Thioredoxin reductase and cancer cell growth inhibition by organotellurium antioxidants

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Thioredoxin (Trx) expression is increased in several human primary cancers and the Trx/Trx reductase (TrxR) system therefore provides an attractive target for cancer drug development. Novel organotellurium antioxidants, especially a primitive analog of vitamin E (compound 1d) and compounds 7, 9 and 10—all carrying highly functionalized 4-(dialkylamino)phenyltelluro groups to secure high antioxidative capacity—were found to inhibit TrxR with IC_{50} values in the low micromolar range. Whereas antioxidant 1d also inhibited the growth of MCF-7 human breast cancer cells in culture at a similar level ($IC_{50} = 1.8\ \mu M$), the other TrxR inhibitors were inactive in concentrations below about 10 μM. *Anti-Cancer Drugs* 14:153–161 © 2003 Lippincott Williams & Wilkins.

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Introduction

The thioredoxins (Trxs) are small redox proteins with the conserved redox catalytic sequence Trp–Cys–Gly–Pro–Cys–Lys where the Cys residues undergo reversible NADPH-dependent reduction by selenocysteine-containing flavoprotein Trx reductases (TrxRs) [1,2]. There are two mammalian Trxs. Human Trx-1 is a 105-amino-acid protein found in the cytoplasm and nucleus of cells [3,4]. Through thiol disulfide exchange reduced Trx-1 is able to catalyze the selective reduction of key Cys residues in proteins resulting in alterations in enzymatic and protein DNA-binding properties. Trx-2 is a 166-amino-acid protein with a 60-amino-acid *N*-terminal sequence that directs Trx-2 to the mitochondrion [5,6] where it protects against oxidant damage [7]. The Trx-1 and mitochondrial Trx-2 systems appear to be completely separate, and they have their own subcellular location and their own TrxR reductases and protein redox acceptors [8].

Redox activity is essential for the biological effects of Trx-1. Transfection of cancer cells with a redox inactive mutant Trx-1 does not transform cells, inhibits cell growth, potentiates apoptosis and blocks tumor formation by the cells in immunodeficient *scid* mice [9]. We and other investigators have shown that Trx-1 protein levels are significantly elevated in several human primary cancers including gastric cancer (50% of cases), colon cancer (55%), pancreatic cancer (41%), liver cancer (52%) and prostate cancer (63%) [3,10–15]. Our work with primary gastric cancer has shown a highly significant correlation between increased Trx-1 expression and inhibited apoptosis [3]. We have recently shown in patients with non-small cell lung cancer that increased Trx-1 expression is an independent prognostic factor for decreased patient survival [16]. These findings firmly establish Trx-1 as an important cancer drug target.

Inhibiting TrxR provides an alternative way of inhibiting the activity of Trxs as a target for anticancer drug development [17,18]. The irreversible inactivation of the reduced form of TrxR (but not of the oxidized one) with low-molecular-weight electrophilic species such as 1-chloro-2,4-dinitrobenzene [19], 1,3-bis-(2-chloroethyl)-1-nitrosourea [20] and various quinonoid compounds [21] suggests that the selenol moiety of the selenocysteine residue is the target of inactivation, but this has not been conclusively demonstrated. Although effective, these compounds are not expected to find use as drugs because of their unselective alkylation of other enzymes and biomolecules including DNA. Certain alkyl 2-imidazolyl disulfides were recently shown to be competitive inhibitors of the reduction of oxidized Trx by TrxR [22,23]. Probably, the catalytic site of TrxR as well as Trx itself are reversibly thioalkylated by these agents. Some of the compounds also show *in vitro* antitumor activity against human tumor xenografts in *scid* mice. However, the reactive nature of these disulfides makes it likely that they also react with other thiol-containing targets in the cell.

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Previously, we have studied the TrxR-inhibiting capacity of organotelluriums and found that lipid- and water-soluble compounds inhibited the enzyme in low micromolar concentrations and the growth of MCF-7 and HT-29 human cancer cells in culture at the 5–10 μmol level [24,25]. Although the chemical events responsible for TrxR inhibition are still not known, our studies have indicated that compounds with good antioxidant capacity (hydroperoxide-decomposing and chain-breaking activity [26–28]) are the most efficient inhibitors. With this in mind, previously reported as well as new organotellurium antioxidants were prepared and evaluated for their capacity to inhibit TrxR and cancer cell growth in culture.

Materials and methods

TrxR/Trx assay

TrxR/Trx-dependent insulin-reducing activity was measured in an incubation with a final volume of 60 μl containing 100 mM HEPES buffer, pH 7.2, 5 mM EDTA (HE buffer), 1 mM NADPH, 1.0 μM human placental TrxR, 0.8 μM Trx, 2.5 mg/ml bovine insulin and inhibitor. Incubations were for 30 min at 37°C in flat-bottom 96-well microtiter plates. The reaction was stopped by the addition of 50 μl of 6 M guanidine–HCl, 50 mM Tris, pH 8.0 and 10 mM DTNB, and the absorbance measured at 412 nM.

TrxR assay

Assays of TrxR were carried out in flat-bottom 96-well microtiter plates. TrxR activity was measured in a final incubation volume of 60 μl containing HE buffer, 10 mM DTNB, 1.0 μM TrxR, 1 mM NADPH and inhibitor. Compounds were diluted in HE buffer and added to the wells as 20-μl aliquots, and TrxR was then added, also as 20-μl aliquots in HE buffer. To ensure uniform coverage of the bottom of the well the plate was spun briefly at 3000 *g*. To start the reaction, NADPH and DTNB were added as a 20-μl aliquot in HE buffer and the plate was moved to the plate reader which had been preheated to 37°C. The optical density at 412 nm was measured every 10 s and initial linear reaction rates measured.

Growth inhibition assay

Compound cytotoxicity was measured using modifications of the MTT assay as described by Mosmann [29] and Carmichael [30]. Human MCF-7 breast cancer cells were seeded at 3000 cells/well into 96-well plates in Dulbecco's minimum essential medium supplemented with 10% fetal bovine serum. After 16 h at 37°C and 5.5% CO_2 in air, drugs were added to the wells at concentrations ranging from 0.1 to 20 μM. The cells were further incubated for 72 h after which 40 μl/well of a 2.5 μg/μl solution MTT solution was added and an additional 3 h, 37°C, 5.5% CO_2 in air incubation was performed. At the end of the incubation time, the untransformed MTT was removed from each well by aspiration and 150 μl/well of dimethyl sulfoxide was added. The plate was shaken to ensure full solubilization of the formazan dye followed by dual optical density readings of 595 and 655 nm using a multiwell microplate spectrophotometer (Molecular Devices, Menlo Park, CA). Cytoviability of control cells was considered to be 100%. For the treated cells viability was expressed as a percentage of control cells. All determinations were carried out in triplicate.

Preparation of organotellurium compounds

Compounds **1a–1d** [28], bis(4-hydroxyphenyl)telluride (**2**) [31], *N*,*N*-dimethyl-4-aminophenyl-3-phenoxypropyl telluride (**13**) [32], *N*,*N*-di(2-carbomethoxyethyl)aniline (**5a**) [33] and *N*-(2-carbomethoxyethyl)aniline (**5b**) [33] were synthesized according to literature methods. All other chemicals were commercially available and used without further purification. THF was distilled under nitrogen from sodium/benzophenone. ^{1}H and ^{13}C spectra were recorded at 400 and 100 MHz, respectively. Mass spectra were recorded by direct inlet on a Finnigan MAT GCQ using EI ionization or on a Finnigan Thermoquest AQA (ESI 10 eV, probe temperature 300°C). M^+, $(M\text{-}H)^-$ and $(M\text{-}2H)^{2-}$ ions are given for ^{130}Te.

4-(4-Carbomethoxybutoxy)phenyl 4-hydroxyphenyl telluride (3a)

Methyl 5-bromovalerate (0.456 ml, 3.18 mmol) was added to a well-stirred suspension of **2** (1.00 *g*, 3.19 mmol) and anhydrous K_2CO_3 (0.59 *g*, 4.25 mmol) in dry acetone (50 ml) at room temperature. After 20 h of stirring at reflux under an N_2 atmosphere (TLC monitoring), the mixture was cooled to room temperature. After concentration under reduced pressure, H_2O (100 ml) and CH_2Cl_2 (100 ml) were added, the organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (50 ml × 2). The combined organic phases were washed with brine (100 ml), dried ($MgSO_4$), filtered and concentrated to give a yellow solid. Purification by flash chromatography (pentane:CH_2Cl_2 = 1:1 and then CH_2Cl_2) and subsequent recrystallization from EtOH afforded the title compound (0.87 g, 64%) as a white solid: m.p. 91°C; ^{1}H-NMR ($CDCl_3$) δ 1.80 (quint., J = 7.2 Hz, 4 H), 2.39 (t, J = 7.2 Hz, 2 H), 3.67 (s, 3 H), 3.93 (t, J = 6.0 Hz, 2 H), 6.69 (d, J = 8.7 Hz, 2 H), 6.73 (d, J = 8.7 Hz, 2 H), 7.56 (d, J = 8.7 Hz, 2 H), 7.60 (d, J = 8.7 Hz, 2 H); ^{13}C-NMR ($CDCl_3$) δ 21.6, 28.5, 33.6, 51.7, 67.2, 104.2, 107.0, 115.9, 116.8, 139.7, 139.9, 155.8, 159.0, 174.2; MS *m/z* (relative intensity) 430 (M^+, 5), 428 (5), 186 (22), 115 (100).

Bis[4-(4-Carbomethoxybutoxy)phenyl)] telluride (3b)

The title compound was obtained from the reaction of **2** (1.5 g, 4.78 mmol) and methyl 5-bromovalerate (2.0 ml, 14.3 mmol), employing the procedure described for the preparation of **3a**. Purification by flash chromatography (pentane:CH_2Cl_2 = 1:1 and then CH_2Cl_2) and

subsequent recrystallization from EtOH furnished the title compound (1.87 g, 72%) as a white solid: m.p. 57°C; ^{1}H-NMR ($CDCl_3$) δ 1.80 (quint, $J = 6.0$ Hz, 8 H), 3.39 (t, $J = 6.6$ Hz, 4 H), 3.67(s, 6 H), 3.94 (t, $J = 6.6$ Hz, 4 H), 6.74 (d, $J = 8.8$ Hz, 4 H), 7.60 (d, $J = 8.8$ Hz, 4 H); ^{13}C-NMR ($CDCl_3$) δ 21.6, 28.5, 33.6, 51.5, 67.2, 104.2, 115.9, 139.7, 159.0, 173.8; MS *m/z* (relative intensity) 544 (M^+, 5), 542 (7), 149 (52), 115 (100).

4-(4-Carboxybutoxy)phenyl 4-hydroxyphenyl telluride (4a)

A solution of $LiOH \cdot H_2O$ (0.049 g, 1.17 mmol) in H_2O (10 ml) was added dropwise to a well-stirred solution of the ester **3a** (0.10 g, 0.234 mmol) in THF (15 ml). After 20 h stirring at room temperature under N_2 atmosphere, the solution was cooled and acidified with pre-cooled concentrated HCl. The resultant precipitate was filtered, washed with H_2O and dried to furnish the title compound (260 mg, 86%) as a white solid: m.p. 127–129°C; ^{1}H-NMR (DMSO-*d6*) δ 1.59–1.65 (m, 2 H), 1.66–1.71 (m, 2 H), 2.26 (t, $J = 7.2$ Hz, 2 H), 3.92 (t, $J = 6.1$ Hz, 2 H), 6.66 (d, $J = 8.2$ Hz, 2 H), 6.80 (d, $J = 8.2$ Hz, 2 H), 7.50 (d, $J = 8.2$ Hz, 2 H), 7.51 (d, $J = 8.2$ Hz, 2 H), 9.61 (br s, 1 H), 11.99 (br s, 1 H); ^{13}C-NMR (DMSO-*d6*) δ 21.1, 28.0, 33.2, 67.0, 101.5, 104.2, 115.6, 117.0, 138.8, 140.0, 157.6, 158.5, 174.2; MS *m/z* (relative intensity) 416 (M^+, 13), 314, 312 (9), 186 (100).

Bis[4-(4-Carboxybutoxy)phenyl] telluride (4b)

This was prepared according to the procedure for **4a** from **3b**: yield 94% of a white solid: m.p. 129–131°C; ^{1}H-NMR (DMSO-*d6*) δ 1.59–1.71 (m, 8 H), 2.26 (t, $J = 7.2$ Hz, 2 H), 3.93 (t, $J = 6.2$ Hz, 2 H), 6.81 (d, $J = 8.5$ Hz, 4 H), 7.55 (d, $J = 8.5$ Hz, 4 H); ^{13}C-NMR (DMSO-*d6*) δ 21.1, 28.0, 33.2, 67.1, 103.9, 116.0, 139.3, 158.6, 174.2; MS *m/z* (relative intensity) 516 (M^+, 14), 514 (13), 414 (7), 286 (14), 186 (100).

Bis[4-(*N*,*N*-di(2-carbomethoxyethyl)amino)phenyl] telluride (7a)

Under N_2 a solution of *N*,*N*-di(2-carbomethoxyethyl)aniline (**5a**, 10.0 g, 37.7 mmol) in ether (10 ml) was added dropwise to a slurry of $TeCl_4$ (5.08 g, 18.9 mmol) in dry ether (100 ml) at 0°C. After stirring overnight, the ether was decanted and the viscous oily product was washed with ether (100 ml × 3). The orange viscous oil was dissolved in CH_2Cl_2 (100 ml) and a solution of $Na_2S \cdot 9H_2O$ (3.00 g, 37.7 mmol) in H_2O (100 ml) was added with stirring. The two-phase mixture was stirred for 1 h at ambient temperature. Solid $NaHCO_3$ was added to neutralize the aqueous phase to pH 7, and the reaction mixture was filtered through Celite. The filtrate was transferred to a separatory funnel, the organic phase was separated, and the aqueous layer extracted with CH_2Cl_2 (50 ml × 2). The combined extracts were washed with brine, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. ^{1}H-NMR revealed the dark red viscous oil to contain a mixture of starting aniline, diaryl ditelluride and the corresponding diaryl telluride (45% ditelluride). Some of the crude product (2.3 g) was dissolved in dioxane (50 ml) and electrolyte copper powder (1 g) was added. The mixture was stirred at reflux until the solution had turned pale yellow (4 h). After cooling, the insoluble material was removed by filtration through Celite and the solvent was removed under reduced pressure. Purification by flash chromatography (pentane:ethyl acetate = 2:3) afforded the title compound (0.91 g, 75%) as an orange viscous oil; ^{1}H-NMR ($CDCl_3$) δ 2.50 (t, $J = 7.4$ Hz, 8H), 3.56 (t, $J = 7.4$ Hz, 8H), 3.60 (s, 12H), 6.48 (d, $J = 9.0$ Hz, 4H), 7.51 (d, $J = 9.0$ Hz, 4H); ^{13}C-NMR ($CDCl_3$) δ 32.1 46.7, 51.7, 99.7, 113.6, 139.9, 146.37, 172.3; MS *m/z* (relative intensity) 657 (M^+, 5), 528 (100), 455 (68), 265 (38), 192 (68).

Bis[4-(*N*-(2-carbomethoxyethyl)amino)phenyl] telluride (7b)

Reaction of $TeCl_4$ (7.53 g, 27.9 mmol), sequentially with *N*-(2-carbomethoxyethyl)aniline (**5b**, 10.0 g, 55.8 mmol) and $Na_2S \cdot 9H_2O$ (12.3 g, 55.8 mmol), as described for the preparation of 7a, afforded (the crude contained 45% ditelluride), after treatment with copper, the title compound in 64% yield as light yellow crystals (from EtOH): m.p. 93-95°C; ^{1}H-NMR ($CDCl_3$) δ 2.59 (t, $J = 6.4$ Hz, 4H), 3.42 (q, $J = 6.4$ Hz, 4H), 3.68 (s, 6H), 6.46 (d, $J = 8.8$ Hz, 4H), 7.52 (d, $J =$ Hz, 4H); ^{13}C-NMR ($CDCl_3$) δ 33.6, 39.1, 51.7, 100.4, 114.1, 139.7, 147.3, 172.6; MS *m/z* (relative intensity) 486 (M^+, 16), 356 (100), 283 (50), 179 (23), 106 (62).

Bis[4-(*N*,*N*-di(2-carboxyethyl)amino)phenyl] telluride tetrasodium salt (8a)

Compound **7a** (0.84 g, 1.28 mmol) was dissolved in methanol (6 ml) and aqueous NaOH (2 ml, 10%) was added. The reaction mixture was stirred at reflux for 2 h. Evaporation of the solvent under vacuum furnished a white solid. The solid was taken up in MeOH and filtered. Ether was added to the filtrate, precipitating the product as a white solid (786 mg, 91%); ^{1}H-NMR (D_2O) δ 2.35 (t, $J = 7.6$ Hz, 2H), 3.49 (t, $J = 7.6$ Hz, 2H), 6.69 (d, $J = 8.8$ Hz, 2H), 7.57 (d, $J = 8.8$ Hz, 2H), ESMS *m/z* 300.0 $(M\text{-}2H)^{2-}$.

4-[*N*,*N*-Di(2-carbomethoxyethyl)amino]phenyl 3-phenoxypropyl telluride (9a)

$NaBH_4$ (0.106 g, 2.81 mmol) was added to a solution of crude bis[4-(*N*,*N*-di(2-carbomethoxyethyl)amino)phenyl] ditelluride (**6a**, 1 g, 1.28 mmol) (2.68 g crude product from the preparation of telluride **7a**) in EtOH (20 ml) and THF (10 ml) under N_2 at ambient temperature. The mixture was stirred until colorless (20 min). A solution of

3-phenoxypropyl bromide (0.60 g, 2.8 mmol) in EtOH (5 ml) was added and the resulting mixture was stirred for 4 h. The solution was poured into H_2O (100 ml), extracted with CH_2Cl_2 (50 ml × 3), and the combined extracts were washed with brine, dried ($MgSO_4$), filtered and concentrated. Purification by flash chromatography (pentane:ethyl acetate = 2:3) afforded the title compound (1.48 g, 96%) as an orange viscous oil; ^{1}H-NMR ($CDCl_3$) δ 2.22 (quint, J = 7.3 Hz, 2H), 2.58 (t, J = 7.4 Hz, 4H), 2.92 (t, J = 7.3 Hz, 2H), 3.64 (t, J = 7.4 Hz, 4H), 3.68 (s, 6H), 3.99 (t, J = 6.1 Hz, 2H), 6.52 (d, J = 9.0 Hz, 2H), 6.86 (dt, J = 1.0, 8.8 Hz, 2H), 6.92 (tt, J = 1.0, 7.3 Hz, 1H), 7.25 (dd, J = 7.3, 8.8 Hz, 2H), 7.63 (d, J = 9.0 Hz, 2H); ^{13}C-NMR ($CDCl_3$) δ 4.4, 31.3, 32.1, 46.6, 51.7, 68.6, 95.5, 113.3, 114.5, 120.6, 129.3, 141.4, 146.6, 158.8, 172.3; MS *m/z* (relative intensity) 529 (M^+, 94), 527 (83), 456 (51), 454 (43), 394 (63), 392 (57), 321 (50), 319 (45), 265 (93), 192 (100).

4-[*N*-(2-Carbomethoxyethyl)amino]phenyl 3-phenoxypropyl telluride (9b)

Reaction of crude ditelluride **6b** (1.00 g, 1.63 mmol) (2.33 g of crude product from the preparation of telluride **7b**) sequentially with $NaBH_4$ (0.136 g, 3.59 mmol) and 3-phenoxypropyl bromide (0.774 g, 3.59 mmol), as described for the preparation of **9a**, afforded the title compound (1.20 g, 83%) as a light yellow solid: m.p. 66°C; ^{1}H-NMR ($CDCl_3$) δ 2.2 (quint, J = 6.9 Hz, 2H), 2.61 (t, J = 6.4 Hz, 2H), 2.91 (t, J = 7.3 Hz, 2H), 3.4 (q, J = 6.4 Hz, 2H), 3.70 (s, 3H), 3.98 (t, J = 6.1 Hz, 2H), 4.09 (t, J = 6.1 Hz, 1H), 6.45 (d, J = 8.7 Hz, 2H), 6.86 (dd, J = 1.1, 8.8 Hz, 2H) 6.92 (tt, J = 1.1, 7.3 Hz, 1H), 7.26 (dd, J = 8.8 Hz, 2H), 7.59 (d, J = 8.7 Hz, 2H); ^{13}C-NMR ($CDCl_3$) δ 4.4, 31.2, 33.6, 39.1, 51.8, 68.7, 96.1, 114.0, 114.5, 120.6, 129.4, 141.3, 147.6, 158.9, 172.6; MS *m/z* (relative intensity) 443 (M^+, 48), 441 (47), 308 (44), 306 (40), 179 (100).

4-[*N*,*N*-Di(2-carbomethoxyethyl)amino]phenyl 3-phenoxybutyl telluride (9c)

Reaction of crude ditelluride **6a** (0.5 g, 0.64 mmol) (1.34 g of crude product from the preparation of telluride **7a**) sequentially with $NaBH_4$ (0.053 g, 1.4 mmol) and 4-phenoxybutyl bromide (0.292 g, 1.28 mmol), as described for the preparation of compound **9a**, furnished the desired product (0.59 g, 86%) as a light yellow viscous oil; ^{1}H-NMR ($CDCl_3$) δ 1.81–1.97 (m, 4H), 2.58 (t, J = 7.2 Hz, 4H), 2.83 (t, J = 7.2 Hz, 2H), 3.65 (t, J = 7.2 Hz, 4H), 3.68 (s, 6H), 3.93 (t, J = 6.2 Hz, 2H), 6.53 (d, J = 8.9 Hz, 2H), 6.86 (d, J = 1.1, 8.8 Hz, 2H), 6.92 (tt, J = 1.1, 7.3 Hz, 1H), 7.23–7.28 (m, 2H), 7.63 (d, J = 8.9 Hz, 2H); ^{13}C-NMR ($CDCl_3$) δ 8.4, 28.5, 31.3, 32.1, 46.7, 51.9, 67.1, 95.5, 113.5, 114.6, 120.6, 129.4, 141.4, 146.6, 159.1, 172.5; MS *m/z* (relative intensity) 543 (M^+, 20), 541 (23), 470 (9), 468 (8), 394 (10), 392 (11), 321 (10), 319 (11), 192 (14), 149 (100).

4-[*N*-(2-Carbomethoxyethyl)amino]phenyl 4-phenoxybutyl telluride (9d)

Reaction of crude ditelluride **6b** (1.00 g, 1.63 mmol) (2.33 g of crude product from the preparation of telluride **7b**) sequentially with $NaBH_4$ (0.136 g, 3.59 mmol) and 4-phenoxybutyl bromide (0.824 g, 3.59 mmol), as described for the preparation of 9a, afforded the title compound (1.34 g, 90%) as a light yellow oil; ^{1}H-NMR ($CDCl_3$) δ 1.81-196 (m, 4H), 2.61 (t, J = 6.4 Hz, 2H), 2.82 (t, J = 7.2 Hz, 2H), 3.54 (q, J = 6.4 Hz, 2H), 3.70 (s, 3H), 3.93 (t, J = 6.4 Hz, 2H), 4.09 (t, J = 6.4 Hz, 1H), 6.46 (d, J = 8.7 Hz, 2H), 6.86 (dd, J = 1.1, 8.8 Hz, 2H) 6.92 (t, J = 1.1, 7.3 Hz, 1H), 7.26 (dd, J = 7.3, 8.8 Hz, 2H), 7.59 (d, J = 8.7 Hz, 2H); ^{13}C-NMR ($CDCl_3$) δ 8.2, 28.2, 31.3, 33.6, 39.1, 51.8, 67.0, 96.1, 113.9, 114.5, 120.5, 129.4, 141.4, 147.5, 159.0, 172.7; MS *m/z* (relative intensity) 457 (M^+, 16), 411 (33), 327 (66), 282 (51), 255 (50), 197 (100).

4-[*N*,*N*-Di(2-carboxyethyl)amino]phenyl 3-phenoxypropyl telluride (10a)

Treatment of compound **9a** (1.3 g, 2.466 mmol) with $LiOH \cdot 2H_2O$ (0.413 g, 9.865 mmol), as described for **4a**, furnished the title compound (1.11 g, 90%) as an orange viscous oil; ^{1}H-NMR ($CDCl_3$) δ 2.22 (quint, J = 6.8 Hz, 2H), 2.61 (t, J = 6.9 Hz, 4H), 2.93 (t, J = 6.8 Hz, 2H), 3.63 (t, J = 6.9 Hz, 2H), 3.98 (t, J = 6.9 Hz, 2H), 6.56 (d, J = 8.9 Hz, 2H), 6.85 (dd, J = 1.1, 8.8 Hz, 2H), 6.92 (tt, J = 1.1, 7.3 Hz, 1H), 7.25 (dd, J = 7.3, 88 Hz, 2H), 7.63 (d, J = 8.9 Hz, 2H), 9.38 (br s, 2H); ^{13}C-NMR ($CDCl_3$) δ 4.5, 31.3, 32.4, 47.2, 68.7, 97.1, 114.4, 114.6, 120.6, 129.4, 141.1, 146.4, 158.8, 177.9; ESMS *m/z* 500.4 $(M\text{-}H)^-$.

4-[*N*,*N*-Di(2-carboxyethyl)amino]phenyl 3-phenoxybutyl telluride (10b)

Treatment of compound **9c** (0.37 g, 0.684 mmol) with $LiOH \cdot 2H_2O$ (0.115 g, 2.73 mmol), as described for **4a**, furnished the title compound (0.33 g, 94%) as an orange viscous oil; ^{1}H-NMR ($CDCl_3$) δ 1.81–1.89 (m, 4H), 2.63 (t, J = 7.0 Hz, 4H), 2.84 (t, J = 6.9 Hz, 2H), 3.65 (t, J = 7.0 Hz, 4H), 3.76 (t, J = 6.9 Hz, 2H), 3.94 (t, J = 6.9 Hz, 2H), 6.58 (d, J = 8.9 Hz, 2H), 6.86 (dd, J = 1.1, 8.8 Hz, 2H), 6.93 (tt, J = 1.1, 7.3 Hz, 1H), 7.26 (dd, J = 7.3, 88 Hz, 2H), 7.64 (d, J = 8.9 Hz, 2H), 9.38 (br s, 2H); ESMS *m/z* 514.4 $(M\text{-}H)^-$.

Results and discussion

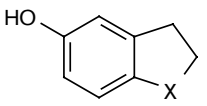
Design of organotellurium antioxidants

Divalent organotellurium compounds possess unique antioxidative properties. They can act both as hydroperoxide decomposers and as chain-breaking donating antioxidants. In addition, since the tetravalent organotelluriums resulting from these actions are reduced to the

divalent state by a variety of mild reductants, they have the capacity to act in a truly catalytic fashion [26–28]. Thus, in their hydroperoxide-decomposing action they mimic the glutathione peroxidase enzymes and in their chain-breaking capacity vitamin E. Certain principles for design of potent antioxidants have emerged from the vast amount of data accumulated concerning the antioxidative properties of organotelluriums. Nucleophilic attack of tellurium is probably involved in the rate-determining step in catalytic hydroperoxide reduction, whereas one-electron donation is critical for the chain-breaking activity. Thus, compounds with high electron density on the heteroatom should be sought for. Among the various classes of diorganyl tellurides, alkyltellurides are therefore likely to be more active than aryltellurides and aryltellurides should preferably carry electron-donating substituents. On the other hand, alkyltellurides are known to undergo oxidative decomposition much more readily than aryltellurides. As a rule, steric hindrance around tellurium should be avoided. However, certain groups with the capacity to coordinate to tetravalent tellurium have been reported to accelerate oxidation of tellurium(II) to tellurium(IV) [34,35]. Structural features which confer water solubility also seem to increase the antioxidative properties of organotelluriums [31,36]. With these guidelines in mind, known as well as new organotellurium antioxidants were prepared and evaluated for their capacity to inhibit TrxR and cancer cell growth in culture.

Preparation of organotellurium compounds

2,3-Dihydrobenzo[*b*]tellurophene-5-ol (**1d**) has recently been prepared as a primitive organotellurium analog of vitamin E [28]. It is an example of a cyclic alkyl aryl telluride where the aryl moiety contains an additional electron donating substituent. Its antioxidant profile was recently assessed in comparison with the corresponding oxygen (**1a**), sulfur (**1b**) and selenium analogs (**1c**). As judged from the inhibited rate of lipid peroxidation, R_{inh}, in a two-phase system, the antioxidant capacity increases (**1a** < **1b** ~ **1c** < **1d**) as one traverses the group of chalcogens. The reduction potentials for the proton coupled oxidation of the compounds decrease with increasing chalcogen substitution (**1a** = **1b** > **1c** > **1d**). It was therefore interesting to evaluate these compounds for TrxR inhibition to see the effect of the chalcogen.



1a X=O
1b X=S
1c X=Se
1d X=Te

Bis(4-hydroxyphenyl) telluride (**2**) is a readily available diaryl telluride with excellent antioxidative properties [37]. In an effort to increase its water solubility, one or both of its phenolic groups were alkylated with ω-bromoesters. Treatment of compound **2** with an excess of K_2CO_3 in acetone at reflux temperature, followed by addition of either 1 or 2.5 equivalents of methyl 5-bromovalerate and continuous heating at reflux for 20 h, afforded the desired products **3a** and **3b** in 64 and 72% yields, respectively (Scheme 1). Basic hydrolysis of the esters with 10% aqueous LiOH in 1:1 MeOH/H_2O furnished the corresponding acids **4a** and **4b** in almost quantitative yields.

A dialkylamino group is mesomerically more electron donating than a hydroxyl group. However, such a group does not usually confer water solubility at neutral pH. In order to increase solubility, organotellurium compounds were prepared where the *N*-alkyl groups had a carboxylate group at the terminus [36].

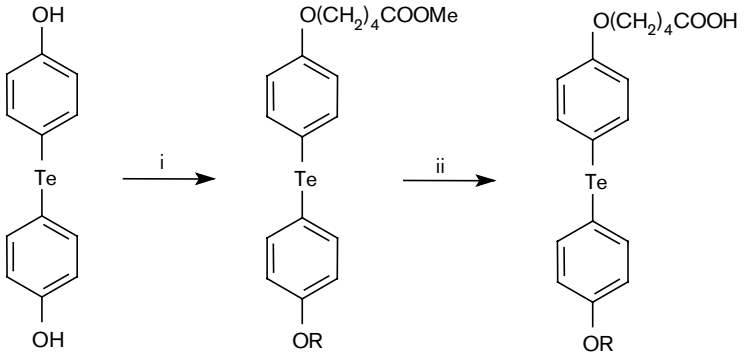
The first step in the synthesis of compounds **8a** and **10a** involved electrophilic aromatic substitution on *N*,*N*-di(2-carbomethoxyethyl)aniline (**5a**) with $TeCl_4$ at 0°C (Scheme 2). After stirring overnight, subsequent addition of an aqueous solution of $Na_2S \cdot 9H_2O$ followed by neutralization with $NaHCO_3$, and extraction with dichloromethane, resulted in a mixture of starting aniline (**5a**), diaryl ditelluride (**6a**) and the corresponding diaryl telluride (**7a**) (45% ditelluride). Detelluration of the ditelluride (**6a**) in the mixture with copper powder afforded telluride **7a** in 75% yield after flash chromatography. Basic hydrolysis with 10% aqueous NaOH in MeOH furnished the corresponding tetra-acid tetrasodium salt **8a** in 91% yield. Reduction of the aforementioned mixture with $NaBH_4$, to form the tellurolate, and addition of 3-bromopropyl phenyl ether furnished alkyl aryl telluride **9a**. Addition of 4-bromobutyl phenyl ether to the tellurolate similarly afforded telluride **9c**. Basic hydrolysis of the esters with 10% aqueous LiOH furnished the corresponding diacids **10a** and **10b** in 90 and 94% yields, respectively.

The synthesis of compound **7b** involved the same protocol as for the synthesis of **7a**, with the secondary amine *N*-(2-carbomethoxyethyl)aniline (**5b**) replacing the tertiary amine (**5a**) (Scheme 2). The synthesis of aryl alkyl tellurides **9a** and **9b** followed the protocol outlined above (Scheme 2).

TrxR and cancer cell growth inhibition

TrxR activity was measured as the increase in absorbance at 405 nm which occurs as added dithionitrobenzoic acid (DTNB) is reduced by thiols produced in an incubation containing Trx, TrxR, NADPH, insulin and the inhibitor (Table 1: TrxR/Trx activity). This assay reflects the

Scheme 1



2

3a R=H
3b R=$(CH_2)_4COOMe$

4a R=H
4b R=$(CH_2)_4COOH$

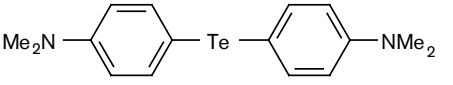
Reagents and conditions: (i) K_2CO_3, methyl 5-bromovalerate, acetone, 20 h, reflux; (ii) LiOH, MeOH, H_2O, 1 N HCl.

combined effects of the inhibition of Trx and TrxR. For most of the inhibitors tested, TrxR activity was also measured in incubations of DTNB, TrxR, NADPH and inhibitor, and measured as the initial increase in absorbance at 405 nm (Table 1: TrxR activity). This assay reflects the inhibition of TrxR only. Often, the IC_{50} values (concentrations required to inhibit thionitrobenzoate formation by 50%) obtained using the two methods were rather similar.

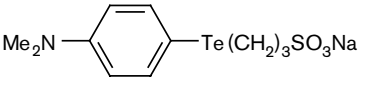
The effect on cancer cell growth was determined for MCF-7 breast cancer cells. Inhibition data expressed as IC_{50} values (concentration required to inhibit cell growth by 50%) are shown in Table 1.

Out of the four cyclic aryl alkyl chalcogenides **1**—all primitive analogs of vitamin E—only the tellurium compound **1d** showed interesting inhibition characteristics. It inhibited TrxR/Trx and TrxR with IC_{50} values of 5.8 and 1.2 μM. This observation is in line with previous findings in the series of diaryl chalcogenide inhibitors that the presence of tellurium in the molecule is essential for TrxR inhibition. It is also noteworthy that the inhibition data for the series of compounds **1** nicely reflect their capacity to decompose hydrogen peroxide as assessed by the coupled reductase assay [28].

Our attempts to modify the solubility of the potent antioxidant bis(4-hydroxyphenyl)telluride (**2**) by attaching one or two ω-carboxyalkyl groups to the phenolic hydroxyls proved rather unsuccessful. Whereas the parent compound **2** had an $IC_{50} = 5.7\ \mu$M [24], the best analog (**4b**) inhibited TrxR/Trx with an IC_{50} of 17.9 μM. As compared with the corresponding methyl esters **3a** and **3b**, the tellurium containing carboxylic acids **4a** and **4b** seem to be the better inhibitors.



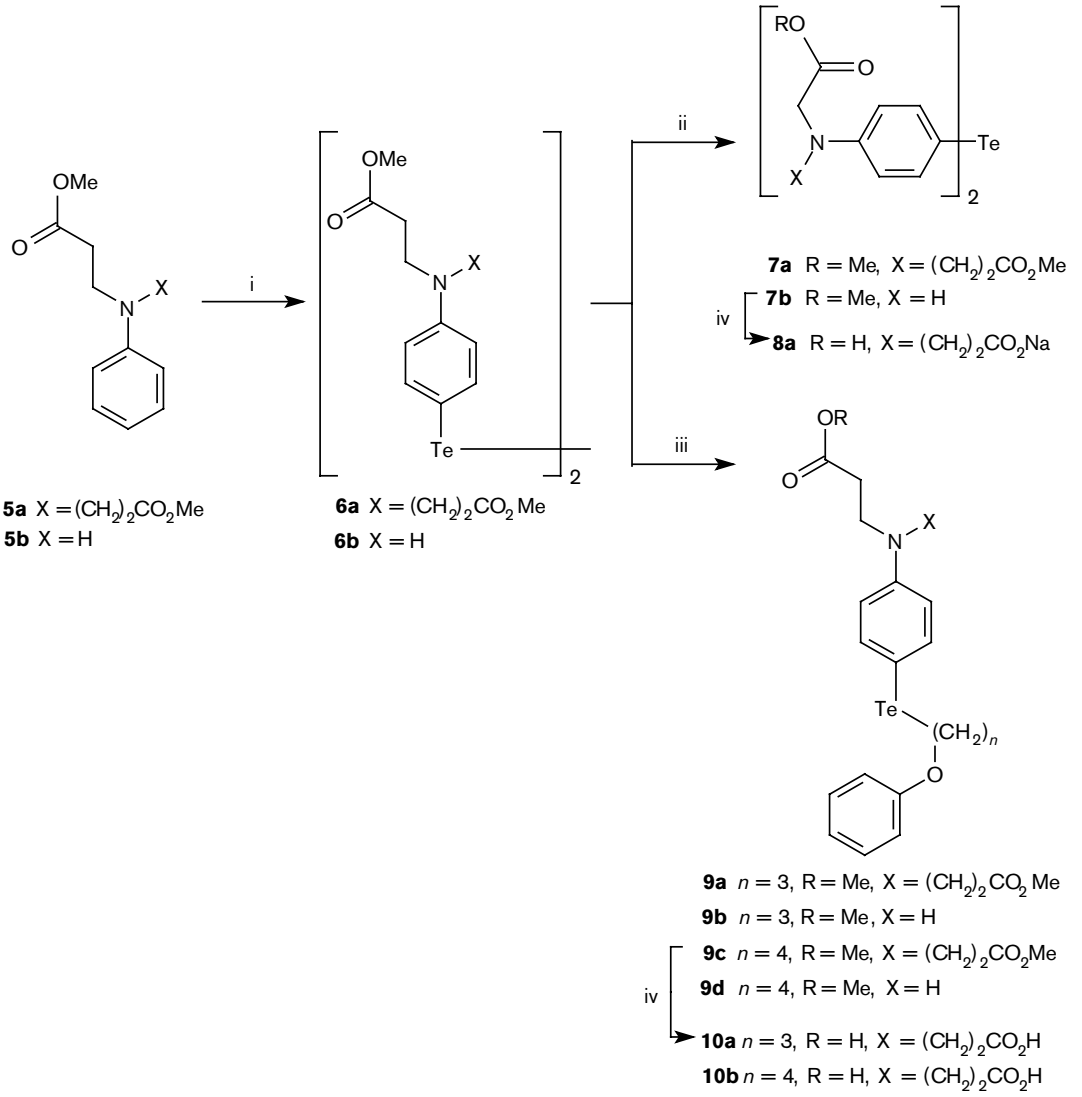
11



12

Organotellurium compounds **7–10** were designed and synthesized in an effort to elaborate on the 4-(dialkylamino)phenyltelluro scaffold of organotellurium antioxidants in order to obtain compounds with better inhibition characteristics. Compounds of this type previously evaluated for TrxR/Trx inhibiting capacity include the lipophilic diaryl telluride **11** ($IC_{50} = 8.2\ \mu$M [24]) and the more hydrophilic aryl alkyl telluride **12** ($IC_{50} = 42\ \mu$M [24]). Replacement of the four methyl groups in compound **11** for 2-(carbomethoxy)ethyls (**7a**) indeed improved TrxR/Trx inhibiting capacity significantly ($IC_{50} = 2.4\ \mu$M). The secondary amine **7b**, carrying only two *N*-2-(carbomethoxy)ethyl substituents, was a similarly efficient inhibitor ($IC_{50} = 1.6\ \mu$M). However, since the IC_{50} values from the TrxR assay were significantly larger for compounds **7a** and **7b** (22.0 and 8.4 μM, respectively), these compounds are probably Trx inhibitors rather than inhibitors of TrxR. As expected, the more hydrophilic tetracarboxylate **8a** turned out to be a better TrxR inhibitor than the corresponding tetraester ($IC_{50} = 4.8\ \mu$M). The 3-phenoxypropyl and 4-phenoxy-

Scheme 2



5a X = $(CH_2)_2CO_2Me$
5b X = H

6a X = $(CH_2)_2CO_2Me$
6b X = H

7a R = Me, X = $(CH_2)_2CO_2Me$
7b R = Me, X = H
8a R = H, X = $(CH_2)_2CO_2Na$

9a *n* = 3, R = Me, X = $(CH_2)_2CO_2Me$
9b *n* = 3, R = Me, X = H
9c *n* = 4, R = Me, X = $(CH_2)_2CO_2Me$
9d *n* = 4, R = Me, X = H

10a *n* = 3, R = H, X = $(CH_2)_2CO_2H$
10b *n* = 4, R = H, X = $(CH_2)_2CO_2H$

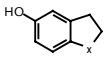
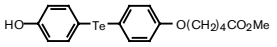
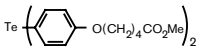
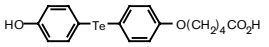
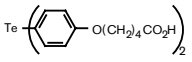
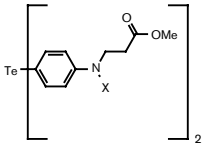
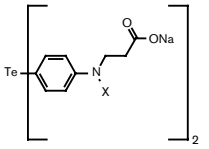
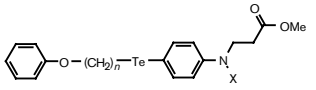
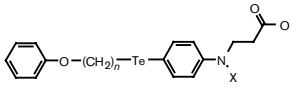
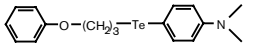
Reagents and conditions: (i) $TeCl_4$, $Na_2S \cdot 9H_2O$; (ii) Cu powder; (iii) $NaBH_4$, phenoxyalkyl bromide; (iv) aq. LiOH or aq. NaOH.

butyl groups were introduced into the 4-(dialkylamino)-phenyltelluro scaffold with the hope to facilitate oxidation of the heteroatom by stabilizing tetravalent tellurium via intramolecular (five- and six-membered rings, respectively) coordination of oxygen. Judging from the inhibition data for most compounds **9** and **10**, this approach was largely successful (compounds **9a**, **9c**, **9d** and **10a** all show TrxR/Trx IC_{50} values in the range of 1–2 μM and TrxR IC_{50} values in the range of 2–7 μM). In this series of compounds there is no indication that carboxylic acid derivatives are any more potent inhibitors than the corresponding methyl esters.

For comparison, alkyl aryl telluride **13** (Table 1), carrying a 3-phenoxypropyl group in the alkyl moiety, but retaining the 4-dimethylaminophenyl group in the aryl motif, was also included in the study. It showed similarly good inhibition characteristics in the TrxR assay ($IC_{50} = 1.6\,\mu$M) as the more hydrophilic compounds **9** and **10**. Strangely enough, it turned out to be a much poorer inhibitor in the combined TrxR/Trx assay. Although less pronounced, similar inhibition characteristics were also observed for compounds **10b** and **8a**. This unusual result could indicate binding to Trx outside the active cysteine residue.

The MCF-7 human breast cancer cell line was chosen for its relatively high level of Trx gene expression. Out of the compounds tested for inhibition in culture (Table 1), only compound **1d** showed interesting activity

Table 1 **Inhibition of TrxR and cancer cell growth by organotellurium antioxidants**

Structure	Compound number	IC_{50} (μM)		
		TrxR/Trx[a]	TrxR[b]	MCF-7[c]
	**1a** X = O	> 50	> 50	–
	1b X = S	> 50	> 50	–
	1c X = Se	> 50	> 50	–
	1d X = Te	5.8	1.2	1.8
	**3a**	32.3	–	–
	**3b**	22.6	–	–
	**4a**	20.8	–	–
	**4b**	17.9	–	–
	**7a** X = $(CH_2)_2CO_2Me$	2.4	22.0	> 50
	7b X = H	1.6	8.4	> 50
	**8a** X = $(CH_2)_2CO_2Na$	10.8	4.8	> 50
	**9a** $n = 3$, X = $(CH_2)_2CO_2Me$	1.6	1.8	> 50
	9b $n = 3$, X = H	26.8	–	26.4
	9c $n = 4$, X = $(CH_2)_2CO_2Me$	2.0	2.4	> 50
	9d $n = 4$, X = H	2.0	7.2	41.8
	**10a** $n = 3$, X = $(CH_2)_2CO_2H$	1.0	2.4	41.2
	10b $n = 4$, X = $(CH_2)_2CO_2H$	10	1.6	> 50
	**13**	18	1.6	10.6

[a]Inhibition of TrxR in the presence of Trx and insulin.
[b]Inhibition of TrxR.
[c]Inhibition of human MCF-7 breast cancer cell growth.

($IC_{50} = 1.8\,\mu M$). Although many of the other antioxidants were similarly good TrxR inhibitors, they were relatively inactive at killing cells ($10.6 < IC_{50} < 50\,\mu M$). The reason for this is as yet unclear. Cellular uptake could be restricted by the many polar groups (ester, acid, ether) present in these materials. The failure of several other excellent but polar (containing sulfopropyl groups) organotellurium inhibitors of TrxR to kill MCF-7 cells in culture was recently observed [25].

We have found that several kinds of potent organotellurium antioxidants inhibit TrxR efficiently already at low micromolar concentrations. However, the mechanism of inhibition is as yet obscure. According to a tentative mechanistic hypothesis previously forwarded by us, the oxidized organotellurium compound [presumably a tellurium(IV) oxide] could conceivably serve to crosslink (oxidize) active site selenocysteine residues or thiols crucial for enzyme activity. In an effort to test this hypothesis, antioxidant **1d** was pretreated with a stoichiometric amount of *t*-butylhydroperoxide (an agent which is known to rapidly oxidize diorganyl tellurides to the corresponding telluroxides) immediately before assessment of its TrxR/Trx inhibiting capacity. However, it turned out that the *in situ* prepared telluroxide inhibited the enzyme with a higher (more than 2-fold)

IC_{50} value than recorded for telluride **1d**. This clearly suggests that the telluroxide is not crucial for the events responsible for TrxR inhibition by organotelluriums. In addition to two-electron oxidation, organotelluriums are also known to undergo one-electron oxidation by various oxidizing agents. Maybe it is this capacity which make them act as inhibitors of TrxR.

In summary, we have shown that several classes of potent organotellurium antioxidants are good inhibitors of TrxR (IC_{50} values in the low micromolar range). Although one relatively non-polar primitive analog of vitamin E efficiently inhibited growth of MCF-7 cells in culture ($IC_{50} = 1.8\,\mu M$), most of the other, more functionalized antioxidants, did not inhibit cell growth in concentrations below $10\,\mu M$.

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