Organoselenium and Organotellurium Compounds: Toxicology and Pharmacology

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1. Introduction

1.1. Chemistry of Organoselenium Compounds

Organoselenium compounds have found such wide utility because of their effects on an extraordinary number of very different reactions, including many carbon−carbon bond formations, under relatively mild reaction conditions. Furthermore, organoselenium compounds can usually be used in a wide variety of functional groups, thus avoiding protection group chemistry.[1] Most organoselenium methodologies proceed stereo- and regioselectively in excellent yields. Although, the first organoselenium compound was prepared by Wöhler and Siemens in 1847,[2] only in the early 1970s did the chemistry of organoselenium become a versatile tool in organic chemistry.[3] After that, the organoselenium chemistry developed rapidly, mainly in the area of selenocarbohydrates, selenoamino acids, and selenopeptides. The selenium group can be introduced in an organic substrate via both nucleophilic and electrophilic reagents. Organoselenium anions are a powerful nucleophile and usually they are prepared in situ because of their sensitivity to air oxidations.[4] They can be prepared from diaryl diselenides by reaction with reduction agents, of which $NaBH_4$ is the most used,[5] from insertion of elemental selenium into lithium and Grignard reagents,[6] and from diorganoyl diselenides by reduction using alkali metals[7] or alkali hydrides.[8] On the other hand, organoselenium compounds containing some leaving groups such as Cl, Br, or O_2-CCF_3 can serve as soft extremely reactive electrophiles. Some of the most efficient sources of electrophile selenium are the organoselenyl halides.[9] Diaryl diselenides can be used as an electrophile reagent but only to powerful nucleophile attack. After being introduced in an organic substrate, the organoselenium group can easily be removed by selenoxide syn elimination[10] and [2,3] sigmatropic rearrangement.[11] In addition, the carbon−selenium bond can also be replaced by a carbon−hydrogen,[12] carbon−halogen,[13] carbon−lithium,[14] or carbon−carbon bond.[15] Thus, in general, organoselenium species can be efficiently introduced, manipulated, and removed in a variety of ways under mild conditions.

1.2. Chemistry of Organotellurim Compounds

Organotellurium chemistry is a very broad and exciting field with many opportunities for research and development of applications. Regarding the odor, toxicity, and instability, we believed, unlike most, that no highly specialized techniques are required in the handling of organotellurium compounds and work with these compounds is very similar to work with any other class of chemical compounds such as organoselenium, organosulfur, organotin, and organophosphorus. The first organotellurium compound was reported more than 150 years ago with the synthesis of diethyl tellurides by Wöhler in 1840.[16] The area of organotellurium chemistry was slow to develop, but in the last 30 years, numerous books and reviews have appeared to cover the thousands of papers dealing with organotellurium compounds.[17] The organotellurium group can be easily introduced

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into a variety of organic substrates by either nucleophilic or electrophilic tellurium species. The nucleophilic organotellurium species can be produced from elemental tellurium by reaction with organolithium[18] or Grignard reagents.[19] Organotellurols can also be prepared by reduction of diorganoyl ditellurides.[20] On the other hand, the electrophilic organotellurium species can be prepared by reaction of an organic substrate with tellurium tetrachloride[21] or by halogenolysis of diorganoyl ditellurides.[22] The application of organotellurium compounds in organic synthesis has become attractive because of their chemio-, regio-, and stereoselectivity reactions. Most synthetic transformations using organotellurium compounds have involved use of the *Z* and *E* vinylic tellurides. *Z*-Vinylic tellurides have been easily obtained by hydrotelluration of alkynes by either sodium tellurolate ($BuTeTeBu/NaBH_4/EtOH$)[23] or lithium tellurolate ($Te^0/BuLi/THF$).[24] Conversely, *E*-vinylic tellurides have been obtained by stereospecific cis hydrometalation of the alkynes, followed by transmetalation of the *E*-vinylorganometallic complexes formed with organotellurenyl halide.[25] Of the two diastereoisomers, the *Z*-vinylic tellurides have been employed more frequently as intermediaries because of their better availability. One of the most important reactions of vinylic tellurides is transmetalation.[26] The vinyl organometallic obtained in this way can react with carbonyl compounds,[27] α,β-unsaturated systems,[28] or epoxides.[29] Recently a new application of vinylic tellurides utilizing palladium-catalyzed cross-coupling was reported.[30] In this case, they behave as aryl or vinyl carbocation equivalents. They react in a manner similar to vinylic halides or triflates in the Sonogashira,[31] Heck,[32] Suzuki,[33] and Stille[34] cross-coupling reactions with palladium as a catalyst. In addition, we have recently reported the synthesis of polyacetylenic acids isolated from *Heisteria acuminata* by using a vinylic telluride coupling reaction to demonstrate the great applicability of these compounds in natural product synthesis.[35]

Joao B. T. Rocha was born in Rio de Janeiro, Brazil, in 1964. He received his B.S. degree in biology at the Federal University of Rio Grande do Sul, Porto Alegre, Brazil, in 1987. Dr. Rocha received his Ph.D. degree working under the direction of Prof. D. O. Souza in 1996. In 1989, he joined the chemistry department at Federal University of Santa Maria, RS, Brazil, where is presently University Professor of Biochemistry. In 1997, he received a CNPq Postdoctoral Fellowship to work with Prof. L. De Meis at Federal University of Rio de Janeiro. His current research interests include the development of new in vitro and in vivo methods to test the toxicity and potential therapeutic use of simple organochalcogenes. The toxicity studies are centered on the reactivity of thiol-containing low-weight molecular compounds and proteins, such as ALA-D and LDH with selenium and tellurium organocompounds. The potential antioxidant activities of organocalcogenides are also tested in experimental models of diseases such as diabetes and diskynesia tardia.

The advance in the area of synthesis and reactivity of organoselenium and organotellurium compounds, as well as the discovery of the toxic properties of selenium compounds in the 1930s and the subsequent discovery that selenium is an essential trace element in the diet, has prompted intense studies of the biological properties of both organic and inorganic selenium compounds.[36]

In this review, we shall cover a wide range of toxicological and pharmacological effects in which organoselenium and organotellurium compounds are involved, but the effects of inorganic compounds will

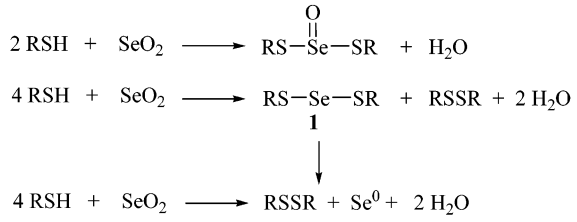
not be discussed here. Since it is not possible to cite all of the findings that have taken place, we apologize to those whose work has been omitted.

2. Toxicology

2.1. Toxicology of Organoselenium Compounds

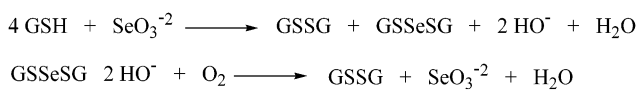
The molecular mechanism underlining organoselenium toxicity is still not completely understood. However, much of our current knowledge of the organoselenium toxicity arose from the experiences with inorganic selenium. Thus, about 60 years ago, Painter proposed that the toxicity of inorganic selenium could be related to the oxidation of thiols of biological importance. In fact, selenite (Se^{4+}) readily oxidizes sulfhydryl groups, producing disulfide and an unstable intermediary named selenotrisulfide, **1** (or (bis(alkylthio) selenide), that subsequently may decompose to elemental selenium.[37] Painter proposed that the reaction between Se^{4+} and thiol groups may take three distinct steps (Scheme 1). However, Painter

Scheme 1



was unable to isolate compounds of the type RS−Se−SR, **1**, from disulfides due to the instability of these intermediaries. Some years later, other groups also demonstrated the formation of selenotrisulfide **1**, which has biological significance.[38] Tsen and Tappel reported that reduced glutathione is catalytically oxidized by selenite and proposed the sequence of reactions shown in Scheme 2 to explain the catalytic

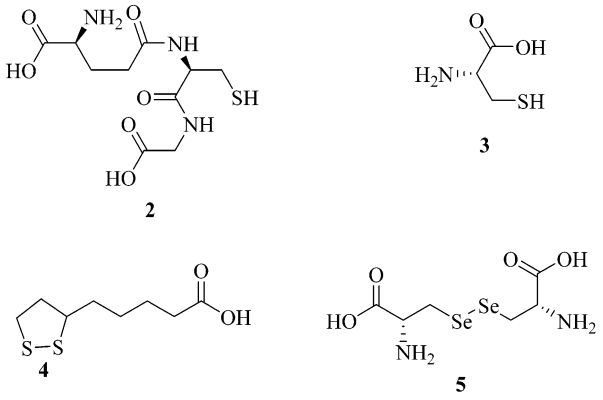
Scheme 2



effect of Se^{4+}.[38c,d] However, whether the catalytic oxidation of thiols really proceeds as indicated in Scheme 2 with the regeneration of selenite in each cycle is open to question.

The fact that selenite was a good catalyst for the oxidation of a variety of biologically significant thiols, including glutathione (GSH) **2**, cysteine **3**, dihydrolipoic acid **4**, and coenzyme A, helped to explain, at least in part, the biochemistry of selenite poisoning, where a significant loss of GSH **2** from the blood and organs of experimental animals was observed (Scheme 3).[39] Therefore, these studies confirmed the early proposition of Painter and established that selenite exerts at least part of its toxic action by catalyzing the oxidation of biologically significant sulfhydryl-containing molecules. However, only about 10 years later, Ganther accomplished the isolation of selenotrisulfide **1** in a pure form.[40] In 1989, Seko and colleagues suggested that sodium selenite reacted

Scheme 3

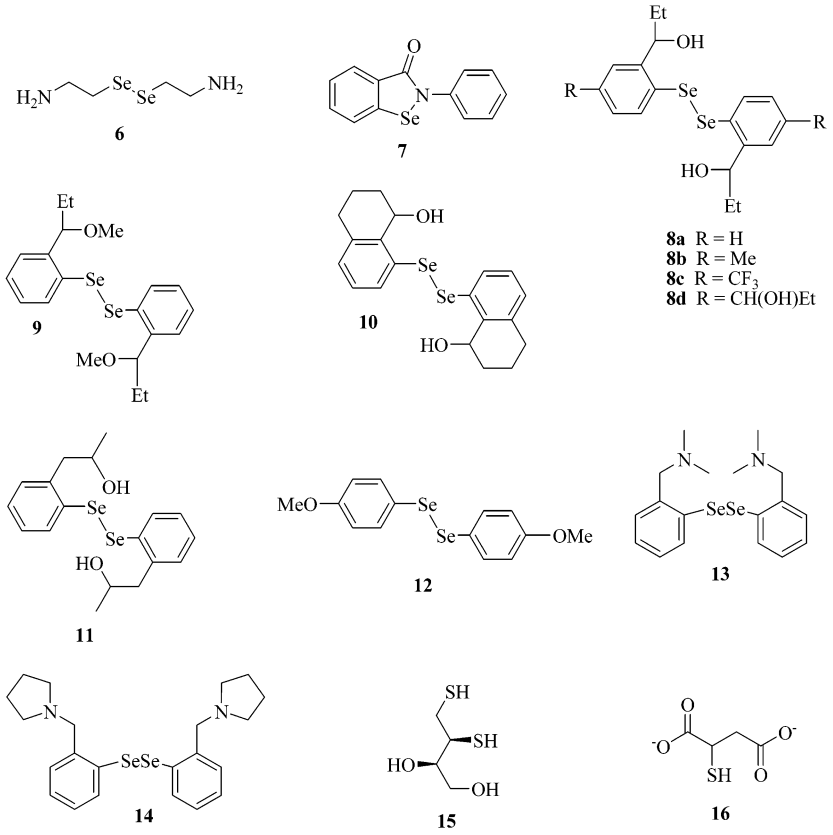


with GSH, forming a reactive superoxide.[41] These studies were extended by Spallholz and colleagues, who showed that other thiols reacted with sodium selenite and selenocystine **5** to produce superoxide and hydroperoxide.[42] According to these authors, the selenium toxicity will be manifested acutely or chronically when oxidative damage exceeds antioxidant defenses. It is interesting to note that Se^{4+} at the same time that it generates reactive oxygen species can oxidize −SH groups by reacting directly with the nucleophilic sulfur atom.

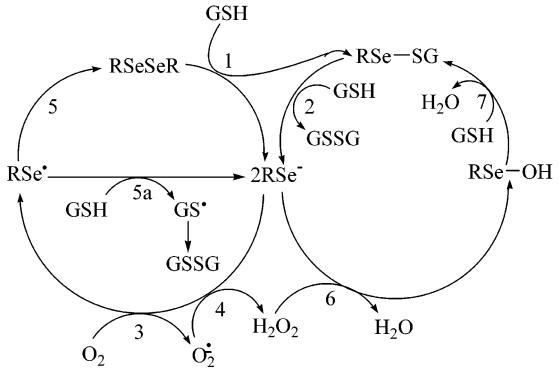
In connection with earlier studies, in 1992 Chaudiere and collaborators carried out an elegant study to demonstrate that thiol oxidase activity may help to explain most of the toxic effects of diorganoyl diselenides and organoselenols.[43] In addition, several model compounds of low molecular weight were studied including selenocystine,[44] selenocystamine **6**,[45] ebselen **7**,[46] oxygen-containing diselenides **8**-**12**,[47] and derivatives of diaryl diselenide such as **13** and **14** (Scheme 4).[48] The Chaudiere study illustrates the ease of one-electron transfers from RSe^- to a variety of reducible substrates in which selenocystamine **6** efficiently catalyzes oxygen reduction in the presence of excess thiol groups from dithiothreitol **15**, GSH, and mercaptosuccinate **16** (Scheme 4).

Selenocystamine was shown to catalyze the oxygen-mediated oxidation of excess GSH to glutathione disulfide at neutral pH and ambient PO_2. This glutathione oxidase activity required the heterolytic reduction of the diselenide bond, which produced 2 equiv of the selenolate ion derivative of selenocysteamine (RSe^-) (Scheme 5, step 1) via the transient formation of a selenylsulfide intermediate (RSe−SG) (Scheme 5, step 2). Formation of RSe^- was the only reaction observed in anaerobic conditions. At ambient PO_2, the kinetics and stoichiometry of GSSG production, as well as those of GSH and oxygen consumptions, demonstrated that RSe^- performed a three-step reduction of oxygen to water. The first step was a one-electron transfer from RSe^- to dioxygen, yielding superoxide and the putative selenyl radical $RSe^\bullet$ (Scheme 5, step 3), which decayed very rapidly to RSe−SeR (Scheme 5, step 5). Some of the selenyl radicals would be reversibly trapped by excess GSH to yield the glutathione radical $GS^\bullet$ (Scheme 5, step 5a), which would decay to GSSG. In the second step, RSe^- reduced superoxide to hydrogen peroxide through a much faster one-electron transfer (Scheme

Scheme 4



Scheme 5



5, step 4) also associated with the decay of RSe• to RSe−SeR. The third step was a two-electron transfer from a RSe^- to hydrogen peroxide, again much faster than oxygen reduction, which resulted in the production of RSe−SG (Scheme 5, step 6), presumably via a selenenic acid intermediate (RSeOH), which was trapped by excess GSH (Scheme 5, step 7).

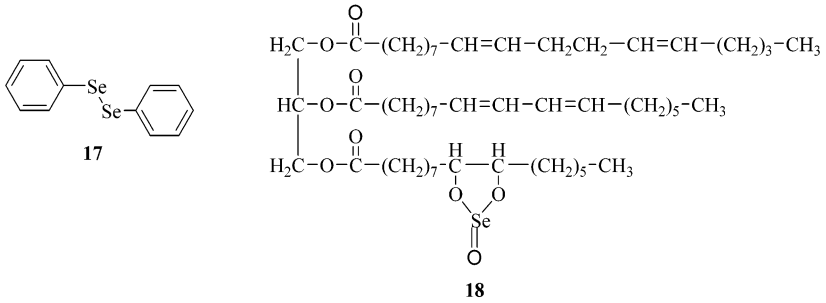
In view of toxicological considerations, toxicity of selenium compounds not only depends on the chemical form and the quantity of the element consumed, but also on a variety of other factors including species, age, physiological state, nutrition and dietary interactions, and the route of administration.[49] Thus, Parnham and Graf have reported that the toxicity of organoselenium compounds in vivo is dependent on the stability of the carbon−selenium bond. In this respect, the C_{sp3}−Se bond is weaker than the C_{sp2}−Se bond, and this is reflected in the higher toxicity of alkyldiselenides in comparison to aryldiselenides in vivo.[36c] Regarding the toxicity of ebselen in vivo, it is also low, and the nonbioavailability of free selenium from ebselen has been demonstrated by the lack of incorporation of [^{75}Se] into hepatic glutathione peroxidase 48 h after administration to mice of 6 μCi [^{75}Se]-labeled ebselen.[46a]

Accordingly, results from Nozawa's group reflect the low subchronic toxicity of ebselen in mice. In this study, the mice's body weight and survival were not affected by intragastric administration of ebselen (36.5 and 365 μmol/kg).[50] Otherwise, in our hands, ebselen was more toxic than diphenyl diselenide **17** (Scheme 6) in rats acutely treated by the intraperitoneal route as compared to the LD_{50} for diphenyl diselenide (1200 μmol/kg)[51] and for ebselen (400 μmol/kg).[52]

In addition, with regard to animal species, ebselen presented similar acute lethal potency in rats and mice when administrated by the intraperitoneal route (LD_{50} values of 400 and 340 μmol/kg, respectively).[52] Conversely, diphenyl diselenide was more toxic in mice than rats when administered by the intraperitoneal route (LD_{50} 210 and 1200 μmol/kg, respectively) and had no acute toxic effects when administrated by the subcutaneous route.[51]

Ebselen has also been demonstrated by our group to induce oxidative injury on the liver of rat pups. In fact, ebselen administered subcutaneously (36.5 μmol/kg) for 21 days on rat pups increased liver thiobarbituric acid reactive species (TBARS) and decreased non-protein thiol groups (NPSH) in hepatic tissue. Besides, simultaneous treatment with methylmercury chloride and ebselen caused a higher increase in hepatic TBARS levels when compared to the treatments with individual compounds.[53] These re-

Scheme 6



17 **18**

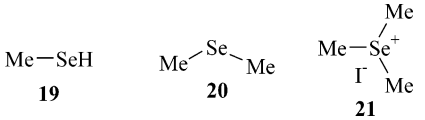
sults indicate that the toxicity of ebselen is related to endogenous thiol depletion, and this can be enhanced by co-administration of thiol blocking agents such as the environmental neurotoxin methylmercury chloride.

Selol **18**, a selenoglyceride compound, has been described as a nontoxic organoselenium compound. Thus, a single dose of selol did not display any toxic effects after parenteral administration, up to 503 μmol/kg subcutaneous, and 100 μmol/kg intraperitoneal (Scheme 6). However, given orally it exhibited high toxicity (LD_{50} = 100 mg/kg).[54] Indeed, data from pharmacokinetic parameters indicated that selol given by the subcutaneous route appears to be a nontoxic organoselenium compound.[55]

Although selenium toxicosis (selenosis) is a rare form of poisoning, it has been reported worldwide in cattle, sheep, pigs, horses, and man.[49] The condition is caused by the ingestion of selenium accumulator plants or by accidental over supplementation or parenteral administration. Since the discussion will center on the toxic effects of organoselenium compounds, selenosis[56] is not completely covered in this review.

In this context, selenium compounds have been described to induce a peripheral demyelinating neuropathy similar to that seen in selenosis, which, in animals, is characterized by a variety of neurological abnormalities. Thus, Gupta and Porter[57] demonstrated that squalene monooxygenase, the second enzyme in the committed pathway for cholesterol biosynthesis, is inhibited by methylselenol **19** (IC_{50} = 95 μM) and dimethylselenide **20** (IC_{50} = 680 μM). The IC_{50} values obtained for these organoselenium compounds were greater than that previously obtained for sodium selenite (37 μM). In the same research, trimethylselenonium iodide **21** was demonstrated to be a weak inhibitor of squalene monooxygenase (IC_{50} > 3 mM) (Scheme 7).[57] Since methylselenol is a potent nucleophile, its inhibitory effect on squalene monooxygenase cannot be explained by a direct oxidation of enzyme thiol groups. Possibly, methylselenol is oxidized to dimethyldiselenide, which acts as the actual inhibitor of squalene monooxygenase.

Scheme 7



19 **20** **21**

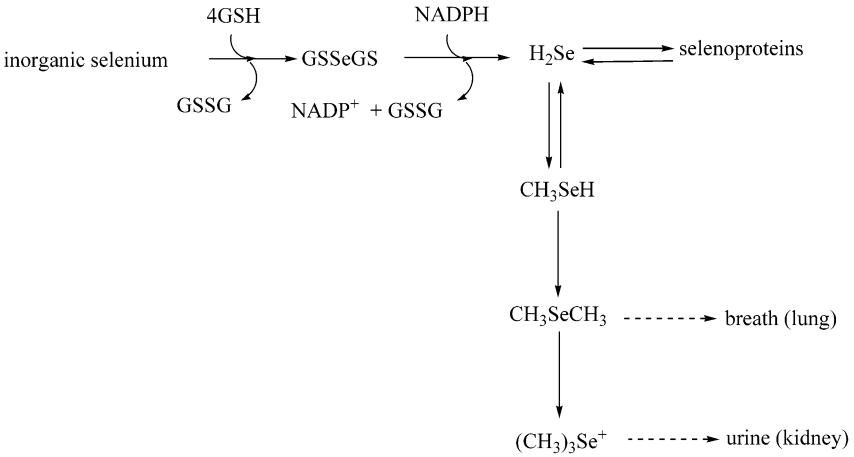
The study of dimethylselenide, methylselenol, and trimethylselenonium iodide is extremely important, because they are metabolites of selenium in man.[58] Chemical identification of dimethylselenide as a product of selenite metabolism in living organisms was accomplished about 70 years ago by Challenger and co-workers.[59] Evidence for production of dimethylselenide from selenate in rodents was obtained about two decades later by McConnell and Portman.[60] These investigators also demonstrated that dimethylselenide was about 2 orders of magnitude less toxic to rats than inorganic selenium and identified the lung as the most important route of dimethylselenide in rats.

Further detailed studies about the metabolism of inorganic selenium, particularly the participation of NADPH-dependent reductases and selenodiglutathione in its transformation to H_2Se, a central metabolite in the assimilatory and excretory pathways of selenium, have been deciphered by Ganther and co-workers during the last 30 years.[61] In short, inorganic selenium(IV) is reduced to H_2Se via selenodiglutathione with the participation of thiols and NADPH-dependent reductases.[61b–d] This reduced form of selenium can be incorporated into protein (assimilatory pathway) or methylated (excretory pathway), giving rise to methylselenol, dimethylselenide, and the trimethylselenonium ion (Scheme 8). In fact, selenium methylation is an important metabolic pathway for selenium fate, particularly its detoxification and excretion. However, dimethylselenide can be toxic to mammals after inhalation and the acute instillation of 0.025−0.10 mg/kg of dimethylselenide to rats causes respiratory inflammation and necrosis.[62]

Furthermore, dimethylselenide is also an important environmental product of selenium formed in the aquatic environment by biomethylation in both fresh and sea waters.[63] The process of biomethylation can also play a part in the evasion of selenium to the atmosphere and can also give rise to a small percentage of dimethyldiselenide and methylselenol in the environment.[64] The contribution of these compounds to the environmental toxicity of selenium is unknown; however, in view of their very low concentrations, they will represent a threat to living organisms only in specific cases of accidental release of excessive inorganic selenium in the environment.

Studies performed by our group demonstrated that exposure for 2 months to high doses (250 μmol/kg, sc) of diphenyl diselenide increased 3 times the total selenium content in the brain. There is also evidence

Scheme 8

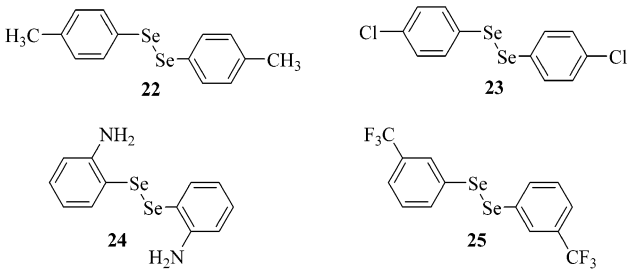


that diphenyl diselenide crosses the blood−brain barrier and brain selenium levels increase in mice after acute and chronic exposure to diphenyl diselenide.[65] These results have supported the hypothesis that the brain is a potential target for the toxicity of highly lipophilic organoselenium compounds and possibly for their pharmacological and therapeutic actions. However, no signs of gross motor impairment were observed in this study.

In connection to this, data from our laboratory have shown that simple organoselenium compounds present convulsant activity. In fact, intraperitoneal, but not subcutaneous, administration of diphenyl diselenide provoked seizures in mice, suggesting that neurotoxicity of diorganoyl diselenides depends on the route of administration. The organoselenium neurotoxic effect could also be related to species, since diphenyl diselenide administration, either intraperitoneal or subcutaneous, produced no seizure episodes in rats.

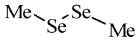
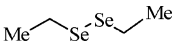
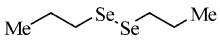
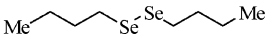
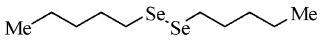
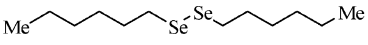
In the same study, we demonstrated that the introduction of functional groups in the aromatic ring of diaryl diselenide (*p*-methyl-diphenyl diselenides **22**, *p*-chloro-diphenyl diselenides **23**, *o*-amino-diphenyl diselenides **24**, and *m*-trifluoromethyl-diphenyl diselenides **25**) abolished the convulsive effect in mice (Scheme 9). The decrease or absence of acute neuro-

Scheme 9



toxicity of these substituted diselenides cannot be attributed to changes in the chemical bond dissociation enthalpies of Se−Se because the introduction of either electron-withdrawing or electron-donating groups causes similar biological response. Thus, it is possible that the introduction of substituent groups causes changes in toxicity by modifying the metabolism of these compounds.

Table 1. Effect of Dialkyl Diselenides on Latency for the Appearance of Seizures Episode in Mice

Entry	dialkyl diselenide	Latency (minutes)[a]
1	 **26**	3
2	 **27**	5
3	 **28**	15
4	 **29**	60
5	 **30**	60
6	 **31**	60

[a] The appearance of seizures episode was recorded up to 60 min. Data are reported as mean of 10 animals.

Conversely, the latency for onset of seizures increased as the aliphatic chain increased from 1 to 3 carbon atoms, **26**−**28** (Table 1, entries 1−3). Furthermore, dibutyl diselenide **29**, dipentyl diselenide **30**, and dihexyl diselenides **31** did not induce seizures in mice (Table 1, entries 4−6).[51] The decrease in the neurotoxicity of aliphatic diselenides as the aliphatic chain increases cannot be explained solely on the basis of changes in the bond dissociation enthalpies of Se−Se, because little modification on this parameter is suggested to occur when the aliphatic chain passes from two to three carbons or more. Thus, it is reasonable to suppose that other factors such as volatility, metabolism, and the molecular size interfere with the neurotoxic properties of these compounds.

Likewise, diphenyl diselenide has been demonstrated to affect a number of neuronal processes. Therefore, diphenyl diselenide increases the basal activity of adenylyl cyclase and inhibits [^{3}H]glutamate,

[^{3}H]MK-801, and unstable [^{3}H]guanylyl-imidodiphate (GMP−PNP) binding to rat synaptic membrane preparations after both in vitro and ex vivo exposure.[66]

Considering the importance of the functioning of the glutamatergic system and the role of the extra cellular glutamate on brain physiology, we examined whether the neuronal effects of diphenyl diselenide could be attributed to changes in the glutamatergic system at the presynaptic level. This study revealed that acute exposure (25 μmol/kg) to diphenyl diselenide and ebselen did not affect [^{3}H]glutamate uptake by brain synaptosomes. However, ebselen, but not diphenyl diselenide, inhibited K^+-stimulated [^{3}H]-glutamate release by brain synaptosomes. As well, data from in vitro experiments showed that diphenyl diselenide and ebselen were able to significantly inhibit vesicular [^{3}H]glutamate uptake in a concentration-dependent manner.[67]

In view of the effects of the organoselenium compounds on glutamatergic neurotransmission in rats, we recently investigated the effects of diphenyl diselenide and ebselen on human platelets. We observed that diphenyl diselenide caused a significant inhibition but ebselen did not interfere in Na^+-independent glutamate binding to platelet membranes. In the same study, diphenyl diselenide and ebselen were demonstrated to inhibit [^{3}H]glutamate uptake into human platelets.[68] Glutamate uptake into synaptosomes, platelets, and synaptic vesicles is mediated by different glutamate transporters; however, they are similarly sensitive to oxidant and sulfhydryl blocking agents.[69]

The inhibitory action of organoselenium and organotellurium compounds on glutamate uptake into these different structures is certainly mediated by the oxidation of essential cysteinyl residues in transporter proteins since dithiothreitol (DTT) decreases their inhibitory action.

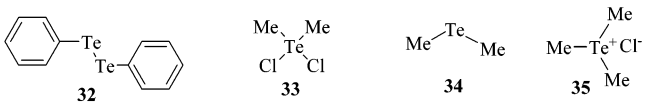
The effects of diphenyl diselenide and ebselen on ^{45}Ca influx into rat synaptosomes were also evaluated. We observed that diphenyl diselenide reduced ^{45}Ca influx into isolated nerve endings when a nondepolarizating condition was used or when 4-aminopyridine (4-AP) was used as a depolarizing agent. In this study, it was also demonstrated that ebselen, at high concentrations, increases ^{45}Ca influx into synaptosomes when a nondepolarizating condition or 4-AP was used. Conversely, ebselen caused a concentration-dependent inhibition on ^{45}Ca influx when KCl was used as a depolarizing agent. One important finding of this investigation is that the inhibitory and stimulatory effects of ebselen on ^{45}Ca influx under depolarizing and nondepolarizating conditions, respectively, are coincident with previous results,[67] showing that ebselen stimulated glutamate release under nondepolarizating conditions and inhibited its release under depolarizing conditions.[70]

2.2. Toxicology of Organotellurium Compounds

Toxicity data on organotellurium compounds are still scarce in the literature. Although some authors have described that organotellurium compounds are less toxic than their selenium derivatives,[17i] consistent data have indicated that organotellurium are more toxic than organoselenium compounds.[51−53,65−68]

Inorganic and organic tellurium compounds are highly toxic to the central nervous system of rodents.[71] Inorganic tellurium(IV) is metabolized by a route similar to that of selenium (Scheme 7),[36m] but in contrast to selenium, the methylated products of tellurium are considered more toxic for mammals.[72] Histological data from our laboratory have shown that mice exposed (sc) to diphenyl ditelluride **32** (Scheme 10) for 1 day (0.5 mmol/kg) or 14 days (2.5, 10, and 18.5 μmol/kg) presented accentuated vacuolization of cellular bodies in the brain.[71c] Organotellurium compounds have been reported as potent inhibitors of squalene monooxygenase, causing a dramatic reduction in the rate of cholesterol biosynthesis and leading to degradation of the myelin sheath. Consequently, a transient demyelination of peripheral nerves occurs. Laden and Porter have described that dimethyltellurium dichloride **33** and dimethyltelluride **34** inhibited purified human squalene monooxygenase (Scheme 10). The IC_{50} value for these methyltellurium compounds was 100 nM and was 100 fold lower than that of sodium tellurite (Na_2TeO_3), indicating a role for hydrophobicity in the enzyme−inhibitor interaction. This study also provides evidence that the organotellurium compounds react with vicinal cysteine sulfhydryl groups on squalene monooxygenase.[72] Since cysteinyl protecting agents such as DTT decrease the inhibitory actions of **33**, **34**, and **35**, it is plausible to suppose that the reaction of reduced cysteinyl residues on squalene monooxygenase with **33** and **34** involves the formation of unstable intermediates, in which the tellurium atom is bonded to sulfhydryl groups on squalene monooxygenase.

Scheme 10



32 **33** **34** **35**

Similarly, Goodrum showed that dimethyltelluride, dimethyltellurium dichloride, and trimethyltelluronium chloride **35** (Scheme 10) inhibited squalene monooxygenase in Schwann cells in culture, but potassium tellurite (K_2TeO_3), either when added to cell cultures or when administered intraperitoneally to rats, was not inhibitory. Dimethyltellurium dichloride was the most potent of these compounds tested, and its neuropathy most resembled that caused by elemental tellurium.[73]

The data from Laden[72] and Goodrum[73] are consistent with the hypothesis that demyelination induced by organotellurium compounds is a result of squalene monooxygenase inhibition and suggest that a dimethyltelluronium dichloride compound may be the neurotoxic species presented to Schwann cells in vivo.

In an attempt to better understand the toxicology of organotellurium compounds, we studied the effect of diphenyl ditelluride on the glutamatergic system. Thus, we found that diphenyl ditelluride inhibits the [^{3}H]glutamate, [^{3}H]MK-801, and total [^{3}H]GMP−PNP binding to rat brain synaptic membrane prepa-

Table 2. Inhibitory Effect of Diphenyl Diselenide and Diphenyl Ditelluride on [^{3}H]Glutamate, [^{3}H]MK-801, and Total [^{3}H]GMP−PNP Binding ex Vivo and in Vitro to Rat Brain Synaptic Membrane Preparations

	diphenyl diselenide[a]		diphenyl ditelluride[a]	
ligand	in vitro (100 μM)	ex vivo (25 μmol/kg)	in vitro (100 μM)	ex vivo (3 μmol/kg)
[^{3}H]MK-801	30	30	50	30
[^{3}H]glutamate	50	30	70	30
[^{3}H]GMP−PNP	40	50	40	60

[a] Data represent percent binding inhibition in relation to control value (100%) (data taken from Nogueira et al., 2001).[66]

rations in vitro and ex vivo after acute in vivo exposure to very low doses of diphenyl ditelluride. This study also suggests that diorganoyl ditellurides are more reactive than structurally related diorganoyl diselenide compounds, essentially due to their higher electronegativity in relation to carbon associated with a larger atomic volume of the tellurium atom. As shown in Table 2, the results from ex vivo experiments are in agreement with these observations, since the dose of diphenyl diselenide used was about 8 times higher than that of diphenyl ditelluride and these doses caused similar reductions in [^{3}H]MK-801, [^{3}H]glutamate, and [^{3}H]GMP−PNP binding. Consequently, diphenyl ditelluride is more toxic than diphenyl diselenide when the dose injected is considered.[66]

However, in vitro the reactivity toward biological systems does not always follow this role, since only [^{3}H]glutamate binding was more sensitive to diphenyl ditelluride than to diphenyl diselenide. [^{3}H]MK-801 and total [^{3}H]GMP−PNP binding were similarly affected by both compounds (Table 2).[66]

In a closely related investigation, our group demonstrated that acute exposure of diphenyl ditelluride did not change [^{3}H]glutamate release and uptake by rat brain synaptosomes. In contrast, data from in vitro experiments indicate that 100 μM of diphenyl ditelluride consistently inhibited [^{3}H]glutamate uptake by brain synaptosomes and synaptic vesicles. The results also suggested that the inhibitory effect on glutamate uptake may be related, at least in part, to the ability of this compound to oxidize thiol groups.[67] Our group has recently extended the studies on the glutamatergic system and demonstrated an inhibitory effect of diphenyl ditelluride on [^{3}H]-glutamate binding and uptake into human platelets.[68]

The effect of diphenyl ditelluride on ^{45}Ca influx into rat brain synaptosomes was investigated, and this compound inhibited ^{45}Ca influx into synaptosomes both when nondepolarizing and when depolarizing conditions were used. Since changes in ^{45}Ca movements can interfere and disrupt a variety of neurophysiologic processes, the high neurotoxicity of diphenyl ditelluride can be linked to its effect on ^{45}Ca fluxes at the presynapse, regardless of the depolarizing condition of the assay.[70]

In addition to its neurotoxic effects, diphenyl ditelluride has been reported as a highly toxic compound. This effect was demonstrated to be independent of the route of administration (intraperitoneal or subcutaneous) in rats (LD_{50} values were <1 μmol/kg). This is also so related to animal species since the LD_{50} for diphenyl ditelluride (ip) was 230-fold lower in rats than in mice ($LD_{50} = 150$ μmol/kg). In fact, mice treated (subcutaneous) with 500 μmol/kg diphenyl ditelluride survived for up to 72 h; however, 30% of the rats that received 0.75 μmol/kg diphenyl ditelluride died. In addition, diphenyl ditelluride administered at a small dose (0.5 μmol/kg) increased 2-fold serum alanine aminotransferase (ALT) and also aspartate aminotransferase (AST) activities in rats, providing evidence for liver and renal toxicity of this organotellurium compound.[52]

2.3. Interaction of Organoselenium and Organotellurium Compounds with δ-Aminolevulinate Dehydratase (δ-ALA-D)

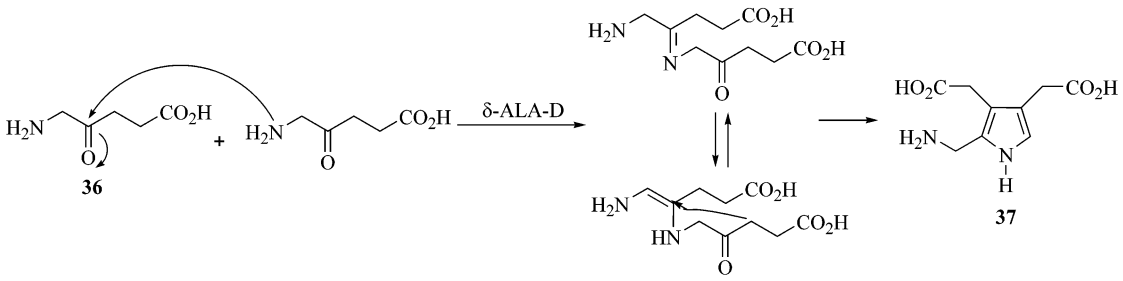
The interaction of organoselenium and organotellurium compounds with δ-ALA-D will be discussed here together, in view of their similar effects on enzyme activity.

As pointed out (see item 2.1), organoselenium and organotellurium compounds can interact directly with low molecular thiols, oxidizing them to disulfides. Reduced cysteinyl residues from proteins can also react with these compounds, which may cause, in the case of enzymes, the loss of their catalytic activity. For instance, δ-aminolevulinate dehydratase or porphibilinogen synthase (δ-ALA-D) is a sulfhydryl-containing enzyme that is extremely sensitive to oxidizing agents.[74] This enzyme catalyzes the asymmetrical condensation of two molecules of 5-aminolevulinic acid, **36**, to form porphibilinogen, **37**, an intermediate in tetrapyrol biosynthesis (Scheme 11).[75] Hence, this enzyme plays a fundamental role in most living aerobic and photosynthesizing organisms by participating in heme and chlorophyll biosynthesis. The mechanism of porphibilinogen synthesis is similar in animals and plants; however, the enzyme obtained from these sources exhibits subtle structural diversity.[75b]

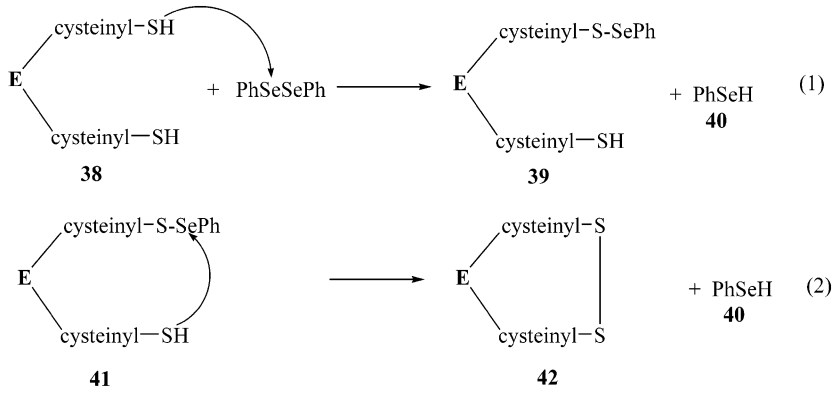
Accordingly, data from our laboratory showed that aminolevulinate dehydratase from plants, in marked contrast to the enzyme from rats, was not inhibited by diphenyl diselenide.[76] The divergent response of the plant enzyme to diphenyl diselenide (and to diphenyl ditelluride and other simple organoselenium and organotellurium compunds) is presumably related to differences in the quantity and spatial proximity of cysteinyl residues in the three-dimensional structure of the enzyme within the plant and the mammal.[77] In fact, the plant enzyme has no cysteinyl residues in close spatial proximity as observed in the active site of the mammal enzyme.[75b] In view of this, we have proposed the mechanism shown in Scheme 12 to explain why the mammal enzyme is inhibited by diphenyl diselenide[78] whereas the plant enzyme is not affected by these compounds.

We realized that the first step in this scheme involves the reaction of enzyme **38** with diphenyl diselenide to give an unstable intermediate of the type E-Cys-S−SePh **39** and selenophenol **40**, probably due to a thiol nucleophilic attack in the selenium atom of diphenyl diselenide. Subsequently, the other cysteinyl residue, due to its close spatial proximity

Scheme 11



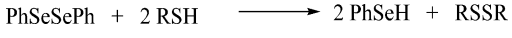
Scheme 12



to the more reactive residue, attacks the sulfur−selenium bond of the intermediate **41**, producing the oxidized enzyme (inactive) **42**, and regenerates a second molecule of selenophenol **40**. Support for this mechanism has also been obtained using low molecular weight thiol-containing molecules. We have observed that dithiothreitol is a better substrate than cysteine or GSH for the oxidation catalyzed by diorganoyl diselenide and diorganoyl ditelluride.[71c] The selenophenol molecules formed after reaction with the thiol group are oxidized back to diphenyl diselenide by atmospheric O_2. The oxidation of selenophenol helps to explain the previous observation that the inhibitory effect of diphenyl diselenide toward rats δ-ALA-D decreases considerably in an anaerobic atmosphere.[76]

Another aspect that must be mentioned here is the presence of a thermolabile factor that catalyzes the thiol/disulfide and selenol/diselenide exchange in rat and fish tissues.[79] Using dithiothreitol sulfhydryl groups as the substrate for the enzyme, we observed that the rate of oxidation of dithiothreitol caused by diphenyl diselenide and *p*-chloro-diphenyl diselenide increased considerably when liver supernatants were added to the reaction medium containing dithiothreitol and the diorganoyl diselenide. Furthermore, heating the tissue fraction for 15 min at 100 °C abolished this effect caused by liver supernatants. Based on these results, we postulated that mammal and fish livers possess a thermolabile factor (possibly an enzyme) that catalyzes the reaction described in Scheme 13. In analogy to the reactions presented

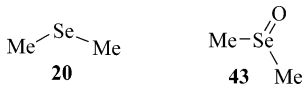
Scheme 13



above, atmospheric oxygen can regenerate diphenyl diselenide. Furthermore, we also presuppose that thiol-containing enzymes (such as δ-ALA-D) can participate in this exchange reaction between thiol and diorganoyl diselenides. Since the selenol formed can be easily oxidized back to diselenide by molecular oxygen, the net product of such an exchange reaction will be the disulfide and diphenyl diselenide. These reactions, catalyzed or not, can have a profound influence on the toxicity of diorganoyl chalcogenides, particularly for selenium- and tellurium-containing compounds.

Furthermore, Goeger and Ganther have demonstrated the monooxygenation of dimethylselenide **20** to the selenoxide derivative **43** (Scheme 14) by flavin-

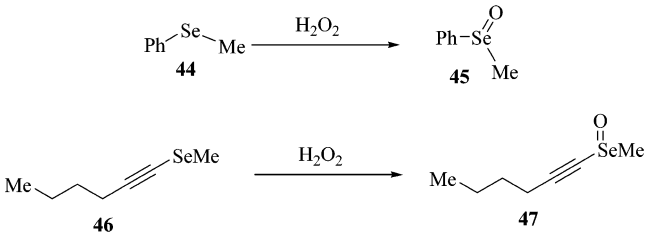
Scheme 14



containing monooxygenase (FMO).[80] Of particular importance, organoyl selenoxides are potent thiol oxidants and can initiate a futile cycle that catalyzes the oxidation of thiols at the expense of NADPH and oxygen.[81]

In this way, additional studies performed by our group have produced convincing evidence that organoyl selenoxides can oxidize sulfhydryl-containing proteins and that mammalian δ-ALA-D is a potential molecular target for organoyl selenides as a consequence of their bioactivation to selenoxides by FMO.[82,74f] Thus, methyl(phenyl)selenide **44** and (hex-1-ynyl)(methyl)selenide **46** did not inhibit δ-ALA-D from a mammalian source, while the (hex-1-ynyl)-(methyl)selenoxide **47** and methyl(phenyl)selenoxide **45**, products of the reaction of these organoselenium compounds with H_2O_2, inhibited the enzyme activity with IC_{50} values in the micromolar range (Scheme 15).

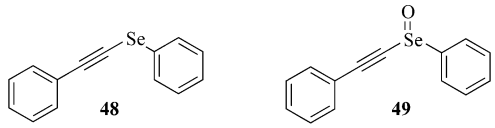
Scheme 15



Moreover, δ-ALA-D from the plant source was also inhibited by organoyl selenoxides, suggesting a possible involvement of thiol groups in a distinct site of the homologous region implicated in Zn^{2+} binding in mammalian δ-ALA-D.[74f]

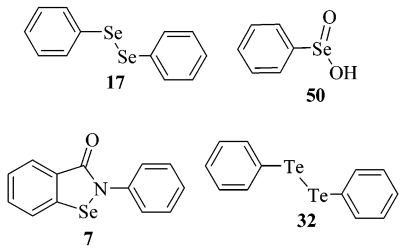
In a closely related study, we demonstrated that, different from previously studied organoyl selenides,[74f] phenyl(2-phenylethynyl)selenide **48** inhibited δ-ALA-D per se. In fact, the compound **48** inhibited mammalian and plant δ-ALA-D with IC_{50} values of 250 and >400 μM, respectively. However, its selenoxide derivative **49** inhibited the enzyme in a stronger manner with IC_{50} values of 45 and 100 μM for the mammalian and plant sources, respectively (Scheme 16).

Scheme 16



Therefore, the organoselenoxides are more potent inhibitors for δ-ALA-D than the parent organoyl selenide compound. Thus, if extrapolated to an in vivo situation, it is reasonable to suppose that the toxicological properties of diphenyl diselenide, in addition to a direct interaction with thiol groups of proteins such as δ-ALA-D, may be related, in part, to its oxidation to seleninic acid **50** after the reaction with endogenous peroxides (Scheme 17).

Scheme 17



We have extended our studies and reported that δ-ALA-D from human erythrocytes was also a target for organoselenium and organotellurium compounds. As a result, diphenyl diselenide, **17**, ebselen, **7**, and diphenyl ditelluride, **32**, inhibited in a concentration-dependent manner δ-ALA-D activity from human erythrocytes (Scheme 17). Similarly, dithiothreitol was able to reactivate and to protect inhibited human δ-ALA-D.[83]

The results, obtained with mammalian δ-ALA-D, suggest that proteins containing cysteinyl residues in close spatial proximity in their three-dimensional structure will react more promptly with organoselenium and organotellurium compounds. Consequently, we can suppose that the cellular toxicity of these compounds may be related, at least in part, to the oxidation of vicinal sulfhydryl groups of target proteins.

3. Pharmacology

3.1. Pharmacology of Organoselenium Compounds

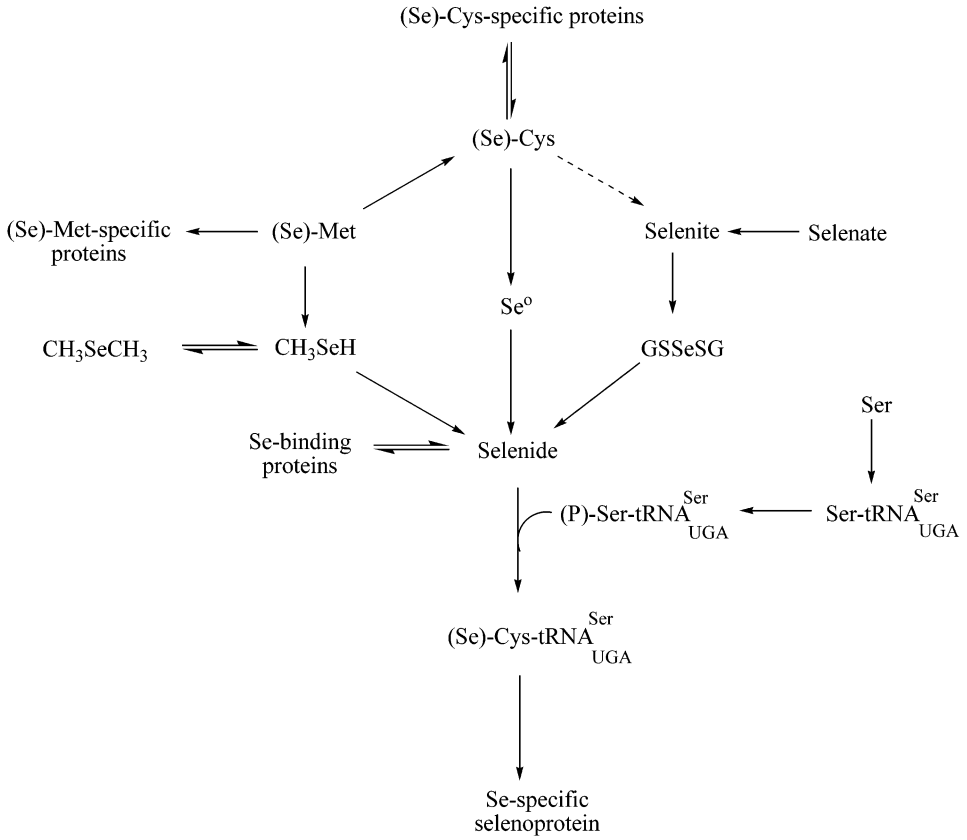
The association between selenium and liver pathology dates from the initial observations of Schwarz and Foltz that selenium, the essential part of the active organic factor 3, could prevent liver necrosis in rats fed a selenium-deficient torula yeast-based diet.[84] This observation led rapidly to the recognition that a number of previously unexplained deficiency diseases in various species of animals were selenium-responsive, and a new chapter in the selenium story began.[85]

The Schwarz and Foltz[84] findings intensified research on the physiological and biochemical role played by selenium in mammals with special emphasis on its interrelationship with sulfur-containing amino acids. In fact, it has been known for many years that selenium in plants and animals was associated with proteins.[86] Furthermore, exogenously administered Na_2SeO_4 became firmly attached to proteins of rats. However, for a long time it was not known whether selenium became bound to proteins because, as pointed out above for sodium selenite, it interacts with reactive thiol groups of a variety of proteins or whether it was reduced and incorporated into proteins as a component of amino acids.[87] Now, it is well established that incorporation of inorganic selenium in proteins involves selenophosphate synthesis from selenide and ATP by a selenophosphate synthetase followed by incorporation of selenium atom into selenocysteine synthesized from seryl-tRNA (Sec) (Scheme 18).[88]

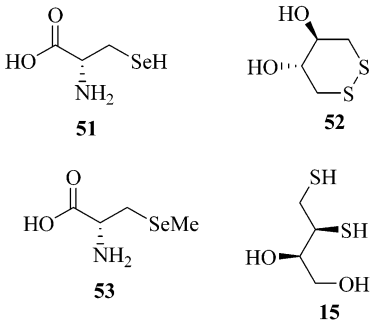
Organoselenium compounds such as selenocystine and a variety of diorgano diselenides can also react with thiols such as cysteine, dithiothreitol, and reduced glutathione to produce selenocysteine, **51**, selenols, and disulfides.[89] In line with these findings, Günther[90] showed that dithiothreitol, a compound with extremely low redox potential, reduced a variety of diorganoyl diselenides, forming selenols and *trans*-4,5-dihydroxy-1,2-dithiane, **52**, the oxidation product of dithiothreitol, **15** (Scheme 19). Some decades ago, the reduction of diorganoyl diselenides to selenol derivatives by reaction with thiols was considered to be of physiological significance.[44b] Indeed, Walter and collaborators hypothesized that selenoamino acids, particularly methylselenocysteine, **53**, could act as reversible catalytically effective biological antioxidants (Scheme 19).[89]

However, it is now apparent that selenium plays its most fundamental role as a component of the active center of the enzymes glutathione peroxidase and phospholipid hydrogen glutathione peroxidase.[91] In fact, all mammalian selenoproteins contain selenium in the form of the amino acid selenocysteine,[92] which is encoded by the UGA triplet. Therefore,

Scheme 18



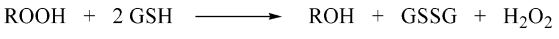
Scheme 19



twenty-two known eukaryotic selenoproteins are now recognized and organized into distinct selenoprotein groups on the basis of the location and functional properties of selenocysteine.[93]

The glutathione peroxidase (EC 1.11.1.19) catalyzes the reduction of a variety of hydroperoxides (ROOH and H_2O_2) using GSH as a reductant, thereby protecting mammalian cells from oxidative damage (Scheme 20).

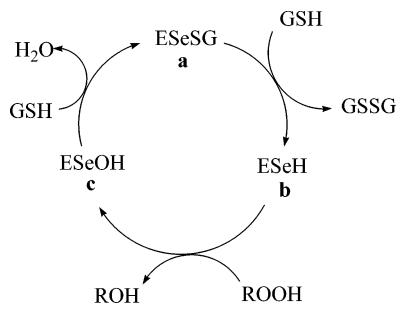
Scheme 20



Moreover, there are at least four glutathione peroxidase isoenzymes identified in mammals, so far, differing in many properties, including their localization, subunit number, global structure, primary structure, and enzymatic properties. Although, their expression is ubiquitous, the levels of which isoform vary, depending on the tissue type. The classical cellular glutathione peroxidase (GPX_1 or cGPX), found in cytosolic space and mitochondria, reduces fatty acid hydroperoxides and H_2O_2. Phospholipid hydroperoxide glutathione peroxidase (GPX_4 or PHGPX), found in most tissues and located in both the cytosol and the membrane fraction, can directly reduce the phospholipid hydroperoxides, fatty acid hydroperoxides, and cholesterol hydroperoxides that are produced in peroxidized membranes and oxidized lipoproteins. Finally, cytosolic glutathione peroxidase (GPX2 or GIGPX) and extracellular glutathione peroxidase (GPX3 or eGPX) are rarely detected in most tissues except for the gastrointestinal tract and kidney.[93b,94]

Considering the importance of glutathione peroxidase activity, the enzymatic catalytic cycle was studied by the Flohe and Wendel groups. Thus, they demonstrated that glutathione peroxidase catalyzes the reduction of H_2O_2 by GSH, following a ping-pong mechanism. The selenol **b** of a reduced selenocysteine molecule (Scheme 21) is oxidized by the hydroperoxides to generate a selenenic acid **c**. The tripeptide GSH then reacts with the selenenic acid **c**, resulting

Scheme 21



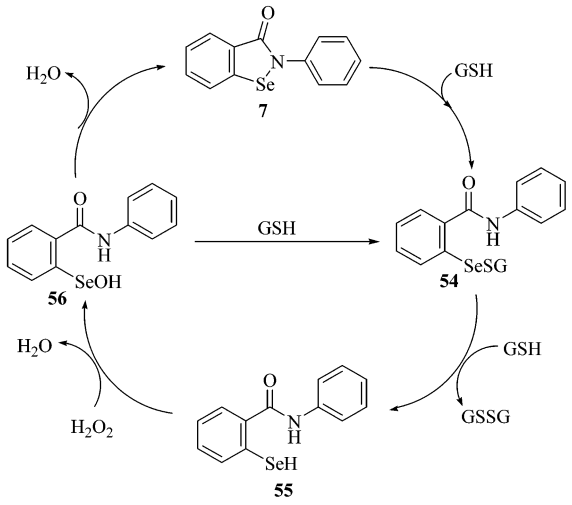
in the corresponding water and selenenyl sulfide **a**. A second molecule of GSH (Scheme 21) attacks the sulfur in the latter species, producing disulfide and regenerating the selenol **b** to complete the catalytic cycle (Scheme 21).[95]

Since glutathione peroxidase catalyses the reduction of a wide variety of hydroperoxides and together with GSH constitutes a powerful cellular defense system against so-called oxidative stress, considerable efforts have been made to find compounds capable of imitating the enzymatic properties of glutathione peroxidase. In this way, several simple synthetic organoselenium compounds with glutathione peroxidase-like activity have been prepared.[36j] As well, semisynthetic enzymes, obtained by enzyme engineering, have been proposed as mimics of glutathione peroxidase.[94,96]

3.1.1. Glutathione Peroxidase-like Activity

Ebselen was the first compound suggested for hydroperoxide-inactivating therapy in the presence of glutathione[46,97] Thus, the catalytic mechanism of ebselen reduction of hydroperoxides by thiols has been widely investigated.[98] In 1988, Maiorino and coworkers performed a kinetic study of the catalysis of the glutathione peroxidase reaction by ebselen and concluded that the mechanism appeared kinetically identical to that of the enzyme reaction, a ter uni ping-pong mechanism (Scheme 22).[99] According to

Scheme 22

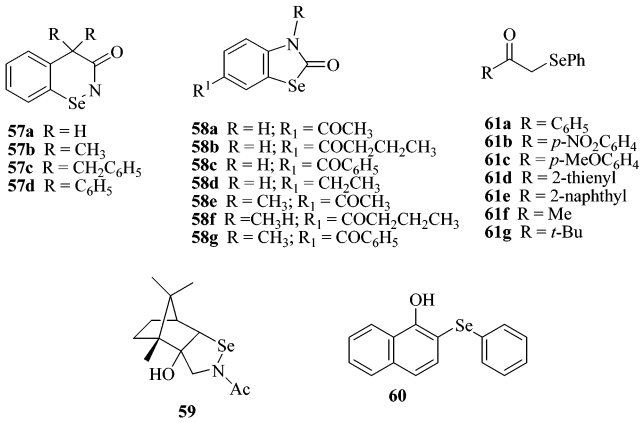


Scheme 22, ebselen, **7**, reacts with the thiols to afford a selenenyl sulfide **54**. The selenenyl sulfide **54** reacts with a second equivalent of GSH to yield a single product that is characterized as selenol **55**. Finally, the selenol reacts with H_2O_2 or organic hydroperoxide to form H_2O and ebselen seleninic acid **56**, which spontaneously produces another molecule of H_2O and turns into ebselen, **7**.

Of note, contrasting to the reaction catalyzed by the enzyme, which contains binding sites conferring specificity for GSH, ebselen and other organoselenium compounds can utilize a variety of thiols,[46,48,100] in addition to GSH, as a substrate.[100a,101]

Based on the recognized glutathione peroxidase-like activity of ebselen, several papers have appeared describing simple synthetic organoselenium compounds that imitate glutathione peroxidase, such as benzoselenazinones **57**,[102] benzoselenazolinones **58**,[103] camphor-derived selenenamide **59**,[104] 2-phenylselenenyl-naphthol **60**,[105] α-(phenylseleny)ketones **61**,[106] (Scheme 23), and oxygen-containing diselenides **8**−**12**

Scheme 23



(Scheme 4).[47] Importantly, data from these researches led to compounds with thiol peroxidase-like activity much higher than that of the original ebselen.

Therefore, the reaction catalyzed by organoselenium compounds is similar to that catalyzed by glutathione peroxidase and is of particular significance for living cells because it decomposes hydrogen peroxide, an intermediate that can give origin to the extremely reactive and toxic product $OH^{\bullet}$. Simple diaryl selenides[107] and ebselen[108] can also reduce phospholipid hydroperoxides, thus protecting biomembranes from peroxidative degradation.

However, in addition to acting as a glutathione peroxidase mimic, simple organoselenium compounds can accelerate the rate of thiol oxidation even in the absence of peroxide by catalyzing the reaction in Scheme 24). Thus, in the presence of oxygen, di-

Scheme 24

organyl selenides are regenerated (Scheme 25). Although the peroxidase-like activity of diorganoyl diselenides may account for their antioxidant properties, the thiol−diselenide exchange catalyzed by organoselenium compounds may contribute to their toxicological properties by oxidizing relevant thiol-containing metabolites and proteins.

Scheme 25

3.1.2. Antioxidant Activity

The rapid growth, in recent years, of the role of reactive oxygen species in pathology has brought with it new ideas for the therapy of a variety of diseases. Thus, the concept that selenium-containing molecules may be better nucleophiles (and therefore antioxi-

dants) than classical antioxidants, has led to the design of synthetic organoselenium compounds.[109] In this way, several reports have appeared describing the antioxidant activity of ebselen[110] and other organoselenium compounds[111] in different experimental models.

As pointed out above, it has been recognized that organoselenium compounds react very efficiently with hydroperoxides. In addition, there is an increasing amount of evidence showing that ebselen and other organoselenium compounds can also serve to protect against peroxynitrite, a potent inflammatory mediator.[112]

In fact, peroxynitrite ($ONOO^-$) is a strong oxidizing and nitrating agent that is produced by the diffusion-limited reaction of nitric oxide and superoxide anion (Scheme 26). The formation of peroxynitrite may be

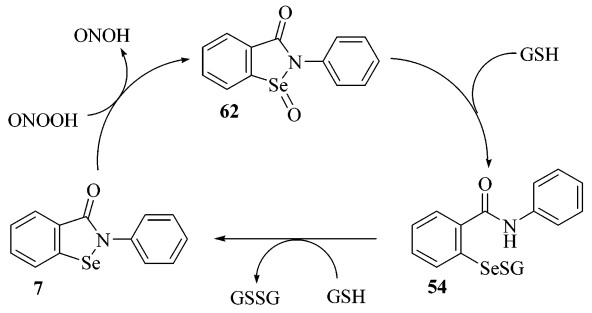
Scheme 26

$$NO^{\bullet} + O_2^{\bullet-} \longrightarrow ONOO^- \longrightarrow \text{oxidation/nitrosation}$$

beneficial in inflammatory reactions in terms of an oxidative destruction of intruding microorganisms. Higher concentrations and an uncontrolled generation of peroxynitrite, however, may result in an unwanted oxidation and the consecutive destruction of host cellular constituents. Taking this into account, ebselen reacts with peroxynitrite efficiently, exhibiting one of the highest second-order rate constants for a low-molecular-weight compound.[112c]

As described in Scheme 27, ebselen, **7**, acts catalytically in reducing peroxynitrite to nitrite in the first step, yielding the selenoxide **62** of the parent molecule, 2-phenyl-1,2-benzisoselenazol-3(2*H*)-one-1-oxide, as the sole selenium-containing product at 1:1 stoichiometry,[112d] followed by the reduction of this selenoxide back to ebselen in two consecutive one-electron reduction steps via the selenodisulfide **54**, utilizing reducing equivalents in the form of glutathione. The analogy to the glutathione peroxidase catalytic cycle is obvious. Further, the mammalian selenoprotein thioredoxin reductase can also reduce ebselen selenoxide **62** at the expense of NADPH.[113]

Scheme 27

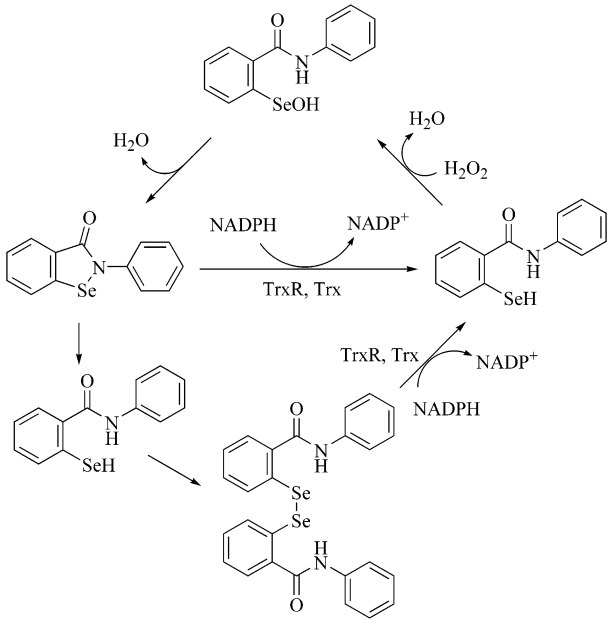


Data from our laboratory have demonstrated that diphenyl diselenide, *p*-chlorodiphenyl diselenide, and ebselen are able to inhibit lipid peroxidation induced by sodium nitroprusside, a substance usually used as an NO generator.[111b] The potency of ebselen was higher than that of diselenides, which may indicate that part of their antioxidant activity is related to a direct interaction with NOO^- formed from NO. These results may indicate that diselenides are not good scavengers of NO generated by lipid peroxidation. Thus, future studies are necessary to establish whether ebselen and diselenides can react directly with NO in addition to NOO^- or whether the antioxidant effects of these organoselenium compounds against sodium nitroprusside-induced lipid peroxidation are mediated by an interaction with lipids from brain and not related to NO itself.

3.1.3. Thioredoxin Reductase

Recent data from the Zhao group strongly suggest that the antioxidant and antiinflammatory effects of ebselen are, to a large extent, due to the reactions with the thioredoxin system (Scheme 28).[114] The

Scheme 28



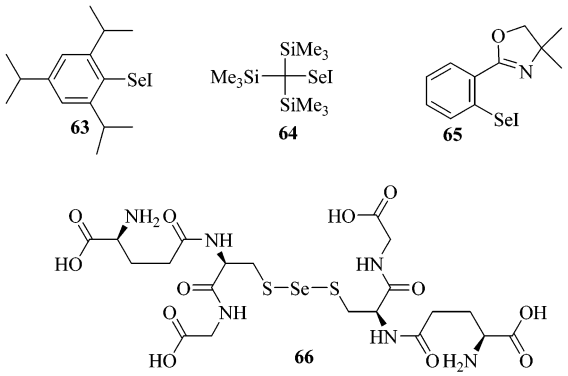
thioredoxin system comprises NADPH, thioredoxin (Trx), and thioredoxin reductase (TrxR). Thioredoxin reductase is a dimeric FAD-containing enzyme that catalyzes the NADPH-dependent reduction of the active-site disulfide in oxidized thioredoxin to give a dithiol in reduced thioredoxin ($Trx(SH_2)$).[93b]

Mammalian thioredoxin reductases are particularly interesting because they are large selenoproteins and their structures show a close homology to glutathione reductase. Besides, thioredoxin reductases have wide substrate specificity,[115] reducing not only different thioredoxins but also sodium selenite,[116] selenodiglutathione, **66**,[117] selenocystine,[118] selenenyl iodides **63**−**65**,[119] and ebselen[114,120] (Scheme 29). Thiredoxin reductase can also reduce the selenenyl sulfide complex of serum albumin−ebselen and release free ebselen as demonstrated by Arteel and collaborators.[113]

3.1.4. Dehydroascorbate Reductase and Thioltransferase-like Activities

Ebselen, in the presence of physiological concentrations of glutathione, has been described as possessing dehydroascorbate reductase (DHA) and thioltransferase (TTase)-like activities.[121] The multiple enzyme-like activities of ebselen as GPX, TTase, and DHA reductase may contribute to the well-documented

Scheme 29



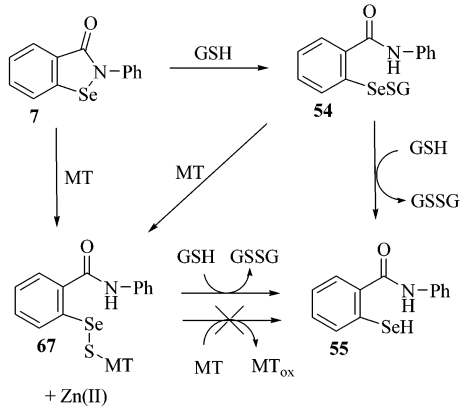
efficacy of ebselen as a potent antiinflammatory and antioxidative agent.

From model studies, using bovine serum albumin, Nomura and co-workers reported that ebselen rapidly binds to albumin in vitro.[122] In addition, the Nikawa group reported that ebselen interacts with glutathione S-transferase and papain by forming selenosulfide bonds.[123] In a related study, the same group suggests that ebselen is transferred from an albumin complex to rat liver cytosolic glutathione S-transferase by their sulfhydryl groups and concludes that this transfer may be necessary for uptake and distribution of ebselen in the cell.[124]

Data regarding the dehydroascorbate reductase and thioltransferase-like activity of simple diorganoyl diselenides and selenides are scarce in the literature. Furthermore, the role of albumin and glutathione-S-transferase on the distribution of diselenide is also not reported. Thus, considering the therapeutic potential of simple diorganoyl diselenides, more studies on the metabolism and distribution of diselenides in plasma are necessary, particularly their interaction with serum albumin.

Furthermore, the data of Jacob and co-workers provide evidence that selenium in the isoselenazole ring of ebselen not only reacts with free thiols, either in the form of glutathione or enzyme active sites,[123,124a] but also reacts with zinc-bound thiolates as in metallothionein or yeast alcohol dehydrogenase.[125] Thus, Scheme 30 illustrates that the high reactivity and

Scheme 30



redox behavior of selenium derivatives toward thiols is retained even when the thiol is bound to zinc. Ebselen, **7**, reacts with thiols (either from GSH or metallothionein) to form a sulfur−selenium bond with the concomitant opening of the isoselenazol ring. Excess thiol then reduces the ebselen selenodisulfide **54** and **67** to its selenol(ate) **55** derivative with formation of a disulfide (GSSG). In analogy to the catalytic cycle of glutathione peroxidase, the selenol(ate) is then oxidized by peroxide to the seleninic acid derivative, which reacts stepwise with two molecules of glutathione to form glutathione disulfide and regenerate the selenol(ate).

3.1.5. Antiinflammatory Activity

The role of reactive oxygen species in tissue injury and particularly in inflammatory disorders has been the subject of intensive investigation.[126] Inflammation and its sequellae are important factors in hydroperoxide-related tissue damage, not only for obviously inflammatory diseases, but also for diseases in which an inflammatory etiological factor is only now becoming apparent.

Thus, intensive search has been made for scavengers and inhibitors of reactive oxygen species with low toxicity. One of the approaches taken in this search has been to investigate synthetic organoselenium compounds, which may be therapeutically beneficial in the treatment of inflammatory diseases.

Therefore, in addition to the ability to reduce hydroperoxides, ebselen has been shown to be an efficient antiinflammatory compound. Ebselen converts leukotriene B_4 (LTB_4) to its biologically inactive 6-trans isomer in humans and porcine leucocytes ($IC_{50} = 4$ and 2.7 μmol/L, respectively).[127] Consistent with the data reported by Kuhl in 1986, ebselen significantly inhibited neutrophil chemotactic and chemokinetic responses to LTB_4 in a dose-dependent fashion.[128]

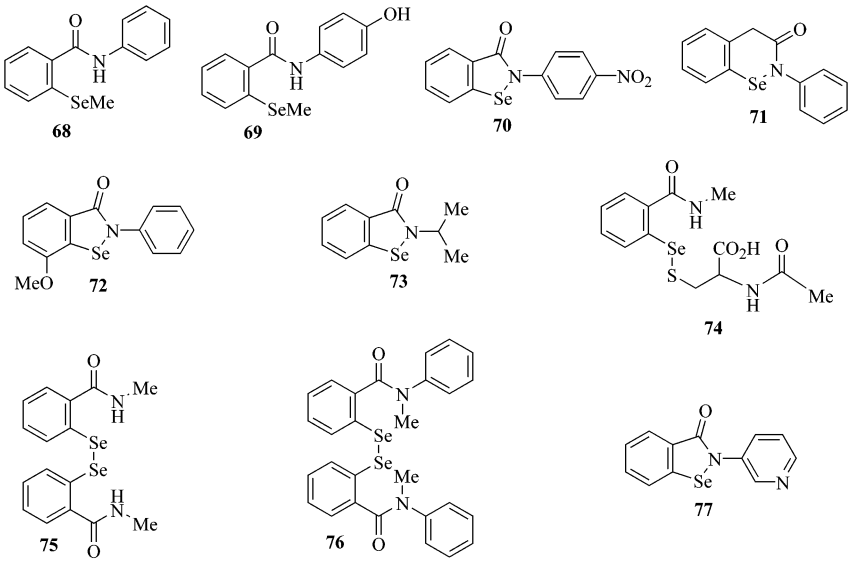
At higher concentrations ebselen also inhibits lipoxygenases, inhibiting 5-hydroxyeicosatetraenoic acid (5-HETE) and LTB_4 formation by ionophore-stimulated rat, pig, and human leucocytes and 12-HETE formation by human platelets.[127,129]

On the other hand, both human recombinant 5- and 15-lipoxygenases purified from rabbit reticulocytes were strongly inhibited in the absence of GSH. Also, a variety of derivatives or structural analogues of ebselen, **68**−**77**, were tested and proved to be either inactive or weaker inhibitors of 15-lipoxygenase (Scheme 31).[130]

On one hand, the pharmacological action of ebselen is thought to originate from its peroxidase activity, which could lower the peroxide tonus required for cycloxygenase and lipoxygenase activation. On the other hand, that this may not be the only target of ebselen is evident from its inhibition of platelet aggregation in aspirin-treated platelets in which a functional cyclooxygenase may be excluded.[131]

From experiments with aspirin-treated human platelets, evidence emerged that ebselen affects intracellular calcium homeostasis by inhibiting inositol-3-phosphate (IP_3)-induced calcium release.[131a] As well, ebselen has been demonstrated to interfere with polymorphonuclear leucocytes (PMNL) oxidative burst to suppress the activity of protein kinase C (PKC) and to inhibit NADPH oxidase.[132]

Scheme 31

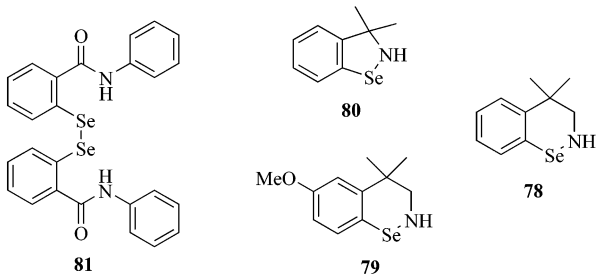


Ebselen has also been shown to inhibit the production by zymosan-stimulated mouse macrophages of reactive oxygen species (ROS).[97] This ebselen in vitro action has been associated with the inhibition on the membrane-bound NADPH oxidase, which generates superoxide anion in both macrophages[133] and neutrophils.[132,134] Besides, ebselen has been described as a selective inhibitor of the endothelial isoform of nitric oxide synthase (NOS) in the rabbit and bovine aorta.[135]

Ebselen is also a potent inhibitor of PMNL adhesion to vascular endothelium and transendothelial migration, both in response to PMNL chemotactic factors and across IL-1 or TNF-α activated endothelial cells in vitro[136] and in vivo.[137]

In addition, other glutathione peroxidase mimics, benzoselenazines **78** and **79** and benzoselenazole **80** derivatives, have been reported to prevent TNF-α and neutrophil-induced endothelial alterations (Scheme 32).[138] Moreover, the mitogenic activity of

Scheme 32



bis-[2-*N*-phneylcarboxamido)phenyl]-diselenide **81** and ebselen was demonstrated, and this effect was correlated with the expression of interleukin-2 receptor in T-lymphocytes (Scheme 32).[139]

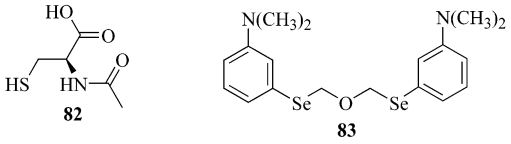
Ebselen was also reported to inhibit cartilage degradation induced by interleukin-1 (IL-1). However, its activity was independent of ebselen glutathione peroxidase activity and of its inhibitory effect on cyclooxygenase and PGE_2 production.[140]

In addition, ebselen was reported to be resistant to HOCl when its glutathione peroxidase-mimetic property was compared with that of glutathione peroxidase. This finding suggests that ebselen might prove more useful as an antiinflammatory agent than the glutathione peroxidase enzyme.[141] With regard to HOCl, it is produced in vivo by the neutrophil-derived enzyme myeloperoxidase, a mechanism highly relevant in inflammatory injuries.

Recently, Joszef and Filep described a novel mechanism by which ebselen, methylselenocysteine, and selenocysteine may affect the inflammatory response. As a result, selenium-containing compounds attenuate $ONOO^-$-mediated nuclear accumulation of the transcription factors NF-κB and activator protein (AP-1) and suppress IL-8 gene expression and production in human leukocytes.[142]

Consistent with these findings, studies in vivo in models in which hydroperoxides play an important role have found that ebselen is a weak inhibitor of the classical carragenan paw edema and adjuvant arthritis in rats, but it is effective as an inhibitor of cobra venom paw edema in rats,[143] glucose oxidase-induced monoarthritis in mice,[144] and H_2O_2-induced foot pad edema.[145] Moreover, Cotgreave and coworkers described the antiinflammatory activity of ebselen but not glutathione and *N*-acetylcysteine, **82**, in experimental models of alveolitis and bronchiolits (Scheme 33).[146] Ebselen has also been effective in

Scheme 33



experimental allergic neuritis,[147] as an inhibitor of late airway response (LAR) and airway inflammation in guinea pigs,[148] to prevent LPO of lung surfactant by stimulated human polymorphonuclear leukocytes,[149] and to inhibit compound 48/80-induced histamine release in rat peritoneal mast cells.[150]

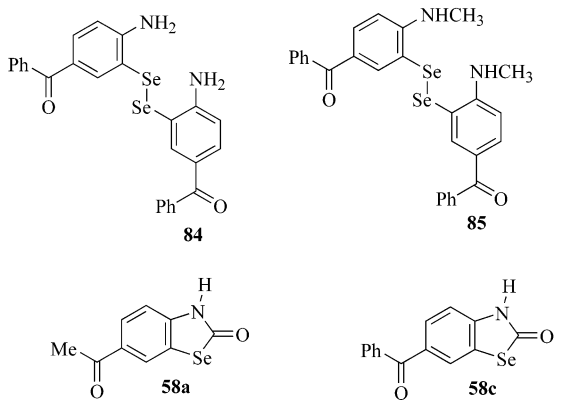
In an attempt to screen new antiinflammatory compounds, the relationship between structure and activity of a series of oxygen-containing aryl se-

lenides, using formalin-induced paw edema in rats, has been studied. Thus it was found that the most potent inhibitor of paw edema was bis-4(dimethylamino-phenylselenomethyl) ether, **83** (Scheme 33).[151]

Our group has also investigated the antiinflammatory and antinociceptive activity of simple diaryl diselenides in vivo, thereupon concluding that diphenyl diselenide displayed the most promising profile in carragenin-induced paw edema. In fact, *p*-methoxyphenyl diselenide and *p*-chlorodiphenyl diselenide were less efficient in inhibiting carrageninduced edema than diphenyl diselenide. As well, *p*-methyldiphenyl diselenide was the weakest inhibitor of paw edema. Besides, diphenyl diselenide demonstrated to be an antinociceptive compound, due to its effective action on experimental models such as tail-flick, formalin, acetic acid-induced abdominal writhing and capsaicin. Interestingly, the antinociceptive and antiinflammatory potency of diphenyl diselenide was higher than that of ebselen.[152]

Investigating benzoselenazolinone derivatives designed to be glutathione peroxidase mimetics, Galet and collaborators reported the potential of these compounds as cyclooxygenase and 5-lipoxygenase inhibitors.[103] As a result, all bis(*o*-amino-phenyl)-diselenides tested exhibited high glutathione peroxidase-like activity, 3 times the activity of ebselen. The most efficient compound was **84** (6-benzoyl derivative), and the least efficient were benzoselenazolinones, which were inactive. From this study, two types of compounds with two sets of structure−activity relationships can also be distinguished. 6-Substituted benzeselazolinones with a benzoyl group are inhibitors of the cyclooxygenase pathway, while all 6-substituted compounds with acetyl, benzoyl, hydroxybenzyl, or benzyl groups are 5-lipoxygenase inhibitors with the condition that the N atom is not substituted. Compound **58a** is selective for the 5-lipoxygenase pathway, and **85** is selective for the cyclooxygenase pathway, whereas **58c** and **84** are found to be active for both tests. Compounds **58c** and **84** were also effective in inhibiting carragenin-induced paw edema in rats. However, only **58c** was an inhibitor of the passive foot anaphylaxis model (Scheme 34).

Scheme 34



3.1.6. Liver Damage

Liver damage is a therapeutic target of selenorganic compounds, as well as the various clinical conditions in which hydroperoxides play a role. In fact, ebselen given orally is an effective inhibitor of galactosamine/endotoxin-induced hepatitis, a model in which inhibition of lipoxygenases is considered to be involved.[153]

An inhibitory action of ebselen on liver damage has also been demonstrated when injury was induced by paracetamol,[154] CCl_4,[155] lipopolysaccharide and *Propionibacterium acnes*,[156] alcohol,[157] ethanol-induced hepatic vasoconstriction,[158] and ischemia−reperfusion injury.[159]

In rat Kupfer cells, ebselen inhibits the production of superoxide anion and nitric oxide.[160] Also, ebselen protects mice against T-cell-dependent, tumor necrosis factor (TNF)-mediated apoptotic liver injury. It renders protection by an NF-κB-dependent mechanism against TNF itself, which is associated with up regulation of IL-10 release[161] and suppresses TNF-α and NO production by lipopolysaccharide-activated Kupfer cells by the modulation of Jun-N-terminal kinase (JNK) and the NF-κB signaling pathway.[162] This pharmacological profile suggests that ebselen has a promising potential in the therapy of diseases that are characterized by an initial overactivation of the immune system. However, more detailed studies in vivo are necessary to determine the safety of ebselen, particularly for liver, since this organ seems to be a target for organoselenium compounds. In fact, exposure to very high doses of diselenides caused hepatotoxicity in rodents.[71c] Furthermore, we recently observed that ebselen at a neuroprotective dose caused an enhancement in the hepatotoxicity of methylmercury in suckling rats.[53] In addition, there is scarce data reported in the literature considering the effects of other diselenides on the liver.

3.1.7. Gastric Mucosal Damage

The major side effect of all nonsteroidal antiinflammatory drugs (NSAIDS) is damage to the gastric mucosa. In this respect, it is of considerable importance that organoselenium compounds have no irritant or damaging effect on the gastric mucosa. Ebselen has been effective in preventing ulceration induced by aspirin, diclofenac,[163] HCl and acidified ethanol,[164] 48/80 compound,[165] ethanol,[166] and water-immersion restraint stress.[167]

Accordingly, diphenyl diselenide has been recently reported by our group as preventing ethanol and indomethacin-induced ulcers, as well as inhibiting gastric acid secretion in pylorus-ligated rats.[168] Ebselen was also reported to inhibit gastric acid secretions in pylorus-ligated rats[167] and H^+,K^+ ATPase in parietal cells,[169] indicating that this antisecretory action is involved in the antiulcer effect of this compound in rats.

Ebselen and diphenyl diselenide protect against mucosal damage by inhibiting the sulfhydryl groups of H^+,K^+ ATPase. This effect on the enzyme responsible for acid secretion can counteract the undesirable side effects caused by NSAIDS . Thus, from the point of view of antiinflammatory drugs, organoselenium compounds are versatile with regard to gastric ulcers because not only do they block the inflammatory cascade, which can impair gastric endogenous protective factors against HCl, but they also inhibit the HCl secretion. Since the therapeutic approach toward a

variety of diseases is changing from a single target to a multitarget one, it would be very useful to evaluate the possible synergism of the association of organoselenium compounds with other NSAIDS with respect to the antiinflammatory response and to the antagonism of gastric ulcer development.

3.1.8. Neuroprotection

With regard to neuroprotection, Ebselen has been suggested to protect against brain damage from different models of permanent focal ischemia,[170] transient focal ischemia,[171] and hypoxia/ischemia-induced neuronal damage.[172] It was also reported that ebselen ameliorated cerebral vasospasm in a canine two-hemorrhage model,[173] inhibited chronic cerebral vasospasm after subarachnoid hemorrhage in primates,[174] protected against cerebral ischemia, and accelerated the recovery during reperfusion.[175] Also, ebselen has been described by others and us to produce an antioxidant effect in different experimental models of neurotoxicity.[108b,110d,111b,176]

Moreover, data from clinical trials have consistently demonstrated that ebselen reduced brain damage in patients with delayed neurological deficits after aneurismal subarachnoid hemorrhage[177] and improved the outcome of acute ischemic stroke, suggesting that ebselen may be a promising neuroprotective agent.[178]

Glutamate neurotoxicity in primary cultures of cerebellar neurons, which is believed to be mediated by *N*-methyl-D-aspartate (NMDA) receptor activation, is significantly reduced by ebselen.[108b] In fact, ebselen reverses dithiothreitol potentiation of NMDA-mediated currents in cultured neurons by acting at the redox-sensitive NMDA receptor site and not by directly antagonizing NMDA-mediated receptor responses.[179] However, it is still not known whether ebselen can block NMDA receptor-mediated responses in vitro and in vivo. Recently, we obtained behavioral evidence suggesting that ebselen does not interfere with the quinolinic acid-induced convulsion but reduces the production of TBARS in the striatum of rats. These results indicate that, in vivo, ebselen does not modulate the activation of NMDA receptor caused by the potent NMDA agonist.

In this context, neuroprotective effects of other glutathione peroxidase mimetics have appeared in the literature in recent years.[180] Thus, diphenyl diselenide has been demonstrated as a neuroprotector agent in a classical model of in vitro ischemia[181] and an inductor of facilitation of long-term object recognition memory.[182] Interestingly, ebselen and diphenyl diselenide blocked the increase in inducible nitric oxide synthase (iNOS) overexpression caused by glucose and oxygen deprivation in rat brain slices. Thus, we can suppose that they inhibited the excessive NO production by iNOS, which occurs after brain ischemia in vitro. This inhibitory effect can be a common molecular mechanism underlying part of the neuroprotection afforded by these compounds after brain ischemia. Furthermore, since iNOS is involved in the inflammatory process,[183] it is possible that organoselenium compounds have part of their antiinflammatory activity mediated by chemically decreasing iNOS overexpression. Regulation of this important molecular pathway can help injured tissues to more efficiently handle the oxidative stress. Thus, in addition to reacting and directly neutralizing NOO^- derived from NO, organoselenium compounds block an early complex molecular pathway that involves repression of the synthesis of an important protein involved in cell death mechanisms.

3.1.9. Chemopreventive Activity

Only 12 years after the proposal that selenium was an essential nutrient, Shamberger and Frost suggested that the element may also be related to cancer risk.[184] After that, a substantial body of research has established that selenium can reduce experimental carcinogenesis. Also, increasing epidemiological points of evidence have shown a protective role of selenium in human cancers.[185]

El-Bayoumy, in the 1980s, was the first to report the use of synthetic organoselenium compounds as chemopreventive agents in laboratory animals. The chronology started with *p*-methoxybenzeneselenol, **86**, an effective inhibitor of benzo[*a*]pirene-induced forestomach tumors in mice[186] and azoxymethane-induced hepato[187] and colon carcinogenesis in rats.[187b] However, this compound was quickly abandoned in favor of benzylselenocyanate, **87** (Scheme 35). In fact,

Scheme 35

86 **87**

benzylselenocyanate, a versatile organoselenium chemopreventive agent in several model systems, has been reported to inhibit the development of azoxymethane-induced colon tumor[188] and 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors during the initiation phase of carcinogenesis in rats.[189] As well, the potential genotoxicity of *p*-methoxybenzeneselenol and benzylselenocyanate was described to induce sister-chromatid exchanges and chromosome aberrations and to inhibit cell proliferation in human blood lymphocytes in vitro.[190]

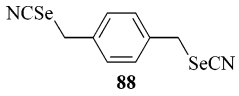
Additional studies on the mode of action of benzylselenocyanate were performed by Foiles and collaborators. Thus, they demonstrated that benzylselenocyanate is capable of reducing the level of polyoma DNA replication induced by NNK-model compounds. Consequently, the data suggest that changes in expression of cellular proteins, which regulate transcription and replication of DNA sequences, are involved in mechanisms by which benzylselenocyanate exerts its chemopreventive effects.[191]

In 1997, data from the Fiala group showed that benzylselenocyanate decreases the level of DNA and RNA modifications produced by 2-nitropropane in the liver. These results provide experimental evidence that denitrification of 2-nitropropane is a detoxication reaction with regard to the genotoxicity of the carcinogenesis and have revealed additional facets to the modes of the action of benzylselenocyanate.[192] Subsequently, they described this organoselenium compound as an inhibitor of DNA cytosine methyltransferase from human colon carcinoma, consistent with the proposal that MTase may be a major mechanism

of chemoprevention by organoselenium compounds at the postinitiation stage of carcinogenesis.[193] Besides, Foiles and collaborators demonstrated that benzylselenocyanate inhibits PKC and PKA activities in cultures of primary human fibroblast.[194]

On the basis of mechanistic and metabolic studies,[195] Reddy and collaborators modified the structure of benzylselenocyanate[196] to develop a more effective and less toxic[197] chemopreventive agent. As a consequence, *p*-phenylenebis(methylene)selenocyanate, **88**, arose as a compound less toxic than benzylselenocyanate (Scheme 36).[198] Thus, the efficiency of *p*-

Scheme 36



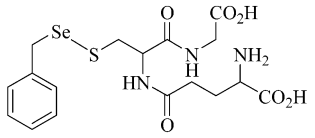
88

phenylenebis(methylene)selenocyanate in experimental models for carcinogenesis at both initiation and postinitiation stages in colon,[199] mammary glands,[200] lung,[201,200a] liver,[201g] intestine,[202] and oral tissues[203] has been widely reported.

In addition to chemepreventive effects, *p*-phenylenebis(methylene)selenocyanate has been reported as cytotoxic to vascular endothelial cells and capable of inhibiting neovascularization in vivo.[204]

As a part of a large-scale investigation directed toward the development of less toxic, but highly effective, organoselenium compounds to be used as chemopreventive agents, a series of novel organoselenium compounds has appeared in the literature. In this fashion, Kawamori and co-workers have proposed benzylselenocyanateglutathione conjugate **89**, an active metabolite mediating the chemopreventive activity of benzylselenocyanate, as an inhibitor of azoxymethane-induced colon carcinogenesis (Scheme 37).[205]

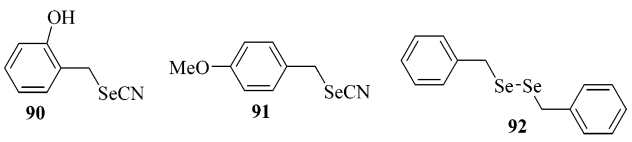
Scheme 37



89

Through the use of the same model of colon cancer, *o*- and *p*-methoxy-benzylselenocyanate, **90** and **91**,[199b] *p*-phenylenebis(methylene)selenocyanate, **88**,[199b] and dibenzyl diselenide, **92,** were demonstrated to possess chemopreventive properties (Scheme 38).[206]

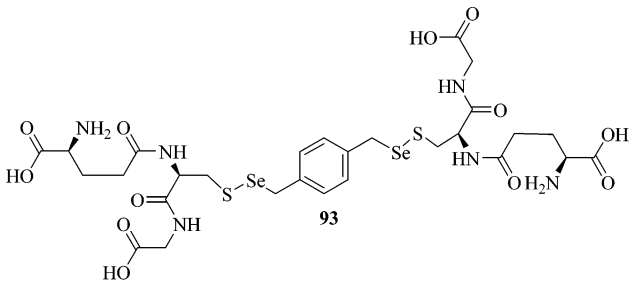
Scheme 38



90 **91** **92**

Moreover, data from the Reddy group have suggested that a glutathione conjugate of *p*-phenylenebis(methylene)selenocyanate, **93**, is effective against colon carcinogenesis when administered during the postinitiation stage and inhibits cyclooxygenase activity. Of note, considering the toxicity associated with selenium, *p*-phenylenebis(methylene)selenocyanate seems to be the least toxic organoselenium chemopreventive agent thus far tested in the experimental colon carcinogenesis (Scheme 39).[207]

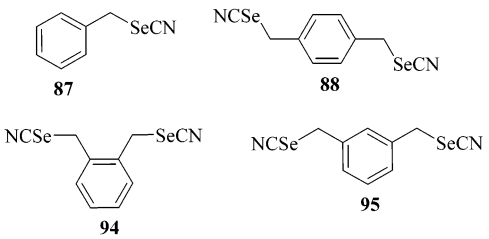
Scheme 39



93

In terms of oral toxicity *p*-phenylenebis(methylene)selenocyanate, **88**, has been reported to be less toxic compound than *o*- and *m*-phenylenebis(methylene)selenocyanate, **94** and **95**, and benzylselenocyanate, **87**. So, these results suggest that the excretion patterns of selenium are significantly different, depending on the position of substitution (Scheme 40).[208]

Scheme 40



87 **88** **94** **95**

Furthermore, structure−activity relationships among the *ortho*-, *meta*-, and *para*-isomers of phenylenebis(methylene)selenocyanate were evaluated in an attempt to design organoselenium analogues with maximal efficacy yet with minimal toxic side effects. The results indicated that *o*- and *m*-phenylenebis(methylene)selenocyanate are equally effective inhibitors of 9,10-dimethylbenz[*a*]anthracene (DMBA)−DNA binding in rat mammary tissues. As well, both isomers are more effective than *p*-phenylenebis(methylene)selenocyanate.[209]

In addition, the effects of dietary *o*-, *m*-, and *p*-phenylenebis(methylene)selenocyanate isomers on the xenobiotic metabolizing enzyme systems have been investigated.[210] The results indicate that individual phenylenebis(methylene)selenocyanate isomers are capable of modulating specific phase I or phase II enzymes or both (CYP2B1, CYP2E1, UDPGT, GST, and GSH peroxidases) involved in the activation or detoxification of chemical carcinogenesis.[211] As well, *p*-phenylenebis(methylene)thiocyanate did not influence these enzyme systems, emphasizing the requirement of selenium in the chemopreventive activity of these compounds.[192,209,211]

The possible mechanisms for inhibition by *p*-phenylenebis(methylene)selenocyanate during the postinitiation phase of carcinogenesis were examined through in vitro and cell culture systems. Thompson and co-workers reported that *p*-phenylenebis(methylene)selenocyanate increased apoptosis and reduced cell numbers in the mouse carcinoma cell line.[212]

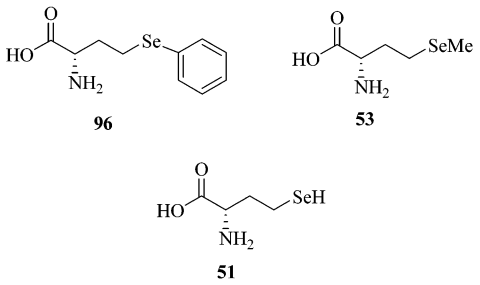
Apoptosis has also been demonstrated to participate in the preventive effect of *p*-phenylenebis(methylene)-selenocyanate on colon[213] and human oral[214] carcinogenesis. Evidence from in vitro assays suggests that *p*-phenylenebis(methylene)selenocyanate inhibits thymidine kinase,[215] protein kinase C and A activities,[194] and topoisomerase II activity,[216] and it selectively modulates inducible cyclooxygenase (COX-2) activity.[217] Other mechanisms that may partially account for the chemoprevention by *p*-phenylenebis(methylene)selenocyanate include the inhibition of DNA, RNA, and protein synthesis[218] and the modulation of Jun N-kinase activities.[219]

Additional studies have shed some light on the mechanisms involved in *p*-phenylenebis(methylene)-selenocyanate chemopreventive effects. This organoselenium compound inhibits AOM-induced colon carcinogenesis by suppressing tyrosine protein kinase and protein kinase C activities and by up-regulating diacylglycerol kinase activity in the colonic mucosa and in tumors in rats.[220]

Furthermore, a recent report provides evidence that *p*-phenylenebis(methylene)selenocyanate and its glutathione conjugate down regulate genes related to CYP450 and induce those related to phase II enzymes, leading to low levels of DMBA−DNA adducts in the rat mammary gland. These results help to elucidate the molecular targets of mammary cancer chemoprevention by *p*-phenylenebis(methylene)selenocyanate in vivo.[221]

Naturally occurring selenium-containing amino acids, such as methylselenocysteine, **53**, and selenocysteine, **51**, have been extensively studied as chemopreventive agents. Thus, the inhibitory capacity of selenocysteine conjugates toward seven of the most important human P450s was examined. The most potent inhibitor was benzylseleno-selenocysteine, **96**, but the majority of the selenocysteine conjugates produced inhibition of CYP1A1 at a micromolar range, suggesting that this effect may contribute to their chemopreventive activity (Scheme 41).[222]

Scheme 41

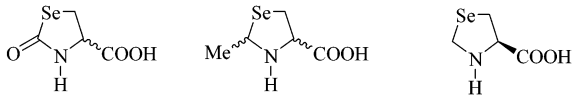


96 **53** **51**

In addition, promising results were observed with the novel selenazolidines **97**−**99**, prodrugs of selenocysteine, as potential selenium delivery agents for cancer chemoprevention. All the selenazolidines exhibited reduced toxicity to V79 cells. Of note, compounds possessing L-stereochemistry were at least as active with respect to selenium-dependent glutathione peroxidase activity as sodium selenite (Scheme 42).[223]

Selenocystine and selenodiglutathione have also been reported to inhibit phorbol ester-induced transformation of epidermal cells. Besides, these organoselenium compounds induce a redox modulation of protein kinase C, compartmentally independent from the cytosolic GSH but intimately connected to an NADPH-dependent reductase system.[224]

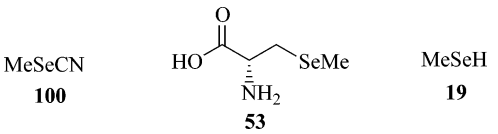
Scheme 42



97a - L **97b** - D **98a** - L **98b** - D **99** - L

Lu and collaborators have demonstrated that selenite inhibits cell growth through predominantly nonspecific genotoxic effects, which are manifested by single-strand DNA breaks and cytotoxicity. In contrast, methylselenocyanate, **100**, and methylselenocysteine, **53**, which are metabolized predominantly to methylselenol, **19**, induced growth inhibition in the absence of DNA single-strand breakage (Scheme 43). Consequently, these findings support

Scheme 43



100 **53** **19**

the hypothesis that selenite and organoselenium compounds have different and distinct modes of action in the growth inhibition of cells in vitro.[225]

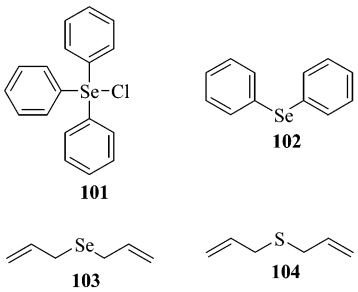
Methylselenocysteine holds effective potential as a chemopreventive agent especially against mammary tumorogenesis.[226] In this way, Sinha convincingly demonstrated that the methylselenocysteine inhibitory effect on the growth of mouse mammary tumor epithelial cells coincides with a specific blocking of cdk2 kinase activity,[227] with the increase of *gadd* gene expression, and with apoptosis.[225b]

Besides, some authors have described that rats supplemented with methylselenocysteine had more 3,2′-dimethyl-4-aminobiphenyl-induced colonic DNA adducts and greater aberrant crypt foci formation in the colon than those supplemented with selenium inorganic. These findings suggest that the supplementation of selenium in the form of methylselenocysteine in the diet does not inhibit the preneoplasic lesions in the colon.[228] Accordingly, methylselenocysteine was unable to inhibit DNA synthesis in mouse mammary epithelial cells.[225b]

In an attempt to design new organoselenium compounds for chemoprevention, triphenylselenonium chloride, **101**, and diphenylselenide, **102**, have been investigated. Thus, triphenylselenonium chloride was found to have a number of desirable attributes for cancer chemoprevention[229] and to be more effective than diphenyl selenide in inhibiting tumor development in two experimental animal models (Scheme 44).[229,230] In this way, studies performed by El-Bayoumy and co-workers demonstrated that diallyl selenide **103** was 300 times more active than diallyl sulfide **104** in inhibiting mammary cancer in rats (Scheme 44).[231]

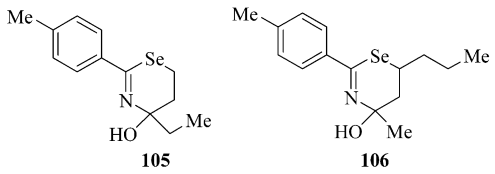
Consistent with the idea of developing new organoselenium compounds with chemopreventive activity, Wu and collaborators studied 1,3-selenazine

Scheme 44



derivatives **105** and **106**. The results show that **105** and **106** inhibit human gastric adenocarcinoma cells by the induction of apoptosis (Scheme 45).[232]

Scheme 45



Recently, Gasparian and collaborators demonstrated that an apoptotic concentration of methyl seleninic acid inhibited IκB kinase activation and IκB-α phosphorylation and degradation induced by α-TNF or LPS in two prostate cancer cell lines, suggesting that selenium may target the NF-κB activation pathway to exert its cancer protective effect.[233]

In parallel to a number of pharmacological properties described, ebselen has been studied as an antitumor compound. In fact, Engman and collaborators demonstrated that ebselen inhibits cell growth in humans, including breast and colon cancer cells.[234] A similar effect on cell growth was observed by Yang and co-workers. They demonstrated that ebselen induces apoptosis in the human hepatoma cell line HepG2,[235] and this effect seems to involve its ability to deplete thiols and to alter the mitochondrial permeability transition.[236]

Evidences concerning the direct effect of ebselen on MAP kinase activity in neuronal cells arose from the Yoshimuzi group. Thus, they demonstrated that ebselen specifically inhibits H_2O_2-induced JNK activation but not extracellular-signal-regulated kinase (ERK) $^1/_2$ and p38 activation in PC12 cells. Ebselen was also found to attenuate H_2O_2-induced PC 12 cell death including apoptosis.[237]

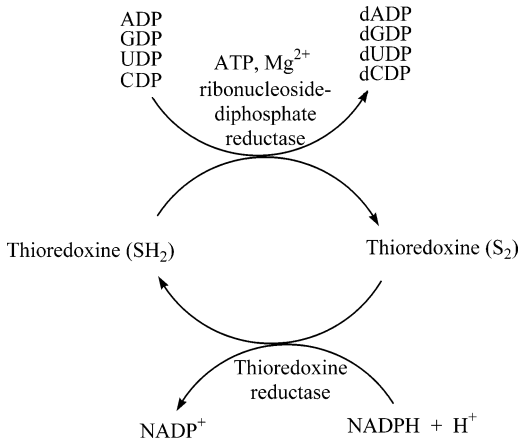
Considering that both apoptotic and nonapoptotic death may occur as a consequence of changes in mitochondrial function; mitochondria are believed to play an important role in neurodegenerative disorders. In this way, ebselen has been reported to prevent mitochondrial damage[238] and antagonize the release of the apoptogenic factor cytochrome *c*, induced by Fe^{2+}/citrate in rat liver mitochondria. Hence, these data are consistent with the hypothesis that ebselen may protect the cell not only as an antioxidant but also by preventing the cascade of apoptotic events that follows release of cytochrome *c*.[239]

In addition to its capacity to provoke cell death, ebselen has been shown to inhibit apoptosis.[240] In fact, ebselen was found to prevent N_2H-induced apoptosis in normal and transformed thymocytes[241] and to protect cells from radiation-induced apoptosis.[242]

In a recent report, Guerin and Gauthier demonstrated that ebselen induced cellular necrosis. In fact, the results clearly show that ebselen induced the rapid necrotic cell death of the murine hybridoma cell line. As well, they suggest that necroses caused by ebselen could not be prevented by the pan-caspase inhibitor Z-VAD-fnk. In the same report, ebselen was found able to completely inhibit caspase activation induced by cycloheximide (CHx) treatment, indicating that the cell death mechanisms triggered by ebselen interfere with the apoptotic death machinery.[243]

From this point of view, the thioredoxin system has been described to be important for cancer cell growth and inhibition of apoptosis. The thioredoxin system was originally studied for its ability to provide reducing equivalents to ribonucleotide reductase, the first unique step in DNA synthesis. Because it provides the reduced form of thiol redoxin for many processes necessary for cell growth and protection against oxidant damage, the enzyme is likely to be an important regulatory protein for both normal and transformed cells (Scheme 46).[244]

Scheme 46

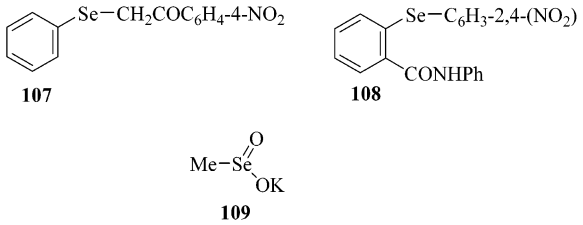


More recently, a number of investigators have been pursuing an approach to the development of antitumor agents that targets the mammalian selenoenzyme thioredoxin reductase. In fact, inhibition of thioredoxin reductase would be expected to be highly detrimental to the growth of tumor cells, especially since the levels of both thioredoxin reductase and thioredoxin have been found to be elevated in tumor cell lines. However, it has been reported that thioredoxin stimulates the proliferation of at least some types of human tumor cells.[245]

In an attempt to investigate new chemopreventive agents, organoselenium compounds have been evaluated as inhibitors of thioredoxin reductase. As a result, ebselen was the most active organoselenium tested on thioredoxin reductase (IC_{50} value of 4.2 μM). In the same study, the inhibitory efficiency of organoselenium compounds **107** and **108** was demonstrated (IC_{50} values of 60.1 and 73.6 μM, respectively). Compound **108** was able to inhibit growth

of cultured tumor cells.[234] Conversely, potassium methylseleninate **109** was found to be a substrate rather than an inhibitor of mammalian thioredoxin reductase (Scheme 47).[246]

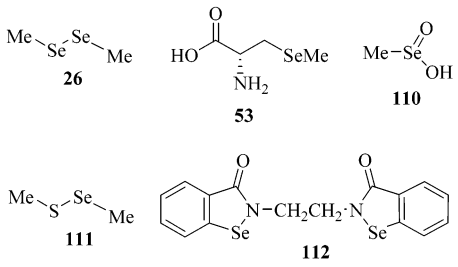
Scheme 47



107 **108** **109**

On the other hand, Ganther and Ip demonstrated that thiroedoxin reductase activity is not affected by supranutritional levels of methylselenocysteine, **53**, and methylseleninic acid, **110**. However, high concentrations of dimethyl diselenide, **26**, and dimethyl selenenylsulfide, **111**, inhibited thioredoxin reductase in vitro (Scheme 48).[247]

Scheme 48



26 **53** **110** **111** **112**

Recently, Shi and co-workers demonstrated that 1,2-[bis(1,2-benzisoselenazole-3(2*H*)-ketone]ethane, **112**, a thioredoxin reductase inhibitor, impedes the proliferation of prostate cancer cells via S phase arrest and apoptosis (Scheme 48).[248]

3.2. Pharmacology of Organotellurium Compounds

3.2.1. Glutathione Peroxidase-like Activity

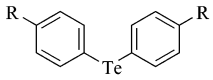
Although the tellurium atom is generally regarded as a toxic metalloid, its role in biological systems has been reviewed.[249] Concerning organotellurium compounds little is known about their biological and pharmacological effects.

As pointed out above (item 3.1.1), glutathione plays a central role in the endogenous antioxidant defense as a reducing agent and nucleophile as well as a substrate for the glutathione peroxidases and transferases. Thus, several reports have been published on glutathione peroxidase-mimetic compounds, which, like the native enzyme forms, rely on the redox cycling of selenium. Similar to organoselenium compounds, organotellurium compounds are readily oxidized from the divalent to the tetravalent state. Consequently, this property makes tellurides attractive as scavengers of reactive oxidizing agents such as hydrogen peroxide, hypochloride, and peroxyl radicals.

In this way, several authors have described that substitution of selenium by tellurium in a series of diarylchalcogenides results in a pronounced increase in antioxidant activity. With this in mind, the development of novel and potent antioxidants plays an important role in targeting therapeutic interventions in several diseases.

In the 1990s, Anderson and collaborators demonstrated potent glutathione peroxidase-like activity of diaryl tellurides **113a**−**l** and contributed to increase the list of synthetic peroxidase catalysts. Thus, the most active diaryl telluride, bis(4-aminophenyl)telluride, **113a**, demonstrated 348%, 530%, 995%, and 900% of the catalytic activity of ebselen for the glutathione-dependent reduction of H_2O_2, *tert*-butyl hydroperoxide, cumene hydroperoxide, and linoleic acid hydroperoxide, respectively (Scheme 49).[250]

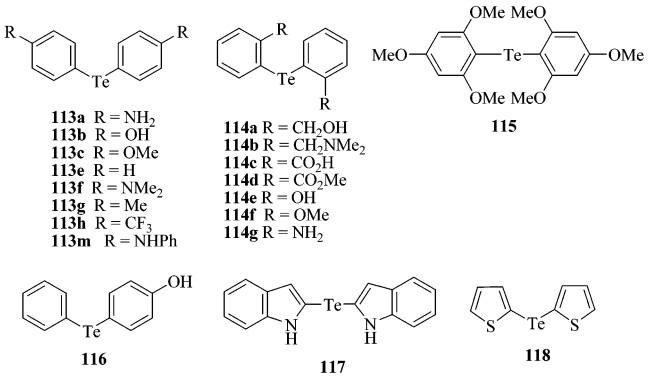
Scheme 49



113a R = NH_2 **113g** R = Me
113b R = OH **113h** R = CF_3
113c R = OMe **113i** R = Cl
113d R = F **113j** R = Br
113e R = H **113l** R = NO_2
113f R = NMe_2

Subsequently, the Engman group reported that a number of diorganoyl tellurides, including some that were preliminarily tested by Andersson, were able to catalyze the reaction of hydrogen peroxide with thiols. Thus, the results with 4,4′-disubstituted diaryl tellurides **113** indicate that the introduction of conjugatively electron-donating substituents (OH, NH_2, NMe_2, NHPh) reduces t_{50} values (time required to reduce the thiol concentration by 50%) as compared to the unsubstituted compound, **113e**. Conversely, the electron-withdrawing CF_3 group in **113h** had the opposite effect. As compared to 4,4′-disubstituted catalysts, the 2,2′-disubstituted compounds were generally less active, probably as a result of steric hydrance. In addition, the hexamethoxy-substituted diphenyl telluride **115** has a lower oxidation potential than any of the 4,4′-disubstituted compounds, **113**. As well, compounds **116** and **113b** presented similar performance, suggesting that only one 4-OH substituent is required to obtain a highly potent catalyst. Finally, the diheteroaryl tellurides **117** and **118** were poorer catalysts than most of the diaryl tellurides tested (Scheme 50).[251]

Scheme 50



113a R = NH_2
113b R = OH
113c R = OMe
113e R = H
113f R = NMe_2
113g R = Me
113h R = CF_3
113m R = NHPh

114a R = CH_2OH
114b R = CH_2NMe_2
114c R = CO_2H
114d R = CO_2Me
114e R = OH
114f R = OMe
114g R = NH_2

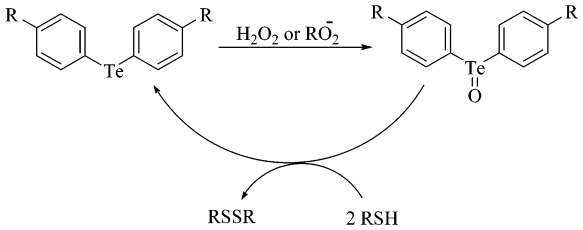
115 **116** **117** **118**

Additional studies performed by the same group described diaryl tellurides carrying substituents in

the *para* position as inhibitors of peroxidation in chemical and biological systems. Therefore, the most active compound, bis-(*p*-(dimethylamino)phenyl telluride, **113f** (IC_{50} = 30 nM), is among the most potent antioxidants in the microsomal system ever reported. This compound also caused a concentration-dependent delay of the onset of the linear phase of microsomal peroxidation stimulated by iron/ADP/ascorbate. In the hepatocyte system, all compounds demonstrated similar protective activity. As for ebselen, the inhibitory effect of these compounds could be ascribed to a chain-breaking or peroxide-decomposing effect or both.[252] In addition, studies on a two-phase model lipid peroxidation have provided further evidence in support of a chain-breaking capacity of these compounds and shown that the effect could be catalytically expressed in the presence of a thiol reducing agent.[253]

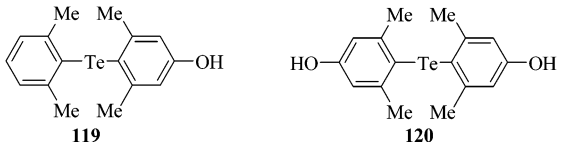
Based on mechanistic studies, diaryl tellurides were pointed to exert an antioxidative effect by deactivating both peroxides and peroxyl radicals under the formation of telluroxides (Scheme 51).[251,252]

Scheme 51



Diaryl tellurides have also been described to retard peroxidation of linolenic acid in methanol. The results clearly showed that the best 4,4′-disubstituted diaryl telluride antioxidants contained hydroxyl, **113b**, amino, **113a**, and phenylamino, **113m**, substituents. The two most active substances presented in this study, diaryl tellurides **119** and **120**, both contain phenolic groups and were found to be equipotent to vitamin E (Scheme 52). Also, the substitution of

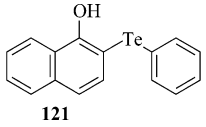
Scheme 52



tellurium in compound **120** for selenium or sulfur produced less active antioxidants. Contrary to the conventional antioxidants examined, diaryl tellurides were found to inhibit peroxidation for a long time, seemingly with an autocatalytic mechanism.[107]

2-Substituted-1-naphthols are the most potent 5-lipoxygenase inhibitors known. Based on this information, a series of 2-substituted-1-naphthol analogues with the benzylic group replaced by sulfur, selenium, and tellurium were prepared. Data obtained with 2-phenyl tellurenyl-1-naphthol, **121**, indicated that this compound inhibits stimulated LTB4 biosynthesis in human neutrophils (Scheme 53). Concerning the antioxidant profile, it was found that the organotellurium derivative **121** had a significantly lower oxidant potential than the other

Scheme 53

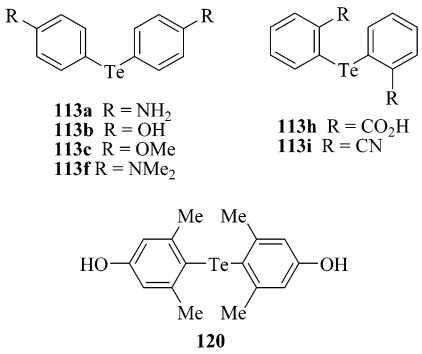


121

compounds. As well, **121** showed much improved antioxidant properties as compared with the parent compound. In the presence of a stoichiometric amount of a thiol reducing agent, compound **121** was shown to act as a catalytic peroxide decomposer and as a catalytic chain-breaking antioxidant.[105]

Diaryl tellurides **113b**, **113c**, **113f**, **113a**, **114h**, **114i**, and **120** have been described to protect against *tert*-butylhydroperoxide (TBH)-induced cell death in lung fibroblast cultures (Scheme 54). Besides, com-

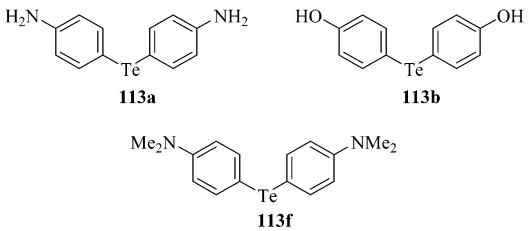
Scheme 54



pounds **113b** and **120** prevented leukocyte-mediated cell damage in Caco-2 cells and protected rat kidney tissue against oxidative damage caused by anoxia and reoxygenation.[254]

Investigating the response of trout erythrocytes exposed to oxidative stress in vitro, Tiano and coworkers found that diaryl tellurides **113a**, **113b**, and **113f** at low concentrations presented a protective effect on DNA damage without modifying the hemolysis rate. Although at low concentrations the protective effect of diaryl tellurides and ebselen was evident, at high concentrations diaryl tellurides **113a**, **113b** and **113f** were not able to protect DNA against breakage. Conversely, compounds **113a** and **113b** drastically increase the level of damage producing a marked genotoxic effect (Scheme 55).[255]

Scheme 55

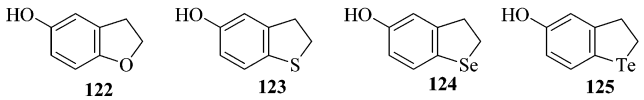


In addition, a cyclodextrinyl ditelluride compound has been pointed out as an excellent glutathione peroxidase-mimic, demonstrated by its high catalytic efficiency. Thus, the investigation on the mitochondria damage system induced by ferrous sulfate/ascorbate reveals that cyclodextrinyl ditelluride is a better antioxidant than ebselen.[256] Additional kinetic

experiments performed by the same group show that, while the spontaneous reduction of *t*-BuOOH by GSH involves free radicals, the same reaction catalyzed by cyclodextrinyl ditelluride does not. These results suggest that the peroxidase activity of cyclodextrinyl ditelluride, is very similar to that of natural glutathione peroxidase, which does not involve free radicals.[257]

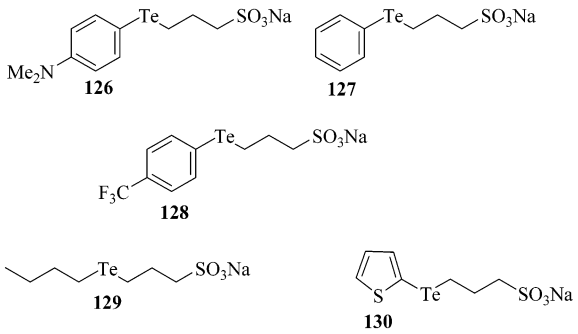
Employing a tandem $S_{RN}1/S_Hi$ sequence for radical-based synthesis, Engman and collaborators have prepared dihydrotellurophenes, **125**, and their seleno analogues (Scheme 56).[258] The same group has extended this study and investigated the antioxidant capacity of these dyhidrotellurophenes, together with the known oxygen, **122**, and sulfur, **123**, analogues, as a function of the chalcogen substituent in the *para* position to the phenolic group. Therefore, results from experimental and redox data demonstrated that the homolytic O−H bond dissociation enthalpies of compounds **122**−**125** were very similar. The antioxidant capacity increases as **125** > **124** = **123** > **122**, based on inhibited rates of peroxidation in a two-phase lipid peroxidation system containing a thiol-reducing agent in the aqueous phase. Also, they demonstrated that the introduction of tellurium, **125**, imposes another antioxidative capacity to the molecule, the ability to catalytically decompose hydrogen hydroperoxides in the presence of a stoichiometric reducing agent.[259]

Scheme 56



Moreover, water-soluble organotellurium compounds **126**−**130** were screened as protectors against peroxynitrite. As a result, 3-[4-(*N*,*N*-dimethylamino)-benzenetellurenyl]propanesulfonic acid sodium salt, **126**, has been demonstrated to efficiently protect against peroxynitrite-induced oxidation in solution (Scheme 58). Thus, it has been suggested that this molecule acts as a catalyst in scavenging $ONOO^-$ in the presence of GSH (Scheme 57). In addition, the ability of the tellurium compounds to effect peroxidation of metallothionein clearly is superior to those of the selenium analogues; thus, compounds **126** and **129** were the most efficient. Compound **128**, which contains an electron-withdrawing substituent, is less reactive than unsubstituted **127** and hence a poor scavenger of peroxynitrite. Similar results are obtained with **130**, which contains a thiophene instead of phenyl substituents on the tellurium atom (Scheme 58).[260]

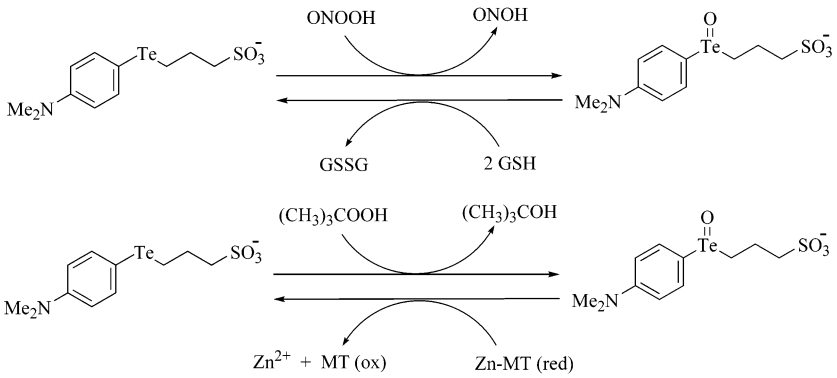
Scheme 58



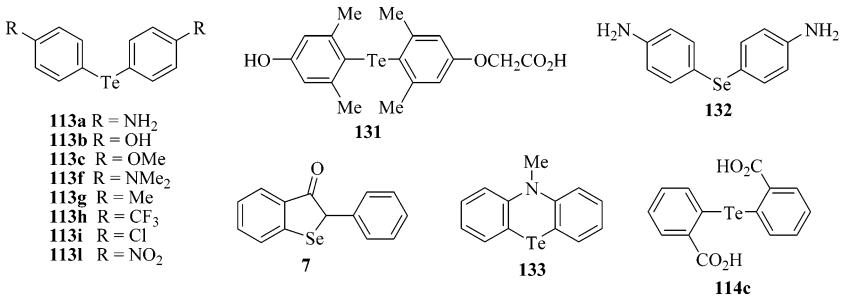
The 3-[4-(*N*,*N*-dimethylamino)benzenetellurenyl]-propanesulfonic acid sodium salt (NDBT), **126,** has also been described to possess significant activity toward hydrogen peroxide, peroxynitrite, and the hydrogen peroxide-induced hydroxyl radical in cortical synaptosomal systems from gerbil brain. In fact, NDBT prevented neuronal death caused by $ONOO^-$ in cultured hippocampal neuronal cells and attenuated $ONOO^-$-induced, luminol-dependent chemiluminescence in red blood cells.[261]

Of note, peroxynitrite (see item 3.1.2), a strong biological oxidant, can be formed in vivo (Scheme 26) and can induce DNA damage as well as initiate lipid peroxidation in biomembranes. Besides, peroxynitrites can cause tyrosine nitration of proteins and inactivate a variety of enzymes. Taking this into account, Briviba and co-workers have demonstrated that organotellurium compounds displaying glutathione peroxidase-like activity effectively protect against peroxynitrite-mediated oxidation and nitration reactions. Thus, among the tested *para*-substituted diaryl tellurides, **113a** offered the most efficient protection against peroxynitrite-mediated oxidation. This compound was 11 times more effective than the methylselenocysteine and approximately 3 times more active than **113b** and **131** in this capacity. As well, **113a** was more active than its selenium analogue **132** and ebselen **7** against peroxynitrite-mediated nitration of tyrosine residues in lysates from human fibroblasts. In addition, compounds **113f**, **113c**, and **113g** showed activity similar to that of selenomethionine. The chloro-, **113i**, nitro-, **113l**, and trifluormethyl-substituted, **113h**, analogues pre-

Scheme 57



Scheme 59



sented only slight protection. Conversely, the heterocyclic organotellurium **133** and **114c** showed no significant activity (Scheme 59).

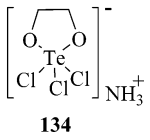
Analogously to what has been shown in Scheme 27, diaryl tellurides act as scavengers of peroxynitrite by an oxygen transfer mechanism similar to that observed with hydrogen peroxide and hydroperoxides.[262]

3.2.2. Chemopreventive Activity

Selenium and tellurium share unique chemical characteristics; however, there are some differences that make tellurium compounds of interest. Motivated by these properties, organotellurium compounds have been investigated as chemopreventive agents.

In 1987, the compound ammonium trichloro(dioxoethylene-O,O′-)tellurate, **134**, coded AS101, was demonstrated for the first time to present immunomodulating properties and, when administered to mice, to mediate antitumor effects.[263] This compound has also been described to stimulate human lymphoid cells to proliferate and produce lymphokines.[264] In fact, AS101 stimulates the production of IL-1, IL-2, colony-stimulating factor (CSF), tumor necrosis factor (TNF), and other cytokines in vitro.[265] Furthermore, Kalechman and co-workers have demonstrated that AS101 protects mice from ionizing radiation (Scheme 60).[266]

Scheme 60



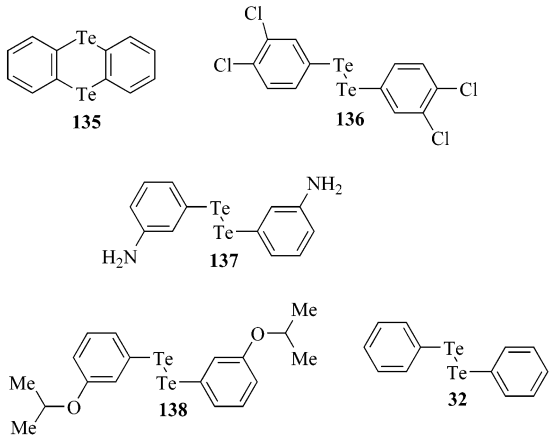
In connection with these studies on AS101, results suggest that restoration by AS101 of T helper activity of lymphocytes from UVB-treated psoriatic patients might result from an increase in DNA repair mechanisms or a stimulation of IL-1 and G-SF secretion, which could restore the function of UVB-damaged cells.[267]

As well, there is evidence that synergism between AS101 and PKC activators involves perturbations in intracellular Ca^{2+} levels, which may in turn facilitate PKC activation and resulting mithogenic/secretory responses.[263a] Thus, studies performed by Rao and collaborators indicated that while co-administration of AS101 with bryostatin 1 (PKC activator) does not lead to further increases in the susceptibility of HL-60 cells to Ara-C-induced apoptosis, AS101 significantly enhances the proliferative and differentiating actions of bryostatin 1 in the cell line.[268]

In a closely related investigation, the same group reported that A23187 (calcium ionophore) administered alone significantly potentiates apoptosis in Ara-C-pretreated HL-60 cells, an effect that is equivalent to that observed with the combination of bryostatin 1 and A23187. Moreover, sequential administration of Ara-C followed by bryostatin 1 and AS101, which mimics the action of A23187, results in substantial reduction of leukemic cell clonogenicity. The significance of these findings is that while induction of apoptosis in leukemic cells is clearly a desirable goal, it is not the only mechanism by which loss of clonogenic potential may occur.[269]

Similar to organoselenium, organotellurium compounds have been described as cytotoxic and capable of inducing apoptotic cell death. Thus, Sailer and coworkers reported that telluranthrene **135** and 1,2-bis(3,4-dichlorophenyl) ditelluride **136** demonstrated bacterial cytotoxicity and a capacity to induce apoptotic cell death in eukaryotic HL-60 cells. The EC_{50} values for telluranthrene **135** and diphenyl ditelluride **32** were 196 and 33 μM, respectively.[270] With the use of the same experimental model, diphenyl ditelluride, **32**, *m*-diaminodiphenyl ditelluride, **137**, and 1,2-bis(3-isopropoxyphenyl) ditelluride, **138**, have also been reported to induce apoptotic cell death (Scheme 61). Moreover, concentration of all three

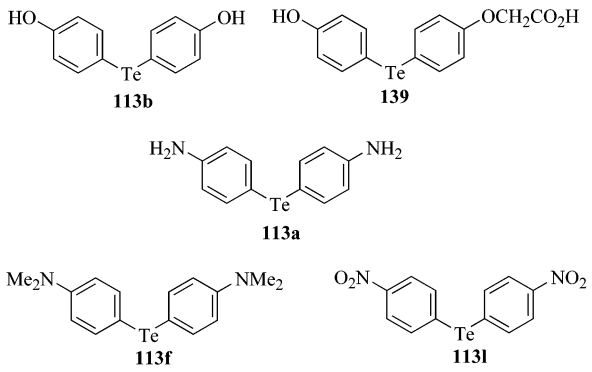
Scheme 61



compounds, as low as 1×10^{-6} M, were able to induce cell death. Finally, on the basis of their abilities to induce apoptosis, diphenyl ditelluride appears to be the most toxic compound tested.[271]

As pointed out, thioredoxin reductase (see item 3.1.3) offers a novel target for anticancer drug

Scheme 62

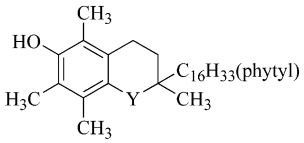


development, which is to regulate the activity of the thioredoxin system as a possible way of inhibiting cancer cell growth. In fact, thioredoxin expression is increased in several human cancers.[272] In this way, Engman and collaborators evaluated a series of organotellurium compounds as selective inhibitors of thioredoxin reductase and their potential antitumor effects (Scheme 62). The results demonstrated that compounds **113b**, **139**, **113a**, and **113f** were good noncompetitive inhibitors of thioredoxin reductase, having IC_{50} values less than 10 μM. The compounds **113b** and **113a** also inhibited growth of human cancer cells in culture with IC_{50} as low as 2 μM. As well, compounds **113b**, **113a**, and **113l** were tested for antitumor activity against MCF-7 breast cancer; however, the antitumor activity was observed only at doses that produced lethality.[234]

The poor solubility in water of the organotellurium compounds previously tested by Engman motivated his group to introduce sulfopropyl groups in an attempt to enhance solubility of these compounds. Thus, diaryl telluride, alkyl aryl telluride, and dialkyl telluride carrying sulfopropyl groups were prepared and were found to be the most efficient tellurium-based inhibitors of thioredoxin reductase ever tested (Scheme 63). The results demonstrated the vastly different inhibiting capacity of the dipotassium salt, **140c**, and the corresponding lithium, **140a**, sodium, **140b**, and tetramethylammonium salts, **140d**. Compounds **141**, **142b**, **143f**, and **143i** presented IC_{50} values lower than 0.8 μM and were the most potent inhibitors of thioredoxin reductase. As well, compounds **142a**, **144**, and **145** demonstrated IC_{50} values between 1 and 4 μM, and **143b**, **143d**, **130**, **143j**, **146a**, **149**, **147**, and **150** were active with IC_{50} values inferior to 14 μM. Finally, some of these water-soluble compounds, including **140a**, **146a**, **149**, and **148**, inhibited HT-29 colon cancer cell cultures at the 5−10 μM level, but the hydrophilicity of the materials seems to restrict their cellular uptake.[273]

Considering these results, Engman and collaborators have extended their study and prepared previously reported, as well as new, organotellurium antioxidants and evaluated them as inhibitors of thioredoxin reductase and cancer cell growth in culture. The results clearly showed that of the four cyclic aryl alkyl chalcogenides **151a**−**d**, all primitive analogues of vitamin E, only the tellurium compound, **151d**, showed interesting inhibition characteristics (Scheme 64). This observation corroborates previous findings in the series of diaryl chalcogenide inhibitors that the presence of tellurium in the molecule is essential for thioredoxin reductase inhibition.[259]

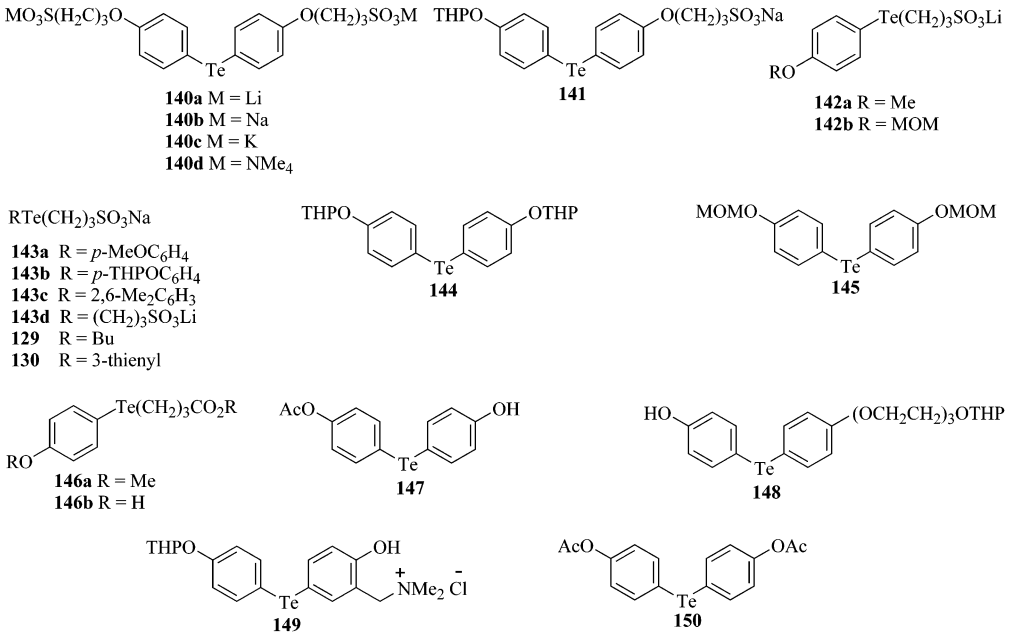
Scheme 64



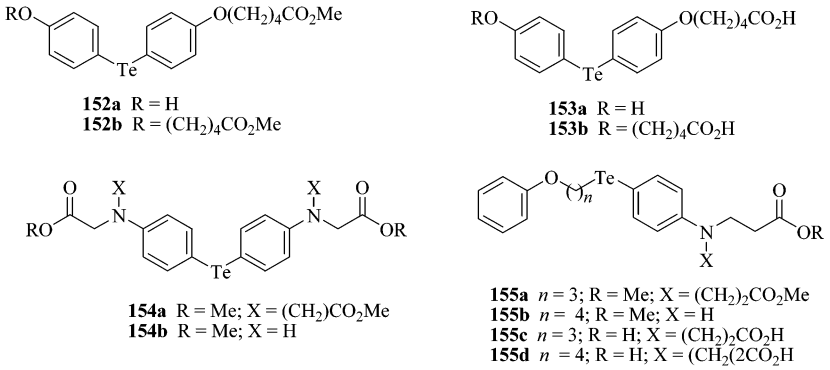
151a Y = O
151b Y = S
151c Y = Se
151d Y = Te

Moreover, as compared with the corresponding methyl esters **152a** and **152b**, the tellurium-containing carboxylic acids **153a** and **153b** were the best inhibitors. Compounds **154a**, **154b**, **155a**, and **155b**

Scheme 63



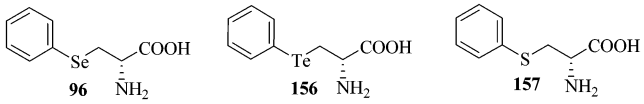
Scheme 65



were potent inhibitors of thioredoxin reductase with IC_{50} values between 2 and 4 μM. Of note, although the IC_{50} values are still in the low micromolar range, the increase of one methyl group in the carbon chain of compound **155d**, as compared to **155c**, decreased 10-fold the ability to inhibit thioredoxin reductase (Scheme 65).[274]

Stimulated by the observed differences between bioactivation of phenylseleniumcysteine, **96**, and the corresponding phenylthiocysteine, **157**, Rooseboom and co-workers evaluated the bioactivation of the corresponding organotellurium cysteine derivative. The results demonstrated that phenyltelluriocysteine, **156**, is bioactivated into its corresponding tellurol (Scheme 67),[275] analogously to what has been

Scheme 66



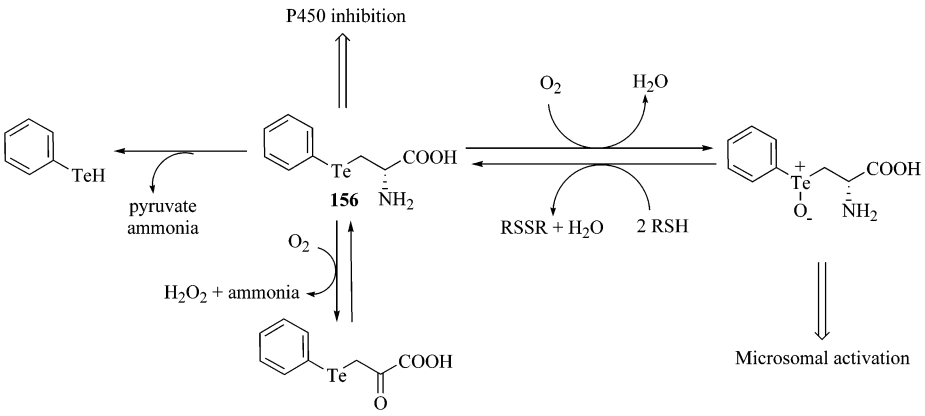
shown previously for phenylselenocysteine, **96** (Scheme 66).[276]

Microsomal activation studies show that the three conjugates seem to show the trend Te ≥ Se ≥ S, whereby phenyltellurocysteine was the strongest inhibitor of several human cytochrome P450 isoenzymes followed by phenylselenocysteine, while phenylthiocysteine was the weakest inhibitor. In addition, cytotoxic studies indicated that phenyltellurocysteine produced GSH depletion and LDH leakage comparable with the relatively nontoxic drug paracetamol, while selenium and sulfur analogues were nontoxic toward rat hepatocytes. So, these authors suggest that phenyltellurocysteine might be a significant novel class of prodrugs to generate biologically active tellurols.[275]

4. Conclusion

In this review, we presented the toxicological and pharmacological effects of a variety of organoselenium and organotellurium compounds, giving, when possible, particular emphasis to the molecular targets of these compounds. With regard to pharmacology, the organoselenium and organotellurium compounds have been described as promising pharmacological agents in view of their unique biological properties. Glutathione peroxidase mimic, antioxidant activity, and thioredoxin reductase inhibition are some of the properties reviewed in this article. On the other hand, little is known about the molecular toxicological effects of organoselenium and organotellurium compounds. Most of our knowledge arose from research on inorganic selenium and tellurium. However, the ability to oxidize sulfhydryl groups from biological molecules can be involved both in their pharmacological properties and in their toxicological effects. In fact, exposure to high doses of organoselenium or to low doses of organotellurium causes the depletion of endogenous reduced glutathione in a variety of tissues. The in vivo organochalcogen-induced thiol depletion does not strictly follow the in vitro reactivity of these organochalcogens toward low molecular weight thiols, emphasizing the necessity of more detailed in vivo studies with a variety of promising simple organochalcogens. We realize that in vitro properties of organochalcogens such as their thiol-peroxidase-like activity should guide future in vivo

Scheme 67



studies. From these studies, a more rational and embodied chemical hypothesis could be constructed about the reactivity of organochalcogens toward widespread endogenous thiols, particularly glutathione and specific thiol-containing proteins. Thus, the design of compounds that cause low depletion of glutathione and react with specific targeted proteins, controlling specific metabolic pathways, will represent an important progress in understanding the field of organochalcogen compounds. Furthermore, the development of new organochalcogens with higher thiol-peroxidase activity that can use other nontoxic thiol reducing agents, such as *N*-acetylcysteine instead of glutathione, will permit the investigation of the co-administration of organochalcogens and thiols as a formulation for antioxidant therapy. Definitely, much more remains to be done in the toxicology and pharmacology of organoselenium and organotellurium, and in the next few years, we will see many new, exciting findings in this field. Thus, we hope, with this review to have been able to give a satisfactory and valuable tool in the study of the toxicology and pharmacology of these compounds.

5. Acknowledgments

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