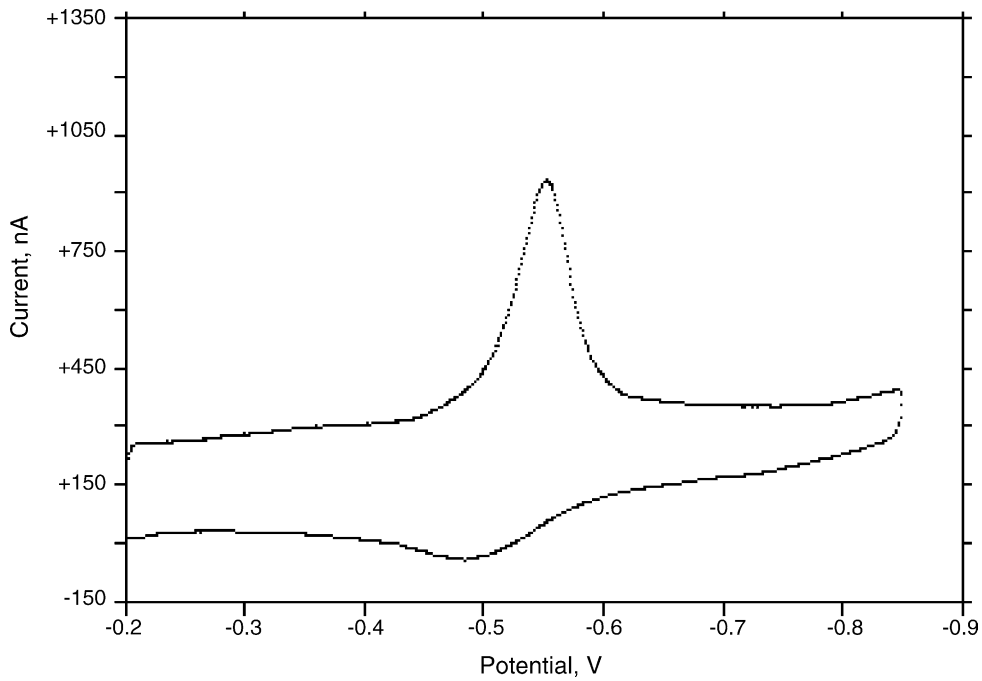
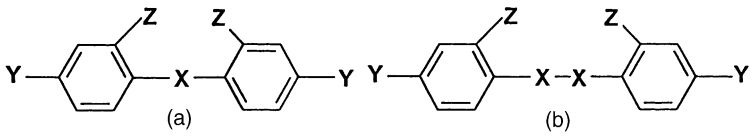


Fig. 1. Cyclic voltammogram of the Zn_1–ZnF peptide of Sp1. The electrochemical potential of the zinc finger peptide (20 μM) was measured at a scan rate of 500 mV/s using an SSE and a DME working electrode. The voltammogram display follows the U.S. convention.

Table 1
Comparison and correlation between organochalcogen structure, biological activity, and E_{pa}



Compound	X	Y	Z	IC_{50} (nM)	E_{pa} (mV)
(a)					
1	Se	OCH_3	H	636	+753[a]
5	Te	OH	H	16	+299[a]
6	Te	OCH_3	H	35	+368[a]
Ebselen	–	–	–	110	+1044[a]
(b)					
2	Se	H	H	31	+1141[b]
3	Se	H	NH_2	19	+733[b]
4	Se	OCH_3	H	21	+1000[a]
7	Te	OCH_3	H	18	+578[a]
8	Te	H	H	18	+653[b]

Zn_1–ZnF (5 μM) was incubated with H_2O_2 (250 μM) and catalyst at 37° for 10 min. Catalase (1 nM) was added followed by DTNB (100 μM). Thiol concentrations were determined at 412 nm ($\varepsilon_{412} = 13{,}600\ M^{-1}\ cm^{-1}$). Cyclic voltammograms of organochalcogens (50–100 μM) were recorded using a SSE and a GCE. The scan rate used was 200 mV/s. Experimental error: 10% (N = 3).

[a] Literature value vs. SSE [20].

[b] Literature value vs. SSE [6].

time. Only 5 nM concentrations of each catalyst were used showing the potential viability of these compounds at 37° for at least 1 hr. The ditelluride **7** appears to function at a faster rate than the monotelluride **6**, in accordance with their IC_{50} values.

3.3. Correlation of redox potential with structure and activity

The electrochemical redox potentials of these catalysts were measured vs. SSE in an attempt to find a correlation with the pro-oxidant activity and structure. The CV of all of these compounds showed one irreversible oxidation peak (E_{pa}) with the exception of **5**, which has two distinct redox couples, one at +277 mV and the other at +792 mV (E_{pa} = +299 and +821 mV, E_{pc} = +255 and +763 mV).

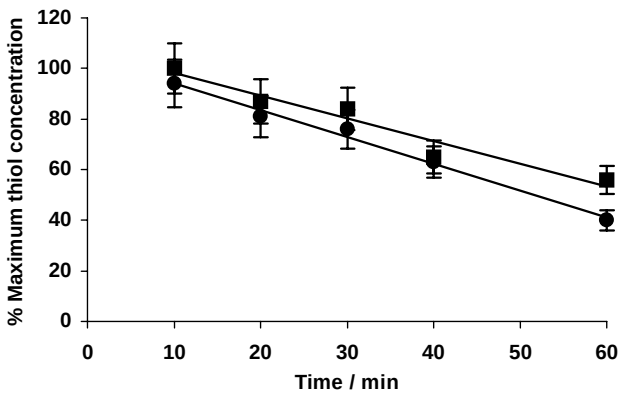


Fig. 3. Time course analysis of tellurides **6** and **7**. A linear relationship is found for **6** (■) and **7**(●) over 60 min. Each compound (5 nM) was incubated with H_2O_2 (250 μM) to demonstrate the viability of these compounds at 37° for at least 1 hr. DTNB (100 μM) was used to measure thiol reactivity at each time interval. Experiments were repeated in triplicate with a standard error of 10%.

A general trend was observed whereby easily oxidizable compounds with low oxidation potentials had higher activities (lower IC_{50} values) in the zinc finger oxidation assay. This interesting trend is pronounced among the dichalcogens (**2–4**, **7**, and **8**) (Table 1, b). It was also observed that the monochalcogens have a lower E_{pa} than their corresponding dichalcogens (**1**, **4**, **6**, and **7**), indicating a structural influence over E_{pa} values. The tellurides have lower E_{pa} values ranging from +300 to +650 mV compared to the selenides, the latter are harder to oxidize with E_{pa} values between +700 and +1200 mV. Direct substitution of a selenide for a telluride increases catalytic activity (**2** and **8**, **4** and **7**) and by almost 20-fold in the case of **1** and **6**. The dichalcogen catalysts (**4** and **7**) appear to be more active in thiol oxidation than their corresponding monochalcogens (**1** and **6**). The presence of either an electron donating amino or methoxy group on the phenyl ring of the diselenides also enhances their activity (**2–4**).

3.4. Sensitization of cells to the effects of H_2O_2

Rat adrenal PC12 cells were subjected to an oxidatively stressed environment, provided by hydrogen peroxide (200 μM), in order to mimic tumor progression [29]. **5** was initially selected on the basis of activity results from the *in vitro* assay for further testing. Figure 4 shows the damaging effects on cells caused by increasing concentrations of the monotelluride **5** (1 nM–25 μM) in the presence of H_2O_2 (200 μM). It must be especially noted that this compound has no toxic effects on the cells in the *absence* of H_2O_2. As the compounds are soluble in methanol, this

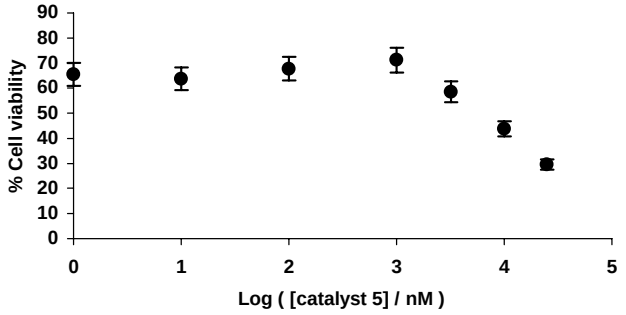


Fig. 4. Cell death caused by catalyst **5**. Concentration-dependent reduction in cell viability on treatment with varying amounts of **5** (1 nM–25 μM) in the presence of H_2O_2 (200 μM). Experimental details are given in Section 2.

solvent alone was tested as a control and found to have no effect on cell viability at 2%. Figure 5 shows the effects of organochalcogens on PC12 cells with and without H_2O_2 (200 μM) and also their effects on HUVECs. Ebselen and the diselenide **4** appear not to have an effect on PC12 cell viability in either the absence or presence of H_2O_2. Compounds **5**–**7**, on the other hand, show an increasing impact on PC12 cell viability that was reduced to 44, 20, and 12%, respectively. Unlike **5**, compounds **6** and **7** are toxic by themselves in the absence of H_2O_2 (57 and 17% cell viability, respectively). The human endothelial cells appear resistant to ebselen, **4** and **5** (80, 100, and 92%, respectively), whereas **6** and **7** are toxic and reduce HUVECs survival to 3 and 12%, respectively.

4. Discussion

Cancerous cells survive because the rate of cell proliferation overtakes the rate of cell death as the cells become resistant to apoptotic stimuli. Our findings shed light on a new approach for the development of novel chemotherapeutic agents, which might be able to specifically target the malignant neoplasm and retard the rate of proliferation, either by a necrotic or apoptotic mechanism. The agents manipulate the ''harmful'' condition known as oxidative stress, where antioxidant activity is low, to promote cancer cell death.

The studies carried out in this paper lend preliminary support to the idea of ''redox sensitizing''. They involve the initial characterization and evaluation of a range of organochalcogen compounds using electrochemical analysis and an *in vitro* oxidation assay, followed by further tests on a tumor growth model under oxidative stress, which yielded encouraging results.

Electrochemical analysis of the Sp1 zinc finger shows a redox potential of this thiol-containing motif at −524 mV vs. SSE (i.e. $E^{\circ\prime} = -284$ mV vs. NHE). This is slightly more negative than that of GSH ($E^{\circ\prime}$(GSSG/2GSH) = −250 mV vs. NHE; ref. [30]), explaining the ability for the substitution of GSH by Zn_1–ZnF, when antioxidant levels are low. The redox potentials (vs. NHE) of some other thiol-containing proteins, such as mammalian protein disulfide isomerase ($E^{\circ\prime} = -127$ mV), glutaredoxin-3 ($E^{\circ\prime} = -198$ mV), and bacterial DsbA ($E^{\circ\prime} = -125$ mV) are higher with respect to zinc fingers, such as Sp1 and also GSH. As a consequence, it would be thermodynamically favorable for the organochalcogen catalysts to oxidize the transcription factors before oxidizing these enzymes [31–33]. On the other hand, it is possible that thioredoxin ($E^{\circ\prime} = -270$ mV) with a redox potential within 15 mV of the Sp1 zinc finger [34] may also act as a target for these catalysts.

Our *in vitro* results show that GPx mimics catalyze the (per)oxidation of the thiols within a zinc finger motif of Sp1 in the presence of H_2O_2. The advantage of these mimics is that they lack the substrate specificity of GPx itself and oxidize various thiol moieties in the presence of an oxidizing ''substrate''. All the catalysts showed activities in the nanomolar range. It has been possible to find an interesting correlation between the electrochemical

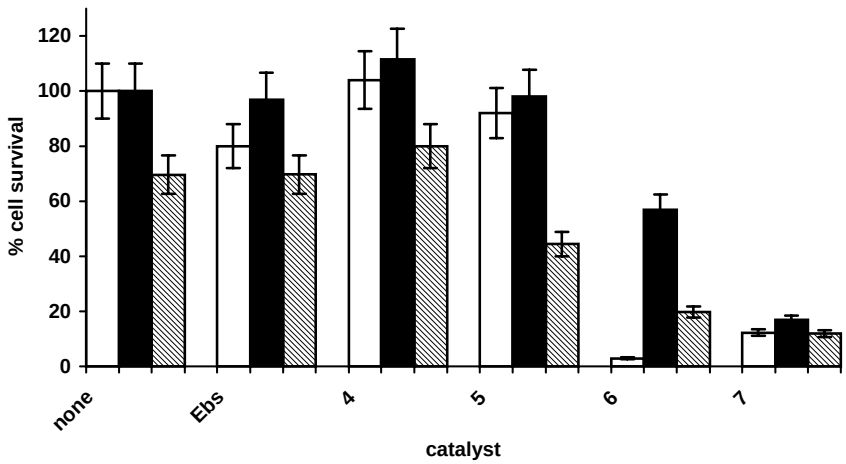


Fig. 5. Cytotoxicity of a selection of organochalcogens. Compounds **4**–**7** (10 μM) were incubated with HUVECs (□), PC12 cells (■), and PC12 cells with H_2O_2 (▨, 200 μM) overnight at 37° and 5% CO_2. Cell viability was established using the MTT assay. The result presented as an average (mean ± SD).

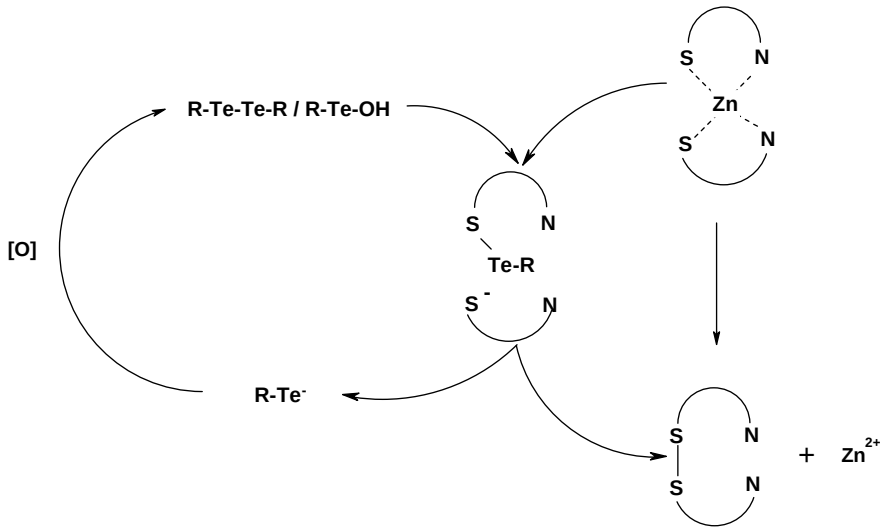


Fig. 6. Proposed redox mechanism for dichalcogen catalysts. A detailed discussion of these mechanisms is given in the text.

potentials and *in vitro* activity of these compounds (Table 1), reflecting the important effects of their chemical structure on biological activity [20].

In general, lower E_{pa} values suggest that the monotellurides should be more active than their ditelluride analogs, however, the *in vitro* thiol peroxidation assay shows similar activity. This may be explained by the possibility that dichalcogens undergo different reaction pathways. Monochalcogens are restricted to atom transfer redox reactions. Dichalcogen species, on the other hand, can also undergo electron transfer and thiol exchange reactions (Fig. 6), allowing the thiol peroxidation reaction to potentially proceed by several pathways.

The electrochemical and *in vitro* data described above assisted in selecting catalysts to be studied in cell culture, the results of which support our notion of catalysts that are sensitive to their redox environment. In the presence of H_2O_2, **5**–**7** are able to promote PC12 cell death. Tellurides **6** and **7** both exhibit catalytic activity *in vitro* and also dramatically reduce cell viability with **7** exhibiting greater activity. Unfortunately, **6** and **7** also show toxicity on both PC12 cells and the non-tumorogenic HUVECs. It was not surprising that ebselen (with a relatively high IC_{50} value) did not show any activity against the undifferentiated PC12 cell line. The selenium analog of **7**, **4**, was also evaluated against cancer cells as its *in vitro* activity gave an IC_{50} value of 21 nM. It failed, however, to significantly promote cell death. The high activity of this diselenide in the *in vitro* assay together with its non-toxicity to both PC12 cells in the absence of H_2O_2 and HUVECs, suggests that **4** could provide an ideal framework for further structural development. Interestingly, a similarity in the toxicities of the redox catalysts towards HUVEC and non-stressed PC12 cells can be observed. This eliminates the possibility of any particular characteristic of the PC12 cell line, which may influence their interaction with the catalysts.

The most active and effective of all the compounds studied is the monotelluride **5** with an IC_{50} of 16 nM *in vitro*, no toxic effect on non-tumorogenic HUVECs and unchallenged PC12 cells, and a strong toxic effect on tumorogenic, *oxidatively stressed* PC12 cells. The results for this compound clearly support the idea of redox sensitizing with catalysts. While **5** shows little toxicity on its own, it uses the oxidative stress environment to inflict damage that goes beyond the toxic effects exerted by the stressor (H_2O_2) on its own.

5. Conclusion

In summary, we have been able to provide preliminary evidence for potential anticancer catalysts whose activity is controlled by the redox environment which they are placed in. They should therefore mediate cell death only in the presence of oxidative stress—a condition found in many cancer cells. In accordance with drug development to maximize effectiveness and minimize side reactions, our initial studies suggest that novel catalysts only need to be administered in small quantities to display a high degree of efficiency and are non-toxic to normal cells.

The redox potential of these compounds assists in predicting their activity, mechanism of action, and future progress as drug candidates. Our results allow us to predict that selenium, which is a naturally occurring essential trace element within the body, could provide an ideal scaffold for further design of novel organoselenium compounds as non-toxic anticancer agents. Although showing similar catalytic activity in the peroxidation of the zinc finger fragment, the organoselenides evaluated in this study were unable to effectively sensitize PC12 cells to hydrogen peroxide. This may be due to selenium metabolism within the cells rendering the compounds inactive, an area for future pharmacological investigation.

In addition to potential anticancer activity, the ability of these catalysts to function according to their environment confers them with intrinsic antioxidant properties as they utilize ROS in their catalytic cycle. Under a normal reducing cellular environment, they might therefore be useful in other oxidative stress-related diseases, such as inflammation, rheumatoid arthritis, and Alzheimer's disease [20,35]. These initial findings only provide a foundation for the development of redox catalysts. We are fully aware that the notion of ''redox sensitizers'' raises a wealth of more questions. For example, what would be the effects of the anticipated treatment on cancer patients simultaneously suffering from other oxidative stress-related diseases? Future studies will show if this potential lead can be turned into effective prototype drugs that might be able to sense their redox environment and act accordingly.

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