Characteristics of Selenazolidine Prodrugs of Selenocysteine: Toxicity and Glutathione Peroxidase Induction in V79 Cells

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Novel selenazolidine prodrugs of selenocysteine are being developed as potential selenium delivery agents for cancer chemoprevention and other clinical uses. The 2-unsubstituted compound, selenazolidine-4(*R*)-carboxylic acid (L-SCA), and the 2-oxo- and 2-methyl analogues possessing D-stereochemistry (D-OSCA and D-MSCA, respectively) were synthesized and chemically characterized. L/D pairs, along with other organoselenium compounds and common inorganic forms, were studied in cultured V79 cells to understand their inherent toxicity and their ability to induce selenium-dependent glutathione peroxidase (GPx) activity, which indicates the provision of biologically available selenium. All of the selenazolidines were much less toxic to the cells than was sodium selenite ($IC_{50} \approx 17\ \mu M$) or the parent selenolamines, L- or D-selenocystine ($IC_{50} \approx 34$ or $39\ \mu M$, respectively); OSCA was less toxic than MSCA. The stereoisomers of OSCA produced very different IC_{50} values (L-OSCA, ∼451 μM; D-OSCA, >3000 μM), while the IC_{50} values derived for the stereoisomers of MSCA were of the same order of magnitude (L-MSCA, ∼79 μM; D-MSCA, ∼160 μM). Compounds possessing L-stereochemistry were at least as active with respect to GPx induction as was sodium selenite (2.2-fold increase at 15 μM). L-Selenocystine produced a 4.2-fold increase in GPx activity at 30 μM, while L-SCA produced a 5.9-fold increase, followed by L-OSCA (4.6-fold) and L-MSCA (2.1-fold), all at 100 μM. Compounds possessing D-stereochemistry showed minimal ability to induce GPx activity (D-selenocystine, 1.0-fold increase; D-OSCA, 1.4-fold increase; D-MSCA, 1.3-fold increase).

Introduction

The element selenium, first described by Berzelius in 1817, was considered a poisonous substance for centuries. It was not until the 1950s that a nutritional role for selenium was appreciated in grazing animals and other livestock. In the 1970s, selenium was recognized as an essential micronutrient in human physiology, largely through its participation in the antioxidant defense system.[1,2]

Selenium is attracting considerable attention aside from its role as a micronutrient. Among other interesting pharmacological effects, selenium has exhibited exciting activity as a cancer chemopreventive agent in a variety of organs.[3−8]

Several selenium-containing compounds are used as part of vitamin/mineral supplementation products to ensure nutritional adequacy. However, new uses of selenium, such as in chemoprevention, require the element to be looked at as a pharmacological agent, whose clinical utility may require long-term administration at supranutritional levels. Current commercially available selenium supplements rely on inorganic forms such as sodium selenite and sodium selenate. While these forms have demonstrated value in replenishing depleted selenium levels, they have a narrow therapeutic index, and their metabolic conversion to H_2Se (the “central selenium pool”; see Figure 1) depletes glutathione (GSH) and may exhibit unacceptable toxicity when long-term or high-dose administration in humans is the ultimate goal. The toxicity of inorganic forms of selenium is well-known from environmental exposures, as well as laboratory studies.[9−11] Toxicity seems to be at least partially mitigated, and the therapeutic window is enhanced in many instances by using an organic form of selenium. Selenomethionine, usually as one component of “selenized” yeast grown in high-selenium media, is another common form found in commercial supplements. Again, selenomethionine is able to provide selenium to correct nutritional deficiency, but one of its major metabolic fates is the nonspecific incorporation

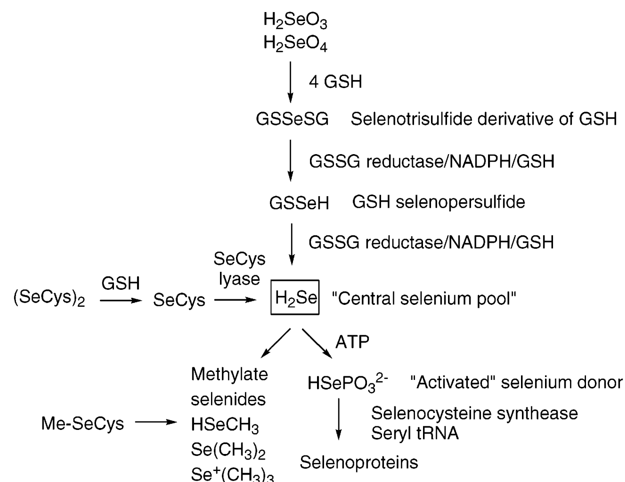

Figure 1. Metabolic fate of common forms of selenium: SeCys, selenocysteine; $(SeCys)_2$, selenocystine; Me-SeCys, Se-methyl-L-selenocysteine; GSH, glutathione.

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Chart 1

1a L-OSCA
1b D-OSCA
2a L-MSCA
2b D-MSCA
3 L-SCA

Scheme 1

L-OSCA, 1a
5-Oxo-L-prolinase
L-MSCA, 2a
H_2O
L-SeCys
Selenocysteine β-lyase
Se^o + Alanine → H_2Se
L-Proline dehydrogenase
L-SCA, 3

into proteins in place of methionine.[12−14] This decreases the therapeutic availability of the selenium, sequestering it inappropriately in protein. Selenocysteine, on the other hand, is subject to a single enzymatic cleavage step catalyzed by selenocysteine β-lyase[15] (Figure 1) that releases the selenium to the central pool. This pool is then used for the translational insertion of selenocysteine into protein (i.e., the production of a variety of selenoproteins), as well as for the generation of methylated metabolites. Both of these types of products may contribute to selenium-mediated cancer chemoprevention.

Unfortunately, selenocysteine is chemically unstable and difficult to handle, which has undoubtedly deterred its study and use. We are applying a prodrug approach to design forms of selenocysteine that possess reduced inherent toxicity and improved physicochemical properties. The prodrugs have been designed to overcome the barriers that exist with selenocysteine and represent chemically superior delivery forms for the amino acid and hence for the element itself.

Our prodrug approach for selenocysteine is based on an analogous system developed for prodrugs of the thiol amino acid cysteine.[16,17] The prodrugs are condensation products of selenocysteine and carbonyl compounds, which produce selenazolidine ring structures (Chart 1). We have previously reported the synthesis of the 2-oxo- and 2-methylselenazolidine-4(*R*)-carboxylic acids (L-OSCA (**1a**) and L-MSCA (**2a**), respectively).[18] Herein, we report the synthesis and chemical characterization of the 2-unsubstituted compound, selenazolidine-4(*R*)-carboxylic acid (L-SCA, **3**), as well as analogues of the other selenazolidines prepared from D-selenocystine, D-OSCA (**1b**), and D-MSCA (**2b**).

Depending on the carbonyl compound used in selenazolidine synthesis, which subsequently determines substitution at the C-2 position, physicochemical properties and biochemical attributes of the prodrugs can be modified. MSCA was designed to undergo nonenzymatic ring opening and hydrolysis to liberate the free amino acid (Scheme 1), in analogy with the thiol-containing compound.[16] No stereochemical preference for selenocysteine release is expected from the L/D-MSCA pair, although downstream biochemical events, such as lyase-mediated release of selenium from the amino acid backbone (Scheme 1), would be expected to exhibit stereoselectivity. OSCA, on the other hand, is hypothesized to require the action of 5-oxo-L-prolinase to release selenocysteine (Scheme 1), also in analogy to the thiol-containing compound.[19] In this case, the L-form is expected to be preferred by the enzyme over the D-form for release of the amino acid. Downstream events would then be identical to those seen after selenocysteine release from MSCA (although rates of release may differ). L-SCA is expected to require the action of L-proline dehydrogenase to release selenocysteine (Scheme 1), in analogy to thioproline.[20,21]

Preliminary biochemical studies of these compounds were performed in cultured V79 cells to begin to understand their properties and potential clinical utility. The toxicity of L- and D-selenocystine, the L- and D-selenazolidines, and other more common selenium-containing agents was measured by cell viability determinations after 1, 2, or 3 days of exposure. The induction of cellular selenium-dependent glutathione peroxidase (GPx) activity was also determined as a measure of the biological availability of the selenium.

Results and Discussion

Chemistry. The D-forms of selenocystine and the two selenazolidines were prepared using the same procedures recently reported for the L-forms.[18] All physical characterizations showed virtual, if not actual, identity between the stereoisomer pairs with the exception of equal but opposite signs for optical rotation values.

^1H and ^{13}C NMR spectra of L-SCA were consistent with other selenazolidines, as well as with thioproline. As previously reported,[18] the substituents at the 2-position exert significant influence on the chemical shift of the selenium atom. The ^{77}Se spectra of both L- and D-OSCA show single peaks at a chemical shift of ~1350 ppm. L- and D-MSCA show two distinct ^{77}Se peaks at a chemical shift of ~320 ppm due to the presence of a pair of diastereomers at C-2. The spectrum of L-SCA, with its two hydrogen atoms at C-2, showed a ^{77}Se peak at a chemical shift of ~215 ppm. Mass spectral data from L-SCA showed the characteristic six-line pattern of selenium, with a molecular ion at m/z 182.0, based on ^{80}Se, the major isotope (49.6% natural abundance).[22]

It is important to note for ultimate compound development that prodrug dissociation not only releases the desired selenocysteine (Scheme 1) but also the carbonyl compound (or a derivative) used in its construction. For the prototype compounds studied here, OSCA produces CO_2 as a byproduct, MSCA releases acetaldehyde, and SCA liberates formaldehyde.

Biological Evaluation. The selenium-containing agents chosen for comparison to the novel selenazolidines represent some “gold standards” in the field (sodium selenite, sodium selenate, and L-selenomethionine), as well as two additional agents with reports of interesting cancer chemopreventive activity. 1,4-Phenylene-bis(methylene)selenocyanate (*p*-XSC) has been extensively studied with interesting results in several tumor models.[23−27] Se-methyl-L-selenocysteine was included because of growing evidence that monomethylated forms of selenium (i.e., CH_3SeH) may be impor-

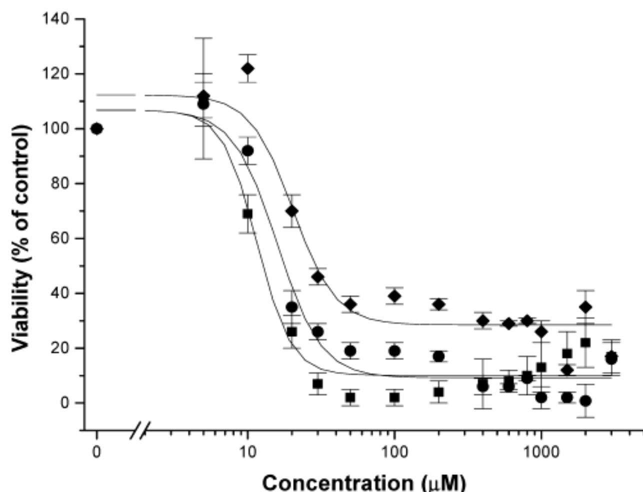

Figure 2. Viability of V79 cells (as a percent of untreated controls) after treatment with sodium selenite for 1 (♦), 2 (●), and 3 (■) days. Each point represents the mean ± SEM of eight replicates.

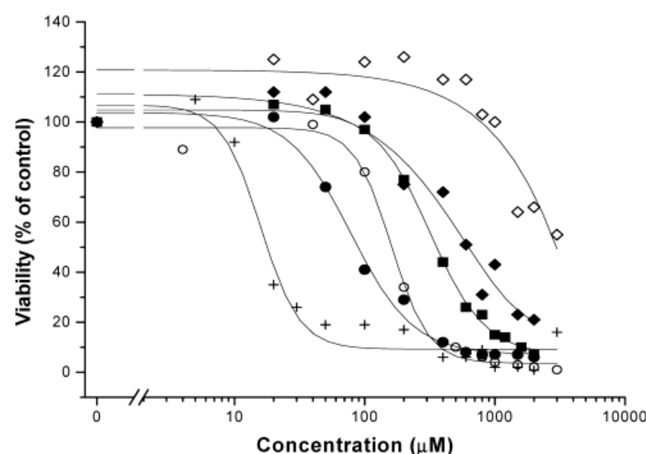

Figure 3. Graphical representation of viability data at day 2 for selected compounds: sodium selenite (+), L-OSCA (◆), D-OSCA (◇), L-MSCA (●), D-MSCA (○), and L-SCA (■). Each point represents the mean of eight replicates. Error bars are omitted for clarity (coefficient of variation averaged less than 11%).

tant in cancer chemoprevention.[28−31] Se-methyl-L-selenocysteine and other derivatives (i.e., methylseleninic acid[32−34]) serve as direct precursors for methylselenol (Figure 1). This represents an interesting alternative hypothesis to the long-held view that elevated selenoprotein expression and/or activity is required for chemoprevention because these forms appear to contribute little, if any, to the central pool and hence to selenoprotein biosynthesis.

Figure 2 presents toxicity data for sodium selenite at three different time points to illustrate common results from the time course experiments. Both efficacy (plateau value) and potency (IC_{50} value) increased significantly between days 1 and 2, but no significant differences were observed between 2- and 3-day time points.

Figure 3 shows the toxicity profile at 2 days of exposure for selected compounds in graphical form, and Table 1 presents derived IC_{50} values at 2 days for all compounds to facilitate comparison. Sodium selenite was among the most toxic compounds, as measured by the MTS cell survival assay ($IC_{50} \approx 17$ μM); sodium selenate treatment resulted in a much higher IC_{50} (~292 μM). The isomers of selenocystine were both quite toxic as well ($IC_{50} \approx 35$ μM). The L- and D-pair of MSCA prodrugs produced toxicity of the same general order of magnitude (IC_{50}(L-MSCA) ≈ 79 μM; IC_{50}(D-MSCA) ≈ 160 μM), which would be expected if both prodrugs undergo identical nonenzymatic ring opening and hydrolysis, releasing the free selenol. This suggests that the free selenol contributes to the toxicity of the selenazolidines. On the other hand, the L- and D-pair of OSCA prodrugs showed very different toxicity values (IC_{50}(L-OSCA) ≈ 451 μM; IC_{50}(D-OSCA) > 3000 μM). This result is consistent with the idea that the enzymatic release of the free selenol is preferred for the L-form, with the D-form being biologically inert. Within the L-selenazolidines, MSCA produced the lowest IC_{50} value followed by SCA (~317 μM) and OSCA. If toxicity is assumed to reflect release of the free selenol, these results suggest that L-MSCA is a more efficient release form than L-OSCA. This is consistent with the nonenzymatic release mechanism hypothesized for 2-alkylselenazolidines, in contrast to the enzymatic release mechanism hypothesized for 2-oxoselenazolidines. The remaining organoselenium compounds resulted in varying levels of toxicity to the V79 cells. The other amino acid based compounds showed low toxicity (IC_{50}(L-selenomethionine) ≈ 267 μM; IC_{50}(Se-methyl-L-selenocysteine) ≈ 472 μM). In contrast, *p*-XSC was very toxic in this system with an IC_{50} of ~12 μM.

Table 1. Cytotoxicity of Selenium Agents and Their Effect on GPx Activity in V79 Cells

treatment	IC_{50} [a] (μM)	GPx activity[b] (fold induction ± SEM at 100 μM) (unless otherwise noted)
Na_2SeO_3	17	2.2 ± 0.2 (at 15 μM)
Na_2SeO_4	292	1.9 ± 0.1
L-selenocystine	34	4.2 ± 0.4 (at 30 μM)
D-selenocystine	39	1.0 ± 0.1 (at 30 μM)
L-OSCA	451	4.6 ± 0.4
D-OSCA	>3000	1.4 ± 0.1
L-MSCA	79	2.1 ± 0.2
D-MSCA	160	1.3 ± 0.1
L-SCA	317	5.9 ± 0.6
Se-methyl-L-selenocysteine	472	1.5 ± 0.1
p-XSC	12	1.5 ± 0.1 (at 20 μM)
L-selenomethionine	267	1.8 ± 0.1

[a] IC_{50} = concentration producing 50% cell survival after 2 days of exposure, derived from regression analysis of viability vs compound concentration graphs. [b] Untreated control values averaged 17.8 ± 1.2 (mean ± SEM) mU/mg protein.

Table 1 also presents GPx activity in V79 cells after treatment with the selenium-containing compounds. The data are presented as the fold increased activity over untreated control values (17.8 ± 1.2 mU/mg protein) at 100 μM supplemental selenium (unless otherwise noted) to facilitate comparison. The concentration at which maximal induction was observed varied with each compound and did not correlate with IC_{50} (r^2 = 0.0295; data not shown). Two general patterns were observed in GPx activity as a function of agent concentration (data not shown). Treatment with sodium selenite resulted in a spike in enzyme activity (2.2-fold) at 15 μM, followed by a sharp decrease probably due to cytotoxicity. In contrast, a plateau in enzyme activity was observed after treatment with the selenazolidines. For example, L-MSCA produced increased GPx activity

between 1.8- and 2.2-fold over the concentration range 100−400 μM. GPx activity has been previously observed to plateau with increased selenium administration, even as chemoprevention increases.[35] This is one reason that it is no longer thought to play an important role in selenium-mediated chemoprevention.

Both inorganic forms, sodium selenite and sodium selenate, produced a modest increase in GPx activity of approximately 2-fold. *p*-XSC showed little GPx induction (1.5-fold), as did L-selenomethionine (1.8-fold) and Se-methyl-L-selenocysteine (1.5-fold). L-Selenocystine and the L-selenazolidines were at least as active as the benchmark compound, sodium selenite, and usually were considerably more active (L-MSCA, 2.1-fold; L-selenocystine, 4.2-fold; L-OSCA, 4.6-fold; L-SCA, 5.9-fold). The D-forms of selenocystine and the selenazolidine prodrugs produced minimal increases in enzyme activity (<1.5-fold). This suggests that a stereochemically sensitive determinant of activity is present at some point in the pathway. One likely candidate is the lyase-mediated cleavage of the carbon−selenium bond.

It has been reported that cell culture media and conditions provide very little selenium to the cells, which correlates with extremely low baseline activity of GPx.[36,37] In fact, selenium in culture media is usually derived from the particular serum employed; no selenium is present in the D-MEM product as purchased. The media formula used here contained approximately 500 nM selenium (chemical form unknown), as measured by atomic absorption spectrometry.

Previous studies in a variety of cell lines have demonstrated the responsiveness of GPx to selenium supplementation in vitro.[36−42] While direct comparisons with these studies are difficult because of variations in cell lines, chemical form of selenium, concentration, timing, and enzyme assay conditions, our data demonstrated that the selenazolidine prodrugs of L-selenocysteine are able to provide biologically available selenium to cells.

Conclusions

Promising results were observed with the novel prodrugs of selenocysteine in preliminary work in cultured mammalian cells. The selenazolidines exhibited reduced toxicity to V79 cells, as measured by an MTS viability assay, compared to sodium selenite. Toxicity seemed to depend on the release of the free selenol, with little stereochemical preference for spontaneous release (MSCA) but with a distinct difference when an enzymatic pathway for release is required (OSCA). The selenium from the prodrugs of L-selenocysteine (but not of D-selenocysteine) was biologically available, as measured by the 2.1- to 5.9-fold increase in selenium-dependent GPx activity, indicating a stereochemical preference for this biological endpoint. The combination of low toxicity and the ability to deliver selenium point to the promise of using selenazolidine prodrugs of L-selenocysteine as new selenium delivery agents. While L-MSCA showed the lowest ability to induce GPx activity, its nonenzymatic release mechanism may be advantageous especially in situations of liver disease or other conditions that compromise the activity of enzymes required to biotransform L-SCA and L-OSCA.

Studies in the A/J mouse model are underway to investigate basic biochemical properties of the prodrugs in vivo, as well as to investigate their ability to serve as cancer chemopreventive agents against tobacco-induced lung carcinogenesis.

Experimental Section

Chemistry. All reagents were purchased from Sigma Chemical Company (St. Louis, MO), Aldrich Chemical Co. (Milwaukee, WI), or Fisher Scientific (Pittsburgh, PA). Melting points were determined on a Laboratory Devices USA Mel-Temp II melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were collected using either a Varian Unity 400 or 500 MHz FT-NMR spectrometer, as noted. D_2O was used as the NMR solvent, and the water peak was set to δ 4.7 ppm in the ^{1}H spectra. Owing to solubility problems, it was occasionally necessary to add one drop of 30% NaOD to the D_2O solution. ^{77}Se NMR spectra used dimethyl selenide (Aldrich) as an external standard set to δ 0.0 ppm. Infrared (IR) spectral analyses were done using KBr pellets on a Perkin-Elmer 1600 series FT-IR spectrophotometer. Fast atom bombardment (FABMS) or electron impact (EIMS) mass spectrometry analyses were carried out on a Finnegan MAT 95 in the Department of Chemistry at the University of Utah. Elemental analysis was conducted by Galbraith Laboratories (Knoxville, TN). Polarimetry experiments were conducted on a JASCO model DIP-370 digital polarimeter. Thin-layer chromatography (TLC) was carried out using Whatman (Clifton, NJ) flexible backed 60 Å silica gel plates, with a layer thickness of 0.25 mm, using iodine vapors for visualization.

L-Selenocystine, 2-Oxoselenazolidine-4(*R*)-carboxylic Acid (L-OSCA, 1a), and 2-(*R*,*S*)-Methylselenazolidine-4(*R*)-carboxylic Acid (L-MSCA, 2a). L-Selenocystine, L-OSCA, and L-MSCA were synthesized and characterized as described.[18] Briefly, selenocystine is prepared from stereochemically defined chloroalanine and elemental selenium. The selenocystine is then transiently reduced with sodium borohydride and reacted with the carbonyl donor of choice: 1.1′-carbonyldiimidazole to produce OSCA or acetaldehyde to produce MSCA.

D-Selenocystine, 2-Oxoselenazolidine-4(*S*)-carboxylic Acid (D-OSCA, 1b), and 2-(*R*,*S*)-Methylselenazolidine-4(*S*)-carboxylic Acid (D-MSCA, 2b). D-Selenocystine, D-OSCA, and D-MSCA were synthesized following similar procedures to the L-forms.[18]

D-Selenocystine: 49% yield; mp 172−174 °C (dec); TLC *n*-BuOH/H_2O/acetic acid (3:2:1), R_f = 0.32.; ^{1}H NMR (D_2O/NaOD, 500 MHz) δ 3.6 (dd, J = 5, 7 Hz, 1H, H-α), 3.3 (dd, J = 5, 12 Hz, 1H, H-β1), 3.2 (dd, J = 7, 12 Hz, 1H, H-β2); ^{13}C NMR (D_2O/NaOD, 125 MHz) δ 181.0 (COOH), 56.3 (C-α), 35.8 (C-β); ^{77}Se NMR (D_2O/NaOD, 95.3 MHz) δ 288.1; IR (KBr) ν_{max} 3500, 3000 cm^{-1}; FABMS [M$^+$ + 1] *m*/*z* 336.9 (^{80}Se); $[\alpha]^{25}_D$ +27.3° (*c* 0.5, 0.1 N NaOH). Anal. Calcd for $C_6H_{12}N_2O_4Se_2$: C, 21.6; H, 3.63; N, 8.38. Found: C, 21.4; H, 3.77; N, 8.32. CAUTION: The preparation of selenocystine produces H_2Se gas. The exhaust from this reaction was forced through two lead acetate traps, each containing 25 g of lead acetate in 300 mL of water for 2 h. As an added precaution, a respirator rated for H_2S was routinely used during reaction workup.

D-OSCA: 33% yield; mp 145−146 °C (dec); TLC *n*-BuOH/H_2O/acetic acid (3:2:1), R_f = 0.65; ^{1}H NMR (D_2O, 500 MHz) δ 4.5 (dd, J = 6, 8 Hz, 1H, H-4), 3.8 (dd, J = 8, 10 Hz, 1H, H-5a), 3.6 (dd, J = 6, 10 Hz, 1H, H-5b); ^{13}C NMR (D_2O, 125 MHz) δ 181.8, 164.4 (COOH, C-2), 60.1 (C-4), 32.0 (C-5); ^{77}Se NMR (D_2O, 95.3 MHz) δ 1352.8; IR (KBr) ν_{max} 3300, 3000, 1700 cm^{-1}; FABMS [M$^+$ + 1] *m*/*z* 195.9 (^{80}Se); $[\alpha]^{25}_D$ +68.1° (*c* 0.5, water). Anal. Calcd for $C_4H_5NO_3Se$: C, 24.8; H, 2.60; N, 7.22. Found: C, 25.2; H, 2.69; N, 7.34.

D-MSCA: 60% yield; mp 139−140 °C (dec); ^{1}H NMR (D_2O, 400 MHz) diastereomer A δ 5.1 (q, J = 7 Hz, 1H, H-2), 4.6 (t, J = 7 Hz, 1H, H-4), 3.5−3.3 (m, 2H, H-5), 1.7 (d, J = 7 Hz, 3H, CH_3); ^{1}H NMR (D_2O, 400 MHz) diastereomer B δ 4.9 (q, J = 7 Hz, 1H, H-2), 4.3 (dd, J = 7, 11 Hz, 1H, H-4), 3.5−3.3 (m,

2H, H-5), 1.7 (d, $J = 7$ Hz, 3H, CH_3); ^{13}C NMR (D_2O, 100 MHz) diastereomer A δ 171.7 (COOH), 66.4 (C-4), 50.7 (C-2), 24.2 (C-5), 18.8 (CH_3); ^{13}C NMR (D_2O, 100 MHz) diastereomer B δ 171.5 (COOH), 64.9 (C-4), 50.7 (C-2), 24.2 (C-5), 21.1 (CH_3); ^{77}Se NMR (D_2O, 76.2 MHz) δ 321.5, 317.0; IR (KBr) ν_{max} 3300, 2950, 1720 cm^{-1}; FABMS [$M^+ + 1$] m/z 195.9 (^{80}Se); $[\alpha]^{25}_D$ +83.8° (c 0.5, water). Anal. Calcd for $C_5H_9NO_2Se$: C, 31.0; H, 4.67; N, 7.22. Found: C, 31.4; H, 4.71; N, 7.35.

Selenazolidine-4(*R*)-carboxylic Acid (L-Selenaproline, L-SCA, 3). The procedure of DeMarco[43] was modified as follows. L-Selenocystine (0.13 g, 0.39 mmol) was suspended in a flask containing 0.05 N NaOH (5 mL) and ethanol (1.5 mL) that had been degassed under vacuum. Sodium borohydride (0.05 g, 1.3 mmol) was added slowly over about 10 min. The reaction mixture was stirred for an additional 20 min until it became colorless and was then placed in an ice bath. The pH was adjusted to 5−6 with 6 M HCl. Formaldehyde solution (37%, 0.3 mL, 3.5 mmol) was added slowly over 30 min, and the reaction mixture was stirred for 3 h. The mixture was concentrated under vacuum to about 2 mL, and the resulting solution was loaded on a 2 cm × 8 cm silica gel column, which was washed with ethyl acetate (300 mL) under argon. The eluant was dried under vacuum to give 0.1 g, 0.55 mmol (70%), of white product: mp 160−162 °C (dec) (lit.[43] 160−164 °C); ^{1}H NMR (D_2O, 400 MHz) δ 4.5 (t, $J = 7$ Hz, 1H, H-4), 4.4 (d, $J = 9$ Hz, 1H, H-2a), 4.3 (d, $J = 9$ Hz, 1H, H-2b), 3.4 (dd, $J = 7, 11$ Hz, 1H, H-5a), 3.3 (dd, $J = 7, 11$ Hz, 1H, H-5b); ^{13}C NMR (D_2O, 100 MHz) δ 170.8 (COOH), 64.4 (C-4), 36.9 (C-2), 23.2 (C-5); ^{77}Se NMR (D_2O, 76.2 MHz) δ 215.3; IR (KBr) ν_{max} 3300, 1700 cm^{-1}. EIMS [$M - H$]$^+$ m/z 182.0 (^{80}Se); $[\alpha]^{25}_D$ −28.0° (c 0.5, water). Anal. Calcd for $C_4H_7NO_2Se$: C, 26.7; H, 3.92; N, 7.78. Found: C, 26.3; H, 4.09; N, 7.87.

Biological Evaluation. General Cell Culture Procedures and Supplies. V79 Chinese hamster lung fibroblasts (V79-4) were purchased from American Type Culture Collection (Manassas, VA). Sigma Chemical Company (St. Louis, MO) was the source of the following supplies: powdered Dulbecco's modified minimum essential media (D-MEM), sodium bicarbonate, Hanks' balanced salt solution, trypsin (1:250), phosphate-buffered saline (PBS), and antibiotic/antimycotic solution (100X). Sodium dodecyl sulfate was purchased from BioRad Laboratories (Richmond, CA). Fetal Clone I serum was obtained from HyClone Laboratories (Logan, UT). Cell survival was measured by the MTS method using a Cell Titer 96 AQ_{ueous} One Solution Cell Proliferation kit from Promega (Madison, WI). GPx activity was determined using the BioxyTECH GPx 340 assay kit from *Oxis*Research (Portland, OR). Sodium selenite and sodium selenate were obtained from Sigma. L-Selenomethionine and Se-methyl-L-selenocysteine were purchased from Acros Organics (Morris Plains, NJ). *p*-XSC was generously provided by Dr. Pramod Upadhyaya, University of Minnesota Cancer Center.

The cells were maintained in a humidified 5% CO_2/air atmosphere at 37 °C in D-MEM containing 3.7 g/L sodium bicarbonate, 10% Fetal Clone I serum, and 0.1% antibiotic/antimycotic solution. Cultures were passaged every 3 days. Cell numbers were determined using a Coulter Counter, model Z1 (Beckman Coulter, Fullerton, CA) particle counter.

Protein concentration was measured with a Bradford assay (Sigma kit). A SPECTRAmax 250 microplate spectrophotometer with SOFTmax PRO software (Molecular Devices Corp., Sunnyvale, CA) was used for the MTS and Bradford assays and the GPx activity measurements. The selenium content of the cell culture media was measured using a Perkin-Elmer Optima 3100 XL atomic absorption spectrometer (Perkin-Elmer, Shelton, CT) after overnight digestion with 90% nitric acid/10% perchloric acid.

Cell Viability by MTS Assay. Wells of a 96-well plate were seeded with a cell number appropriate for the number of days in culture: 1×10^4 cells (1 day), 2.5×10^3 cells (2 days), or 1×10^3 cells (3 days). The plates were incubated overnight to allow the cells to adhere. The media was removed from each well and was replaced with 0.2 mL of freshly made solutions (pH 7−7.5) of the following drugs in complete media at various concentrations (0−3000 μM): sodium selenite, sodium selenate, L- and D-selenocystine, L- and D-OSCA, L- and D-MSCA, L-SCA, Se-methyl-L-selenocysteine, *p*-XSC, and L-selenomethionine. The cells were incubated with the treatment solutions for 1, 2, or 3 days. At the appropriate time point, the media was removed and cell viability was determined by adding 0.12 mL of a 1:5 dilution of MTS tetrazolium dye in media. The plates were incubated for 2 h, and 10% SDS (0.025 mL) was then added to quench the reaction. The plates were stored at 4 °C overnight. Prior to taking absorbance measurements, the plates were warmed slightly at 37 °C (10−15 min). The absorbance at 490 nm was determined, and the value corresponding to the blank well was subtracted from each sample measurement. Mean absorbance values (±SEM) of treated cells were compared to untreated controls (100% viability); $N = 8$. Differences in cell viability as a function of time were analyzed by a one-way ANOVA followed by a Tukey−Kramer multiple comparisons test. Statistical significance was set at $p < 0.05$. IC_{50} values were derived from regression analysis of the viability vs concentration plots (for the 2-day exposure) to facilitate comparison.

GPx Activity. Culture flasks (25 cm^2) containing 10 mL of media were seeded with 5.0×10^5 cells for each treatment group as well as untreated controls. The flasks were then incubated at 37 °C overnight to allow adhesion of cells. The media was removed and was replaced with 10 mL of freshly made solutions (pH 7−7.5) of the following compounds in complete media at the concentrations indicated (μM): sodium selenite (0−30), sodium selenate (0−400), L- and D-selenocystine (0−60), L- and D-OSCA (0−400), L- and D-MSCA (0−400), L-SCA (0−400), Se-methyl-L-selenocysteine (0−400), *p*-XSC (0−20), and L-selenomethionine (0−400). Controls received 10 mL of fresh media. After incubating for 48 h with treatment solutions, the media was removed and fresh media was added (10 mL). The flasks were incubated for an additional 24 h. The media was poured off, and the cells were washed twice with ice-cold phosphate-buffered saline.

The adherent cells were removed from the flask with a rubber policeman and were sonicated at 4 °C (three 10 s pulses with 30 s intervals; Branson 2201 sonicator, Branson Ultrasonics, Corp., Danbury, CT) in 1 mL of cold Tris-HCl buffer (50 mM, pH 7.5, containing 5 mM EDTA and 1 mM 2-mercaptoethanol). The lysed cells were centrifuged (5000*g*, 20 min) at 4 °C. The supernatant was assayed for GPx activity using *tert*-butyl hydroperoxide to initiate the reaction, monitoring the rate of NADPH consumption at 340 nm over a 3-min period. Conversion to mU of enzyme activity used 0.00622 mM^{-1} cm^{-1} as the extinction coefficient for NADPH at 340 nm. The protein concentration of the supernatant was determined using a Bradford assay. Results were expressed as the mean GPx activity ± SEM as mU/mg protein; $N = 9$. Fold induction at 100 μM (unless otherwise noted) over untreated control cultures was then calculated to facilitate comparison. Untreated control activity averaged 17.8 ± 1.2 mU/mg protein.

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