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THE ANTIMUTAGENICITY OF 2-SUBSTITUTED SELENAZOLIDINE-4-(R)-CARBOXYLIC ACIDS.

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Abstract

Selenium can have cancer chemopreventive activity, although the mechanism of action has not been well defined. Selenazolidine-4-(R)-carboxylic acids (SCAs) were devised as prodrugs of L-selenocysteine, to provide selenium in a form and at a concentration commensurate with cancer chemopreventive activity. In the present study, a series of selenazolidines has been evaluated in the *Salmonella typhimurium* TA98 tester strain and all were found to possess antimutagenic activity. There was little difference between the seven selenazolidines in their effectiveness against either benzo[a]pyrene (B[a]P) or 3,6-bis(dimethylamino)acridine (acridine orange), agents which differ in their requirement for mammalian enzyme bioactivation for mutagenicity. Antimutagenic activity against acridine orange was dependent on selenazolidine concentration, and EC50 values were in the 5–10 μ M range. At 25 μ M, the concentration tested in common for the two mutagens, the selenazolidines were more effective antimutagens against acridine orange than against B[a]P, with reductions in mutant frequency ranging from 54–71% for B[a]P and 79–93% for acridine orange. Efficacy against B[a]P was not enhanced when the concentration was increased to 50 μ M. The similarity in efficacy among the selenazolidines against B[a]P mutagenicity, contrasted with inter-compound differences in their ability to inhibit S9 CYP1A activity. The CYP1A Ki values ranged from a low of 63 μ M (2-[2'-hydroxyphenyl]SCA) to a high of 1.1 mM (2-cyclohexylSCA), but all were above the concentration required to inhibit mutagenicity by 50%. Thus, all the SCAs possess antimutagenic activity against both B[a]P and acridine orange, the efficacy varies little between the individual selenazolidines, and for B[a]P, the efficacy is not proportional to the inhibitory effect on the mutagen bioactivating enzyme.

Keywords

Salmonella typhimurium TA98; selenazolidine-4-(R)-carboxylic acids; selenocysteine prodrugs; antimutagenic; benzo[a]pyrene; acridine orange; CYP1A inhibition

1. Introduction

The antimutagenic property of selenium against chemical mutagens was first established [1] soon after the widespread use of mutant *Salmonella typhimurium* strains and bioactivating mammalian liver homogenates to detect such activity [2]. Since that time, the antimutagenic effect of selenium, largely in the form of inorganic selenite, has been observed with numerous chemicals including 3,6-bis(dimethylamino)acridine (acridine orange) and 7,12-dimethylbenz

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[a]anthracene [3], benzo[a]pyrene (B[a]P) [4,5], 3-methylcholanthrene and 3-methylcholanthrylene [5], mainstream and sidestream cigarette smoke condensate [6], and N-methyl-N-nitrosourea and N-methyl-N'-nitro-N-nitrosoguanidine [7,8]. The selenite inhibition of B[a]P mutagenicity was attributed to its inhibitory effect on arylhydrocarbon hydroxylase (CYP1A) activity [5,9] an action which decreases the bioactivating metabolism to mutagenic metabolites [4,10]. In animals, selenite was an effective chemopreventive agent against 7,12-dimethylbenz[a]anthracene-induced mammary tumors in mice [11–13] and rats [14] but was not effective against 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced mouse lung adenomas [15,16] or azaserine-induced rat pancreatic and hepatic neoplasms [17]. Seleno-amino acids and their derivatives have also been investigated for cancer chemopreventive properties but were not effective against all classes of mutagens tested [13,16,18–21]. In the only published antimutagenesis study for these organic selenium compounds, neither selenomethionine nor Se-methylselenocysteine were effective against 2-amino-3-methylimidazo[4,5]quinoline [22]. Of the several organoselenium compounds that have been demonstrated to have tumor reducing activity against a variety of carcinogens in rats and mice [15,23–34], to our knowledge no *in vitro* studies have been conducted to detect antimutagenic activity that could explain the observed anticarcinogenic effect.

An L-selenocysteine-prodrug approach was conceived as a way to supply the supranutritional selenium requirement necessary for cancer chemopreventive activity [35] without toxicity. As a prelude to *in vivo* evaluation, we pursued an investigation of antimutagenic properties of selenazolidine-4-(R)-carboxylic acid (SCA) and six 2-substituted derivatives (Figure 1).

2. Materials and methods

2.1 Materials

The *Salmonella typhimurium* TA98 bacterial strain was obtained from ATCC (Manassas, VA). B[a]P, acridine orange, 7-ethoxyresorufin, and NADPH were obtained from Sigma (St Louis, MO). SCA was synthesized as described by Short et al., [36], 2-oxoSCA and 2-methylSCA as described by Xie et al., [35] and the four other 2-substituted selenazolidines were synthesized in a similar manner to 2-methylSCA except the appropriate carbonyl compound was substituted for acetaldehyde in the synthesis. S9 mix consisted of filter-sterilized NADPH (1.25 mM) and hepatic S9 fraction (4 mg protein/ml) prepared from male Sprague Dawley rats treated three days previously with a single dose (25 mg/kg, i.p. in corn oil) of Aroclor 1254.

2.2 Cytotoxicity

The cytotoxicity of the selenazolidines was determined under two exposure conditions; bacterial growth in complete broth [37] and bacterial colony growth on nutrient broth agar plates. In the former, *Salmonella typhimurium* TA98 (1×10^6 cfu) were grown in Oxoid Nutrient Broth No. 2 in 96-well plates in the presence and absence of the selenazolidine compounds (10–200 μ M). After 15 hours at 37°C, the absorbance was determined at 570 nm. In the latter, with conditions that mimic those of the revertant mutagenesis/antimutagenesis assay described below, an appropriate dilution (10^{-5} to 10^{-7}) of the overnight bacteria culture in LB medium, and selenazolidine (0, 10, 25, or 50 μ M), was mixed with 2.5 ml of warm 0.6% top agar (NaCl/agar), added to nutrient broth agar plates and incubated at 37°C. The colony/plate evaluation was also performed following 30 minutes of incubation of the bacteria in the presence of 100 μ M acridine orange or 20 μ M B[a]P and S9 mix. Colonies on triplicate plates were counted after 24 hours.

2.3 Mutagenicity and antimutagenicity

For assessing the mutagenic properties of the selenium compounds, 0.1 ml of a 16-hour overnight *Salmonella typhimurium* TA98 culture grown in LB medium was incubated with the

selenium compound at 37°C for 30 minutes in phosphate buffer (pH =7.4) in the presence and absence of S9 mix. The incubate (0.6 ml) was then added to top agar containing growth-initiating concentrations (50 µM) of histidine and biotin, and poured on to minimal glucose agar plates. Revertant colonies were counted after incubation at 37°C for 2 days. Background (spontaneous) revertant colonies were also counted, from incubations without selenazolidines. Revertant colonies seen with 20 µM B[a]P incubated with S9 mix were performed alongside as a positive control.

For assessing the antimutagenic properties of the selenium compounds, 0.1 ml of the overnight LB culture used in the agar plate cytotoxicity evaluation (Section 2.2) was incubated (37°C for 30 minutes) with either B[a]P (20 µM) together with S9 mix, or acridine orange (100 µM), with the selenium compound (0, 5, 10, 25 or 50 µM) added prior to plating. The data were expressed as the percentage reduction of revertant colonies relative to the appropriate control (acridine orange or B[a]P without selenazolidine) control. All mutagenesis and antimutagenesis determinations were performed in triplicate utilizing two independent overnight *Salmonella typhimurium* TA98 cultures.

2.4 Cytochrome P4501A Inhibition

Rat hepatic S9 fraction used in the mutagenesis/antimutagenesis assays was incubated (50 µg protein/ml) with 7-ethoxyresorufin (0.025-0.4 µM) with and without the selenazolidine (50 µM). The cytochrome P450-catalyzed product (resorufin) formation rate was monitored (Ex, 544 nm; Fl, 612 nm) following the addition of NADPH (4 mM). Enzyme inhibition kinetics were analyzed using SigmaPlot Enzyme Kinetics Module 1.1 software (SPSS Inc, Chicago, IL).

2.5 Statistical analysis of data

Statistical analyses were performed using ANOVA, followed by Fisher's protected least significant difference multiple range test. Differences were considered significant at P values of < 0.05.

3. Results and Discussion

3.1 Cytotoxicity of selenazolidine-4-(R)-carboxylic acids

As a necessary prerequisite for mutagenic and antimutagenic activity determinations, the cytotoxicity of the SCAs towards *Salmonella typhimurium* TA98 was investigated. In the liquid broth protocol, two compounds, 2-(2'-hydroxyphenyl)SCA and to a lesser extent the unsubstituted SCA, showed statistically significant cytotoxicity, but only when present at 100 µM (Table 1). Several of the compounds showed an enhancement of viability at the lower concentrations (12.5 – 50 µM), approximately 20% for 2-cyclohexyl- and 2-butyl-derivatives, but these were not statistically significant. In the colony/plate assay, significant increases in the number of colonies, ranging from 16% to 29% were seen at either 25 or 50 µM for all compounds except SCA (Table 2). Whether the selenazolidines are providing additional selenium necessary for maximal growth or are acting through additional or alternative mechanisms is not known. The effect appears not confined to bacteria as in other studies, growth stimulation has also been observed for eukaryotic (murine and rat hepatoma) cells in culture [38]. In the colony/plate assay, no selenazolidine exhibited any cytotoxicity at the 25 and 50 µM concentrations evaluated, an effect that would have resulted in a decrease in the number of colonies. Concentrations at or below 50 µM were therefore selected as appropriate for antimutagenesis studies since the outcome would not likely be confounded by overt cytotoxicity concerns. However, antimutagenesis studies first required investigation of whether there is toxicity when selenazolidines and mutagens are present together, and also whether the selenazolidines alone have any mutagenic properties.

When bacteria were exposed to B[a]P, S9 mix and selenazolidines at 25 and 50 μM , and evaluated for colony formation in the presence of adequate histidine, the result (Table 3) was similar to the effects seen with selenazolidines alone (Table 2). There was no diminution in the number of colonies (i.e. toxicity), and for most selenazolidines, there was a statistically significant increase in colony numbers in at least one of the plating densities of each of the duplicate experiments. When bacteria were exposed to acridine orange in the absence of S9 mix, and 10 and 25 μM of each of the selenazolidines, the effects on colony numbers were quite similar to the above with one very noticeable exception (Table 4). In the presence of acridine orange, SCA at 25 μM was toxic. This toxicity was not seen with either component alone at the concentrations employed, nor with co-exposure of bacteria to B[a]P and SCA, and therefore represents a detrimental combination unique to acridine orange and SCA. This toxicity precluded evaluation of any antimutagenic effect of SCA at 25 μM on the mutagenicity of acridine orange.

3.2 Mutagenicity of selenazolidine-4-(R)-carboxylic acids

When examined for mutagenic activity at or below the non-toxic concentrations, none of the selenazolidines were mutagenic with or without aerobic incubation with the S9 mix (Table 5). Mutagenicity is defined as a doubling over the spontaneous or background number, together with selenazolidine concentration dependency, in the number of revertant colonies. As in the liquid-medium viability assay (Table 1), both SCA and 2-(2'-hydroxyphenyl)SCA were toxic to the bacteria at 100 μM and any mutagenic effects at this high concentration could not be evaluated.

3.3 Antimutagenicity of selenazolidine-4-(R)-carboxylic acids against B[a]P

Without exception, all the selenazolidines significantly reduced B[a]P mutagenicity (Table 6). The reduction in the number of revertant colonies ranged from 40% to 82% but overall there was no clear concentration dependency. Although there was variation in the level of reductions between experiments (cultures), the mean selenazolidine reduction in B[a]P mutagenicity was 61%. With a high of 68% (2-phenylSCA and 2-butylSCA) and a low of 49% (2-[2'-hydroxyphenyl]SCA), there was little differentiation between the compounds. If the absence of concentration dependency at the 25 and 50 μM selenazolidine concentrations tested represents a plateau or saturation effect, this indicates that the selenazolidines are less efficacious against B[a]P than against acridine orange where antimutagenic effects reached ~90 % for many of the derivatives (see below).

3.4 Effect of selenazolidine-4-(R)-carboxylic acid derivatives on CYP1A metabolism

When compounds are antimutagenic for mutagens that require bioactivation, one of the possible mechanisms of action is the inhibition of the bioactivation reaction. The antimutagenic effect of inorganic selenite towards B[a]P was attributed to this mechanism [5,9]. Accordingly, the ability of the selenazolidines to affect S9 fraction 7-ethoxyresorufin O-deethylase activity, a reaction catalyzed by CYP1As was therefore investigated (Table 7). The CYP1As are the enzymes largely responsible for B[a]P metabolism to mutagenic metabolites. Across a range of CYP1A substrate (i.e., 7-ethoxyresorufin) concentrations, the 2-(2'-hydroxyphenyl)-derivative followed by the 2-oxo- and 2-phenyl-derivatives were the most inhibitory. The unsubstituted compound showed activation. Both the activation and the inhibitions by a fixed concentration (50 μM) of the selenazolidines were independent of the enzyme substrate concentration with the exception of 2-butylSCA. Accordingly, the best-fit mechanism of inhibition by the selenazolidines was noncompetitive for all except the 2-butyl-derivative where a competitive mechanism prevailed. The K_i values for the noncompetitive inhibitors indicated that with the exception of 2-(2'-hydroxyphenyl)SCA ($K_i = 63 \mu\text{M}$) they were relatively weak inhibitors ($K_i = 179\text{--}1106 \mu\text{M}$).

3.5 Antimutagenicity of selenazolidine-4-(R)-carboxylic acid derivatives against acridine orange

Acridine orange does not require bioactivation to be mutagenic. Accordingly, selenazolidine antimutagenic activity was evaluated in the absence of S9 mix. The optimal non-toxic concentration of acridine orange for mutagenicity (100 μ M; data not shown) was used to evaluate the antimutagenic effect of the SCAs. All the SCAs reduced acridine orange mutagenicity and with the exception of SCA, in a concentration dependent manner (Table 8). There was little difference in antimutagenicity between compounds. The interpolated EC50 values for those showing concentration dependency ranged only from 4.8 μ M (2-phenylSCA) to 9.9 μ M (2-(2'-hydroxyphenyl)SCA). Intervening EC50 values were 6.8 μ M (2-cyclohexylSCA), 7.1 μ M (2-butylSCA), 7.5 μ M (2-oxoSCA), and 9.5 μ M (2-methylSCA).

The selenazolidines were more effective antimutagens against acridine orange than against B[a]P. Given that interference with bioactivation of B[a]P appears not to explain the antimutagenic effects, the mechanistic basis for this is not known. It could reflect a differential effect of the selenocompounds on interactions of the proximate mutagens with DNA, intercalation in the case of acridine orange and adduct formation for many of the B[a]P metabolites. It could also reflect a differential effect on the misreading of the modified DNA during replication.

3.6 Summary

All seven organoselenium compounds, SCA and six 2-substituted derivatives, were found to have antimutagenic activity. The antimutagenic activity was present for B[a]P, a carcinogen that requires oxidative bioactivation for mutagenicity, and for a DNA-intercalating mutagen requiring no activation (acridine orange). Antimutagenic activity against acridine orange was dependent on selenazolidine concentration. EC50 values ranged from 4.8 to 9.9 μ M. In contrast to effects with acridine orange, there was no apparent concentration dependency to antimutagenicity against B[a]P. Based on the ineffectiveness of six of seven selenazolidines to inhibit the B[a]P-activating CYP1A activity to any major extent at concentrations demonstrating antimutagenic activity, as well as the demonstrated greater antimutagenic activity towards acridine orange which requires no bioactivation, it is concluded that the selenazolidines do not exert their effect through this mechanism. At 25 μ M, the selenazolidine concentration tested in common for the two mutagens, the reduction in mutant frequency ranged from a mean low of 53.5% (2-cyclohexylSCA) to a mean high of 70.6% (2-methylSCA) for B[a]P. Against acridine orange, the range of reductions was approximately 25% higher, with a low of 78.7% (2-oxoSCA) and a high of 92.6% (2-cyclohexyl SCA) (Table 9). Thus there was only 17-18% difference between the seven individual selenazolidines in their antimutagenic effectiveness against either B[a]P or acridine orange. However, the rank order for reduction of mutant frequency was different for each mutagen. 2-CyclohexylSCA was the least effective compound against B[a]P but among the most effective (2-butylSCA, 2-cyclohexylSCA, 2-phenylSCA) against acridine orange. 2-MethylSCA was the most effective compound against B[a]P but among the least effective (2-oxoSCA, 2-methylSCA, 2-(2'-hydroxyphenyl)SCA) against acridine orange.

There are few published studies on the antimutagenicity of organoselenium compounds with which to compare our observations. Seleno-amino acids were ineffective against 2-amino-3-methylimidazole[4,5]quinoline *in vitro* [22]. Antimutagenicity studies with organoselenium compounds synthesized and investigated by other research groups and known to possess chemoprevention activity against several mutagens/carcinogens in animal models would have considerable merit, and could provide the foundation for the utility of such testing in selecting lead compounds for long term and costly *in vivo* chemoprevention investigations. Results of the present selenazolidines, based on the antimutagenic activity of selenazolidines at nontoxic

concentrations, suggest these compounds should be tested for chemopreventive properties in animal studies

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R**H****=O****—CH₃****—CH₂—CH₂—CH₂—CH₃**

abbreviations

SCA (selenazolidine-4-(R)-carboxylic acid)

OSCA, 2-oxoSCA

MSCA, 2-methylSCA

BSCA, 2-butylSCA

PhSCA, 2-phenylSCA

ChSCA, 2-cyclohexylSCA

PhOHSCA, 2-(2'-hydroxyphenyl)SCA

Fig 1.

Structure of Selenazolidine-4-(R)-carboxylic acids.

Table 1
Selenazolidine Cytotoxicity to *Salmonella typhimurium* TA98 in Liquid Medium.

	Viability at selenazolidine concentrations of:			
	12.5 μ M	25 μ M	50 μ M	100 μ M
Compound ^a				
SCA	121.8 $\pm$ 1.6	115.3 $\pm$ 5.1	108.4 $\pm$ 5.4	62.4 $\pm$ 5.6 ^b
OSCA	99.2 $\pm$ 20.8	97.4 $\pm$ 24.7	107.9 $\pm$ 18.3	98.7 $\pm$ 12.3
MSCA	115.3 $\pm$ 7.1	121.6 $\pm$ 3.6	114.5 $\pm$ 1.2	105.9 $\pm$ 3.0
BSCA	125.3 $\pm$ 12.0	119.8 $\pm$ 8.3	123.1 $\pm$ 8.7	107.3 $\pm$ 5.8
ChSCA	124.5 $\pm$ 10.3	120.4 $\pm$ 15.8	121.2 $\pm$ 9.5	116.7 $\pm$ 19.4
PhSCA	101.1 $\pm$ 15.0	99.5 $\pm$ 5.7	98.7 $\pm$ 6.1	93.4 $\pm$ 2.8
PhOHSCA	100.0 $\pm$ 4.3	96.8 $\pm$ 5.0	87.6 $\pm$ 1.4	36.5 $\pm$ 15.0 ^b

^aCompound abbreviations are as shown in Figure 1.

^b significantly different (p<0.05) from bacteria grown in the absence of any added selenium compound. Cytotoxicity assays were performed in quadruplicate and are expressed (mean $\pm$ SEM) as % control (i.e., no selenazolidine).

Table 2
Effect of Selenazolidines on *Salmonella typhimurium* TA98 Viability as Assessed by Plate Colony Formation.

	Viability at selenazolidine concentrations of:	
	25 μ M	50 μ M
	colonies/plate and (% no selenazolidine)	
Compound ^a		
None		82.7 $\pm$ 0.3 (100%)
SCA	90.3 $\pm$ 4.3 (109%) ^b	88.3 $\pm$ 3.7 (107%)
OSCA	106.0 $\pm$ 1.5 (128%) ^b	106.0 $\pm$ 1.7 (128%) ^b
MSCA	98.3 $\pm$ 2.3 (119%) ^b	105.0 $\pm$ 3.6 (127%) ^b
BSCA	100.7 $\pm$ 1.5 (122%) ^b	95.7 $\pm$ 7.8 (116%) ^b
ChSCA	107.0 $\pm$ 1.5 (129%) ^b	89.3 $\pm$ 0.3 (108%)
PhSCA	104.0 $\pm$ 2.7 (126%) ^b	104.3 $\pm$ 3.5 (126%) ^b
PhOHSCA	91.3 $\pm$ 4.3 (110%)	100.7 $\pm$ 0.8 (122%) ^b

^a Compound abbreviations are as shown in Figure 1.

^b significant (p<0.05) change from the number of colonies/plate (mean $\pm$ SEM) seen in the absence of added selenazolidine Assays were performed in triplicate with a 10⁻⁶ dilution of an overnight *Salmonella typhimurium* TA98 culture.

Table 3
Effect of Selenazolidines, B[a]P, and S9 mix on *Salmonella typhimurium* TA98 Viability

	Experiment #1		Experiment #2	
	10 ⁻⁵ dilution	10 ⁻⁶ dilution	10 ⁻⁶ dilution	10 ⁻⁷ dilution
Colonies/plate; mean ± SEM and (% of no S9 mix, B[a]P and selenazolidine)				
Compound ^a				
At 0 μM	340 ± 2.3 (99%)	66.7 ± 1.3 (104%)	77.7 ± 2.0 (108%)	13.3 ± 0.3 (114%)
At 25 μM:				
SCA	344 ± 1.3 (100%)	99 ± 0.6 (155%) ^b	72 ± 3.0 (100%)	12.3 ± 0.9 (105%)
OSCA	340 ± 1.9 (99%)	93 ± 6.0 (145%) ^b	79 ± 0.9 (110%)	14.0 ± 0.6 (120%) ^b
MSCA	336 ± 2.7 (98%)	87 ± 3.0 (136%) ^b	78 ± 2.4 (108%)	14.3 ± 0.3 (122%) ^b
BSCA	326 ± 9.0 (95%)	59 ± 3.2 (92%)	79 ± 3.3 (110%)	13.7 ± 0.3 (117%) ^b
ChSCA	348 ± 1.5 (101%)	56 ± 0.3 (88%)	72 ± 1.5 (100%)	12.7 ± 0.9 (109%)
PhSCA	343 ± 2.7 (100%)	71 ± 2.1 (111%)	87 ± 3.7 (121%) ^b	16.7 ± 0.3 (143%) ^b
PhOHSCA	351 ± 3.0 (102%)	76 ± 8.0 (119%) ^b	80 ± 3.5 (111%)	12.7 ± 0.7 (109%)
At 50 μM:				
SCA	343 ± 1.0 (100%)	100 ± 2.1 (156%) ^b	72 ± 2.7 (100%)	12.0 ± 0.6 (103%)
OSCA	342 ± 1.5 (100%)	89 ± 1.5 (139%) ^b	80 ± 1.3 (111%)	14.0 ± 0.6 (120%) ^b
MSCA	346 ± 1.0 (101%)	102 ± 2.4 (159%) ^b	79 ± 5.0 (110%)	13.7 ± 0.7 (117%) ^b
BSCA	343 ± 4.2 (100%)	100 ± 6.2 (156%) ^b	80 ± 2.3 (111%)	13.7 ± 0.3 (117%) ^b
ChSCA	344 ± 2.5 (100%)	95 ± 7.1 (148%) ^b	71 ± 2.1 (99%)	14.7 ± 0.7 (126%) ^b
PhSCA	345 ± 2.3 (101%)	88 ± 4.1 (138%) ^b	87 ± 4.5 (121%) ^b	15.7 ± 0.9 (134%) ^b
PhOHSCA	340 ± 4.3 (99%)	95 ± 0.3 (148%) ^b	79 ± 4.2 (110%)	13.0 ± 0.6 (111%)

^aCompound abbreviations are as shown in Figure 1.

^bsignificant (p<0.05) change (% indicated in parentheses) from colonies seen in the absence of added S9 mix, 20 μM B[a]P and selenazolidine which were 343 ± 2.7 and 64.0 ± 0.6 for 10⁻⁵ and 10⁻⁶ dilutions in experiment (culture) #1; 72.0 ± 3.5 and 11.7 ± 0.9 for 10⁻⁶ and 10⁻⁷ dilutions in experiment (culture) #2 (n = 3).

Table 4
Effect of Selenazolidines and Acridine Orange on *Salmonella typhimurium* TA98 Viability

	Experiment #1		Experiment #2	
	10 ⁻⁶ dilution	10 ⁻⁷ dilution	10 ⁻⁶ dilution	10 ⁻⁷ dilution
Colonies/plate; mean $\pm$ SEM and (% of no acridine orange and selenazolidine)				
Compound ^a				
At 0 μ M	71.0 $\pm$ 1.0 (93%)	6.7 $\pm$ 0.3 (84%)	93.3 $\pm$ 1.5 (94%)	10.0 $\pm$ 0.6 (91%)
At 10 μ M:				
SCA	75 $\pm$ 0.0 (106%)	8 $\pm$ 1.0 (119%)	98 $\pm$ 0.7 (105%)	11 $\pm$ 0.3 (110%)
OSCA	75 $\pm$ 0.6 (106%)	9 $\pm$ 0.7 (134%)	101 $\pm$ 3.2 (109%)	13 $\pm$ 0.6 (130%) ^b
MSCA	78 $\pm$ 0.9 (110%)	9 $\pm$ 1.0 (134%)	102 $\pm$ 2.9 (110%)	13 $\pm$ 0.3 (130%)
BSCA	78 $\pm$ 1.5 (110%)	8 $\pm$ 0.7 (119%)	104 $\pm$ 4.4 (112%)	12 $\pm$ 0.7 (120%)
ChSCA	88 $\pm$ 1.7 (124%) ^b	9 $\pm$ 1.0 (134%)	118 $\pm$ 2.0 (127%) ^b	13 $\pm$ 0.0 (130%) ^b
PhSCA	79 $\pm$ 0.6 (111%)	12 $\pm$ 0.0 (179%) ^b	104 $\pm$ 3.8 (112%)	14 $\pm$ 0.3 (140%) ^b
PhOHSCA	81 $\pm$ 0.6 (114%)	8 $\pm$ 0.3 (119%)	106 $\pm$ 4.2 (114%)	11 $\pm$ 0.3 (110%)
At 25 μ M:				
SCA	29 $\pm$ 2.7 (41%) ^b	4 $\pm$ 0.6 (60%) ^b	73 $\pm$ 3.9 (78%) ^b	9 $\pm$ 0.3 (90%)
OSCA	73 $\pm$ 1.2 (103%)	12 $\pm$ 0.3 (179%) ^b	99 $\pm$ 1.2 (106%)	16 $\pm$ 0.6 (160%) ^b
MSCA	74 $\pm$ 2.3 (104%)	9 $\pm$ 0.3 (134%)	100 $\pm$ 2.3 (108%)	11 $\pm$ 0.9 (110%)
BSCA	79 $\pm$ 4.9 (111%)	9 $\pm$ 0.0 (134%)	107 $\pm$ 4.3 (115%)	13 $\pm$ 0.7 (130%)
ChSCA	75 $\pm$ 2.0 (106%)	11 $\pm$ 0.7 (164%) ^b	104 $\pm$ 2.7 (112%)	15 $\pm$ 1.2 (150%) ^b
PhSCA	75 $\pm$ 2.6 (106%)	11 $\pm$ 0.9 (164%) ^b	99 $\pm$ 0.6 (106%)	14 $\pm$ 0.9 (140%) ^b
PhOHSCA	75 $\pm$ 0.3 (106%)	8 $\pm$ 1.2 (119%)	100 $\pm$ 3.3 (108%)	12 $\pm$ 1.2 (120%)

^aCompound abbreviations are as shown in Figure 1.

^bsignificant (p<0.05) change (% indicated in parentheses) from colonies seen in the absence of both 100 μ M acridine orange and selenazolidine, which were 76.0 $\pm$ 5.8 and 8.0 $\pm$ 1.2 for 10⁻⁶ and 10⁻⁷ dilutions in experiment (culture) #1; 99.3 $\pm$ 2.7 and 11.0 $\pm$ 0.6 for 10⁻⁶ and 10⁻⁷ dilutions in experiment (culture) #2 (n = 3).

Table 5
Determination of the Mutagenic Activity of Selenazolidines.

	Selenazolidine mutagenicity at:			
	10 μ M	25 μ M	50 μ M	100 μ M
<i>Revertant colonies/plate (mean $\pm$ SEM)</i>				
Compound and incubation ^a				
SCA				
+S9 mix	20.1 $\pm$ 0.8	21.9 $\pm$ 0.8	26.1 $\pm$ 2.2	-
- S9 mix	12.9 $\pm$ 0.8	14.1 $\pm$ 0.8	18.9 $\pm$ 0.8	-
OSCA				
+S9 mix	20.4 $\pm$ 1.1	25.2 $\pm$ 2.3	24.3 $\pm$ 2.3	29.7 $\pm$ 1.4
- S9 mix	14.1 $\pm$ 0.9	18.6 $\pm$ 2.4	18.3 $\pm$ 2.1	19.5 $\pm$ 3.2
MSCA				
+S9 mix	17.4 $\pm$ 0.8	20.4 $\pm$ 0.5	24.3 $\pm$ 1.8	23.4 $\pm$ 1.5
- S9 mix	17.4 $\pm$ 0.8	19.8 $\pm$ 1.1	18.3 $\pm$ 1.1	17.1 $\pm$ 1.8
BSCA				
+S9 mix	22.5 $\pm$ 0.7	27.0 $\pm$ 2.5	27.9 $\pm$ 0.8	29.1 $\pm$ 0.8
- S9 mix	19.2 $\pm$ 0.6	20.1 $\pm$ 0.7	21.9 $\pm$ 0.8	21.0 $\pm$ 1.4
ChSCA				
+S9 mix	33.9 $\pm$ 0.8	33.0 $\pm$ 1.4	44.4 $\pm$ 3.4	36.0 $\pm$ 2.8
- S9 mix	30.9 $\pm$ 0.8	32.1 $\pm$ 1.6	34.8 $\pm$ 2.2	30.9 $\pm$ 2.2
PhSCA				
+S9 mix	36.9 $\pm$ 0.8	39.0 $\pm$ 1.4	39.9 $\pm$ 2.2	36.9 $\pm$ 1.6
- S9 mix	32.7 $\pm$ 2.6	33.0 $\pm$ 2.4	33.9 $\pm$ 2.2	30.0 $\pm$ 1.5
PhOHSCA				
+S9 mix	39.0 $\pm$ 1.4	40.5 $\pm$ 2.6	38.1 $\pm$ 0.8	-
- S9 mix	36.0 $\pm$ 1.4	37.5 $\pm$ 1.9	30.0 $\pm$ 1.2	-

^aCompound abbreviations are as shown in Figure 1.

Background (spontaneous) revertant colonies were 30.0 ± 1.7 . Positive control (20 μ M B[a]P incubated with S9 mix) revertant colonies was 300 ± 0.8 , n = 9.

Table 6
Effect of Selenazolidines on B[a]P Mutagenicity

	B[a]P mutagenicity at selenazolidine concentrations of:			
	25 μ M Experiment #1	50 μ M Experiment #1	25 μ M Experiment #2	50 μ M Experiment #2
<i>Revertant colonies/plate; mean $\pm$ SEM and (% reduction in B[a]P mutagenicity)</i>				
Compound ^a				
none		349.0 $\pm$ 20.3 (0%)		376.3 $\pm$ 5.9 (0%)
SCA	206.0 $\pm$ 15.6 (41%) ^b	160.7 $\pm$ 6.4 (54%) ^{bc}	89.3 $\pm$ 4.6 (76%) ^b	66.7 $\pm$ 2.4 (82%) ^b
OSCA	206.0 $\pm$ 2.9 (41%) ^b	137.0 $\pm$ 4.0 (61%) ^{bc}	141.7 $\pm$ 8.7 (62%) ^b	166.3 $\pm$ 2.4 (56%) ^b
MSCA	132.0 $\pm$ 9.5 (62%) ^b	119.7 $\pm$ 5.2 (66%) ^b	115.3 $\pm$ 11.9 (69%) ^b	172.7 $\pm$ 12.7 (54%) ^{bc}
BSCA	114.3 $\pm$ 1.2 (67%) ^b	110.0 $\pm$ 4.2 (68%) ^b	166.7 $\pm$ 11.6 (56%) ^b	78.0 $\pm$ 1.5 (79%) ^{bc}
ChSCA	138.3 $\pm$ 6.1 (60%) ^b	121.7 $\pm$ 2.0 (65%) ^b	155.0 $\pm$ 7.2 (59%) ^b	160.7 $\pm$ 5.2 (57%) ^b
PhSCA	117.3 $\pm$ 2.7 (66%) ^b	110.7 $\pm$ 3.5 (68%) ^b	124.7 $\pm$ 13.9 (67%) ^b	103.0 $\pm$ 17.0 (73%) ^b
PhOHSCA	163.0 $\pm$ 8.0 (53%) ^b	126.3 $\pm$ 7.2 (64%) ^b	224.7 $\pm$ 3.2 (40%) ^b	225.7 $\pm$ 4.7 (40%) ^b

^a Compound abbreviations are as shown in Figure 1.

^b significant ($p < 0.05$) reduction (% indicated in parentheses) from revertant colonies seen with 20 μ M B[a]P (+S9 mix) in the absence of added selenium compound. Spontaneous revertant colonies for TA98 alone in the absence of S9 mix were 17.7 ± 0.3 and 18.0 ± 1.2 in experiments #1 and #2, respectively. In experiment #1, the number of revertant colonies in the presence of S9 mix was 19.0 ± 1.5 .

^c significant difference ($p < 0.05$) between SCA concentrations.

Table 7
Inhibition of Rat Hepatic S9 Fraction 7-Ethoxyresorufin O-Deethylase Activity by Selenazolidines.

	% change in deethylase activity by 50 μ M selenazolidine at 7-ethoxyresorufin (μ M):					Inhibition	
	0.025	0.050	0.100	0.200	0.400	Type	R ²
Compound ^a							
SCA	+17.0	+22.5	+17.4	+12.5	+15.3	activation	
OSCA	-25.8	-20.7	-23.2	-17.3	-24.7	noncompetitive	.991
MSCA	-13.2	0.0	-9.9	-12.1	-9.1	noncompetitive	.986
BSCA	-17.5	-11.5	-6.3	-13.4	-3.2	competitive	.993
ChSCA	0.0	-7.9	-2.3	-9.5	-2.9	noncompetitive	.987
PhSCA	-21.3	-19.4	-19.8	-20.8	-16.7	noncompetitive	.990
PhOHSCA	-46.3	-42.4	-45.9	-43.2	-44.3	noncompetitive	.987

^aCompound abbreviations are as shown in Figure 1.

Numbers are the mean of duplicate determinations.

Table 8
Effect of Selenazolidines on Acridine Orange Mutagenicity

	Acridine orange mutagenicity at selenazolidine concentrations of:				
	10 μ M Experiment #1	25 μ M	10 μ M Experiment #2	25 μ M	5 μ M Experiment #3
<i>Revertant colonies/plate; mean $\pm$ SEM and (% reduction in acridine orange mutagenicity)</i>					
Compound ^a					
none		213.0 $\pm$ 4.5 (0%)		299.7 $\pm$ 4.5 (0%)	219.0 $\pm$ 7.5 (0%)
SCA	80.3 $\pm$ 2.3 (62%) <i>b</i>	-	85.7 $\pm$ 2.9 (71%) <i>b</i>	-	79.7 $\pm$ 0.9 (64%) ^b
OSCA	48.0 $\pm$ 1.5 (77%) <i>b</i>	45.0 $\pm$ 1.2 (79%) <i>b</i>	80.7 $\pm$ 4.3 (73%) <i>b</i>	70.7 $\pm$ 0.9 (76%) <i>b</i>	137.3 $\pm$ 4.1 (37%) ^b
MSCA	85.7 $\pm$ 6.2 (60%) <i>b</i>	40.7 $\pm$ 3.3 (81%) <i>bc</i>	121.3 $\pm$ 5.4 (60%) <i>b</i>	64.3 $\pm$ 3.0 (79%) <i>bc</i>	146.7 $\pm$ 1.3 (33%) ^a
BSCA	49.0 $\pm$ 7.5 (77%) <i>b</i>	18.0 $\pm$ 2.7 (92%) <i>bc</i>	71.3 $\pm$ 4.1 (76%) <i>b</i>	26.7 $\pm$ 2.2 (91%) <i>bc</i>	132.0 $\pm$ 2.9 (40%) ^b
ChSCA	39.3 $\pm$ 0.3 (82%) <i>b</i>	18.3 $\pm$ 1.5 (91%) <i>bc</i>	67.3 $\pm$ 1.9 (78%) <i>b</i>	22.7 $\pm$ 1.2 (92%) <i>bc</i>	132.0 $\pm$ 6.1 (40%) ^b
PhSCA	44.7 $\pm$ 1.8 (79%) <i>b</i>	16.0 $\pm$ 1.5 (92%) <i>bc</i>	80.7 $\pm$ 4.9 (73%) <i>b</i>	39.0 $\pm$ 3.1 (87%) <i>bc</i>	105.7 $\pm$ 2.3 (52%) ^b
PhOHSCA	65.7 $\pm$ 2.4 (69%) <i>b</i>	41.7 $\pm$ 1.2 (80%) <i>bc</i>	100.7 $\pm$ 4.9 (66%) <i>b</i>	72.3 $\pm$ 4.7 (76%) <i>bc</i>	184.7 $\pm$ 3.3 (16%) ^b

^a Compound abbreviations are as shown in Figure 1

^b significant (p<0.05) reduction (% indicated in parentheses) from revertant colonies seen with 100 μ M acridine orange in the absence of added selenium compound. Spontaneous revertant colonies for TA98 alone were 18.3 $\pm$ 0.3, 30.0 $\pm$ 0.6, and 23.3 $\pm$ 0.9 in experiment #1, #2, and #3, respectively.

^c significant difference (p<0.05) between within-experiment SCA concentrations.

Table 9
Effects of Selenazolidines (25 μ M) on B[a]P and Acridine Orange Mutant Frequency.

Mutant Frequency with: *				
B[a]P		Acridine orange		
mutant frequency and (% of control [no selenium])				
Compound ^a	Expt 1	Expt 2	Expt 1	Expt 2
None	5.23 (100)	4.84 (100)	3.00 (100)	3.21 (100)
SCA	2.08 (40)	1.24 (26)	-	-
OSCA	2.22 (42)	1.79 (37)	0.62 (21)	0.71 (22)
MSCA	1.52 (29)	1.48 (31)	0.55 (18)	0.64 (20)
BSCA	1.94 (37)	2.11 (44)	0.23 (8)	0.25 (8)
ChSCA	2.47 (47)	2.15 (44)	0.24 (8)	0.22 (7)
PhSCA	1.65 (32)	1.43 (30)	0.21 (7)	0.39 (12)
PhOHSCA	2.14 (41)	2.81 (58)	0.56 (19)	0.72 (23)

* calculated from mutant colonies (Tables 6, and 8) / survival colonies at 10^{-6} (Tables 3, and 4)