Amino Acids (2014) 46:459–470
DOI 10.1007/s00726-013-1639-1

ORIGINAL ARTICLE

Se-(2-aminoalkyl)selenocysteines as biochemical redox agents. A tool to contrast cell injury induced by aflatoxin B_1 in HepG2 cells

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Received: 11 April 2013 / Accepted: 7 December 2013 / Published online: 19 December 2013

Abstract *Se*-(2-aminoalkyl)selenocysteines were shown to have a chemoprotective activity towards HepG2 cells, contrasting the cell damage of aflatoxin B_1. The results of this study suggest that our newly synthesized seleno-diamino acids are apparently endowed with a potent protective potential against cell damage caused by AFB_1 similar to, or even higher than, that exerted by the reference compound *Se*-Me-SeCys. The protective effect does not seem to be absolute, i.e., merely determined by the presence of the chalcogen atom, but rather strictly related to the molecular structure of the new compounds tested. From this point of view, *Se*-(2-aminoalkyl)selenocysteines may represent a new class of biochemical redox agents fruitfully exploitable to contrast aflatoxin toxicity, at the same time a sound medical application and an economically relevant agricultural issue.

Keywords Sec conjugates · GPx mimics · Aflatoxin B_1 · Chemoprevention · Nutraceuticals

Dedicated to the memory of Prof. Marcello Tiecco.

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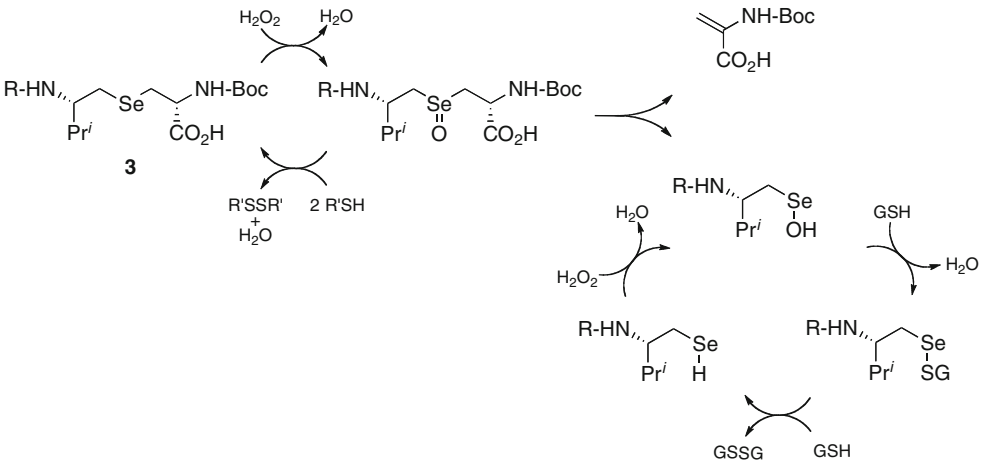
Introduction

"*Allium chemistry*" identifies a broad area of interest that includes a plethora of sulfur- and selenium-containing compounds naturally occurring in garlic, onion, and other genus *Allium* plants (Block 2010). Due to the outcomes of several epidemiological studies (Agarwal 1996; Le Bon and Siess 2000; Amagase 2006), such compounds (e.g., *S*-allylcysteine, alliin, diallylsulfides, diallyldisulfides, *Se*-methylselenocysteine and many others), have gained significant credit as chemopreventive agents (Yang et al. 2001; el-Bayoumy et al. 2006; Roman et al. 2013).

Several reports from the recent literature indicate that organo-chalcogen compounds interfere with the cellular redox mechanism through the modulation of the oxidative stress (OS). Such a modulation may exert pleiotropic effects, depending on the target cells. For example, some selenium-containing organic compounds can counteract OS by producing, often through a multi-step process, biologically active species that eventually reduce the ROS (reactive oxygen species) within catalytic cycles mimicking the action of the selenoenzyme glutathione peroxidase (GPx). On the other hand, redox modulating compounds may induce selective cytotoxicity in specific cells, including cancer cells (Jamier et al. 2010; Du et al. 2013).

The awareness that selenium- (and, even more, tellurium)-containing molecules may work better than classical,



Scheme 1 Oxidation of *Se*-(2-aminoalkyl)selenocysteines (e.g., compound **3**) with formation of catalytically active selenols by fragmentation of the resulting selenoxides

mostly radical scavenging, antioxidants has led to the design and synthesis of a multitude of new organoselenium compounds (el-Bayoumy et al. 1995; Mugesh et al. 2001; Jacob et al. 2003; Sanmartín et al. 2009; Bhabak and Mugesh 2010; Plano et al. 2010, 2011; Haratake et al. 2011; Santi et al. 2011; Satheeshkumar and Mugesh 2011; Storkey et al. 2011; Martins et al. 2013; Saluk et al. 2013). In any case, natural as well as synthetic organic compounds containing one or more selenium atom(s) exhibit different GPx activity, and sometimes different chemopreventive efficacy, depending upon both oxidation state and substitution of the selenium atom(s), that are responsible for their biochemical behavior (Mugesh et al. 2001; Drake 2006; Nogueira and Rocha 2011).

Not long ago some of us reported (Caputo et al. 2007, 2010) the synthesis of new selenium-containing diamino acids (such as compound **3**) that actually can be regarded as selenocysteines carrying a 2-aminoalkyl residue linked to the selenium atom. Within the wide scenery of organoselenium compounds, this sort of selenocysteine derivative appears to be rather interesting, notwithstanding that these compounds were basically monoselenides and, as such, would not be expected to have great value as GPx mimcs. As a matter of fact, it has been reported (Iwaoka and Kumakura 2008) that monoselenides undergo oxidation to the corresponding selenoxides in a reversible process where the selenoxides are reconverted to selenides, even at a higher rate. Otherwise, the molecular architecture of our selenocysteine derivatives implies that their selenoxides, once formed can eliminate rapidly a stable dehydroalanine residue (Scheme 1) and give rise to selenenic acids which are promptly reduced to selenols (the catalytically active species for GPx enzyme) by a generic thiol (that within the cellular environment would likely be represented by reduced glutathione, GSH) (Gieselman et al. 2002).

Other selenocysteine conjugates reported so far bear mostly aromatic residues linked to the selenium atom and, in such a context, our seleno-diamino acids gain special value due to the presence of an amino group at C-2 of the alkyl-selenium moiety. It is, in fact, well documented that either nitrogen- or oxygen-containing groups, suitably positioned toward the selenium atom to afford non-covalent intramolecular *Se···Het* interactions (Mugesh and du Mont 2001; Mukherjee et al. 2010), influence significantly the competition between reduction and beta-elimination of the intermediate selenoxide, thus modulating "by internal chelation" (Phadnis and Mugesh 2005; Bayse and Allison 2007) the activity of selenocysteines. They can be therefore regarded as pro-drugs, able to release in situ the active R-selenol species well known for their pharmacological activity.

To the best of our knowledge no examples of 2-aminoalkyl conjugates of selenocysteine were investigated so far for chemopreventive activity in cells, and the leading compounds tested were mostly aromatic conjugates bearing one nitrogen group on the benzene ring (usually non-interacting with the selenium atom), beside some sporadic aliphatic (allyl, propyl) derivatives (Andreadou et al. 1996; Ip et al. 1999).

On the basis of such evidence we decided to check the possible chemopreventive action of 2-aminoalkyl derivatives of selenocysteine towards the hepatic cell damage induced by aflatoxin B_1 (AFB_1) that is known to be cytotoxic and originate liver pathologies (IARC 1993), thus representing a risk factor for human liver cancer (Wogan et al. 2011). In particular, the toxicity of AFB_1 in HepG2 cells appeared to represent an appropriate experimental model owing to the interference of AFB_1 molecule with the redox balance in living cells, enhancing OS (Shen et al. 1995).

It is worth pointing out that many natural and synthetic compounds are already known to be able to contrast the cytotoxic damage induced by AFB_1 (Aboobaker et al. 1994; Gradelet et al. 1997; Gonzàlez de Mejìa et al. 1997;

Galvano et al. 2001). Ebselen, the first ever synthetic GPx mimic studied extensively in biological systems, is still one outstanding example of a broadly recognized selenium-based protective agent (Yang et al. 2000), whereas there is scientific evidence that garlic (*Allium sativum*) is itself an efficient dietary component capable to contrast cell injury induced by AFB_1, likely due to the cocktail of sulfur and selenium compounds it contains, many of which have been independently shown to prevent AFB_1 cytotoxicity (el-Mofty et al. 1994; Sheen et al. 2001; Guyonett et al. 2002; Berges et al. 2004).

The results obtained (vide infra), albeit being of a preliminary nature, seem to disclose that *Se*-(2-aminoalkyl)selenocysteine conjugates may indeed represent a new class of biochemical redox agents which in the medium term may be exploited to contrast the toxicity of aflatoxins. Such an activity would at the same time provide the basis for a sound medical application and also address an economically relevant agricultural issue (Khlangwiset and Wu 2010).

Materials and methods

The redox catalysts used in this study were synthesized and studied with a combination of chemical, biochemical and cell culture assays to evaluate their redox behavior and their protective effects against cell damage caused by AFB_1.

Chemistry and biochemistry

Inorganic substances, organic reagents, and solvents were commercially obtained, pure compounds (Fluka, Aldrich) and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on Varian Inova (500 MHz) and Gemini (200 MHz), Bruker DRX (400 MHz) spectrometers: chemical shifts are in ppm (δ) and J coupling constants in Hz. Low resolution MS spectra were recorded on Thermo-Finnigan LXQ linear trap instrument. Optical rotations were measured with Jasco 1010 polarimeter: $\lambda = 589$ nm, 1.0 dm cell, $CHCl_3$ unless otherwise specified. All compounds for which analytical and spectroscopic data are quoted were homogeneous by TLC and HPLC, and solids were crystallized. Elemental analyses were performed on a Perkin–Elmer Series II 2400, CHNS analyzer. TLC analyses were performed using silica gel plates (E. Merck silica gel 60 F-254) visualized by UV light, iodine vapors, or ninhydrin spray. Column chromatography was carried out on silica gel (E. Merck, 70–230 mesh).

Unless stated otherwise, all experiments described (except synthetic chemistry) were performed in triplicate.

*(2R,2′S)-2-(Boc)-Amino-3-(2′-(Boc)-amino-4′-methylpentylselanyl)propanoic acid (**3**)*

Prepared according to a reported general selenoalkylation procedure (Caputo et al. 2007) with slight modifications. Commercial Boc-diprotected L-selenocystine (**1**) (0.5 g; 1 mmol) was suspended in absolute ethanol (10 mL) under argon atmosphere. Solid $NaBH_4$ (0.2 g; 5 mmol) was added in one portion and the mixture was stirred at room temperature for 20 min, until clear and colorless. (*S*)-1-Iodo-4-methylpentan-2-(Boc)-amine (**2**) (Caputo et al. 1995) (0.6 g; 2 mmol) dissolved in tetrahydrofuran (4 mL) was then added portion wise and stirring was continued for 12 h. The reaction was quenched by addition of aq 10 % NH_4Cl (10 mL), and the mixture was shaken with ethyl acetate and brine until neutral. The organic layer was dried (Na_2SO_4) and the solvents were evaporated under reduced pressure. The residue was chromatographed ($CHCl_3$/CH_3OH, 97:3) to afford **3** as colorless oil (66 % yield).

One analytical sample: $[\alpha]_D^{25} = 5.8$ ($c = 0.9$) [5.5 ($c = 1.0$ in CH_2Cl_2)]. ^{1}H NMR (400 MHz): δ 0.92 (m, 6H, H-5′), 1.31 (m, 2H, H-3′), 1.45 (s, 9H, Boc), 1.46 (s, 9H, Boc), 1.64 (m, 1H, H-4′), 2.73 (m, 2H, H-1′), 3.01 (m, 2H, H-3), 3.83 (m, 1H, H-2′), 4.70 (m, 1H, NH), 4.91 (m, 1H, H-2), 5.46 (m, 1H, NH). ^{13}C NMR (100 MHz): δ 22.1, 22.8, 24.8, 28.2, 28.3, 43.6, 46.4, 54.6, 69.1, 80.0, 155.1, 155.2, 171.0. Calculated for $C_{19}H_{36}N_2O_6Se$ (467.5): C, 48.82 %; H, 7.76 %; N, 5.99 %; found: C, 48.86 %; H, 7.72 %; N, 5.94 %.

*(2R,2′S)-2-(Boc)-Amino-3-(2′-(Boc)-amino-4′-methylpentylthio)propanoic acid (**4**)*

Prepared from commercial Boc-Cys-OH and iodide **2** according to a reported general thioalkylation procedure (Bolognese et al. 2006).

One analytical sample: $[\alpha]_D^{25} = 11.7$ ($c = 1.3$). ^{1}H NMR (400 MHz): δ 0.94 (t, 6H, $J = 6.5$, H-5′), 1.44–1.58 (m, 21H, H-4′, H-3′, Boc), 2.68 (m, 2H, H-1′), 3.09 (m, 2H, H-3), 3.77 (m, 1H, H-2′), 4.52–5.17 (m, 2H, H-2, NH), 5.59 (bs, 1H, NH). ^{13}C NMR (100 MHz): δ 21.7, 23.1, 24.8, 27.3, 27.9, 28.0, 36.5, 39.5, 42.9, 49.2, 53.1, 80.2, 155.5, 172.9. Calculated for $C_{19}H_{36}N_2O_6S$ (420.56): C, 54.26 %; H, 8.63 %; N, 6.66 %; found: C, 54.20 %; H, 8.68 %; N, 6.64 %.

*(2R,2′S)-2-(Fmoc)-Amino-3-(2′-amino-4′-methylpentylselanyl)propanoic acid (**6**)*

Prepared from commercial L-selenocystine and iodide **2**, as reported above for the preparation of compound **3**. The crude reaction product, after evaporation of the solvents under vacuum, was redissolved in tetrahydrofuran (10 mL)

and treated with FmocOSu (0.3 g, 1 mmol) under standard conditions. The final product, (2*R*,2′*S*)-2-(Fmoc)-amino-3-(2′-Boc-amino-4′-methylpentylselanyl)propanoic acid (**5**), after column chromatography ($CHCl_3$/CH_3OH, 97:3) was obtained in 72 % yield.

One analytical sample: $[\alpha]_D^{25} = 29.6$ ($c = 1.1$). ^{1}H NMR (500 MHz): δ 0.93 (t, 6H, $J = 6.5$, H-5′), 1.36–1.57 (m, 10H, H-4′, Boc), 1.68 (m, 2H, H-3′), 2.70 (m, 2H, H-1′), 3.19 (m, 2H, H-3), 3.80 (m, 1H, H-2′), 4.25 (t, 1H, $J = 6.9$, C*H*-Fmoc), 4.38 (m, 2H, CH_2-Fmoc), 4.62 (bd, 1H, $J = 8.6$, NH), 4.81 (m, 1H, H-2), 6.01 (bd, 1H, $J = 7.0$, NH), 7.32 (t, 2H, $J = 6.6$, H-arom.), 7.40 (t, 2H, $J = 7.8$, H-arom.), 7.63 (bd, 2H, H-arom), 7.77 (d, 2H, $J = 7.8$, H-arom). ^{13}C NMR (100 MHz): δ 20.1, 23.9, 22.5, 28.3, 42.3, 46.9, 51.3, 55.9, 66.7, 80.2, 119.9, 125.1, 126.9, 127.2, 141.5, 148.0, 154.9, 156.0, 177.5. Calculated for $C_{29}H_{38}N_2O_6Se$ (589.58): C, 59.08 %; H, 6.50 %; N, 4.75 %; found: C, 59.05 %; H, 6.52 %; N, 4.72 %.

Removal of the Boc protecting group from chromatographically pure **5**, with formic acid under standard conditions (Gandhi and Singh 2008), afforded the final compound **6** (88 %) which was pure enough to be used without further purification in the subsequent assays.

One analytical sample: $[\alpha]_D^{25} = 31.8$ ($c = 0.9$). ^{1}H NMR (400 MHz): δ 0.90 (t, 6H, $J = 6.4$, H-5′), 1.46 (m, 1H, H-4′), 1.70 (m, 2H, H-3′), 2.71 (m, 1H, H-1′a), 2.90–3.15 (m, 3H, H-1′b, NH_2), 3.19–3.54 (m, 3H, H-2′, H-3), 4.22 (m, 1H, H-2), 4.29 (m, 1H, C*H*-Fmoc), 4.42 (m, 2H, CH_2-Fmoc), 6.22 (bs, 1H, NH), 7.30 (m, 2H, H-arom.), 7.39 (t, 2H, $J = 7.4$, H-arom.), 7.60 (d, 2H, $J = 7.4$, H-arom), 7.76 (d, 2H, $J = 7.4$, H-arom). ^{13}C NMR (100 MHz): δ 22.2, 24.2, 22.7, 29.6, 42.6, 47, 51.5, 55.8, 66.8, 119.9, 125.0, 127.0, 127.6, 141.1, 143.8, 155.8, 175.6. Calculated for $C_{24}H_{30}N_2O_4Se$: C, 58.89 %; H, 6.18 %; N, 5.72 %; found: C, 58.92 %; H, 6.15 %; N, 5.74 %.

*(S)-4-Methyl-1-(phenylselanyl)pentan-2-(Boc)-amine (**7**)*

Commercial diphenyldiselenide (0.3 g, 1 mmol) was suspended in absolute ethanol (10 mL) under argon atmosphere. Solid $NaBH_4$ (0.2 g, 5 mmol) was added in one portion and the mixture stirred for 20 min, until clear and colorless. The iodide **2** (0.6 g, 2 mmol) dissolved in tetrahydrofuran (4 mL) was then added. After 10 min under reflux, the reaction mixture was cooled to room temperature and quenched by addition of aq 10 % NH_4Cl (10 mL). The mixture was then shaken with ethyl acetate and brine until neutral. The organic layer was dried (Na_2SO_4) and the solvents were evaporated under reduced pressure. The residue was chromatographed (petroleum ether/ethyl acetate, 98:2) to obtain **7** as colorless oil (75 %).

One analytical sample: $[\alpha]_D^{25} = -3.3$ ($c = 0.9$) [−0.7 ($c = 1.0$ in CH_2Cl_2]. ^{1}H NMR (400 MHz): δ 0.96 (t, 6H, $J = 6.4$, H-5), 1.33–1.56 (m, 10H, H-4, Boc), 1.62 (m, 2H, H-3), 3.12 (m, 2H, H-1), 3.95 (m, 1H, H-2), 4.60 (bs, 1H, NH), 7.23–7.33 (m, 3H, H-arom), 7.53–7.60 (m, 2H, H-arom). ^{13}C NMR (50 MHz): δ 22.7, 23.3, 28.8, 30.1, 35.5, 44.8, 48.9, 79.6, 126.9, 127.4, 130.9, 133.2, 155.6. Calculated for $C_{17}H_{27}NO_2Se$ (356.4): C, 57.30 %; H, 7.64 %; N, 3.93 %; found: C, 57.24 %; H, 7.68 %; N, 3.91 %.

The compound **7** had been formerly mentioned by Narayanaperumal et al. (2010), unfortunately without detailing experimental/spectroscopic data.

PhSH assay: the activities of the compounds under investigation as oxidation catalysts were estimated according to the method reported by Iwaoka and Tomoda (1994) using thiophenol (PhSH) as a methanol-soluble alternative to glutathione. To a solution of 1 mM PhSH in CH_3OH (890 μL) a solution (10 μL) of each compound under investigation (100 μM in dimethylsulfoxide) was added. The reaction was initiated by adding 2 mM H_2O_2 (100 μL) and monitored at $\lambda = 305$ nm for 30 min at 25 °C. The initial rates (v_0) were calculated from the first 5–10 % of the reaction. Negative controls included the compounds in the presence of either H_2O_2 or PhSH only. *Se*-Me-SeCys was used as positive control in the assay.[1]

DPPH assay: the radical scavenging activities were estimated according to the method reported by Blois (1958). Methanolic solutions at different concentrations of each of the compounds tested and 50 μM 1,1-diphenyl-2-picrylhydrazyl (DPPH) in CH_3OH (200 μL) were incubated for 20 min in the dark at 25 °C. After that, their absorbance were measured at λ = 517 nm versus a blank containing exclusively DPPH. The activities were expressed as inhibition percentages and calculated as: radical scavenging activity % = $[(A_0 - A_1)/A_0 \times 100]$, where A_0 is the absorbance of the blank and A_1 the absorbance of the single samples examined. The measures were validated by using in parallel ascorbic acid as a positive control under the same conditions.

[1] Ebselen has been broadly used as reference compound in the measure of GPx activity. The rather recent elucidation (Sarma and Mugesh 2005, 2008) of the mechanism(s) involved in its GPx cycle, however, has put in evidence that Ebselen can react with aryl thiol substrates and leads to side species that are responsible for over/under-estimation of GPx activities. Therefore, in our earlier experiments PhSeSePh was preferred as reference compound, due to its catalytic cycle which involves the formation of the selenol species PhSeH (Nogueira et al. 2004; Sausen de Freitas et al. 2010; Nogueira and Rocha 2011). Nevertheless, also in view of the considerably higher activity of PhSeSePh (Table 1), we decided eventually to refer to *Se*-CH_3-selenocysteine (*Se*-Me-SeCys), monoselenide whose structure is much closer to that of our *Se*-(2-aminoalkyl)selenocysteines.

FRAP assay: the ferric reducing antioxidant power (FRAP) was estimated according to the method described by Benzie and Strain (1996) with several modifications. Fresh FRAP reagent was prepared by mixing 2.5 mL of a solution of 2,4,6-tris(2-pyridyl)-1,3,5-*s*-triazine (TPTZ) (1.0 mmol) in 40 mM hydrochloric acid (10 mL) with aq 20 mM Fe(III) (2.5 mL) and 0.3 M acetate buffer (pH 3.6; 25 mL). The final solution contained 1.67 mM Fe(III) and 0.83 mM TPTZ. A sample (300 µL) was warmed up to 37 °C and its absorbance ($A_{0\ min}$) was read at λ 593 nm. Afterwards, methanolic solutions (at different concentrations) of the compounds to be tested (10 µL) and water (30 µL) were added to the FRAP reagent and incubated in the dark at room temperature for 10 min. The relative absorbances ($A_{10\ min}$) were read at $\lambda = 593$ nm. The final values were expressed as $\Delta A = A_{10\ min} - A_{0\ min}$.

Biology

In vitro cytotoxic and protective activities

For assaying the cytotoxic and the protective, biological activities of the compounds, cells were inoculated into 96-well microtiter plates at a density of 5×10^3 cells/well in 100 µL of culture medium with or without the addition of increasing concentrations of the compounds under investigation. To evaluate the protective effects against cell damage caused by AFB_1, the latter was added to the cultures after 20 h pre-incubation with different concentrations of the compounds to assay and after washing cells three times with fresh medium prior to addition of AFB_1. Cells were then cultured for a further 48 h. During the entire experimental phases, cells were incubated at 37 °C and 5 % CO_2 in culture medium. Control cultures received equivalent volumes of culture medium and vehicle. At the end of the incubation times, the number of total cells and number of viable cells were evaluated by microscopic analysis in a haemocytometer chamber, using the Trypan Blue exclusion test as a viability assay. The effects of the compounds on the metabolic activity were examined by the MTS-test colorimetric method, using a commercial kit (MTS, Cell Titer 96 Aqueous One Solution, Promega, Madison, WI, USA). The assay was performed according to the manufacturer's protocol, by directly adding 20 µL of "CellTiter 96 Aqueous One Solution Reagent" to the culture wells at the end of the incubation period. After further 2–3 h incubation, absorbance was read at $\lambda = 490$ nm. These methods are widely used to assess cytotoxicity of compounds, as also shown by some of us (Balestrieri et al. 2011; Bonaccorsi et al. 2012; Cordero et al. 2012).

Calculation of dose–response indexes

For determining cell injury caused by AFB_1, the metabolic activity inhibitory concentrations nn (ICnn, AFB_1 concentration able to cause reduction of the formazan product formation, MTS assay, by nn %) were calculated according to the best-fit curve, y value versus log x, where y is the value of the examined function and x is the AFB_1 concentration. Results from at least three different determinations were used to calculate the dose–response curve. The nn % level was chosen to ensure that values lie within the concentration range utilized.

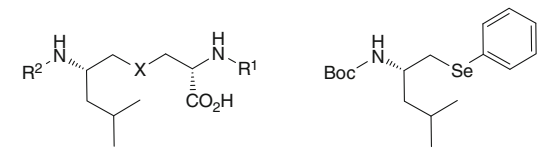
Statistical analysis

Data analysis was performed using the SPSS statistical software system (version 17.0 for Windows, Chicago, IL, USA). Comparison of means among sample groups was carried out using Hochberg's GT2 post hoc multiple comparison One-way ANOVA test. Differences were considered significant at $p < 0.05$ and highly significant at $p < 0.001$.

Results and discussion

This work arises as a continuation of our previous studies on the reactions of thio-alkylation (Bolognese et al. 2006) and seleno-alkylation (Caputo et al. 2007) of enantiomerically pure 2-aminoalkyl iodides originating from naturally occurring α-amino acids (Caputo et al. 1995). The present results show that the seleno-diamino acids obtained in the seleno-alkylation, *Se*-2-aminoalkyl derivatives of selenocysteine, may carry out a chemopreventive action towards the hepatic cell damage induced by AFB_1, which is known to be cytotoxic and may cause liver pathologies (IARC 1993).

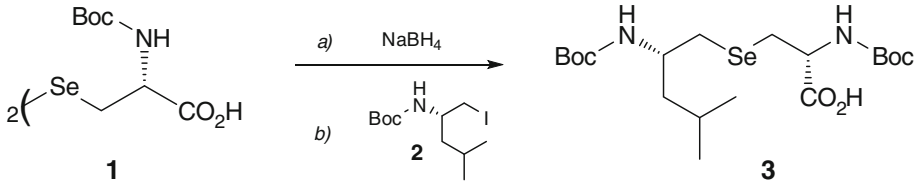
The seleno-diamino acid **3** was our main target to undertake a preliminary assessment of the biochemical and biological properties of the class of compounds to which it belongs. It was synthesized as shown in Scheme 2 (see also the "Materials and methods" section), by coupling of Boc-Sec-OH selenide (prepared in situ from commercial diselenide **1**) with 2-(Boc)-aminoalkyl iodide **2** (obtained from Boc-Leu-OH, Caputo et al. 1995), according to our already mentioned general seleno-alkylation procedure.



4 X=S, R^1=R^2=Boc
5 X=Se, R^1=Fmoc, R^2=Boc
6 X=Se, R^1=Fmoc, R^2=H

7

Scheme 2 Seleno-alkylation of 2-(Boc)-aminoalkyl iodide **2**. Synthesis of seleno-diamino acid **3**



Beside compound **3** some more analogues (namely, compounds **4**, **6**, and **7**) were also prepared.

In consideration of the predictable antioxidant activity of organoselenium compounds, prior to investigating any hepatic chemopreventive capacities in HepG2 cells we submitted our chalcogen-compounds to preliminary assays in vitro, to ascertain their supposed antioxidant properties.

Three different assays have been reported above: DPPH and FRAP assays led all to negative results, thus allowing us to rule out any major (and maybe counter-productive) antioxidant activity performed by either sequestering oxygen-based radicals or donating electrons to oxidized species (e.g., reactive oxygen metabolites, ROM). A negligible radical inhibition in the DPPH assay (9 % at 500 μM conc.) was observed for compound **6**, but it can be confidently ascribed to the possible interference of some 9H-fluorene radical originating from the Fmoc group.

The thiophenol, PhSH, assay was used as predictive measure of activity in cell culture (Giles et al. 2003). It is a standard catalytic assay that spectrophotometrically follows the formation of diphenyl disulfide (PhSSPh) from PhSH in the presence of H_2O_2 and catalyst. Increased rates of disulfide formation are indicative of catalytic activity (Iwaoka and Tomoda 1994). The results obtained, that are shown in Table 1, deserve some comments.

Overall, the increases in the baseline rate of 2- or even 3-fold were quite significant and similar to the activity of other selenium agents tested by us and others in the past (Collins et al. 2005; Pariagh et al. 2005; Doering et al. 2012). Compound **3**, in particular, appears to be rather active. This compound is therefore of special interest as a possible catalytic redox modulator able to interfere with cellular redox processes and signaling in the presence of (elevated levels of) H_2O_2 (see below). Otherwise, compound **4**, its sulfur analogue, did not show thiol peroxidase activity under our conditions. Indeed such a lower activity of the sulfur compounds would not be entirely unexpected, as GPx-like catalytic activity tends to increase for analogues in the order of sulfur < selenium < tellurium. But then, the value of 0.75 (Table 1) does not indicate that compound **4** is less active (or inactive at all) than the other compounds tested. As a matter of fact it inhibits (slows down) the oxidation of PhSH by H_2O_2 and this may be accounted for by (partial) oxidation of **4** (likely to its corresponding sulfoxide) that consumes the hydrogen peroxide available for the oxidation of PhSH. In fact, the relative negative control experiment (compound **4** + H_2O_2, 305 nm) showed a constant absorbance line paralleling the abscissa axis, although at a value >0 (=0.41), probably due to the formation of a side species that in the final experiment might be responsible for a slight (considering the stoichiometric ratio of the reactants involved) reduction of the hydrogen peroxide available for the oxidation of PhSH.

Table 1 Thiol-peroxidase activity of compounds tested

Compound	PhSH activity[a]
3	3.00 ± 0.40
4	0.75 ± 0.01
6	1.67 ± 0.69
7	1.33 ± 0.11
Ph-Se-Se-Ph	4.28 ± 0.12
Se-Me-SeCys	2.08 ± 0.11

[a] Catalyzed/uncatalyzed reaction rate ratio

The other selenium-containing compounds tested as part of this study showed similar increases in the initial rate of PhSH oxidation, which implies that apart from the sulfur/selenium issue, there is no clear structure-activity relationship emerging, at least as far as the few compounds under investigation are concerned.

The relationship between molecular structure and biological activity of our chalcogen-compounds was therefore investigated further in cell culture, firstly by comparing the cytotoxic effect exerted by the synthesized compounds using morphological analysis and a conventional viability assay. In fact, in this phase methods such as MTS- or MTT-based assays were excluded to avoid false results due to direct or indirect interactions of high concentrations of compounds with intrinsic reductive potential with the bioreductive reaction of these assays (Bruggisser et al. 2002; Wang et al. 2010). For dose–response studies on cytotoxicity, mother solutions of compounds **3**, **4**, **6**, **7,** and *Se*-Me-SeCys were diluted in experimental culture medium to reach the final increasing concentrations of 10, 50, and 100 μM. The obtained samples were added to adherent HepG2 cells, seeded 24 h before at 5×10^3/well into 96-well microtiter plates, in 100 μL total volume and incubated at 37 °C in a CO_2 incubator for a further 72 h. As a control, the same cells were exposed to the vehicle

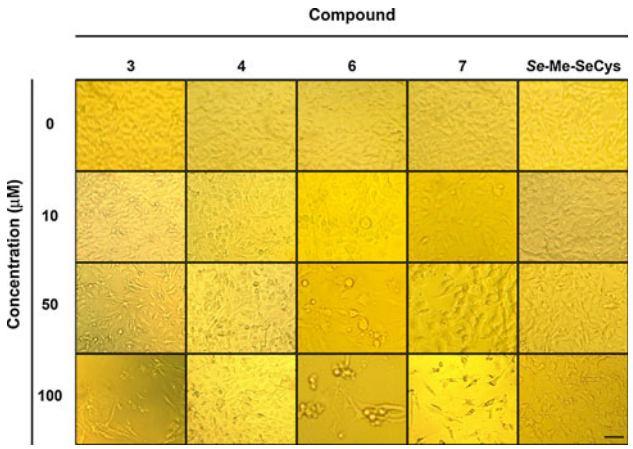


Fig. 1 Effects of chalcogen-compounds on HepG2 cell cultures. Cell cultures were examined after 72 h treatment for changes in morphology and growth, directly in the culture well, under an inverted microscope at the same magnification (original magnification, 100×). Panels show representative fields selected from experimental samples of one out of three experiments with similar results. The *scale bar*, reported only in the *lower/right panel*, represents 50 μM

alone (DMSO), in amounts corresponding to the higher employed for dissolving the compounds. At the end of the incubation time, cell morphological changes in various samples were observed under an inverted microscope. The cells were then recovered by brief trypsinization and immediately re-suspended in serum containing medium to stop further protease action. The total number of recovered cells and the percentage of viable cells was then evaluated by microscopy analysis of stained cells using a standard Trypan Blue exclusion test. Preliminary experiments indicated that no significant change of dead cell percentage could be detected with any of the compounds assayed at concentrations lower than those utilized in these dose–response experiments. The qualitative, morphological analysis under inverted microscope revealed that no changes with respect to control samples were practically observed for all the compounds assayed at the lower concentration of 10 μM (Fig. 1).

Conversely, remarkable differences, both in terms of monostrate confluence and in morphology of cells, were observed at the higher concentrations of 50 and 100 μM for all the compounds assayed except, apparently, for compound **4**. In fact, the sulfur-containing analogue of selenodiamino acid **3** exerted only mild, morphologically detectable, inhibitory effects on cell growth at the higher concentration of 100 μM. Noticeably, dramatic morphological changes caused by compound **6** at 50 μM and, even higher, at 100 μM, suggested that this compound was endowed with an high biologically active potential. Quantification of the cytotoxic effects of the compounds under study, as assayed by total and dead cell counts, in agreement with the qualitative observations, generally confirmed the dose-dependency of the exerted effects. In fact, no significant changes in total cell number were observed for all the compounds assayed at the concentration of 10 μM (Fig. 2a). Otherwise, dose-dependent significant or highly significant changes, with remarkable decreases in cell counts, were detected at the higher concentrations for all the selenium containing compounds, including *Se*-Me-SeCys, except for compound **6** at 50 μM. No significant change was observed in cultures exposed to the sulfur-containing compound **4**. In order to ascertain whether decreases in total cell numbers were associated only to inhibitory effects on cell growth or also to specific cytotoxic effects, the above reported results were compared with those concerning cell viability, as assayed by Trypan Blue staining, in samples from cultures subjected to the same experimental conditions. Results, reported in Fig. 2b, showed a very good coincidence between % total cells and % viable cells for compound **6** and *Se*-Me-SeCys, suggesting that these compounds may act mainly as growth-inhibitory, i.e. cytostatic agents rather than as cytotoxic agents, even at the higher concentrations assayed. In contrast, compounds **3** and **7** showed a similar capacity to act as highly cytostatic as well as moderately cytotoxic agents, at concentrations of 50 μM and higher. Interestingly, compound **4**, i.e., the only compound that did not apparently modify cell morphology or number, nevertheless significantly reduced viable cell numbers at the concentrations of 50 and 100 μM, and hence exhibited a limited cytotoxic potential (Fig. 2b).

Altogether, these results clearly indicate that all the compounds under investigation at a concentration of 10 μM did not exert any cytotoxic or cytostatic effect but, at the same time, that our chalcogen-compounds were still endowed with a potent, dose-dependent biological activity. Based on these results, the concentration of 10 μM was considered as suitable for successive studies on possible protective effects against cell injury caused by AFB_1.

To fully set up the optimal experimental conditions for evaluating a possible protection by our novel chalcogen-compounds against AFB_1 in HepG2 cells, we then performed dose–response experiments using concentrations of aflatoxin in the range between 1 and 100 μM. Culture conditions were the same as described above for measuring the cytotoxicity of the chalcogen-compounds, but at the end of a 48 h incubation time, cell damage induced by AFB_1 was detected directly in the cultures subjected to different experimental conditions by adding the MTS reagent to the culture wells and by determining color changes, as described in "Materials and methods". To exclude a possible direct effect of AFB_1 on the MTS reaction, the MTS reagent was also added to some control wells containing equal volumes of culture medium with the

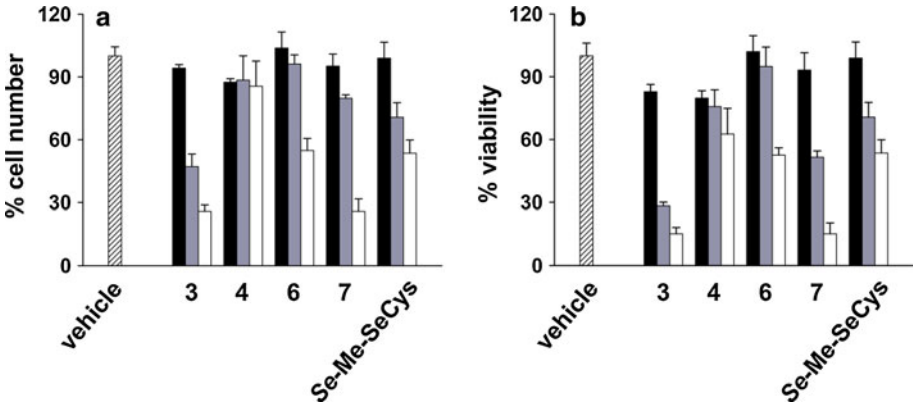


Fig. 2 Quantification of the effects of chalcogen-compounds on growth and viability of HepG2 cells. Cells were treated for 72 h with control vehicle (vehicle), or 10 μM (*black*), 50 μM (*gray*), 100 μM (*white*), compound **3**, compound **4**, compound **6**, compound **7**, and *Se*-Me-SeCys (**SeMC**). After trypsinization, all cells, including the floating ones, were recovered and total cell number (**a**) and viable cell number (**b**) were evaluated by microscopy analysis in a haemocytometer chamber, using the Trypan Blue exclusion test. Results are expressed as percentage cell number (total number of cells in the experimental sample/mean total number of cells in the control samples ×100) (**a**) or percentage viability (number of viable cells in the experimental sample/mean number of viable cells in the control samples ×100) (**b**). Each data point represents the mean and the standard deviation from three different determinations obtained in one out of three experiments with similar results. Comparisons of the means between treated samples and controls, by Hochberg's GT2 post hoc multiple comparison One-Way ANOVA test, were as follows. **a** 10 μM, all comparisons = not significant (NS); 50 μM, **3** $p < 0.001$, **4** = NS, **6** = NS, **7** $p = 0.004$, **SeMC** $p = 0.006$; 100 μM, **3** $p < 0.001$, **4** = NS, **6** $p < 0.001$, **7** $p < 0.001$, **SeMC** $p < 0.001$. **b** 10 μM, **3** $p = 0.004$, all other comparisons = NS; 50 μM, 3 $p < 0.001$, 4 $p = 0.033$, 6 = NS, **7** $p < 0.001$, **SeMC** $p = 0.004$; 100 μM, 3 $p < 0.001$, 4 $p = 0.033$, 6 $p < 0.001$, **7** $p < 0.001$, **SeMC** $p < 0.001$

Table 2 Cytotoxic activity of AFB_1 on HepG2 cell line

Cytotoxic agent	IC_{30} (μM)[a]	IC_{40} (μM)[a]	IC_{50} (μM)[a]
AFB_1	6.36 (10.28/ 3.90)[b]	11.12 (17.74/ 6.97)[b]	19.46 (31.94/ 11.86)[b]

[a] Inhibitory concentrations (IC), defined as the concentrations required to decrease metabolic activity after a 48 h treatment, as assessed using the MTS assay, by 30 % (IC_{30}), 40 % (IC_{40}) and 50 % (IC_{50}), respectively. Results represent the mean values obtained from three independent determinations in one of two experiments with similar results. Within parenthesis, mean + SD/mean − SD, are also reported

[b] Pearson's r value = 0.97

different concentrations of AFB_1, as in the experimental samples, but without cells. No color change was observed in these wells at any of the concentrations of AFB_1 assayed. Results, expressed as IC_{30}, IC_{40} and IC_{50} values, showed that AFB_1 actually caused, as expected, a well-detectable cell injury in a dose-dependent manner (see Table 2). Based on these results a concentration of 10 μM AFB_1, i.e., a concentration close to that capable to reduce the metabolic activity of the cells by 40 %, was considered as suitable for successive, cell protection experiments.

We next focused our attention on the possible protective effects of our chalcogen-compounds, by directly investigating their effects on cell injury caused by AFB_1. To this purpose, the biological activity of the seleno-diamino acids **3**, **6**, **7** and that of compound **4** and of *Se*-Me-SeCys was assayed by performing further experiments in which HepG2 cells pre-incubated for 20 h with 10 μM of the compounds were then washed three times with fresh medium and exposed to 10 μM AFB_1 for a further 48 h. At the end of the incubation time, the effects on the cells were first measured by determining the mitochondrial metabolic activity using the MTS assay. Preliminary experiments showed that none of the compounds assayed significantly modified by themselves absorbance values of the MTS reaction when added to the culture medium, at the concentration utilized of 10 μM, either in the presence or in the absence of the cells.

The results shown in Fig. 3a represent the mean values of three determinations for each compound tested, obtained in one representative experiment out of the two performed with similar results, and are expressed as % to vehicle treated control. No significant change was observed, as expected, in all groups treated with chalcogen-compounds alone with respect to the vehicle treated group. Conversely, a highly significant inhibitory effect of AFB_1 on cell metabolic activity was observed, in agreement to preliminary experiments, indicating a reduction of about 40 % with respect to control cells. Compound **7** did not exert any protective effect, but rather further reduced MTS values in a significant manner. All other compounds assayed significantly (compound **4**) or highly significantly improved values related to the metabolic activity, with compound **3**, which was also the most active compound in the thiophenol-based catalysis assay, exerting a potent protective effect on this parameter that was even higher than that exerted by *Se*-Me-SeCys.

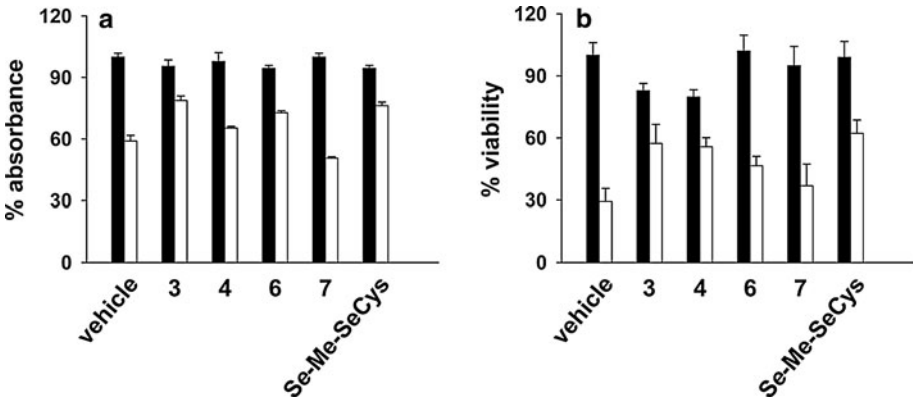


Fig. 3 Quantification of the protective effects of chalcogen-compounds towards cytotoxicity induced by AFB_1 in HepG2 cells. Cells were pretreated for 20 h with control vehicle or 10 μM compound **3**, compound **4**, compound **6**, compound **7**, and *Se*-Me-SeCys (**SeMC**) before washing and addition of vehicle (*black*) or 10 μM AFB_1 (*white*) for a further 48 h. Cell metabolic activity and viability were then assessed by the MTS assay (**a**) or by the Trypan Blue exclusion test (**b**), respectively. Results are expressed as percentage absorbance (absorbance value in the experimental sample/mean absorbance value in the control, vehicle treated, samples × 100) (**a**) or percentage viability (number of viable cells in the experimental sample/mean number of viable cells in the control, vehicle treated, samples × 100) (**b**). Each data point represents the mean and the standard deviation from three different determinations obtained in one out of three experiments with similar results. Comparisons of the means by Hochberg's GT2 post hoc multiple comparison One-Way ANOVA test, were as follows: **a** vehicle group without AFB_1 versus all groups without AFB_1, all comparisons = not significant (NS); vehicle group without AFB_1, versus all groups with AFB_1, all comparisons $p < 0.001$; vehicle group with AFB_1, versus **4** with AFB_1 $p = 0.015$, versus **7** with AFB_1 $p = 0.007$, all other comparisons $p < 0.001$; **b** vehicle group without AFB_1 versus all groups without AFB_1, all comparisons = not significant (NS); vehicle group without AFB_1, versus all groups with AFB_1, all comparisons $p < 0.001$; vehicle group with AFB_1, versus **3** with AFB_1 $p = 0.003$, versus **4** with AFB_1 $p = 0.006$, versus **6** and **7** with AFB_1 = NS, all other comparisons $p < 0.001$

Levels of protection of chalco-diamino acids against cell injury caused by AFB_1 were also evaluated by determining cell viability, according to the Trypan Blue exclusion test, in cultures that underwent the same treatment under the same experimental conditions as described above for those which have been investigated in the MTS assay. Results indicate that, in this case, pre-treatment with all the compounds assayed actually increased levels of viable cells with respect to the dramatic inhibitory effect exerted by AFB_1. Nevertheless, changes in levels of viability caused by compounds **6** and **7** were not statistically significant. Conversely, treatment with compounds **3**, **4**, and *Se*-Me-SeCys all caused significant difference in % viable cells, showing a remarkable increase of viability, with *Se*-Me-SeCys exerting a slightly higher protective effect with respect to compound **3**, against cell death caused by AFB_1.

These results suggest that our newly synthesized seleno-diamino acids are apparently endowed with a potent protective potential against cell damage caused by AFB_1 that is at least similar to, or even higher than, that exerted by *Se*-Me-SeCys. However, this protective effect is not absolute, but rather strictly related to the molecular structure of the compounds.

Interestingly, compounds particularly active in the thiophenol-based catalysis assay, such as compound **3**, also appear to act as better cell protectants compared to "poor" GPx-like catalysts (e.g., sulfur compound **4**). Nonetheless, there are exceptions. In fact, the sulfur-containing analogue of seleno-diamino acid **3**, i.e. compound **4**, was able to contrast cell damage and loss caused by AFB_1 apparently better than the other selenium containing compounds, and despite the fact that it was considerably less active in the thiophenol assay. This suggests that the protective effect could be related in some way to the specific molecular moiety of these diamino acids rather than to the presence of selenium. As a matter of fact, both the selenide and sulfide may act as one-shot sacrificial reducing agents which become oxidized to the sulfoxide/selenoxide or even the sulfone/selenone. As there is no extensive catalysis, both, the sulfur and selenium analogues may behave similarly in cell culture. Indeed, the advantage of selenium to outperform sulfur as antioxidant is usually based on the fact that the selenium analogue is catalytic, due to its ability to react spontaneously as selenoxide back to selenide (sulfur in form of a sulfoxide cannot do this). If this step of the reaction becomes less important, or does not occur, as in the case of a sacrificial, non-catalytic antioxidant, then the sulfur and selenium analogues may well act as equally good antioxidants, as both are able to interfere with OS by reacting with and sequestering ROS.

Conclusions

Together, the results presented above indicate that cells pretreated with a non-toxic concentration of chalcogen-

diamino acids were efficiently protected against cell damage caused by AFB_1, presumably attributable to a reduction in the oxidative insult. Interestingly, the seleno-diamino acid **3** exerted a chemo-protective effect that was slightly higher than that shown by the reference compound *Se*-Me-SeCys. The presence of selenium in the molecular structure of the compound, however, seems not to be always mandatory.

Besides, chemoprotection seems not attributable to a direct action of chalcogen-diamino acids on AFB_1, as proved by the ability of AFB_1 to fully conserve its cytotoxic potential when exposed to chalcogen-diamino acids before addition to the cells (data not shown).

In addition to their capacity, when administered to the cells in pre-treatment at non-toxic concentrations, to contrast, at least partially, cytotoxic effects of AFB_1, our seleno-diamino acids **3**, **6**, and **7** if administered at the higher concentrations of 50–100 μM seemed to be endowed with a potent cytotoxic potential. In particular, while compound **6**, similarly to the reference compound *Se*-Me-SeCys, seems to preferentially act as a cell growth inhibitor, compounds **3** and **7** seem to be relatively good inducers of cell death, especially when used at the higher concentration. Thus, seleno-diamino acid **3**, i.e. the compound showing the higher protective activity against cell death caused by AFB_1 at the concentration of 10 μM, also seems to exert the higher cytotoxic effect towards HepG2 carcinoma cells when used at higher concentrations. This apparent paradox should not be surprising based on existing data on the biological functions of selenium showing a dose-dependent shift from antioxidant to pro-oxidant effects for this element (reviewed by Lee and Jeong 2012). Moreover, the cytotoxicity we observed in HepG2 cells could be related to complex and only partly understood mechanisms underlying the selective induction of cell death by redox modulating agents (Saidu et al. 2013a, b).

In conclusion, further investigations are required to clarify the chemical and biochemical mechanisms underlying the protective effects exerted by chalcogen-diamino acids towards AFB_1-induced cell damage as well as to characterize growth inhibition and cell death induced by the same compounds at higher concentrations. Nonetheless, our results confirm that this family of compounds is endowed with a potent biological activity, greatly encouraging future studies to understand the actual potential of compounds of this family as chemoprotective agents and/or cytostatic or even cytotoxic redox modulators, against both OS and cancer.

Acknowledgments The authors acknowledge that part of this work was performed (by A. N.) at Heinrich-Heine University, Düsseldorf (Germany), under the skilled guidance of Prof. Dr. Helmut Sies. Financial support to A. N. by the Scuola di Dottorato Agrisystem of the Catholic University of the S. H. (Italy), to A. M. by the University of Messina (Italy), and to C. J. by the University of Saarland (Germany) is gratefully acknowledged. R. C. and A. M. contributed equally to the study. All authors discussed the results and commented on the manuscript. C. J. wishes to acknowledge funding via the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement 215009 RedCat. ^1H and ^{13}C NMR spectra were performed at Centro Interdipartimentale di Metodologie Chimico-Fisiche, University of Napoli Federico II. Varian Inova 500 MHz instrument is property of Consorzio Interuniversitario Nazionale la Chimica per l'Ambiente (INCA).

Conflict of interest The authors have declared no conflict of interest.

References

Aboobaker VS, Balgi AD, Bhattacharya RK (1994) In vivo effect of dietary factors on the molecular action of aflatoxin B_1: role of non-nutrient phenolic compounds on the catalytic activity of liver fractions. In Vivo 8:1095–1098

Agarwal KC (1996) Therapeutic actions of garlic constituents. Med Res Rev 16:111–124

Amagase H (2006) Clarifying the real bioactive constituents of garlic. J Nutr 136:716S–725S

Andreadou I, Menge WMPB, Commandeur JNM, Worthington EA, Vermeulen NPE (1996) Synthesis of novel *Se*-substituted selenocysteine derivatives as potential kidney selective prodrugs of biologically active selenol compounds: evaluation of kinetics of β-elimination reactions in rat renal cytosol. J Med Chem 39:2040–2046

Balestrieri E, Pizzimenti F, Ferlazzo A, Giofrè SV, Iannazzo D, Piperno A, Romeo R, Chiacchio MA, Mastino A, Macchi B (2011) Antiviral activity of seed extract from *Citrus bergamia* towards human retroviruses. Bioorg Med Chem 19:2084–2089

Bayse CA, Allison BD (2007) Activation energies of selenoxide elimination from *Se*-substituted selenocysteine. J Mol Model 13:47–53

Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70–76

Berges R, Siess MH, Arnault I, Auger J, Kahane R, Pinnert MF, Vernevaut MF, le Bon AM (2004) Comparison of the chemopreventive efficacies of garlic powders with different alliin contents against aflatoxin B_1 carcinogenicity in rats. Carcinogenesis 25:1953–1959

Bhabak KP, Mugesh G (2010) Functional mimics of glutathione peroxidase: bioinspired synthetic antioxidants. Acc Chem Res 43:1408–1419

Block E (2010) Garlic and other alliums: the lore and the science. Royal Society of Chemistry, Cambridge. ISBN 0854041907

Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181:1199–1200

Bolognese A, Fierro O, Guarino D, Longobardo L, Caputo R (2006) One-pot synthesis of orthogonally protected enantiopure *S*-(aminoalkyl)cysteine derivatives. Eur J Org Chem 15:169–173

Bonaccorsi P, Marino-Merlo F, Barattucci A, Battaglia G, Papaianni E, Papalia T, Aversa MC, Mastino A (2012) Synthesis and biological evaluation of a new class of glycoconjugated disulfides that exhibit potential anticancer properties. Bioorg Med Chem 20:3186–3195

Bruggisser R, von Daeniken K, Jundt G, Schaffner W, Tullberg-Reinert H (2002) Interference of plant extracts, phytoestrogens and antioxidants with the MTT tetrazolium assay. Planta Med 68:445–448

Caputo R, Cassano E, Longobardo L, Palumbo G (1995) Synthesis of enantiopure *N*- and *C*-protected homo-β-amino acids by direct homologation of α-amino acids. Tetrahedron Lett 36:167–168

Caputo R, Capone S, Della Greca M, Longobardo L, Pinto G (2007) Novel selenium-containing non-natural diamino acids. Tetrahedron Lett 48:1425–1427

Caputo R, Della Greca M, de Paola I, Mastroianni D, Longobardo L (2010) Novel sulfur and selenium containing bis-α-amino acids from 4-hydroxyproline. Amino Acids 38:305–310

Collins CA, Fry FH, Holme AL, Yiakouvaki A, Al-Qenaei A, Pourzandc C, Jacob C (2005) Towards multifunctional antioxidants: synthesis, electrochemistry, in vitro and cell culture evaluation of compounds with ligand/catalytic properties. Org Biol Chem 3:1541–1546

Cordero FM, Bonanno P, Khairnar BB, Cardona F, Brandi A, Macchi B, Minutolo A, Grelli S, Mastino A (2012) (−)-(1*R*,2*R*,7*S*,8a*R*)-1,2,7-Trihydroxyindolizidine [(−)-7*S*-OH-lentiginosine]: synthesis and proapoptotic activity. Chem Plus Chem 77:224–233

Doering M, Diesel B, Gruhlke MCH, Viswanathan UM, Mániková D, Chovanec M, Burkholz T, Slusarenko AJ, Kiemer AK, Jacob C (2012) Selenium- and tellurium-containing redox modulators with distinct activity against macrophages: possible implications for the treatment of inflammatory diseases. Tetrahedron 68:10577–10585

Drake EN (2006) Cancer chemoprevention: selenium as a prooxidant, not an antioxidant. Med Hypotheses 67:318–322

Du P, Viswanathan UM, Khairan K, Buric T, Saidu NEB, Xu Z, Hanf B, Bazukyan I, Trchounian A, Hannemann F, Bernhardt I, Burkholz T, Diesel B, Kiemer AK, Schäfer K-H, Montenarh M, Kirschd G, Jacob C (2013) Synthesis 1 of amphiphilic, chalcogen-based redox modulators with in vitro cytotoxic activity against cancer cells, macrophages and microbes. MedChemComm. doi:10.1039/c3md00204g

el-Bayoumy K, Upadhyaya P, Chae YH, Sohn OS, Rao CV, Fiala E, Reddy BS (1995) Chemoprevention of cancer by organoselenium compounds. J Cell Biochem Suppl 22:92–100

el-Bayoumy K, Sinha R, Pinto JT, Rivlin RS (2006) Cancer chemoprevention by garlic and garlic-containing sulfur and selenium compounds. J Nutr 136:864S–869S

el-Mofty MM, Sakr SA, Essawy A, Abdel GH (1994) Preventive action of garlic on aflatoxin B_1-induced carcinogenesis in the toad Bufo regularis. Nutr Cancer 21:95–100

Galvano F, Piva A, Ritieni A, Galvano G (2001) Dietary strategies to counteract the effects of mycotoxins: a review. J Food Protect 64:120–131

Gandhi S, Singh VK (2008) Synthesis of chiral organocatalysts derived from aziridines: application in asymmetric aldol reaction. J Org Chem 73:9411–9416

Gieselman MD, Zhu Y, Zhou H, Galonic D, van der Donk WA (2002) Selenocysteine derivatives for chemoselective ligations. Chem Bio Chem 3:709–716

Giles GI, Giles NM, Collins CA, Holt K, Fry FH, Lowden PAS, Gutowski NJ, Jacob C (2003) Electrochemical, in vitro and cell culture analysis of integrated redox catalysts: implications for cancer therapy. Chem Commun 16:2030–2031

Gonzàlez de Mejìa E, Gomez MR, Pina GL (1997) Antimutagenic activity of natural xanthophylls against aflatoxin B_1 mutagenicity. Environ Mol Mutagen 30:346–353

Gradelet S, Astorg P, Le Bon AM, Berges R (1997) Modulation of aflatoxin B_1 carcinogenicity, genotoxicity and metabolism in rat liver by dietary carotenoids: evidence for a protective effect of CYP1A inducers. Cancer Lett 114:221–223

Guyonett D, Belloir C, Suschetet M, Siesse MH, le Bon AM (2002) Mechanism of protection against aflatoxin B_1 genotoxicity in rats treated by organosulfur compounds from garlic. Carcinogenesis 23:1335–1341

Haratake M, Sakano T, Fuchigami T, Nakayama M (2011) Thiol-targeted introduction of selenocysteine to polypeptides for synthesis of glutathione peroxidase mimics. Metallomics 3:702–709

IARC (1993) Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. Monogr Eval Carcinog Risks Hum, Lyon (F) vol 56

Ip C, Zhu Z, Thompson HJ, Lisk D, Ganther HE (1999) Chemoprevention of mammary cancer with *Se*-allylselenocysteine and other seleno-amino acids in the rat. Anticancer Res 19:2875–2880

Iwaoka M, Kumakura F (2008) Applications of water-soluble selenides and selenoxides to protein chemistry. Phosphorus Sulfur Silicon Relat Elem 183:1009–1017

Iwaoka M, Tomoda S (1994) A model study on the effect of an amino group on the antioxidant activity of glutathione peroxidase. J Am Chem Soc 116:2557–2561

Jacob C, Giles GI, Giles NM, Sies H (2003) Sulfur and selenium: the role of oxidation state in protein structure and function. Angew Chem Int Ed 42:4742–4758

Jamier V, Ba LA, Jacob C (2010) Selenium- and tellurium-containing multifunctional redox agents as biochemical redox modulators with selective cytotoxicity. Chem Eur J 16:10920–10928

Khlangwiset P, Wu F (2010) Costs and efficacy of public health interventions to reduce aflatoxin-induced human disease. Food Addit Contam A 27:998–1014

Le Bon AM, Siess MH (2000) Organosulfur compounds from allium and the chemoprevention of cancer. Drug Metabol Drug Interact 17:51–79

Lee KH, Jeong D (2012) Bimodal actions of selenium essential for antioxidant and toxic pro-oxidant activities: the selenium paradox. Mol Med Report 5:299–304

Martins IL, Miranda JP, Oliveira NG, Fernandes AS, Gonçalves S, Antunes AMM (2013) Synthesis and biological activity of 6-selenocaffeine: potential modulator of chemotherapeutic drugs in breast cancer cells. Molecules 18:5251–5264

Mugesh G, du Mont WW (2001) Structure activity correlation between natural glutathione peroxidase (GPx) and mimics: a biomimetic concept for the design and synthesis of more efficient GPx mimics. Chemistry 7:1365–1370

Mugesh G, du Mont WW, Sies H (2001) Chemistry of biologically important synthetic organoselenium compounds. Chem Rev 101:2125–2179

Mukherjee AJ, Zade SS, Singh HB, Sunoj RB (2010) Organoselenium chemistry: role of intramolecular interactions. Chem Rev 110:4357–4416

Narayanaperumal S, Gul K, Kawasoko CY, Singh D, Dornelles L, Rodrigues OED, Braga AL (2010) Transition metal oxide nanopowder and ionic liquid: an efficient system for the synthesis of diorganyl selenides, selenocysteine and derivatives. J Braz Chem Soc 21:2079–2087

Nogueira CW, Rocha JBT (2011) Organoselenium and organotellurium compounds: toxicology and pharmacology. Patai's Chem Func Groups. doi:10.1002/9780470682531.pat0567

Nogueira CW, Zeni G, Rocha JBT (2004) Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem Rev 104:6255–6285

Pariagh S, Tasker KM, Fry FH, Holme AL, Collins CA, Okarter N, Gutowskib N, Jacob C (2005) Asymmetric organotellurides as potent antioxidants and building blocks of protein conjugates. Org Biol Chem 3:975–980

Phadnis PP, Mugesh G (2005) Internally stabilized selenocysteine derivatives: syntheses, ^{77}Se NMR and biomimetic studies. Org Biomol Chem 3:2476–2481

Plano D, Baquedano Y, Ibáñez E, Jiménez I, Palop JA, Spallholz JE, Sanmartín C (2010) Antioxidant-prooxidant properties of a new organoselenium compound library. Molecules 15:7292–7312

Plano D, Ibáñez E, Calvo A, Palop JA, Sanmartín C (2011) Novel library of seleno-compounds as kinase modulators. Molecules 16:6349–6364

Roman M, Jitaru P, Barbante C (2013) Selenium biochemistry and its role for human health. Metallomics. doi:10.1039/c3mt00185g

Saidu NEB, Asali IA, Czepukojc B, Seitz B, Jacob C, Montenarh M (2013a) Comparison between the effects of diallyl tetrasulfide on human retina pigment epithelial cells (ARPE-19) and HCT116 cells. Biochim Biophys Acta 1830:5267–5276

Saidu NEB, Touma R, Asali IA, Jacob C, Montenarh M (2013b) Diallyl tetrasulfane activates both the eIF2α and Nrf2/HO-1 pathways. Biochim Biophys Acta 1830:2214–2225

Saluk J, Bijak M, Nowak P, Wachowicz B (2013) Evaluating the antioxidative activity of diselenide containing compounds in human blood. Bioorg Chem 50:26–33

Sanmartín C, Plano D, Domínguez E, Font M, Calvo A, Prior C, Encío I, Palop JA (2009) Synthesis and pharmacological screening of several aroyl and heteroaroyl selenylacetic acid derivatives as cytotoxic and anti-proliferative agents. Molecules 14:3313–3338

Santi C, Di Lorenzo R, Battistelli B, Scalera C, Tidei C, Dragone V, Incipini L, Di Schino L, Rongoni E, Testaferri L, Tiecco M (2011) Bioinspired use of organoselenium catalysts. ECSOC-15, CD-ROM edition, ISBN: 3-906980-25-1, Published Online: http://www.sciforum.net/presentation/665/

Sarma BK, Mugesh G (2005) Glutathione peroxidase (GPx)-like antioxidant activity of the organoselenium drug Ebselen: unexpected complications with thiol exchange reactions. J Am Chem Soc 127:11477–11485

Sarma BK, Mugesh G (2008) Antioxidant activity of the anti-inflammatory compound Ebselen: a reversible cyclization pathway via selenenic and seleninic acid intermediates. Chem Eur J 14:10603–10614

Satheeshkumar K, Mugesh G (2011) Synthesis and antioxidant activity of peptide-based Ebselen analogues. Chem Eur J 17:4849–4857

Sausen de Freitas A, de Souza Prestes A, Wagner C, Haigert Sudati J, Alves D, Oliveira Porciúncula L, Kade IJ, Rocha JBT (2010) Reduction of diphenyl diselenide and analogs by mammalian thioredoxin reductase is independent of their glutathione peroxidase-like activity: a possible novel pathway for their antioxidant activity. Molecules 15:7699–7714

Sheen L, Wu C, Lii C, Tsai S (2001) Effect of diallyl sulfide and diallyl disulfide, the active principles of garlic, on the aflatoxin B_1-induced DNA damage in primary rat hepatocytes. Toxicol Lett 122:45–52

Shen HM, Ong CN, Shi CY (1995) Involvement of reactive oxygen species in aflatoxin B_1-induced cell injury in cultured rat hepatocytes. Toxicology 99:115–123

Storkey C, Davies MJ, White JM, Schiesser CH (2011) Synthesis and antioxidant capacity of 5-selenopyranose derivatives. Chem Commun 47:9693–9695

Wang P, Henning SM, Heber D (2010) Limitations of MTT and MTS-based assays for measurement of anti-proliferative activity of green tea polyphenols. PLoS One 5(4):e10202. doi:10.1371/journal.pone.0010202

Wogan GN, Kensler TW, Groopman JD (2011) Present and future directions of translational research on aflatoxin and hepatocellular carcinoma. A review. Food Addit Contam Part A 29:249–257. doi:10.1080/19440049.2011.563370

Yang CF, Liu J, Shen HM, Ong CN (2000) Protective effect of Ebselen on aflatoxin B_1-induced cytotoxicity in primary rat hepatocytes. Pharmacol Toxicol 86:156–161

Yang CS, Chhabra SK, Hong JY, Smith TJ (2001) Mechanisms of inhibition of chemical toxicity and carcinogenesis by diallyl sulfide (DAS) and related compounds from garlic. J Nutr 131:1041S–1045S