6954 J. Med. Chem. 2010, 53, 6954–6963
DOI: 10.1021/jm100576z

Journal of Medicinal Chemistry Article

Synthesis and Selective Anticancer Activity of Organochalcogen Based Redox Catalysts

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Received May 11, 2010

Many tumor cells exhibit a disturbed intracellular redox state resulting in higher levels of reactive oxygen species (ROS). As these contribute to tumor initiation and sustenance, catalytic redox agents combining significant activity with substrate specificity promise high activity and selectivity against oxidatively stressed malignant cells. We describe here the design and synthesis of novel organochalcogen based redox sensor/effector catalysts. Their selective anticancer activity at submicromolar and low micromolar concentrations was established here in a range of tumor entities in various biological systems including cell lines, primary tumor cell cultures, and animal models. In the B-cell derived chronic lymphocytic leukemia (CLL), for instance, such compounds preferentially induce apoptosis in the cancer cells while peripheral blood mononuclear cells (PBMC) from healthy donors and the subset of normal B-cells remain largely unaffected. In support of the concept of sensor/effector based ROS amplification, we are able to demonstrate that underlying this selective activity against CLL cells are pre-existing elevated ROS levels in the leukemic cells compared to their nonmalignant counterparts. Furthermore, the catalysts act in concert with certain chemotherapeutic drugs in several carcinoma cell lines to decrease cell proliferation while showing no such interactions in normal cells. Overall, the high efficacy and selectivity of (redox) catalytic sensor/effector compounds warrant further, extensive testing toward transfer into the clinical arena.

1. Introduction

The marked presence of oxidative stress (OS[a]) in various cancer cells has recently received increasing attention.[1,2] Several strategies have been proposed to use the presence of reactive oxygen species (ROS) in these cells to selectively target tumors without damaging healthy tissue. Some of these approaches have been reviewed by us recently.[3] Agents such as certain quinones and arsenic oxide (As_2O_3), for instance, increase levels of reactive oxygen species (ROS) in cells.[4] They also appear to push oxidatively stressed cancer cells over a critical redox threshold while affecting healthy cells (which are naturally lower in ROS) to a lesser extent. Similarly, inhibitors of key antioxidant enzymes, such as 2-methoxyestradiol (2-ME, an inhibitor of superoxide dismutase, SOD), have been employed. 2-ME causes the build-up of superoxide radical anions ($O_2^{\bullet -}$) in human leukemia cells, which are subsequently "overwhelmed" by $O_2^{\bullet -}$ and its follow-on products (e.g., H_2O_2, $HO^{\bullet}$ radical), while normal lymphocytes are not affected.[5,6]

While these agents increase ROS levels in cells indiscriminately, various compounds have been developed that are able to modulate the *existing* intracellular redox balance via catalysis: A catalytic process requires not only a suitable catalyst but also the presence of appropriate substrate(s). Catalysis therefore combines high *efficiency* with *selectivity*. Several recent reports have confirmed the notion of redox catalysts as effective, yet also rather selective agents able to kill cancer cells. For instance, we have used SOD mimics able to generate oxygen-based radicals in order to target carcinoma cells. This approach has provided promising results in murine CT26 colon cancer cells, in culture, and in BALB/c xenograft models[7,8,35]. At the same time, we (and others) have developed mimics of the human enzyme glutathione peroxidase (GPx), which target redox sensitive thiol proteins and enzymes. In the presence of elevated levels of ROS, these chalcogen-based catalysts turn toxic against a range of cultured cells, including Jurkat leukemia cells and human Daudi cells.[11,12]

While most of the earlier studies have been performed with initial generations of agents and solely in cell lines, we are now able for the first time to report the effects of novel catalytic,

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[a] Abbreviations: CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; COX-2, cyclooxygenase 2; DMSO, dimethylsulfoxide; 5-FU, 5-fluorouracil; GSH, reduced glutathione; H_2DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; HUVEC, human umbilical vascular endothelial cells; LPS, lipopolysaccharide; OS, oxidative stress; PB, peripheral blood; PBMC, peripheral blood mononuclear cells; PEITC, β-phenylethyl isothiocyanate; ROS, reactive oxygen species; SOD, superoxide dismutase.

 Published on Web 09/13/2010

chalcogen-containing agents on primary human cell material. Malignant B-cells from CLL, for instance, contain elevated levels of ROS,[13] and our agents increase these ROS levels significantly. Such compounds induce caspase-mediated apoptosis in the malignant lymphocytes, while PBMC from healthy donors (including their normal B-cell fraction) remain largely unaffected. Similarly, certain organotellurium compounds are cytotoxic against myeloid leukemia cells (K562) and cells derived from the most common solid tumor pathologies, such as colorectal carcinoma (HT29), lung cancer (A549), and breast cancer (MCF7), while normal human umbilical vascular endothelial cells (HUVEC) or healthy fibroblasts (NIH 3T3) are less affected.

2. Results

2.1. Selection and Synthesis of Suitable Redox Agents. Our previous studies have indicated that multifunctional redox agents combining two or more redox sites in one molecule are particularly suitable to target (cancer) cells under OS. This is due to the fact that OS is a multicomponent event and agents able to interact with various ingredients of OS are more effective compared to agents with a single target. On the basis of the need to combine several redox centers in one molecule and our recent investigations in cell culture, 10 agents were selected for this study and subsequently synthesized (Figure 1). Compounds **1**−**6** and **10** were already known in the literature and could be synthesized according to established procedures.[12,14−19] The remaining compounds (**7**−**9**), to the best of our knowledge, have not been reported before. Appropriate synthetic procedures were developed for these agents and successfully employed (see Materials and Methods section and Supporting Information). Scheme 1 exemplifies the synthetic pathway for compound **7**. With a few small adjustments, this procedure is also applicable to the other compounds, all of which were obtained in yields between 18% and 95% and in high purity (97% or more as measured by HPLC).

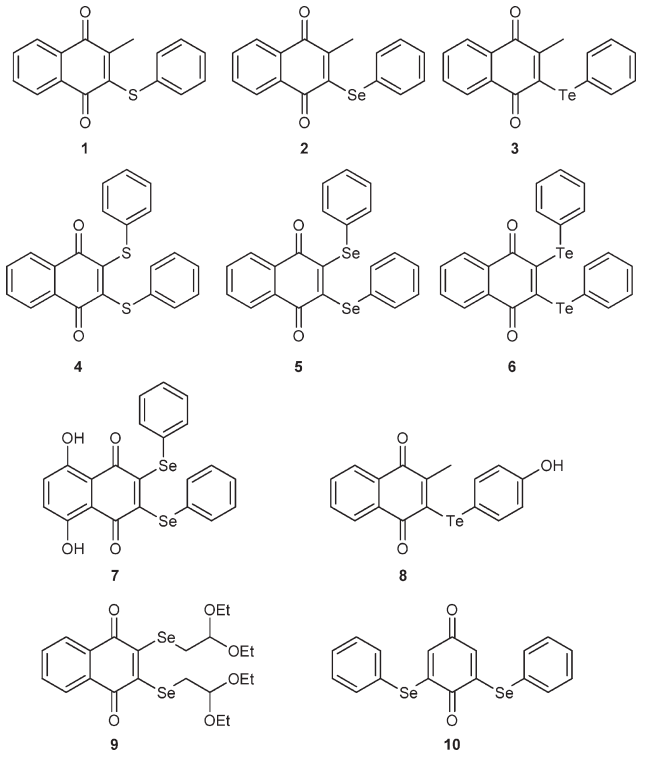
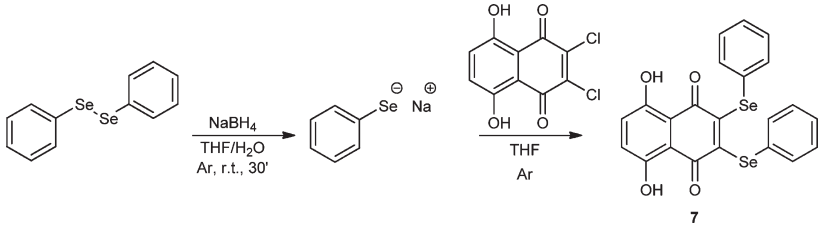


Figure 1. Chemical structures of compounds **1**−**10** which were selected for this study. **1**−**3** and **4**−**6** form a series of S → Se → Te homologous structures with two or more redox centers.

The important, GPx-like catalytic activity of the various compounds was confirmed using the thiophenol (PhSH) assay, a standard catalytic assay that spectrophotometrically follows the formation of diphenyl disulfide (PhSSPh) in the presence of H_2O_2 and catalyst. Increased rates of disulfide formation are indicative of catalytic activity.[12,20] As expected, most compounds had a significant effect on disulfide formation, with **6** increasing the initial rate of the reaction by 2.15-fold, **7** by 1.95-fold, and **3** by 1.54-fold. For comparison, the benchmark organoselenium catalyst ebselen increased the rate by 1.87-fold.

2.2. Initial Efficacy Screen in Human K562 Myeloid Leukemia Cells. In order to gain an initial overview of possible cytotoxicity of the compounds and to narrow down the number of potentially interesting candidates for further, more extensive studies, an initial activity screen in K562 cells, derived from a chronic myelogenous leukemia (CML) in blast crisis, was employed. The results obtained in the K562 viability assay are shown in Figure 2. Among the compounds tested in this assay (compound **10** did not give any reproducible results), compounds **3**, **6**, **8**, and **9** were particularly active at 10 μM, with an average remaining cell viability of just 4.1% for **3**, 15.0% for **6**, 30.0% for **8**, and 8.6% for **9**. The other compounds tested did not show any major effect on cell survival. Compounds **1**, **2**, and **7**, for instance, reduced cell viability by around 10% or less. Apart from enabling a preselection of compounds, the data obtained also point toward an emerging structure−function relationship. Cytotoxicity to some extent reflects the catalytic activity of compounds and generally supports the previously held notion that tellurium agents are considerably more active than their sulfur and selenium analogues. Yet there also appear to be some exceptions, such as selenium compound **9**, which is fairly active in the K562 screen. We

Scheme 1. General Reaction Scheme for the Synthesis of Quinone−Chalcogen Compounds as Exemplified for the Synthesis of Compound **7**[a]



[a] Experimental details are provided in the Supporting Information.

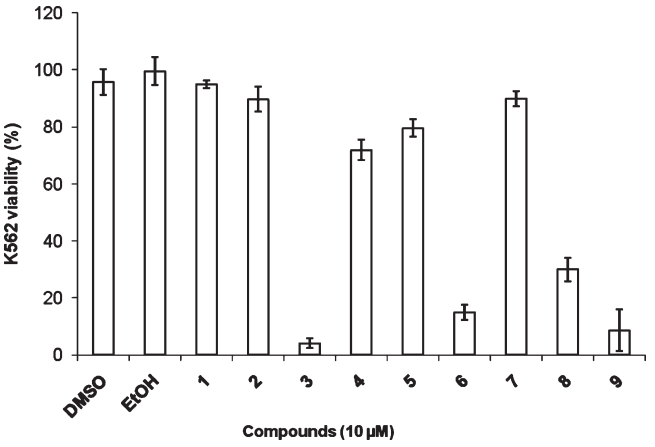


Figure 2. Influence of quinone−chalcogen compounds on K562 chronic myelogenous leukemia cells. These experiments serve as an initial "screen" for biological activity of compounds and provide a basic structure−activity relationship. The results highlight the rather high activity of quinone−tellurium agents in comparison to the sulfur and selenium analogues and identify compounds **3**, **6**, and **9** as the most promising agents for further testing. Error bars represent SD. Since DMSO and ethanol were used to prepare the stock solutions of the compounds, appropriate controls were included.

point out, however, that this effect might also be related to the protected aldehyde (acetal) function: The reactive aldehyde (which can be synthesized but yet is chemically unstable and hence cannot be employed in cell culture) could be liberated in cells and might exert its own toxic effects.

2.3. Evaluation in CLL B-Cells Derived from Peripheral Blood of Leukemia Patients. On the basis of the results obtained in the thiophenol assay and in K562 cells, compounds **3**−**8** were tested in CLL B-cells isolated from the peripheral blood (PB) of leukemia patients paralleled by tests in PBMC from healthy blood donors as controls in at least three independent experiments per assay. Details on cell harvest, readouts, dose titrations, incubation times, and statistics are all described in the Supporting Information. The term "activity" is used here to describe the effect on CLL cells (viability or apoptosis), while "toxicity" describes the effect on healthy PB control cells.

CLL cell viability was determined by intracellular ATP levels. On the basis of three different patient samples, a clearly differential activity across compounds could be observed (Figure 3a). With the exception of compound **8**, all other compounds showed moderate (compounds **3** and **4**) to strong (compounds **5**, **6**, and **7**) activity in reducing CLL B-cell viability at low to submicromolar concentrations. Moreover, at the same concentrations, viability of healthy donor PBMC was largely unaffected by compounds **3**, **5**, and **6**, whereas a high toxicity to healthy control cells was observed for compound **7**. Overall, by comparison of the activity of the tested compounds against CLL to toxicity against healthy donor PBMC, compounds **3**, **5**, and **6** were the most effective in selectively reducing viability of CLL cells. Other agents, such as **4** and **7**, exhibited a considerably lower activity or were only active at higher concentrations (10 μM or more), which are not deemed to be physiologically relevant or clinically feasible for such compounds. Interestingly, **3**, **5**, and **6** are chemically closely related: **3** is a monosubstituted derivative, and **5** is the selenium analogue of **6**.

A subsequent more in-depth study was conducted for the most promising compounds **3**, **5**, and **6** with compounds **4**, **7**, and **8** as comparison. Flow cytometry based annexin V/7AAD

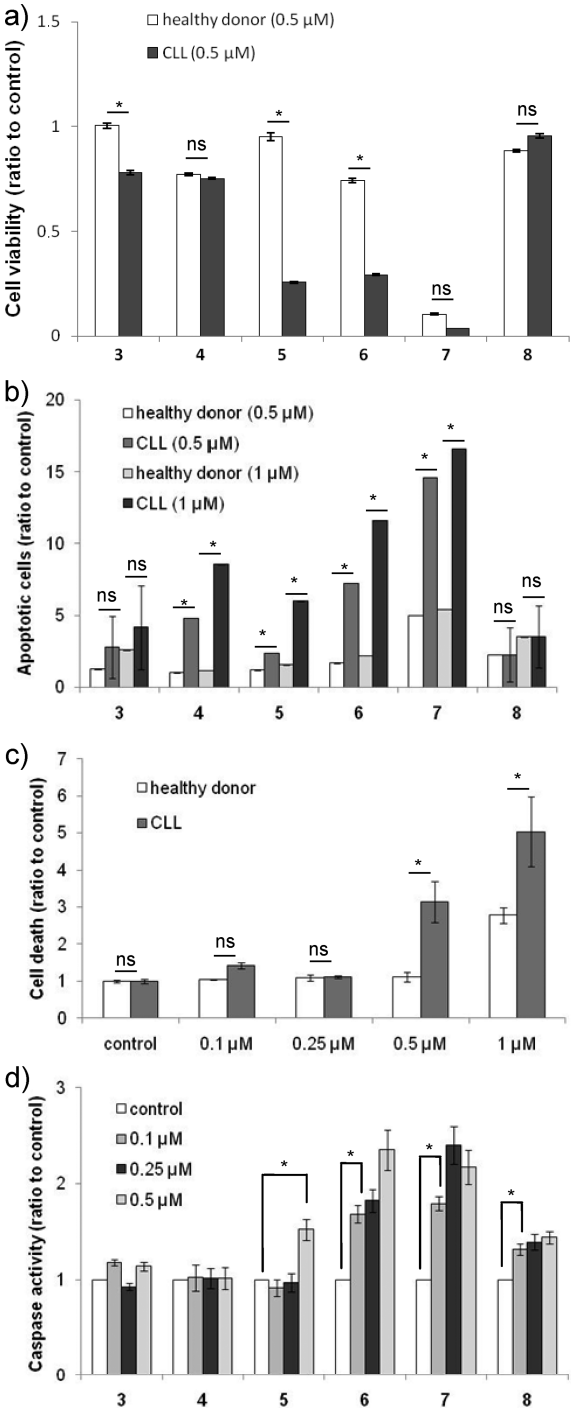


Figure 3. Cytotoxicity of selected compounds against B-cells isolated from peripheral blood of CLL patients and PBMC from healthy donors. Pooled data on three CLL samples in panel a indicate that **3**, **5**, and **6** most selectively reduce cellular ATP levels (measured by a colorimetric plate readout) and thus viability in leukemia cells at low, submicromolar concentrations (0.5 μM). Panel b shows the proportion of dying/dead cells as measured by flow-cytometric annexin V staining after 36 h to be generally higher in CLL cells compared to healthy donor leukocytes after treatment with 0.5 and 1 μM compounds **3**−**8**. Panel c illustrates the concentration-dependent cytotoxic effect for **3** as measured by annexin V staining in flow cytometry after 36 h. (d) Colorimetric caspase activity measurements in the presence of compounds **3**−**8** illustrate that the CLL-selective cell death occurs mostly through apoptosis. In all analyses the error bars represent SD and the data are normalized to cells treated with DMSO only as vehicle control to correct for spontaneous and DMSO-mediated cell death. Asterisks indicate significance levels of $P < 0.05$.

staining to determine the number of dying and dead cells indicated a significantly higher induction of cell death in CLL tumor cells compared to PBMC from healthy donors for most of the compounds studied. This effect was particularly pronounced at 500 nM and 1 μM (Figure 3b). In order to narrow down the concentration threshold, dose titration studies were carried out (Figure 3c). Here, a drastic increase of specific CLL cell death over vehicle control following incubation with compounds was observed starting at 500 nM. This apparent tumor cell "selectivity" at around 500 nM was shared by most compounds (**3**, **4**, **5**, and **6** as well as the selenium compound **7**). As illustrated for **3** in Figure 3c, the ratio for cell death of treated versus untreated cells was 3.17 ± 0.61 at 500 nM and 5.03 ± 0.75 at 1 μM. In contrast, healthy PBMC were significantly less affected by **3** (ratio for cell death of treated versus untreated cells, 1.07 ± 0.19 at 500 nM and 2.70 ± 0.13 at 1 μM). As inferred from this, although dose levels of 1 μM and above further add to the efficacy in the tumor cells, parallel increases in cytotoxicity on healthy leukocytes start to reduce the benefit/hazard ratio. For most compounds, 500 nM therefore remains the concentration with the best discrimination and broadest efficacy across individual CLL samples.

Cellular mechanisms leading to apoptosis were briefly surveyed. Compound **6** significantly increased the intracellular level of caspase-3 activity in CLL B-cells (around 2.5-fold), an effect particularly pronounced at 500 nM and higher (Figure 3d). In contrast, caspase-3 activity did not increase significantly in the PBMC control. This finding points to caspase-mediated induction of apoptosis which could be triggered by a redox event and mediated through ROS and catalysts.

In order to gain a better insight into these mechanisms and to understand why the compounds employed may act *specifically* in CLL tumor cells, the ROS levels in these malignant B-cells were investigated by flow cytometry and compared to a healthy B-cell population. Figure 4a indicates that a significant difference in ROS levels *pre-exists* between CLL patient tumor cells and healthy donor PBMC. The malignant B-cells of CLL show higher ROS levels compared to the normal cell control ($P = 0.001$). This marked difference between malignant and normal B-cells is in excellent agreement with previous reports and may explain the selective response toward the redox-active agents observed in our study.[13,21] In further support of this concept are data derived *from within the same CLL patient*: As it is usually hard to identify remaining normal B-cells within the PB of a CLL patient, we screened multiple samples and identified five cases with a distinctly detectable normal B-cell population as marked by a CD19+5-negative flow cytometry profile compared to the characteristic CD19+5+ phenotype of CLL cells. In all analyzed cases, we found that the normal B-cells showed considerably lower ROS levels than the intraindividual CLL cells. Figure 4b illustrates this by two cases at different disease stages, one with a still small PB tumor cell burden (patient 1, early stage) and another with a more predominant (patient 2, advanced stage) clone, both showing the specifically neoplastic association of elevated ROS.

Interestingly, compounds such as **3** and **6** increase ROS levels even further in CLL cells *but not* in PBMC from healthy donors (Figure 4c). The magnitude of this compound-induced ROS elevation is similar to the degree mediated by strong lymphocyte activating stimuli, such as lipopolysaccharide (LPS). This selectivity between malignant and normal cells is

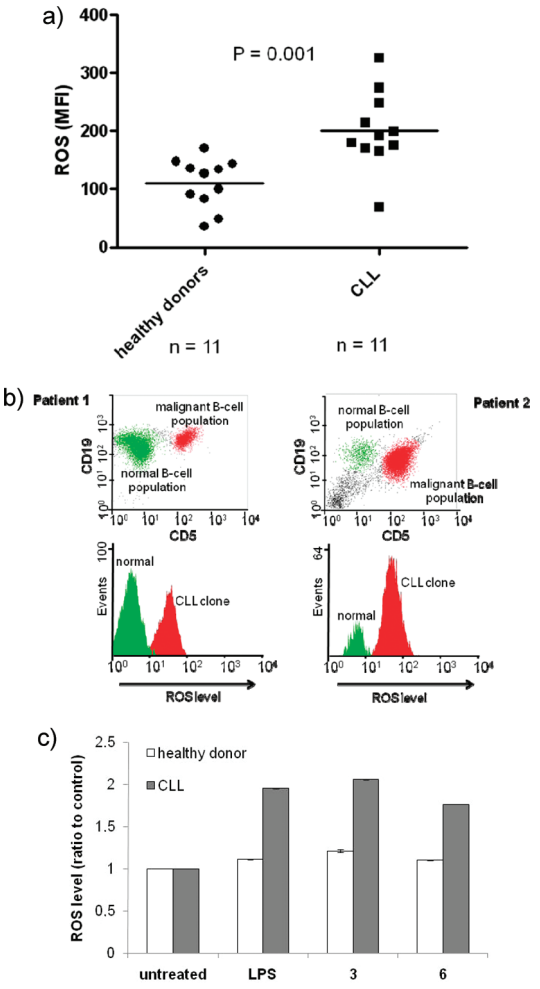


Figure 4. CLL cells show higher baseline and induced levels of overall ROS as measured by mean fluorescence intensity (MFI) in flow cytometry with the FITC labeled probe H_2DCFDA. (a) Pre-existing differences in ROS levels between CLL B-cells and healthy donor PBMC are significant. (b) Data are presented for two representative CLL patients of five cases in which a normal B-cell population could be distinguished from the leukemic clone. In all five cases, markedly elevated inherent ROS level in the malignant CLL B-cell population (CD19+5+) compared to the healthy B-cell population (CD19+5-negative) is found within the same patient. Patient 1 (left) is an early phase case with low-grade PB involvement by CLL, while the PB sample of patient 2 (right) represents a case with a more advanced-stage leukemic burden. (c) Differential increase in ROS levels (measured as in panel b) was triggered by 12 h of incubation with 0.5 μM **3** and **6** in CLL compared to healthy donor PBMC (10 ng/mL lipopolysaccharide, LPS, served as a positive control of a strong lymphocyte stimulant). Both CLL and healthy donor PBMC are individually set to "1" to allow comparison of experimental conditions. Error bars represent SD.

particularly striking. It appears that the agents interconvert pre-existing ROS to more aggressive species or generate ROS that cannot be degraded in CLL cells but are removed in PBMC. In any case, there is a strong effect of **3** and **6** on ROS levels in CLL cells which may explain the increases in caspase-3 activity executing the higher apoptosis observed in these cells. This chain of (bio)chemical events leading from the catalyst and ROS levels to apoptosis is notably absent in PBMC from healthy donors.

In terms of the most efficient and most selective compounds, we noticed that **3** and its analogues are best able to kill CLL B-cells when employed at ~500 nM, the level at

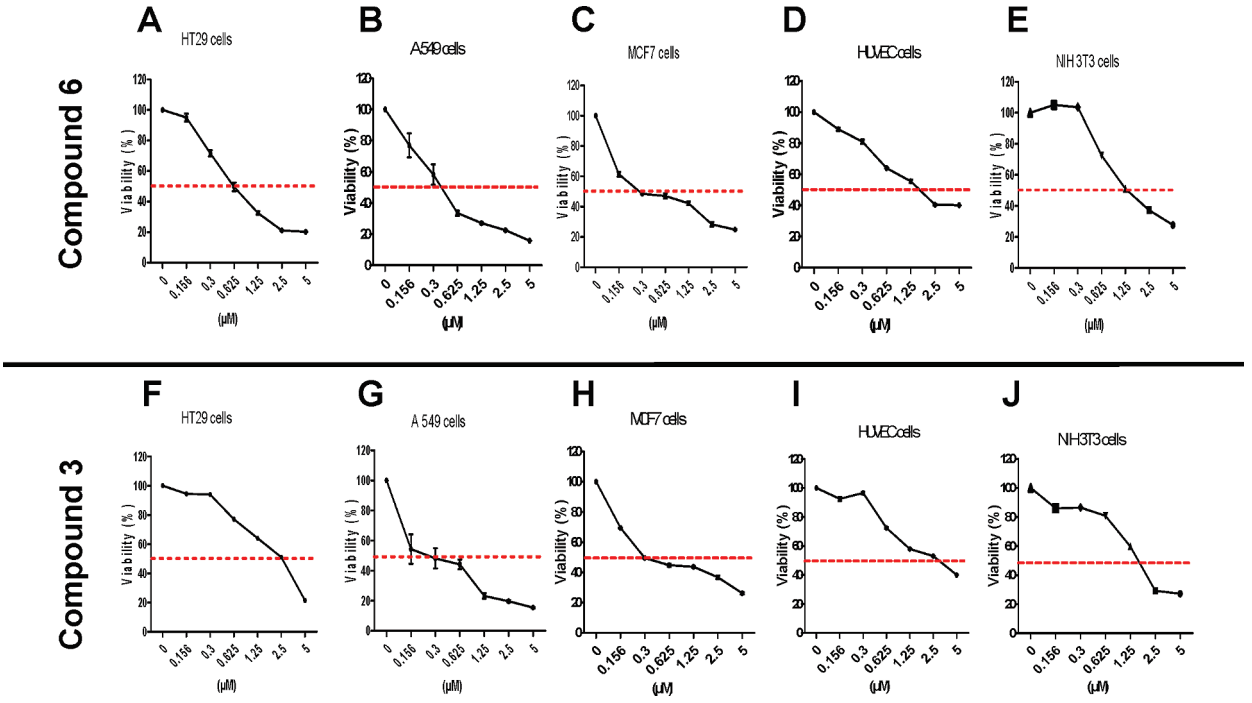


Figure 5. Cytotoxicity of **6** and **3** in cultured HT29 (A, F), A549 (B, G), MCF7 (C, H), HUVEC (D, I), and NIH 3T3 cells (E, J). These cells were incubated with various concentrations of **6** (upper panel) and **3** (lower panel). Cytotoxicity was assayed using the crystal violet method. Results are expressed as a ratio between treated and untreated cells. Error bars represent SEM.

which normal PBMC are significantly less affected. The highest "selectivity" for the leukemia cells seems to be associated with compound **6**.

2.4. Activity of Compounds 3 and 6 against Colon, Lung, and Breast Cancer Cells in Vitro. While the studies involving CLL B-cells from patients point toward certain selectivity of compounds such as **6**, they leave a range of questions unanswered. We have therefore further investigated **3** and **6** and their activities in a second, independent model involving human carcinoma cell lines from various origins.

In the first step, the influence of **3** and **6** on cell viability of HT29 colon carcinoma cells, A549 lung carcinoma cells, and MCF7 breast carcinoma cells was studied, with HUVEC and NIH 3T3 fibroblasts serving as controls (Figure 5). In this cell-based assay, a concentration of **6** between 0.3 and 0.625 μM reduced cell viability of all carcinoma cell lines tested to less than 50%. By contrast, at least 1.25 μM **6** was required to reduce the viability of normal fibroblast NIH 3T3 or endothelial cells (HUVEC) to less than 50%. The apparent selectivity of the tellurium compound for cancer cells was also observed in the case of **3**. 0.3 μM **3** reduced both A549 and MCF7 carcinoma cell proliferation to less than 50%, while 1.25 μM, i.e., a more than 4 times higher concentration of **3**, was required to reduce normal fibroblast NIH 3T3 or endothelial cells (HUVEC) to less than 50%. In contrast, HT29 colon cancer carcinoma cells were more resistant toward **3**, with 2.5 μM **3** required for a 50% or larger reduction in cell viability.

On the basis of these data, **6** was chosen for drug synergy studies in the more resistant HT29 cell line, in combination with 5-fluorouracil (5-FU) and irinotecan, two chemotherapeutic drugs currently used to treat colon cancer[22,23] (Figure 6). Irinotecan (at 15 μM) or 5-FU (at 200 μM), although being used at considerably higher concentration than **6**, only exerted a similar or even lower effect on the proliferation of the human colon cancer cell line HT29 (Figure 6). Moreover, irinotecan and 5-FU acted in concert with **6** to decrease HT29 cell proliferation ($P < 0.05$). In contrast, a rather surprising result was obtained in the normal NIH 3T3 fibroblast cell line: 2 μM **6** did not add its own effect to those of irinotecan and 5-FU to decrease normal fibroblast proliferation. The latter remained unchanged compared to treatment with **6** alone. It therefore appears not only that **6** is very active in carcinoma cells and less active in "healthy" cells but that this phenomenon is even amplified when tumor and normal cells are co-treated with chemotherapeutic compounds such as 5-FU or irinotecan.

As in the case of CLL tumor cells, the activity observed for **6** in HT29 and NIH 3T3 cells may be explained by changes in ROS levels. Similar to the induced ROS increase in CLL cells, compound **6** significantly increased H_2O_2 levels in HT29 *and* NIH 3T3 cells (Figure 7). The different responses of individual cell types toward **6**-induced ROS (HT29 versus NIH 3T3; see above) are likely to be caused by differences in pre-existing levels of ROS, variations in antioxidant defenses, and general sensitivity toward these stressors. Those differences are amplified when irinotecan and 5-FU are co-incubated with **6**: ROS levels increase further but only in tumor cells and not in normal cells. When irinotecan and 5-FU were co-incubated with **6**, enhancement of ROS production was observed in HT29 cells *but not* in NIH 3T3 cells (Figure 7C−F). This observation may explain why **6** acts in concert with chemotherapeutic drugs in order to selectively decrease tumor cell proliferation.

3. Discussion

The results obtained as part of this study have several implications for anticancer drug design. First of all, they confirm the ability of redox catalysts to act against cancer cells in an encouragingly specific fashion. While there has been considerable speculation about this kind of effect in the past,[3] it is now possible to demonstrate and rationalize such activity

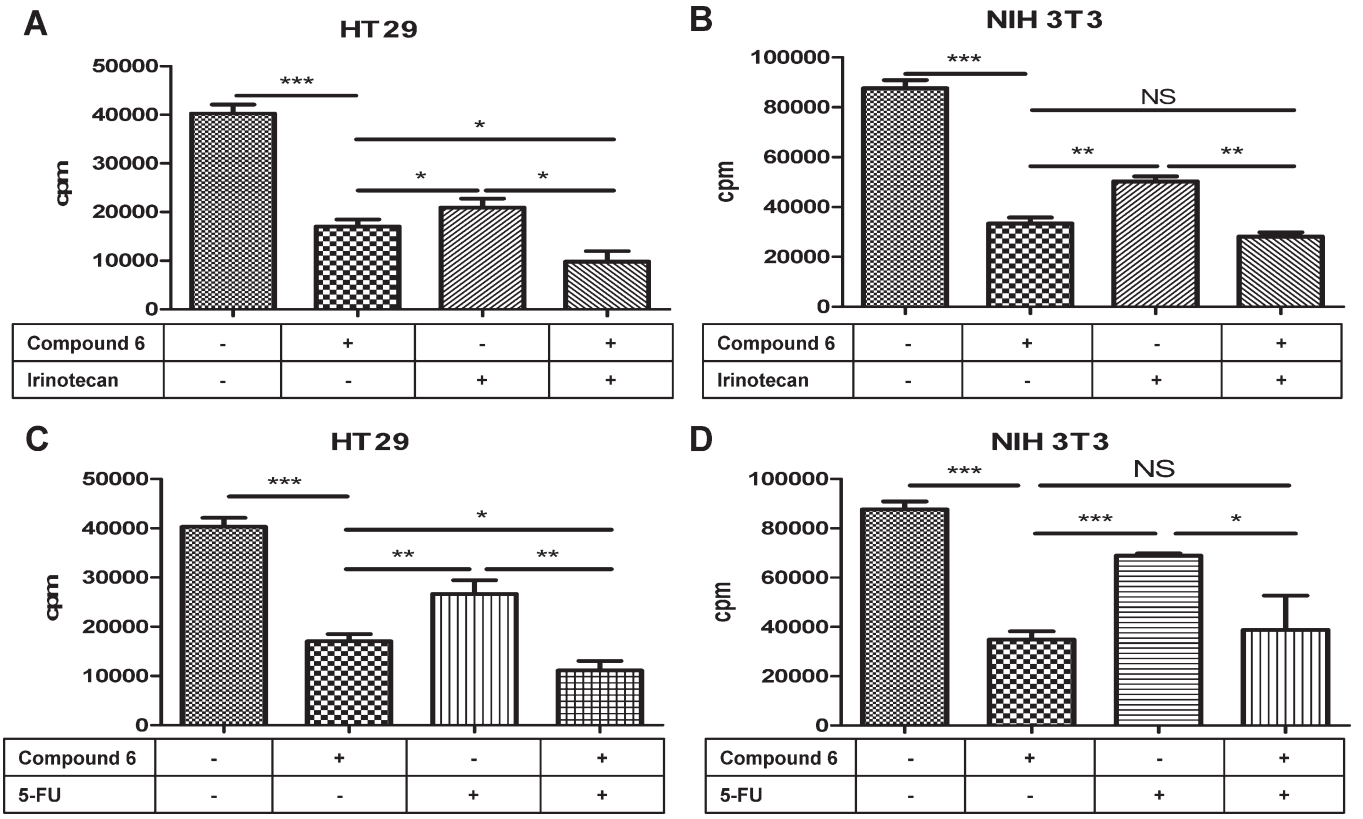


Figure 6. Proliferation rates of HT29 human colon carcinoma cells (A, C) and NIH 3T3 cells (B, D) treated with **6** alone or associated with the chemotherapeutic drug irinotecan (A, B) or 5-FU (C, D). Proliferation rates were measured by thymidine incorporation. Error bars represent SEM. Irinotecan doses were 15 and 2.5 μM for HT29 and NIH 3T3 cells, respectively. 5-FU doses were 200 and 20 μM for HT29 and NIH 3T3 cells, respectively. **6** was used at a dose of 2 μM for HT29 and NIH 3T3 cells.

in two independent models, patient-derived CLL B-cells on the one hand and various carcinoma cell lines on the other.

Second, the selectivity observed is not limited to one particular cancer cell type but can be found in several distinctively different models. There is, for instance, a significant difference between the effects observed in B-cells from patients suffering from CLL leukemia and in PBMC cells from healthy volunteers. At the same time, the results obtained with MCF7, A549, and HT29 carcinoma cells and with NIH 3T3 and HUVEC normal cells point toward a selective behavior against cancerous versus normal cells.

3.1. Catalysis as a Key to Efficiency and Selectivity. Catalysts combine high efficiency at low concentrations with an often astonishing selectivity for specific substrates. There is no reason why catalysts, once placed inside a living cell, should behave differently. This notion has far-reaching implications for the development of anticancer drugs. It is possible, for instance, to synthesize multifunctional redox agents that combine two, three, or even four redox centers in one chemically simple molecule.[24,25] Experimental data collected for such compounds in this and previous studies (by us and others) point toward their ability to modulate the intracellular redox environment with the help of low concentrations of catalysts (between 500 nM and 2 μM were used in the present study).[11,12] Substances such as **6** may, for instance, raise ROS levels over a critical oxidative stress threshold (this activity is associated primarily with the quinone moiety). Above this threshold, cells may become "overwhelmed" by oxidants and enter into apoptosis. We found this type of behavior in CLL B-cells: Apoptosis, which is the mode of choice for promising anticancer agents, is induced by a caspase-3 mediated pathway. This finding also supports the link between mitochondrial redox-mediated signaling and cell death, which is mediated through mitochondrial leakage of apoptotic effectors.

Yet why are redox modulators primarily active in CLL cells and not in normal cells? There are several mechanisms, which may act in concert and can be supported experimentally. First, as we and others[13] have demonstrated, PBMC are further away from the critical "OS threshold" compared to CLL B-cells and hence may be less affected by increases in ROS levels, even if these increases were similar in both cell types (which they actually are not). Second, it appears that normal cells (such as PBMC) feature a more efficient antioxidant defense and hence are able to degrade any ROS formed more efficiently. Third, catalysts may convert ROS already present inside CLL B-cells (but less so in PBMC) into more aggressive oxidants (such as $^{\bullet}$OH radicals) which then induce apoptosis. This mode of action would not require the indiscriminate up-regulation of ROS levels in all cells. Indeed, the selective increase of ROS reactivity triggered by **3** and **6** in CLL cells, but not in PBMC, appears to connect the action of the Te compounds to *pre-existing* levels of ROS. Fourth, the Se or Te redox center is able to catalyze the oxidation of thiol containing proteins and enzymes in the presence of H_2O_2 and possibly also other ROS. This mode of action also takes account of pre-existing levels of ROS; yet unlike the other explanations, it does not require a general increase in either ROS levels or ROS reactivity.

Alternatively, the tellurium moiety, either as telluride or telluroxide, may bind to and subsequently inhibit certain cysteine or selenocysteine containing proteins and enzymes, as postulated in the case of thioredoxin reductase (TrxR).[26]

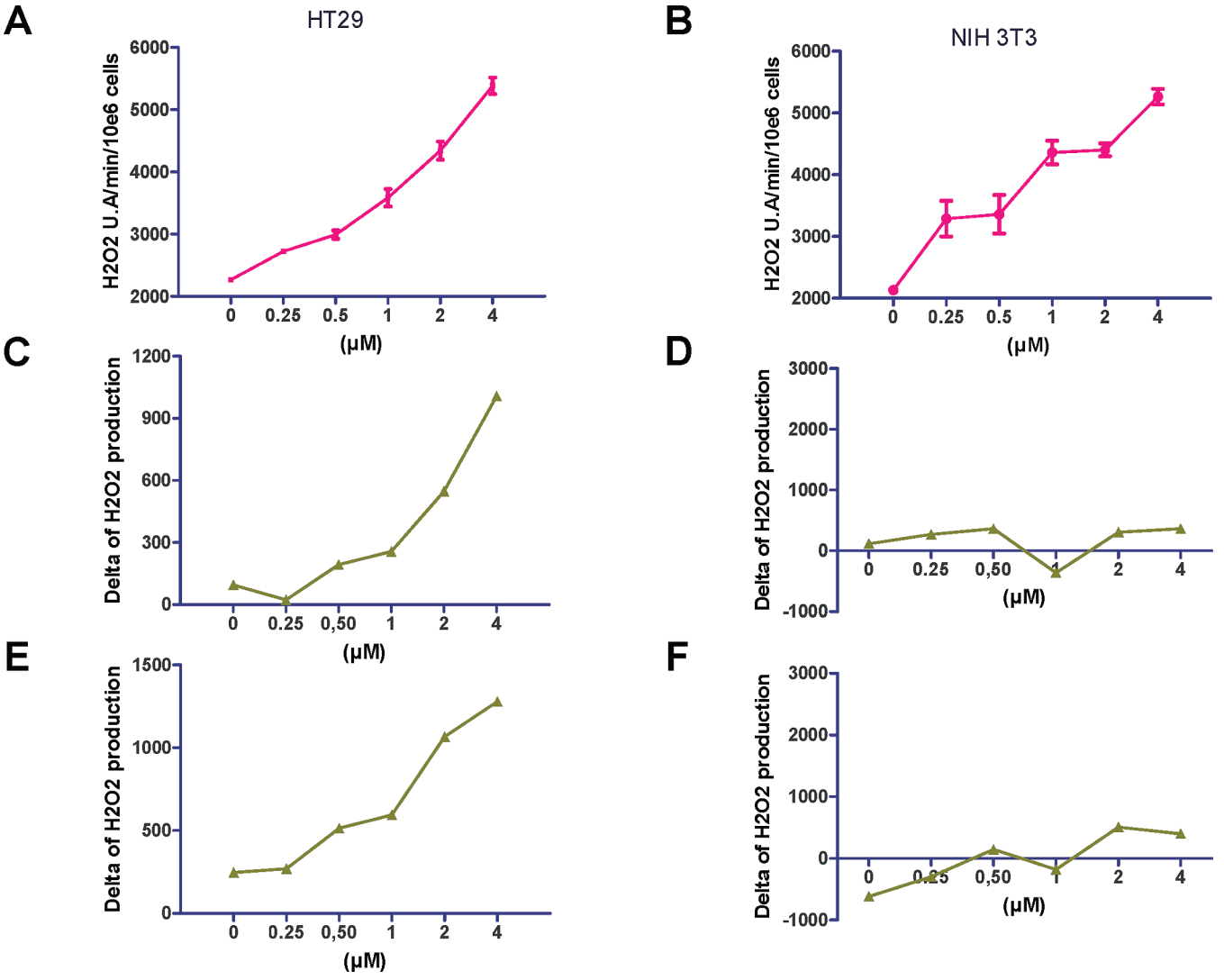


Figure 7. Production of H_2O_2 by HT29 (A) and NIH 3T3 (B) cells incubated with increasing doses of **6**. Production of H_2O_2 by irinotecan-treated HT29 (C) and NIH 3T3 (D) cells incubated with increasing doses of **6**. Production of H_2O_2 by 5-FU-treated HT29 (E) and NIH 3T3 (F) cells incubated with increasing doses of **6**. Note that in parts C−F the *difference* of H_2O_2 production is calculated and shown between cells treated with **6** and irinotecan (C, D) or 5-FU (E, F) and cells treated with **6** alone. The synergistic increases in ROS levels, which are observed in the HT29 cells (C, E), are notably absent in the NIH 3T3 cells (D, F). Error bars represent SEM.

Such an inhibitory action on an antioxidant protein would also result in the modulation of the intracellular redox homeostasis. At the same time, nonredox enzymes, such as proteases, may also be inhibited, enabling the tellurium compounds to cause damage via a redox-independent pathway.

In any case, a variation of ROS levels triggered by such agents appears to be the initial step in a chain of events that leads to cell death via apoptosis. These processes seem to be cell type specific, with increases in ROS levels and cellular responses toward them differing between different cell types. While CLL and healthy PBMC per se differ in their ROS makeup and subsequent build-up, different *responses* toward increased ROS levels are observed in HT29 and NIH 3T3 cell culture: **6** stimulates 3- to 5-fold increases in H_2O_2 levels in all these cells; yet further increases in H_2O_2 levels in the presence of irinotecan or 5-FU are only observed in HT29 cells but not in NIH 3T3 cells.

3.2. Possible Implications for the Therapy of CLL. The activities observed for compounds such as **3** and **6** obviously have implications for future anticancer drug design. First of all, our results may point toward a possible therapeutic approach for the treatment of CLL, a common and traditionally incurable kind of cancer. Besides the frequent reemergence of the leukemic clone, the higher age and comorbidity of patients at diagnosis pose another problematic issue that limits therapeutic options, already in the first-line setting. With the long-term results of modern regimens such as fludarabine-based chemoimmunotherapies becoming available,[27] another debatable issue is the optimal timing of treatment initiation. A "watch and wait" strategy in early stage patients may not always be the best choice, as it provides a wider window for a more aggressive and less therapeutically sensitive clone to emerge. Therefore, milder drugs with a better CLL to normal cell efficacy profile are required to be introduced into early treatments without significantly reducing quality of life in these often still asymptomatic patients. Here, one may recognize a possible benefit of redox catalysts, especially since CLL is one type of cancer known to be associated with OS and appears to respond well to redox modulation.[13,21,28]

Indeed, several redox-based therapies have been discussed in CLL, among them COX-2 inhibitors (SC-58125 and

OSU03012),[21] some of which cause OS in B-cells by increasing levels of ROS and decreasing levels of reduced glutathione (GSH), the major intracellular antioxidant. Such compounds, however, were found to be active in the range of 5−50 μM, which is significantly higher than the concentrations used for the compounds in our studies. Similarly, a recent study has used the isothiocyanate compound β-phenylethyl isothiocyanate (PEITC) to attack fludarabine-resistant CLL B-cells rather efficiently without affecting healthy lymphocytes to the same extent (IC_{50} values of around 5 μM for the CLL cells, 27 μM for the normal cells).[13] In that study, a "redox link" was established, whereby the isothiocyanate caused increased intracellular levels of ROS and decreased levels of GSH, triggered oxidation of mitochondrial cardiolipin, and ultimately caused massive apoptotic cell death. Of note is also that in this study, the tumor cells of pretreated (even after a long period of time) and relapsing patients showed higher ROS levels, which may have implications on the use of redox modifying agents as successful second line therapeutics.

Generally, a closer look at currently available therapies for CLL further underlines the promise of catalytic agents. CLL cells are characterized by a very high apoptotic threshold, which is hard to overcome therapeutically, and usually only achieved by a combination of classical cytostatics with CLL-targeting antibodies. For example, fludarabine, the major single chemotherapeutic component currently used in CLL, only achieves around 30% reduction of CLL B-cell survival in vitro if used at clinically achievable doses (data not shown). The significant reduction of cell survival achieved by our redox modulating compounds, such as **3**, **5**, and **6**, at concentrations as low as 500 nM means that they compare well with such established cytostatics, at least in vitro. These low effective catalyst concentrations are also associated with a reasonably low toxicity against normal PBMC, normal fibroblast lines, and endothelial cell cultures, as shown here for several of the compounds studied.

Furthermore, a possible combination or even synergistic effect of our compounds with fludarabine holds great promise. This is based on the rationale of a redox-mediated cooperation of ROS elevation and selective DNA damage, mutually potentiated through classical cytostatics in combination with our catalytic agents. This might eventually allow subtoxic dosing of the tellurium agents. Nonetheless, potential side effects of the organotellurium compounds used in this study, such as neurotoxicity, cannot be ruled out upfront and require extensive further investigations.

3.3. Combination of Catalyst with Other Chemotherapeutic Drugs: Implications for Tumor Therapy. In cell culture, compound **6** is able to decrease carcinoma cell proliferation and viability per se. In human patients, however, most protocols use a combination of drugs to treat cancer. In order to determine whether catalysts could be used in combination with other chemotherapeutic drugs, we evaluated the efficiency of **6** along with 5-FU and irinotecan (two drugs used to treat colon cancer in humans) in the colon carcinoma cell line HT29. **6** acted together with both 5-FU and irinotecan to significantly decrease HT29 cell proliferation but did not add its own toxicity to irinotecan and 5-FU in normal cells. Those results further demonstrate how catalysts could be used to improve the therapeutic index of chemotherapeutic drugs. This effect was associated with a possible redox-synergistic action of 5-FU, irinotecan, and **6** to increase ROS levels in cancer cells but not in normal cells. Those results are in line with previous reports showing that selective modulation of H_2O_2 levels between normal and tumor cells could be used to increase the therapeutic index of chemotherapeutic drugs. Such a phenomenon could be obtained with SOD mimics like mangafodipir.[7,8] In this case, the selectivity of the SOD mimics was associated with their ability to produce H_2O_2 and related ROS through the dismutation of superoxide radical anions that are overproduced by tumor cells but not by normal cells. In our present model, the catalyst also exploited the differences between endogenous ROS levels to assist chemotherapeutic drugs in order to kill tumor cells while exerting few effects on normal cells. The possible drug synergy between **6** and standard chemotherapeutic drugs requires more extensive studies, such as synergy quantification studies ultimately using the Chou−Talalay method.[29] How this phenomenon could be applied in vivo also requires further investigation.

4. Conclusions

In summary, this study has shown that it is possible to synthesize with comparable ease a range of multifunctional redox catalysts designed to target cancer cells by modulating intracellular levels of ROS. These compounds include tellurium agents, which have been synthesized for the first time, and highlight the huge potential of this often forgotten element and its associated catalytic activity in drug design. There is an urgent demand for more (organo)tellurium compounds and a deeper understanding of the metabolic transformations such compounds may undergo in the human body. Ultimately, it is credible that certain tellurium-based agents could be useful in therapy.[30−33] Existing literature on this topic is very limited, occasionally confusing, and often focused on just a handful of selected inorganic and organic tellurium agents (recently reviewed by Nogueira and colleagues[34]).

Studies in CLL tumor cells and with PBMC from healthy donors as well as in carcinoma cells, normal fibroblasts, and endothelial cells point toward a high, yet selective activity of some of the compounds we have designed, synthesized, and employed here. The biological activity of these compounds seems to be linked to redox modulation and subsequent apoptotic processes. These findings raise a number of interesting questions that need to be followed up in the future. First of all, in-depth studies are now required to elucidate the cellular processes influenced or even triggered by the redox catalysts presented here. It is likely that such processes are "somehow" related to redox events; yet other mechanisms, such as enzyme inhibition or receptor binding, cannot be ruled out at this point.[34] Therapeutically, it is interesting to find out if there are cell-cycle dependent differences in compound activity and if there is synergism with current standard drugs in this disease. From a clinically translational point, further studies need to address which CLL subsets are the most responsive to our agents. There is a desperate need for drugs that induce cell death in a p53 independent fashion to improve outcomes in the prognostically poorest of all CLL patients, namely, those with dysfunctional (deleted and mutated) p53.

Second, selectivity of such compounds needs to be determined by using a wider range of cells. CLL B-cells and a panel of carcinoma cells are reasonable and justifiable model systems that are quite close to the "real life" situation. Nonetheless, other cells, such as macrophages and neutrophils, are also rich in ROS and may provide additional targets for

redox catalysts. There is clearly considerable scope to explore further the effects of redox catalysts on various cells, tissues, and organs.

5. Materials and Methods

5.1. Materials. Chemical reagents were purchased from Sigma-Aldrich-Fluka (Steinheim, Germany) and used without further purification unless stated otherwise. For chemical synthesis, reactions were carried out in distilled water or in laboratory grade solvents at room temperature and under nitrogen atmosphere. Purification was carried out by column chromatography using silica gel (Macherey-Nagel, 50−200 μm diameter) under nitrogen pressure. Melting points were recorded using a digital melting point apparatus (IA9000 series, ThermoFischer Scientific, Rochford, U.K.) and are given without correction. ^{1}H NMR spectra were recorded at 500 MHz, and ^{13}C NMR spectra were recorded at 125 MHz on a Bruker Avance 500 spectrometer. Chemical shifts are reported in δ (ppm), expressed relative to the solvent signal at 7.26 ppm ($CDCl_3$, ^{1}H NMR) and at 77.16 ppm ($CDCl_3$, ^{13}C NMR). Compounds were determined to exceed 95% purity by HPLC recorded on a Bischoff Lambda 1000 UV/vis at 275 nm using a YMC C18 Pro column and methanol/water (85:15) as mobile phase at a flow rate of 1.0 mL/min. IR data were measured on a Bruker Tensor 27 using a golden gate. HRMS were recorded on a Finnigan MAT 95 spectrometer using the Ci positive technique. UV/vis spectra were recorded on a CARY 50Bio spectrophotometer (Varian Inc.), with quartz cells used throughout. All recordings were taken at 18 °C and repeated at least three times.

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.

5.2. Methods. Reactions were carried out under a protective N_2 atmosphere. Full details for all compounds are provided in the Supporting Information.

2,3-Bis(phenylselanyl)-5,8-dihydroxynaphthoquinone 7 was synthesized from diphenyldiselenide (57.3 mg, 0.15 mmol), $NaBH_4$ (32.6 mg, 8.58 mmol), and 2,3-dichloro-5,8-dihydroxy-1,4-napthoquinone (74.8 mg, 0.29 mmol) following the general procedure described in the Supporting Information. Its formation was monitored by TLC (petroleum ether/ethyl acetate, 9:1 v/v): R_f = 0.45. Compound **7** was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (9:1 v/v) as solvent. Yield 27%, deep orange-red crystals, mp 166−167 °C. ^{1}H NMR: 12.30 (s, 2H, H-6, H-7), 7.55−7.53 (m, 4H, H-a), 7.36−7.31 (m, 6H, H-b, H-c) ppm. ^{13}C NMR: 181.3 (2C), 159.0 (2C), 152.0 (2C), 133.4 (4C), 130.7 (2C), 129.4 (4C), 129.3 (2C), 128.1 (2C), 111.7 (2C) ppm. HPLC: t_R = 16.748 min, purity 99.8%. HRMS (m/z): $[M]^+$ calcd for $C_{22}H_{14}O_4Se_2$, 501.9222; found 501.9472.

2-(Hydroxyphenyltellanyl)-3-methylnaphthoquinone 8 was synthesized from 4-hydroxyphenyltellurium trichloride (598.4 mg, 1.83 mmol), $NaBH_4$ (310.1 mg, 8.16 mmol), and 3-bromo-2-methyl-1,4-naphthoquinone (458.0 mg, 1.82 mmol) following the general procedure described in the Supporting Information (in this particular case, a tellurium trichloride instead of ditelluride was used, demanding more reducing agent). Its formation was monitored by TLC (petroleum ether/ethyl acetate, 8:2 v/v): R_f = 0.33. Compound **8** was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (8.5:1.5 v/v) as solvent. Yield 18%, deep purple crystals, mp 161 °C. ^{1}H NMR: 8.06−8.03 (m, 2H, H-5, H-8), 7.74−7.63 (m, 4H, H-a or H-b, H-6, H-7), 6.74−6.71 (m, 2H, H-a or H-b), 5.03 (br s, OH), 1.92 (s, 3H, CH_3) ppm. ^{13}C NMR: 184.8 (C), 181.6 (C), 179.8 (C), 156.7 (C), 153.4 (C), 142.5 (2C), 134.1 (C), 133.5 (C), 132.2 (C), 131.8 (C), 127.2 (C), 127.1 (C), 117.1 (2C), 103.4 (C), 19.6 (C) ppm. HPLC: t_R = 4.692 min, purity 97.3%. HRMS (m/z): $[M]^+$ calcd for $C_{17}H_{12}O_3Te$, 393.9848; found 393.9677.

2,3-Bis[(2,2-diethoxyethyl)selanyl]naphthoquinone 9 was synthesized from 1,2-bis(2,2-diethoxyethyl)diselane (335.5 mg, 0.85 mmol), $NaBH_4$ (129.5 mg, 3.42 mmol), and 2,3-dichloro-1,4-naphthoquinone (97 mg, 0.43 mmol) following the general procedure described in the Supporting Information. Its formation was monitored by TLC (petroleum ether/ethyl acetate, 9:1 v/v): R_f = 0.20. Compound **9** was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (9.7:0.3 v/v) as solvent. Yield 44%, deep orange crystals, mp 68 °C. ^{1}H NMR: 8.06 − 8.02 (dd, J(H,H) = 3.19, 2.56 Hz, 2H, H-5, H-6 or H-7, H-8), 7.67−7.65 (dd, J(H,H) = 3.19, 2.56 Hz, 2H, H-5, H-6 or H-7, H-8), 4.84−4.82 (t, J(H,H) = 5.66 Hz, 2H, H-10), 3.71−3.52 (m, 8H, C_2H_5), 3.42−3.41 (d, J(H,H) = 5.74 Hz, 4H, H-9), 1.17−1.15 (t, J(H,H) = 6.9 Hz, 12H, C_2H_5) ppm. ^{13}C NMR: 178.6 (2C), 149.9 (2C), 133.2 (2C), 132.9 (2C), 127.1 (2C), 102.6 (2C), 62.2 (4C), 32.4 (2C), 15.1 (4C) ppm. HPLC: t_R = 7.420 min, purity 99.2%. HRMS (m/z): $[M]^+$ calcd for $C_{22}H_{30}O_6Se_2$, 550.0372; found 550.0223.

Acknowledgment. This work was supported financially by Saarland University, Germany, the Ministry of Economics and Science of Saarland, DFG Grants JA1741/2-1 and HE3553/2-1, the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement 215009 RedCat, and a Deutsche Krebshilfe Max-Eder Young Investigator's Award (M.H.). N.L. received a stipend from the Cologne University and Bayer Health Care Preferred Partnership Pharmacology Program. IR measurements were performed by A. Engelke in the Wenz laboratory (Saarland University). C.S. was financed by a grant from the Fonds National pour la Recherche (FNR), Luxembourg. M.D.'s research is supported by the "Recherche Cancer et Sang" foundation, by the "Recherches Scientifiques Luxembourg" asbl, by the "Een Häerz fir Kriibskrank Kanner" association, the Action Lions "Vaincre le Cancer" Luxembourg and Televie Luxembourg.

Supporting Information Available: General and specific procedures for the synthesis of the remaining compounds; additional analytical data for **7**, **8**, and **9**; and detailed information regarding the various biological and cell culture assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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