Supporting Information

Synthesis and Selective Anti-Cancer Activity of Organochalcogen Based Redox Catalysts

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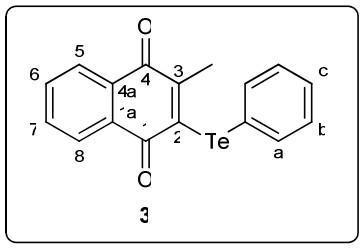
1. General procedure for the synthesis of compounds **1** to **10**

Compounds **1** to **6** and **10** have previously been reported in the literature[1-7] and were synthesized according to the given procedures. In order to improve the yield, a more general procedure for the synthesis of quinone-chalcogen compounds was developed as part of this study and also applied for the synthesis of some of the known compounds in addition to the hitherto unknown compounds **7** to **9**.

As part of this general procedure, the appropriate diselenide/ditelluride (1 eq.) was dissolved in a 1:1 mixture of water and THF (50 mL) under protective (nitrogen or argon) gas. $NaBH_4$ (5 eq.) was added (when thiophenol was used in case of sulfur analogues, $NaBH_4$ was not required). The mixture was stirred until the solution turned colourless (1-3 min). The reaction mixture was stirred for a further 30 min. A solution of a haloquinone (1 eq. for dihaloquinone or 2 eq. for monohaloquinone) in THF (5 mL) was added through the rubber septum without opening the reaction apparatus. The reaction mixture was stirred at room temperature and monitored via thin layer chromatography (TLC). Disappearance of the quinone spot indicated that the reaction was complete (usually between 3 to 10 min). Afterward the solution was stirred for a further 30 min on air. The violet, dark red or orange coloured reaction mixture (depending upon the Te, Se or S counterpart of the product) was diluted with 50 mL of saturated aqueous solution of NH_4Cl and extracted with diethylether or ethyl acetate. The combined organic extracts were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel chromatography using mixtures of petrol ether (40-65°C) and ethyl acetate as specified for each compound below. Yields between 18% and 95% were obtained by this method and compounds were determined to exceed 95% purity by HPLC.

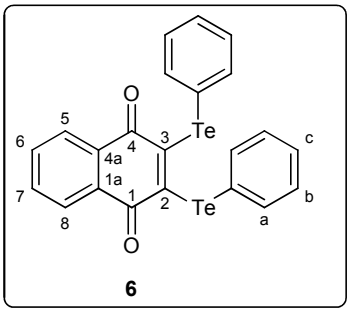
Please note that some of the compounds can be sensitive to oxidation and light. They should therefore be stored under protective gas at low temperature and in the dark.

2. Synthesis of 2-(phenyltellanyl)-3-methylnaphthoquinone **3**



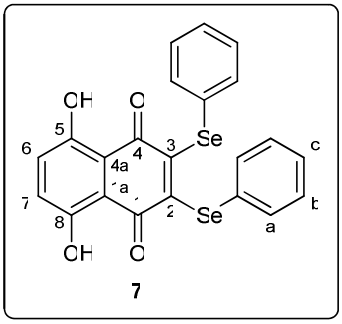
Compound **3** was synthesized from diphenylditelluride (50 mg, 0.12 mmol), $NaBH_4$ (23 mg, 0.61 mmol) and 2-methyl-3-bromo-1,4-napthoquinone (46 mg, 0.18 mmol) following the general procedure. Formation of **3** was monitored by TLC (petrol ether: ethyl acetate, 9:1 v/v): R_f = 0.45. The compound was purified by column chromatography on silica gel (mesh size 40-60 µm) using petrol ether: ethyl acetate (9.5:0.5 v/v) as solvent. Yield 98%, deep purple crystals, mp 89°C. ^{1}H NMR: 8.07-8.05 (m, 2H, H-5, H-8); 7.83-7.81 (m, 2H, H-a or H-b); 7.71-7.64 (m, 2H, H-6, H-7); 7.35-7.32 (m, 1H, H-c); 7.25-7.22 (m, 2H, H-a or H-b); 1.95 (s, 3H, CH_3). ^{13}C NMR: 184.2 (1C), 181.2 (1C), 153.5 (1C), 142.0 (1C), 139.6 (2C), 133.8 (1C), 133.3 (1C), 132.0 (1C), 131.6 (1C), 129.5 (2C), 128.6 (1C), 127.1 (1C), 126.9 (1C), 114.2 (1C), 20.2 (1C) ppm. IR: ν= 1647, 1587, 1558, 1277, 1245, 1176, 952, 736, 694, 649 cm^{-1}. HPLC: t_R 6.571 min, purity 98.8%. HRMS (*m/z*): $[M]^+$ calcd. for $C_{17}H_{12}O_2Te$ 377.9899; found 377.9855; $[M+H]^+$ calcd. 378.9977; found 378.9935. Calculated isotope pattern of Te: m/z (relative abundance %) 369.9868 (7.7), 370.9880 (2.7), 372.9881 (21.1), 375.9882 (93.8), 376.9915 (17.2), 377.9899 (100), 378.9933 (18.4), 379.9966 (1.6); found isotope pattern of Te: m/z (relative abundance %) 368.9602 (1.91), 369.9776 (7.01), 370.9761 (5.18), 372.9816 (23.12), 375.9858 (90.11), 376.9880 (20.71), 377.9855 (100), 378.9935 (24.28), 379.9995 (2.90).

3. Synthesis of 2, 3-bis(phenyltellanyl)naphthoquinone **6**



Compound **6** was synthesized from diphenylditelluride (302 mg, 0.74 mmol), $NaBH_4$ (112 mg, 2.95 mmol) and 2,3-dichloro-1,4-napthoquinone (84 mg, 0.37 mmol) following the general procedure. Its formation was monitored by TLC (petrol ether : ethyl acetate, 9.5:0.5 v/v): R_f = 0.24. **6** was purified by column chromatography on silica gel (mesh size 40-60 μm) using petrol ether : ethyl acetate (9.5:0.5 v/v) as solvent. Yield 25%, deep purple crystals, m.p. 163-164°C. ^{1}H NMR: 7.96-7.94 (m, 2H, H-5, H-6 or H-7, H-8); 7.80-7.78 (m, 4H, H-a or H-b); 7.61-7.59 (m, 2H, H-5, H-6 or H-7, H-8); 7.34-7.31 (m, 2H, H-c); 7.25-7.22 (m, 4H, H-a or H-b). ^{13}C NMR: 179.7 (2C), 154.2 (2C), 139.1 (4C), 133.3 (2C), 132.0 (2C), 129.6 (4C), 128.5 (2C), 127.7 (2C), 118.4 (2C) ppm. IR: ν= 1655, 1568, 1256, 1116, 781, 731, 654 cm^{-1}. HPLC: t_R 12.53 min, purity 99.1%. HRMS (*m/z*): $[M]^+$ calcd. for $C_{22}H_{14}O_2Te_2$, 569.9118; found 569.9187; $[M+H]^+$ calcd. 570.9196; found 570.9188. Calculated isotope pattern of Te: m/z (relative abundance %) 559.9069 (7.7), 560.9071 (12.6), 561.9060 (16.8), 562.9082 (21.1), 563.9071 (56.1), 564.9100 (22.5), 565.9089 (59.8), 566.9122 (14.2), 567.9100 (100), 568.9134 (23.8), 569.9118 (53.3), 570.9152 (12.7), 571.9185 (1.4); found isotope pattern of Te: m/z (relative abundance %) 559.9163 (17.09), 560.9181 (17.28), 561.9150 (37.09), 562.9155 (29.45), 563.9117 (67.96), 564.9149 (34.77), 565.9121 (100), 566.9224 (24.76), 567.9161 (90.34), 568.9227 (21.59), 569.9187 (50.01), 570.9188 (11.65), 571.9195 (1.51).

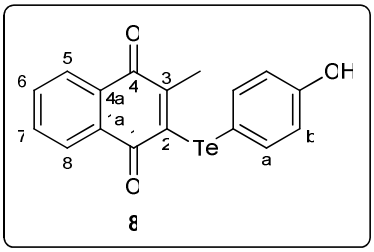
4. 2,3-Bis(phenylselanyl)-5,8-dihydroxynaphthoquinone **7**



Compound **7** has not been reported yet. Its synthesis and basic analytical data are provided in the experimental part of the manuscript. Additional analytical information:

IR: ν = 2918, 2849, 1648, 1588, 1559, 1398, 1177, 1123, 736 cm^{-1}. Calculated isotope pattern of Se: m/z (relative abundance %) 494.9283 (5.6), 495.9257 (17.2), 496.9264 (14.5), 497.9249 (36.3), 498.9256 (30.6), 499.9230 (94.8), 500.9264 (22.5), 501.9222 (100), 502.9256 (23.8), 503.9224 (37.9), 505.9226 (3.6); found isotope pattern of Se: m/z (relative abundance %) 494.9563 (6.86), 495.9487 (20.55), 496.9485 (19.72), 497.9478 (54.57), 498.9545 (45.83), 499.9536 (94.80), 500.9564 (39.93), 501.9472 (100.00), 502.9539 (39.12), 503.9546 (35.47), 505.9578 (5.13).

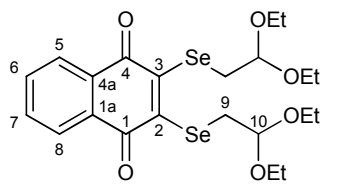
5. 2-(Hydroxyphenyltellanyl)-3-methylnaphthoquinone **8**



Compound **8** has not been reported yet. Its synthesis and basic analytical data are provided in the experimental part of the manuscript. Additional analytical information:

IR: ν = 1655, 1568, 1256, 1116, 781, 731, 654 cm^{-1}. Calculated isotope pattern of Te: m/z (relative abundance %) 385.9817 (7.7), 386.9829 (2.7), 387.9814 (14.3), 388.9831 (21.1), 389.9819 (56.1), 391.9831 (93.8), 393.9848 (100), 394.9882 (18.4), 395.9916 (1.6); found isotope pattern of Te: m/z (relative abundance %) 385.9653 (7.08), 386.9681 (4.92), 387.9660 (14.12), 388.9688 (23.79), 389.9700 (58.90), 391.2749 (0.09), 391.9746 (91.89), 393.1926 (0.19), 393.9677 (100.00), 394.9754 (30.57), 395.9840 (4.62).

6. 2,3-Bis[(2,2-diethoxyethyl)selanyl]naphthoquinone **9**



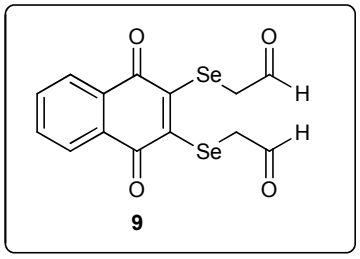
9

Compound **9** has not been reported yet. Its synthesis and basic analytical data are provided in the experimental part of the manuscript. Additional analytical information:

IR: ν = 2975, 2876, 1664, 1644, 1495, 1271, 1118, 1096, 1053, 1021, 1000, 972, 703, 622 cm^{-1}. Calculated isotope pattern of Se: m/z (relative abundance %) 544.0407 (17.2), 545.0414 (14.5), 546.0400 (36.3), 547.0407 (30.6), 548.0380 (94.8), 549.0414 (22.5), 550.0373 (100), 551.0406 (23.8),

552.0375 (37.9), 553.0408 (9.0), 554.0376 (3.6); found isotope pattern of Se: m/z (relative abundance %) 544.0058 (21.15), 545.0101 (17.82), 546.0100 (52.89), 547.0142 (38.45), 548.0191 (91.99), 549.0166 (29.80), 550.0223 (100.00), 551.0254 (24.43), 552.0272 (32.00), 553.0207 (7.87), 554.0304 (3.41).

7. 2,3-Bis[(2-acetaldehyde)selanyl]naphthoquinone **11**



Compound **9** (291 mg, 5.301 mmol) was dissolved in a mixture of 3 mL HCl (1M) and 1 mL THF, and heated at 50°C for 12h. The mixture was poured onto 10 mL saturated aqueous $Cu(OAc)_2$ solution, then extracted with chloroform. The combined organic extracts were evaporated and the crude product obtained was dissolved in 20 mL diethylether and rinsed 3 times with 10 mL H_2SO_4 (2.5 M). The organic phase was dried over Na_2SO_4, filtered and then concentrated under reduced pressure to yield 166 mg of a dark brown solid. ^{1}H NMR of crude **11**: 9.71-9.70 (t, *J*(H,H) = 2.8 Hz, 2H, 2 x CHO); 8.04-8.02 (m, 2H); 7.69-7.68 (m, 2H); 3.94-3.93 (d, *J*(H,H) = 2.9 Hz, 4H, 2 x CH_2). ^{13}C NMR of crude **11**: 194.2 (2C), 178.5 (2C), 149.0 (2C), 134.2 (2C), 132.6 (2C), 127.8 (2C), 38.2 (2C) ppm.

Further purification of the compound resulted in decomposition and/or reaction with components the purification system.

8. Thiophenol assay

The GPx-like catalytic activity of the compounds was measured by monitoring the formation of PhSSPh formed during the oxidation of thiophenol in presence of H_2O_2. 10 µl of compound (100 µM) in DMSO were added to 890 µl methanolic solution of PhSH (1 mM) containing Et_3N (0.05 mM). The reaction was initiated by adding 100 µl H_2O_2 (2 mM) and monitored spectrophotometrically at 305 nm for 25 min at 18°C. Ebselen was used as benchmark compound in this assay.[3, 8]

9. Luciferase assay for K562 cell viability

This assay was used to assess metabolic activity (ATP-quantification) as a measure of cell viability. The percentage of cell death after incubation with the test compounds was determined using the Cell Titer Glo® Luminescent Cell Viability Assay Kit (Promega, Leiden, Netherlands). Assays were performed according to the manufacturer's instructions. Briefly, the assay measures the number of viable cells, based on the quantification of ATP as an indicator of metabolic activity. Lyophilized enzyme/substrate mixture was reconstituted with the buffer provided. Then, an equal volume of the reaction buffer was added to the medium containing K562 cells (untreated or treated with the indicated compounds). The mixture was shaken on a rocking platform for 2 min, then incubated for 10 min in the dark at room temperature. The luminescence signal, proportional to the amount of ATP present, was quantified by using an Orion Microplate Luminometer (Berthold, Pforzheim, Germany), and converted to a number of viable cells according to the manufacturer's instructions. Data were normalized to the control and reported as percentage of viable cells.

10. CLL B-cell and PBMC sample processing and culture

Blood was obtained from both healthy donors of the Cologne University central blood bank and from CLL patients seen at the Cologne University Department of Internal Medicine I. All had provided written informed consent under a protocol approved by the Cologne University Hospital Institutional Review Board according to the regulations of the Declaration of Helsinki. All CLL patients had a confirmed diagnosis using standard diagnostic criteria. Healthy donor PBMC were isolated by Ficoll (resource) density gradient centrifugation of 'buffy coat' material. For lymphocyte isolation from CLL patient samples, a CD19-negative enrichment (Rossette Sep, Stem Cell Technologies, Cell Systems, Germany) prior to density gradient centrifugation was performed. When assessed by flow cytometry (FACSscan; Becton Dickinson, Franklin Lakes, NJ USA), the isolated cells were predominately CLL B-cells (minimum 80% CD19-positive, of which approximately 95% were CD19/CD5 co-positive).

Freshly isolated primary CLL B-cells or healthy controls (PBMC) were cultured in RPMI medium (GIBCO, Germany) with L-glutamine, penicillin, and streptomycin with or without 10% fetal calf serum. The cells were maintained at 37°C in a 95% air / 5% CO_2 (vol/vol) atmosphere. CD40-L (100 ng/mL, Peprotech, Germany) was used as standard supplementation to sustain CLL B-cell cultures at viable conditions.

CLL B-cells and PBMC were treated with different concentrations of indicated compounds ranging from 50 nM to 5 μM for 12 to 36 h followed by the assays indicated. All results shown are representatives of at least three independent experiments.

11. Determination of cell viability

Primary CLL B-cells or PBMC from healthy donors (approximately 1 x 10^6 cells/mL) were treated with either vehicle (DMSO) control or indicated compounds for 24 to 48 h, washed with Dulbecco calcium- and magnesium-free phosphate-buffered saline (PBS), and evaluated for cell viability with the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Germany) according to the manufacturer's protocol with minor modifications. This assay is based on quantification of cellular ATP levels, reflecting metabolic activity. The tests were performed in opaque-walled 96-well plates in triplicates and the luminescence was recorded on a Microplate Luminometer (LB 96V, EG & G Berthold, Germany) with an integration time of 0.1 to 1 s per well.

12. Apoptosis and cell death assay by flow cytometry

Following the cultures and harvests described above, approximately $5x10^5$/mL CLL B-cells or PBMC from healthy donors were evaluated for apoptosis using dual AnnexinV-PE and 7-AAD staining with fluorescence activated cell sorter (FACS Calibur, Becton Dickinson) analysis. Cells stained with AnnexinV only (early apoptosis) or both (AnnexinV and 7-AAD, late apoptosis) were considered positive. Storing and processing of data (10,000 events / condition) were carried out via Cell QuestPro FACSsoftware (Becton Dickinson).

13. Determination of caspase-3/7 activity

The same accrual and analysis of colorimetric readings as above were performed in the caspase-3/7 activity assays estimating the degree of caspase-activation and supplementing the flow-cytometric data on cell survival below. The Caspase-GloTM 3/7 Assay (Promega, Germany) was used. It provides a pro-

luminescent caspase-3/7 substrate, which contains the caspase-3 specific tetrapeptide sequence DEVD. The addition of a Caspase-3/7 reagent results in cell lysis, followed by caspase-mediated cleavage of Z-DEVD and release of aminoluciferin, which is a substrate for the luciferase reaction resulting in luminescence.

14. HT29, A549, MCF7, HUVEC, NIH 3T3 cells culture and sample processing

All chemicals were from Sigma (Saint Quentin Fallavier, France). MCF7 (human breast adenocarcinoma, HTB-22), HT29 (human colon adenocarcinoma, HTB-38), A549 (human lung carcinoma, CCL-185) and NIH 3T3 (mouse fibroblast, CRL-1658) were obtained from American Type Culture Collection (Manassas, VA). Human umbilical vein endothelial cells (HUVEC) were obtained by digestion of umbilical cords with 0.1% collagenase. Cells were cultured in DMEM/Glutamax-I supplemented with 10% heat-inactivated FCS and antibiotics (Life Technologies, Cergy Pontoise, France). All cell lines were routinely tested to rule out Mycoplasma infection of cells.

15. Cellular production of H_2O_2

Cells (2 x 10^4 cells per well) were seeded in 96-well plates (Costar, Corning, Inc., Corning, NY) and incubated for 48 h with various concentrations of **6** alone or in combination with 5-FU or irinotecan, or with culture medium alone. Levels of intracellular H_2O_2 were assessed spectrofluorometrically (Fusion, Packard, Boston, Ma, USA) by oxidation of 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) (Molecular Probes). Cells were washed once with PBS and then incubated with 200 μM H_2DCFDA in PBS for 1 h at 37°C. Excitation and emission wavelengths used were 500 and 605 nm for DHE and 490 and 535 nm for H_2DCFDA, respectively. The number of viable cells was evaluated by the crystal violet assay as described below. The levels of H_2O_2 were calculated in each sample as follows: ROS rate

(arbitrary units/min/10^6 cells) = (fluorescence intensity [arbitrary units] at 60 min – fluorescence intensity [arbitrary units] at 0 min)/(60 min x number of viable cells as measured by the crystal violet assay).

16. *In vitro* cell proliferation and viability assays

MCF7, HT29, A549 carcinoma cell lines and NIH 3T3 (mouse fibroblast) or HUVEC (2×10^4 cells/well) were seeded in 96-well plates and incubated for 24 h in complete DMEM medium with varying amounts of **6** alone or with 5-FU or irinotecan. Cell proliferation was determined by pulsing the cells with [^{3}H]thymidine (1 Ci/well) during the last 16 h of culture. Cell viability was evaluated by the crystal violet assay. In brief, cells were stained in 0.5% crystal violet and 30% ethanol in PBS for 30 min at room temperature. After two washes in PBS, the stain was dissolved in 50% ethanol, and absorbance was measured at 560 nm on an ELISA multiwell reader. Results are expressed as percentage of viable cells compared to untreated cells (which have 100% viability).

17. Detection of cellular ROS levels in blood lymphocytes

Cellular ROS contents were measured by incubating cells (5 x 10^5 cells) with 1 μM CM-H_2DCF-DA for 30 min and analyzed by flow cytometry as described[9]. To ensure the consistency of the assay across variable patient samples, we used a reference cell line (Raji cells) stained under identical conditions as a control for comparable parameter settings.

Statistical Analysis. Statistical significance of differences between experimentally treated groups and untreated controls was analyzed by Student's t test for comparison of means, except for comparison of

non-parametric data (*i.e.* Figure 4a), which were analyzed by Mann-Whitney test. A level of $P < 0.05$ was considered significant. Symbols: *: $P < 0.05$; **: $P < 0.02$; ***: $P < 0.01$; ****: $P < 0.001$ *versus* controls.

18. References

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