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Chemistry of Biologically Important Synthetic Organoselenium Compounds

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I. Introduction

The element selenium was discovered in 1818 by the Swedish chemist Berzelius and was named after the Greek goddess of the moon, Selene.[1] In biology, selenium was long considered as an absolute poison until Schwarz and Foltz identified it as a micronutrient for bacteria, mammals, and birds.[2] After 15 years of empirical studies on selenium deficiency syndromes in experimental animals, selenium biochemistry emerged in 1973 when two bacterial enzymes, formate dehydrogenase[3] and glycine reductase,[4] were reported to contain selenium. At the same time, the biochemical role of selenium in mammals was clearly established by the discovery that it is part of the active site of the antioxidant enzyme glutathione peroxidase (GPx).[5,6] The number of selenoproteins indentified has grown substantially in recent years.[7,8] In prokaryotes, formate dehydrogenases,[9] hydrogenases,[10−12] and glycine reductase[13,14] are a few representative examples in which selenocysteine[15,16] has been verified as the selenium moiety. In contrast, selenium is bound to a cysteine residue in CO dehydrogenase, where it forms a redox active center with cofactor-bound molybdenum.[17] In eukaryotes, iodothyronine deiodinases,[18−21] thioredoxin reductases,[22−27] selenophosphate synthetase,[26] and selenoprotein P[28] represent important classes of selenoenzymes in addition to the well-known glutathione peroxidases.[5,6,29−32] Many books and reviews appeared in the literature describing various biological functions of selenium, including nutritional importance.[33−38]

Although the first synthetic organoselenium compound, diethyl selenide, was prepared by Löwig in 1836,[39] the highly malodorous nature of selenium compounds, difficulties in purification, and the instability of many of the derivatives hampered the early developments. Organoselenium research intensified during the 1970s, when the discovery of several useful new reactions and a variety of novel structures with unusual properties began to attract more general interest in the discipline. Interest in the use of organoselenium compounds in biochemistry started with the findings that organoselenium compounds are much less toxic compared with the inorganic selenium species.[40] Since then, there has been a growing interest in the synthesis of organoselenium compounds with respect to their use in enzymology and bioorganic chemistry.[41] Many of the compounds synthesized at the initial stages were never studied for biological activity beyond initial screening. The pharmacology of synthetic organoselenium compounds that have been subjected to more than just a biological screen was critically evaluated in a review by Parnham and Graf.[42]

During the past decade, a lot of effort has been directed toward the development of stable organoselenium compounds that could be used as antioxidants, enzyme inhibitors, antitumor and anti-infective agents, cytokine inducers, and immunomodulators. In addition, many organoselenium compounds have been studied as biological models that

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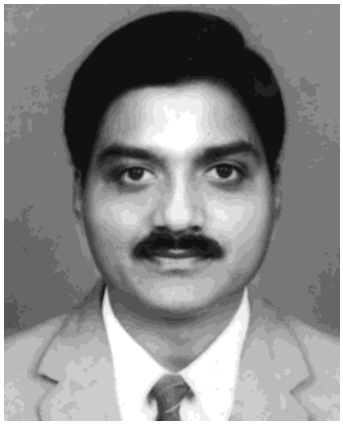
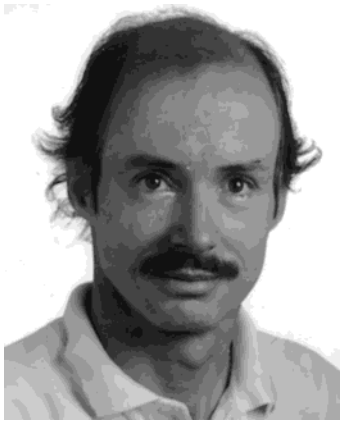


Govindasamy Mugesh, born in 1970 in Tamilnadu, India, received his B.S. (1990) and M.S. (1993) degrees in Chemistry from the University of Madras and Bharathidasan University, respectively. He obtained his Ph.D. (1998) at the Indian Institute of Technology, Bombay, India, under the supervision of Prof. Harkesh B. Singh. He subsequently became Research Associate and continued his research work in the laboratory of Prof. Singh on organochalcogen (S, Se, Te) compounds. He is currently working with the group of Prof. W.-W. du Mont at the Institute of Inorganic and Analytical Chemistry, Technical University of Braunschweig, Germany, as an Alexander von Humboldt Fellow. His research interests include development of new molecular systems for the synthesis of unstable organometallic and biologically interesting compounds.



Wolf-Walther du Mont was born in 1945 in Celle, Germany, and spent his childhood in Würzburg, Germany. He received a Diploma in Chemistry from the University of Würzburg in 1971 and his Ph.D. degree in Inorganic Chemistry from the Technical University of Berlin under the direction of Prof. Dr. Herbert Schumann. After receiving a habilitation grant of the Deutsche Forschungsgemeinschaft in 1977, he was appointed as Assistant Professor at the Institute of Inorganic and Analytical Chemistry, Technical University of Berlin. During this period, he worked on ^{119}Sn Mössbauer spectroscopy with Prof. J. J. Zuckerman, Albany, NY, and Norman, OK (1976 and 1980). In 1981, he joined the Department of Chemistry, University of Oldenburg as Professor and spent 10 years in Oldenburg. Since 1991, he is Professor of Inorganic and Analytical Chemistry at the Technical University of Braunschweig. His current research interests in main group chemistry include organic and supramolecular chemistry of the semiconductor elements and biologically important organoselenium compounds.

are capable of simulating catalytic functions demonstrated by natural enzymes. For example, ebselen [2-phenyl-1,2-benzisoselenazol-3(2*H*)-one] has been shown to act as a GPx mimic and as a scavenger of peroxynitrite.[43−45] Certain photoactive organoselenium compounds have been used as sensitizers in photodynamic therapy (PDT).[46−48] PDT is a promising approach to the treatment of cancer in which a tumor-specific dye is irradiated to produce a cytotoxic

Helmut Sies, born in 1942 in Goslar, Germany, is Professor and Chairman, Department of Physiological Chemistry I, at the Faculty of Medicine, Heinrich-Heine University at Düsseldorf, Germany, since 1979. After studying medicine at Tübingen, Paris, and Munich (M.D., 1967), he received his Habilitation for Physiological Chemistry and Physical Biochemistry at the University of Munich in 1972 and an Honorary Ph.D. from the University of Buenos Aires, Argentina, in 1996. He worked with Britton Chance at the Johnson Research Foundation (Philadelphia, 1969–1970) and was Visiting Professor at the University of California at Berkeley, Department of Biochemistry (Bruce Ames, 1984–1985) and Department of Molecular and Cell Biology as Miller Visiting Professor (Lester Packer, 1992), and at the Heart Research Institute, Sydney (Roland Stocker, 1993). He was President of the Society for Free Radical Research (International) (1998–2000). He was also an Adjunct Professor of Molecular Pharmacology and Toxicology, University of Southern California, Los Angeles (2000). His research interests in biological oxidations include oxidative stress, oxidants, and antioxidants (glutathione, tocopherols, carotenoids, flavonoids, peroxynitrite, and selenium).

species or reaction in or around the cancer cell. Therefore, the design and synthesis of organoselenium compounds with biological activity currently constitute engaging fundamental problems in applied chemistry in both pharmaceutical and academic laboratories. In this review, we would like to give a comprehensive coverage of the above-mentioned aspects of organoselenium compounds.

II. Scope and Limitations

This review covers the scientific literature (but no patent literature) from 1990 to the present, but includes a few significant earlier references where necessary for discussion. The chemistry of selenium compounds is not included if the compounds do not play a crucial role in biologically relevant processes. For example, the application of chiral and achiral organoselenium compounds in organic synthesis has been reviewed by several research groups[49−56] and therefore such types of selenium derivatives are not included in this review. In addition to the biological activities, the synthetic methodologies for some important classes of compounds are described. In a few cases, comparison of the biological activity of organoselenium compounds with their sulfur analogues will be provided.

III. Antioxidants and Antioxidant Defense Enzymes

Aerobic organisms, which derive their energy from the reduction of oxygen, are susceptible to the damaging actions of the small amounts of $O_2^{-\bullet}$, $^{\bullet}OH$, and H_2O_2 that inevitably form during the metabolism of oxygen, especially in the reduction of oxygen by

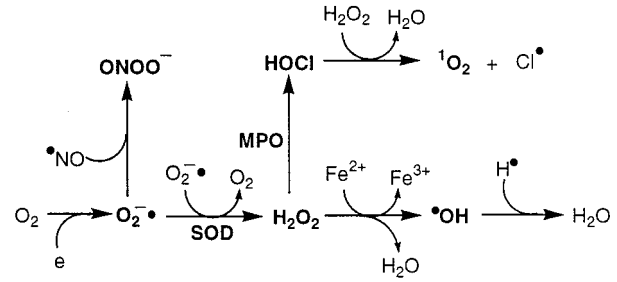


Figure 1.

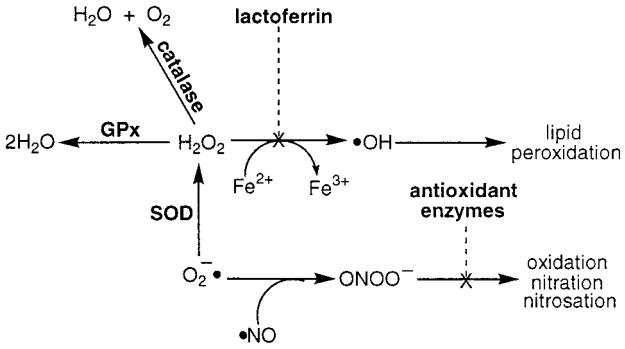


Figure 2.

the electron transfer system of mitochondria. This condition is normally referred to as "oxidative stress".[57,58] The above-mentioned three species, together with unstable intermediates in the peroxidation of lipids, are referred to as reactive oxygen species (ROS).[59] In addition to these species, hypochlorous acid (HOCl), which is generated from H_2O_2 by myeloperoxidase (MPO) in neutrophils, and peroxynitrite ($ONOO^-$), which is generated from superoxide ($O_2^{-\bullet}$) and nitric oxide ($^\bullet NO$), can also be considered as strong biological oxidants (Figure 1). It should be noted that singlet oxygen ($^1O_2$) and the nonradical excited states of oxygen atoms in organic compounds, such as excited carbonyls and dioxetanes, fall into the category of ROS related to oxidative stress.[58] Many diseases such as Alzheimer's disease, myocardial infarction, atherosclerosis, Parkinson's disease, autoimmune diseases, radiation injury, emphysema, and sunburn are linked to damage from ROS as a result of an imbalance between radical-generating and radical-scavenging systems.

Mammalian cells possess elaborate defense mechanisms to detoxify radicals (Figure 2).[57,58,60] The key metabolic steps are superoxide dismutase (SOD)[61] catalysis of the dismutation of superoxide to H_2O_2 and O_2 and the reduction of H_2O_2 to H_2O by glutathione peroxidase[62,63] (GPx) or to $O_2 + H_2O$ by catalase. Since the reaction catalyzed by GPx requires glutathione (GSH) as substrate, the concentration of this reactant is important to ROS detoxification. Similarly, some redox-active metals, such as iron, catalyze the formation of some ROS. This is minimized by keeping the concentrations of these metal ions very low by binding to storage and transport proteins (e.g., ferritin, transferrin, lactoferrin), thereby minimizing $^\bullet OH$ formation. A number of synthetic organoselenium compounds are known to act as antioxidants by reducing H_2O_2 and $ONOO^-$ and also by preventing lipid peroxidation.

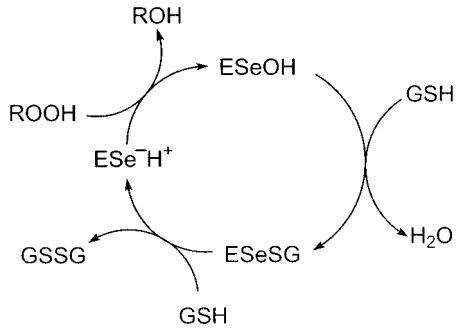


Figure 3.

A. Reduction of Hydroperoxides—GPx Mimics

Glutathione peroxidases (GPx) are antioxidant selenoenzymes protecting various organisms from oxidative stresses by catalyzing the reduction of hydroperoxides at the expense of GSH.[64,65] The GPx superfamily contains four types of enzymes, the classical cytosolic GPx (cGPx), phospholipid hydroperoxide GPx (PHGPx), plasma GPx (pGPx), and gastrointestinal GPx (giGPx), all of which require selenium in their active sites for the catalytic activity.[66−70] The reactivity of these enzymes differs considerably depending upon the hydroperoxides and thiol cofactor. The classical GPx utilizes exclusively GSH as reducing substrate for the reduction of H_2O_2 and a limited number of organic hydroperoxides such as cumene hydroperoxide and *tert*-butyl hydroperoxide. The PHGPx also uses GSH as physiological reducing substrate, but the hydroperoxide substrate specificity is more broad. This enzyme is active on all phospholipid hydroperoxides, fatty acid hydroperoxides, cumene hydroperoxide, *tert*-butyl hydroperoxide, cholesterol hydroperoxides, and H_2O_2.[71] On the other hand, the hydroperoxide substrate specificity of pGPx is more restricted. Although pGPx can reduce H_2O_2 and organic hydroperoxides, it is approximately 10 times less active than the cGPx. In contrast to the cGPx, GSH is a poor reducing substrate for this enzyme. Since the concentration of reduced thiol groups in human plasma is very low, it is quite unlikely that GSH is the reducing substrate for the plasma enzyme. Alternatively, the extracellular thioredoxin reductase, thioredoxin, or glutaredoxin could be reasonable candidates.[72] The catalytic cycle of GPx involves three major steps, as shown in Figure 3.

Since the discovery that ebselen (**1**) mimics the action of GPx,[43,44] several groups have worked toward the design and synthesis of new GPx mimics by modifying the basic structure of ebselen or by incorporating some structural features of the native enzyme. Ebselen is a nontoxic compound at pharmacologically active concentrations, because its selenium is not bioavailable. It is mostly bound to proteins in the form of selenenyl sulfide[44,73] and it is metabolized predominantly into glucuronidated metabolites.[74−76] Another important feature of ebselen is its inability to oxidize GSH in the presence of oxygen, which normally leads to the uncontrolled production of superoxide and other free radical species.[77]

The catalytic cycle of ebselen has been controversial probably due to major differences in working condi-

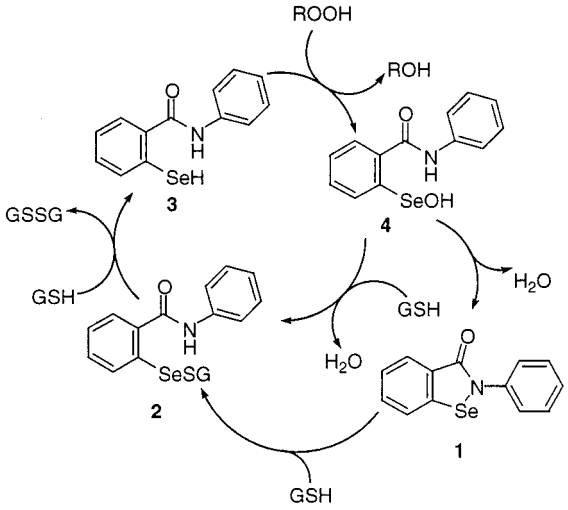


Figure 4.

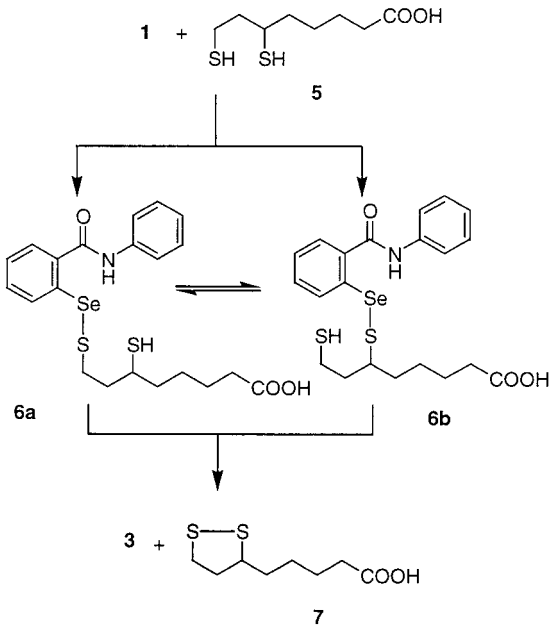


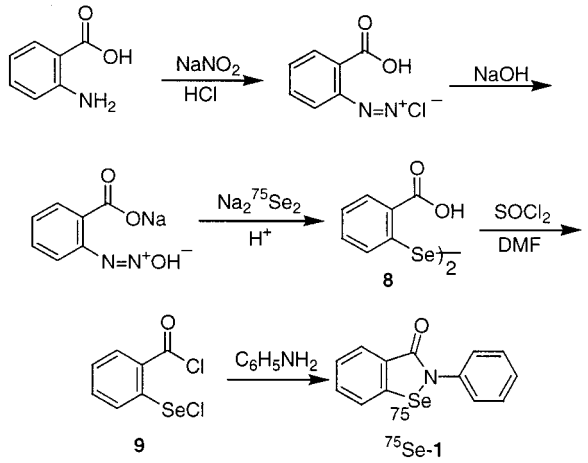
Figure 5.

tions such as solvents, pH, and the nature of hydroperoxides that were used by many research groups to characterize the potential catalytic intermediates.[78−81] Although several mechanisms have been proposed to explain the observed GPx activity of ebselen, the available information reveals a hypothetical catalytic cycle as shown in Figure 4. According to this cycle, ebselen reacts rapidly with GSH to produce the selenenyl sulfide **2**. Compound **2** reacts with a second equivalent of GSH to yield a single product that is characterized as a selenol (**3**). It has been proved independently by Maiorino et al.,[82] Morgenstern et al.,[83] and Cotgreave et al.,[84] that the selenol is the predominant molecular species responsible for the GPx activity of ebselen.

The rate of selenol formation is increased in the presence of dithiols compared to GSH. For example, the replacement of GSH by dihydrolipoate (**5**) improves the peroxidase activity of ebselen (Figure 5).[85] A comparison of the kinetic parameters of ebselen catalysis in the presence of GSH and dihydrolipoate suggests that the selenol formation is not rate-limiting in the presence of the dithiol. The most likely explanation for this is the availability of the second intramolecular nucleophilic thiol group in the vicinity of the electrophilic sulfur atom attached to the selenium in the selenenyl sulfide (**6**).[86]

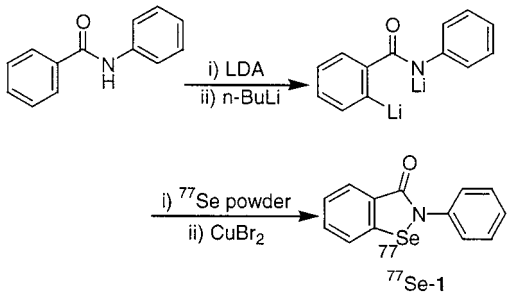
Although ebselen is a major GPx mimic, its synthesis has been a challenging area[87−92] since it was first prepared in 1924 by Lesser and Weiss.[93] In the earliest and most direct approach, 2,2′-diselenobis(benzoic acid) was converted to a selenenyl chloride benzoyl chloride, which was treated with aniline to give ebselen.[89] The most expedient method was reported by Engman et al. and involves ortho-lithiation of benzanilide, selenium insertion, and oxidative cyclization reactions.[90] A free-radical synthesis of ebselen has been achieved by intramolecular homolytic substitution with amidyl radicals.[91,92] The syntheses of ^{75}Se- and ^{77}Se-labeled ebselen have also been reported. In the first case, the ^{75}Se-2,2′-diselenobis(benzoic acid) **8** was initially prepared as the key intermediate that was transformed into a corresponding dichloride **9** before treating with aniline to yield the desired ^{75}Se-ebselen (Scheme 1).[94]

Scheme 1



In the second case, the ^{77}Se-ebselen was prepared in one pot from commercially available benzanilide and enriched elemental ^{77}Se in 76% yield (Scheme 2). The ortho-lithiation of benzanilide with LDA and

Scheme 2



n-BuLi, followed by insertion of ^{77}Se-enriched selenium and $CuBr_2$-mediated cyclization gave the ^{77}Se-ebselen.[95]

The reactivity of ebselen can be altered by modifying the basic structure of ebselen based on substituent effects and isosteric replacements. Substitution of a hydrogen atom with a nitro group in the ortho-

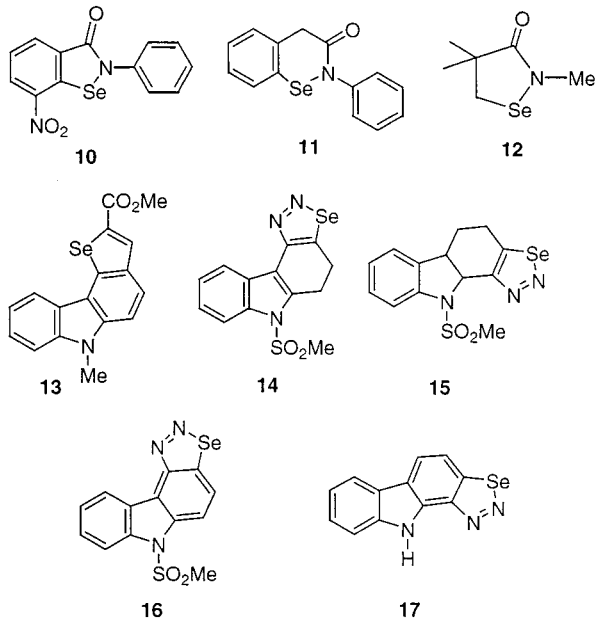


Figure 6.

Table 1. GPx Activity of Compounds 13−17[99]

compound	GPx activity[a]	compound	GPx activity[a]
13	104	**16**	46
14	140	**17**	51
15	246		

[a] Micromole of NADPH oxidized per minute in the presence of 10^{-2} M catalyst.

position to selenium (**10**, Figure 6) has been shown to increase the GPx activity of ebselen.[96] The pharmacological differences can be attributed to the influence of the electronic effects that the nitro substitution causes upon interaction with selenium. The incorporation of a supplementary tetrahedral carbon (−CH_2− group) into the heterocycle led to compound **11**.[97] This compound preserves (i) a Se−C(aromatic) bond to avoid selenium release and to maintain the low toxicity of ebselen, (ii) an Se−N bond, which is responsible for the GPx activity, and (iii) a N−C═O bond to stabilize the selenenamide structure. The selenenamide **12**, without any aromatic substituents, has also been used as a model system for studying the redox chemistry of selenocysteine in GPx.[98] Another good illustration of this heterocycle modification is observed in a recent series of antitumor alkaloid ellipticin analogues (**13**−**17**, Figure 6).[99] The GPx activity of **16** and **17** are lower than that of **14** and **15,** which indicates that the aromatization of the C-cycle is an inhibitory factor for the antioxidant activity of this series of compounds (Table 1).

Other GPx mimics with a direct Se−N bond but without a carbonyl group in the five-membered ring have been synthesized and studied for their activity.[100] Although selenazoline **18** (Figure 7) exhibited moderate GPx activity, compounds **19**−**21**, in which the nitrogen of compound **18** has been further substituted, exhibited much lower activity compared with that of the parent compound **18**. The *N*-acetyl derivative (**20**) was less active than the *N*-ethyl derivative (**19**), due to an attenuation of the nucleophilic character of nitrogen in **19** by the carbonyl group (Table 2).[101]

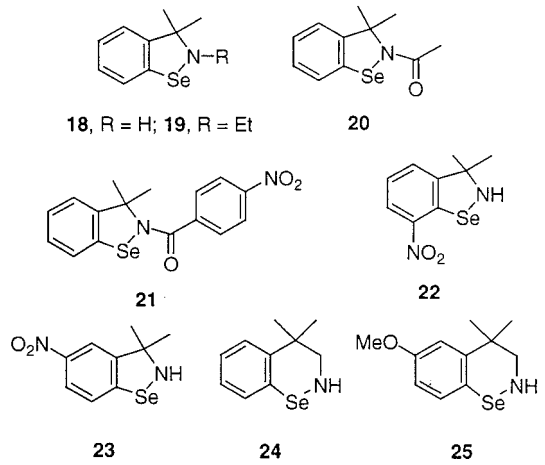


Figure 7.

Table 2. GPx Activity of Compound 18−25[101]

compound	GPx activity[a]	compound	GPx activity[a]
18	8	**22**	5
19	3	**23**	3
20	1	**24**	27
21	>1	**25**	27

[a] Nanomole of NADPH oxidized per minute in the presence of 20 μM catalyst and 2 mM GSH.

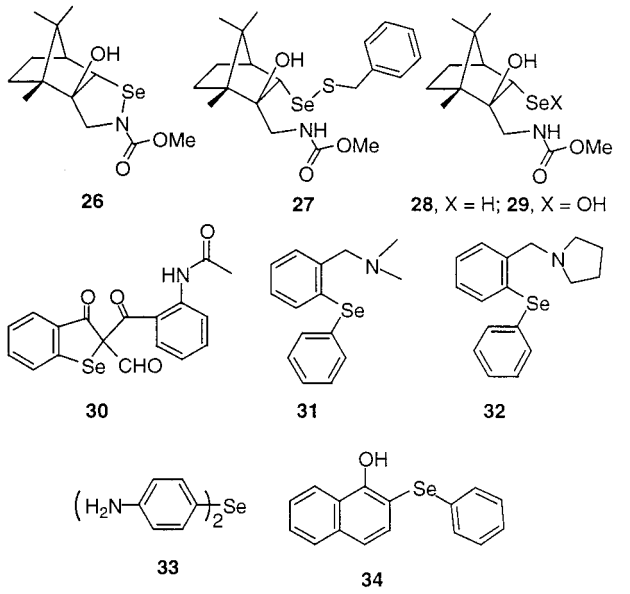


Figure 8.

In contrast to the observation that the introduction of a nitro group in ebselen strongly increases the GPx activity,[96] the introduction of a nitro group in the ortho- or para-position of compound **18** lowered the GPx activity of the parent compound. Compound **24**, containing a six-membered heterocycle, was found to be much more active than the parent compound.[101] Substitution of an electron-donating group in compound **24** did not enhance the activity, as the GPx activity of **25** containing a *p*-methoxy substituent is identical to that of **24**. The camphor-derived cyclic selenenamide **26** (Figure 8) also exhibits GPx-like activity by acting as a procatalyst.[102]

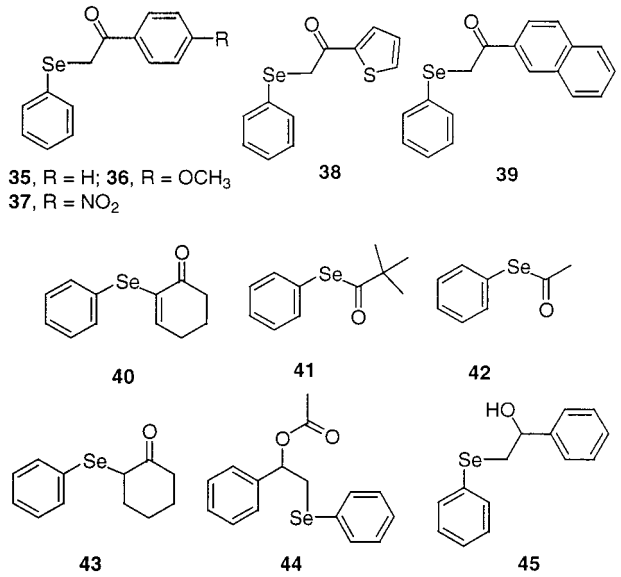


Figure 9.

Table 3. GPx Activity of Compounds 35−45[106]

compound	GPx activity[a]	compound	GPx activity[a]
ebselen (**1**)	70 ± 5	**40**	30 ± 2
35	103 ± 6	**41**	20 ± 1
36	25 ± 2	**42**	20 ± 2
37	157 ± 5	**43**	14 ± 1
38	75 ± 3	**44**	35 ± 3
39	94 ± 4	**45**	13 ± 1

[a] Nanomole of NADPH oxidized per minute using 50 μM catalyst and 1 mM GSH.

Compound **26** functions by reaction with thiol to afford the true catalyst selenenyl sulfide **27**, which undergoes further attack by the thiol to produce selenol **28**. The selenol reduces H_2O_2 to H_2O and forms a selenenic acid **29**, which in turn reacts with additional thiol to regenerate the selenenyl sulfide **27**. In other studies aimed at designing GPx mimics without a Se−N bond, the cyclic compound **30** and open-chain compounds **31**−**34** were tested.[103−105] While compound **30** is only 0.033 times as active as ebselen, the diaryl selenides (**31**−**34**) do not exhibit catalytic activity. However, compounds with a Se−C bond do exhibit significant GPx activity, if the Se−C bond is easily cleaved by GSH. For example, some α-(phenylseleno)ketones and derivatives **35**−**45** exhibit GPx activity by reacting with GSH to produce catalytically active species.[106] All these mimics (**35**−**45**) exert their catalytic activity through a common selenolate $PhSe^-$ intermediate.[107] An electron-withdrawing substituent in the acetophenone moiety (**37**) increases the potency of the catalyst, whereas an electron-donating substituent (**36**) decreases the catalytic activity of the parent compound. Similarly, substitution of the acetophenone aryl group with alkyl groups (**41**, **42**) or acetylation/reduction of the carbonyl group (**44**, **45**) causes a decrease in the catalytic activity of the compounds (Figure 9, Table 3).

The observation that diphenyl diselenide (**46**, Figure 10) exhibits moderate GPx activity and low GSH oxidase activity led to the development of compounds with a −Se−Se− bond.[103] In the presence of a 10-fold excess of GSH, the −Se−Se− bond in **46** is

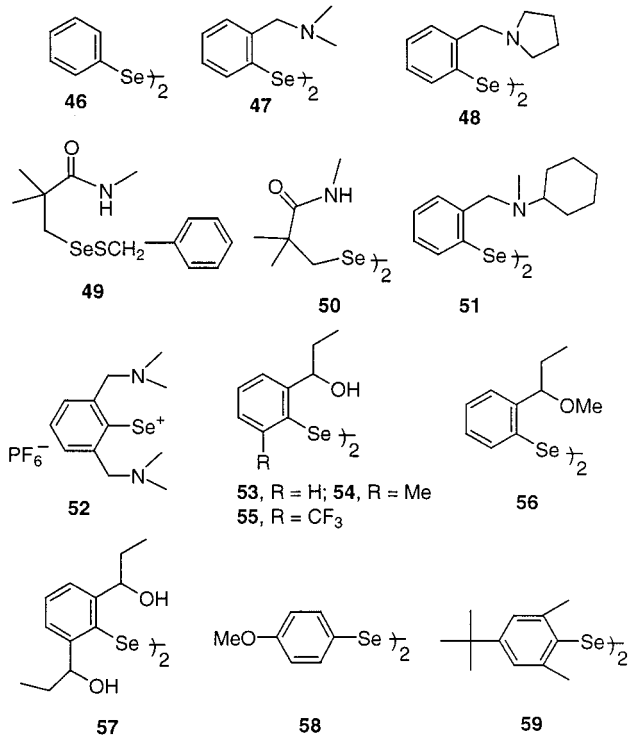


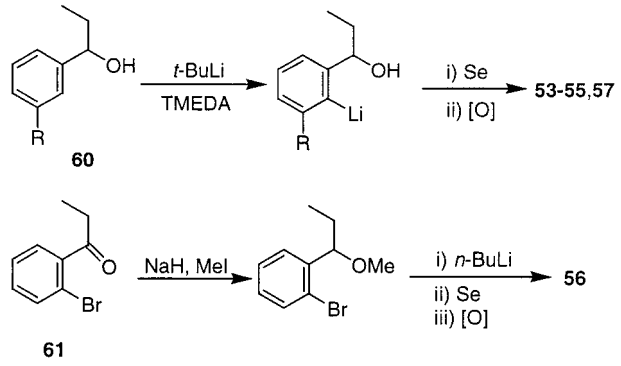
Figure 10.

reduced to form selenol (PhSeH) as the predominant species, which reacts very rapidly with 0.5 equiv of *t*-BuOOH.[101] The reduction of diselenide bond in **46** by GSH may not be a facile reaction, since the redox potential of GSH ($E_0 = -240$ mV) is much higher than that of the −Se−Se− bond ($E_0 = -380$ mV).[108] In general, diselenides were found to be reduced by strongly reducing dithiols such as DTT in aqueous solution at pH 7.6,[109] whereas monothiols cannot reduce diselenides to any significant extent.[110] It has been reported that even $(Sec)_2$-peptides can be only partially reduced with a large excess of GSH. However, this partial reduction by GSH is sufficient to maintain in living cells a concentration of Sec suitable for its incorporation into proteins. For example, the selenium analogue of thioredoxin was efficiently expressed in Cys-auxotrophic *Escherichia coli*. In this case, the $(Sec)_2$ has to be reduced at least to some extent that allows tRNA to charge with Sec.[111]

Further developments have started in accordance with the finding that the active site of GPx may involve in some interactions with other amino acid residues that would alter enzyme activity. The observations by Epp et al.[112] and Maiorino et al.[66] that the catalytically active selenocysteine residue in GPx, which is located at the N-terminal end of helix α_1, may be stabilized by nearby amino acid residues and the findings by Hilvert et al that the GPx activity of semisynthetic enzyme selenosubtilisin is modulated by basic histidine residues (section VIII, Figure 62)[113] led to the development of diorgano diselenides containing heteroatoms in close proximity to the selenium. The first interesting results, having come from Wilson's group, show that the protonated derivatives of diselenides **47** and **48**, each of which possesses a basic amino group near the selenium, exhibit strong GPx activity.[103] The positive effect of amino groups

has been proved by model studies that suggest that the basic amino nitrogen (i) activates the Se−Se bond toward an oxidative cleavage, (ii) activates the selenol intermediate into kinetically more active selenolate, and (iii) stabilizes the selenenic acid form of the catalyst against further oxidation.[114] These results, when combined with the observations of Reich et al. that the selenenyl sulfide (**49**) and diselenide (**50**) derived from selenenamide **12** react with thiol only in the presence of a strong base,[98] provided a solid basis for the importance of ortho-chelating groups. According to these observations, compounds **51** and **52**, having Se···N interactions,[114,115] and **53**−**57**, having Se···O interactions,[116] have been developed. Compound **58**, containing an electron-donating substituent (OMe group) in the para-position, showed high GPx activity. In contrast, compound **59**, containing an electron-donating substituent (*tert*-butyl group), did not show any noticeable activity.[117] However, the exact role of electron-donating or -withdrawing substituents in the diselenides remains to be elucidated. The diselenides containing −OH groups (**53**−**55**, **57**) can be synthesized in one step from chiral alcohol **60** by ortho-deprotonation and treatment with elemental selenium (Scheme 3). Diselenide

Scheme 3



56 is accessible from the bromo precursor, **61**, which is obtained by chiral reduction of the 2-bromo ketone and alkylation of the hydroxy group.[118,119]

The presence of amino groups in close proximity to selenium does not always play a positive role, particularly when the phenyl ring is substituted by a pyridyl ring. For example, compound **62** (Figure 11) has been reported to be an inactive compound.[101] In this particular case, the nature of the −Se−Se− bond cleavage cannot be considered for its inactivity, since the selenols **63** and **64** have also been found to be inactive compounds. Unexpectedly, the pyridine-based diselenides **65**−**67**, selones **68**−**73**, and even the pyridine analogue of ebselen (**74**) were inactive compounds at neutral pH.[101] This suggests that the electron-withdrawing effects of such aromatic structures considerably lower the nucleophilic character of selenium in the reduced intermediates.

Singh et al. recently reported that the redox-active diferrocenyl diselenides **75** and **76** (Figure 12), containing basic amino groups near the selenium, exhibit high GPx activity.[120] Since the activity of these compounds is much higher than that of the phenyl-based diselenides having amino groups and ferrocene-based diselenides having no amino groups, the enhancement in the catalytic activity could be ascribed to the synergistic effect of redox-active ferrocenyl and internally chelating amino groups. The X-ray crystallographic data of **75** and **76** indicate that these compounds do not have any significant Se···N interactions in the solid state, as the observed Se···N distances of 3.697 and 4.296 Å for **75** and 3.98 and 4.12 Å for **76** are greater than the sum of their van der Waals radii (3.54 Å).[117,121] On the other hand, compounds **77**−**79**, having strong Se···N interactions, showed negligible GPx activity under similar experimental conditions.[120] The difference in the activity between the two series of compounds has been shown to correlate with the nature of Se···N interaction in each intermediate of the catalytic cycle.[117]

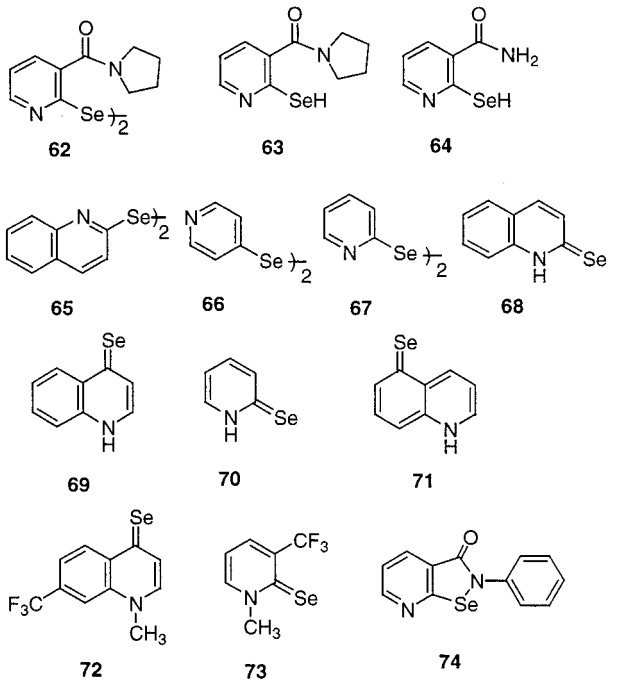


Figure 11.

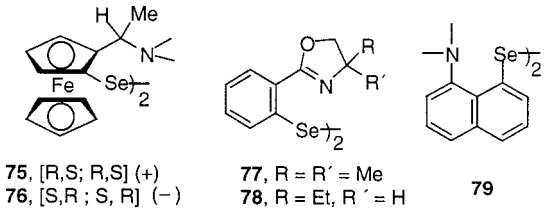
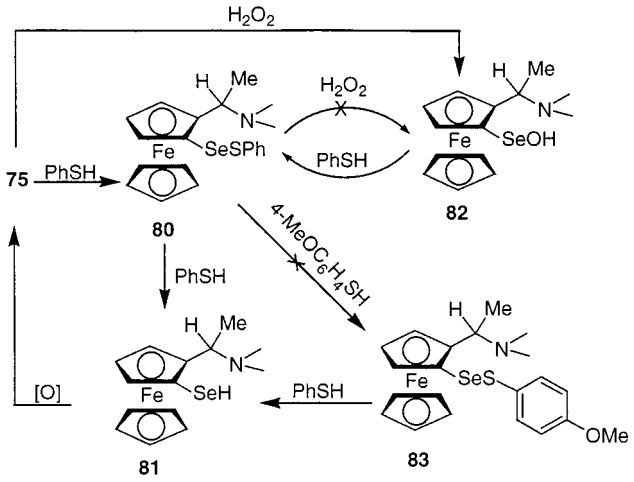


Figure 12.

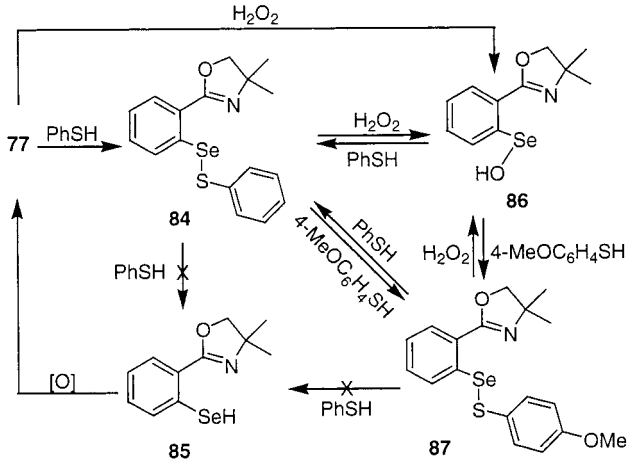
As proved by ^{77}Se NMR studies, compounds **75** and **76** have a catalytic cycle (Scheme 4) similar to the one proposed for natural GPx.[117] While the proximal nitrogen base stabilizes and activates the selenol **81** and selenenic acid **82**, the nitrogen atom does not interact with selenium in the selenenyl sulfide state **80**. On the other hand, the imino nitrogen in compound **77** interacts with selenium in all three intermediates, i.e., selenol **85**, selenium acid **86**, and selenenyl sulfide **84**, thus complicating the catalytic pathway. The strong Se···N interaction in the selenenyl sulfide **84** is expected to be the major factor for the inactivity of **77**, since this leads to a thiol exchange by increasing the electrophilic reactivity of

Scheme 4



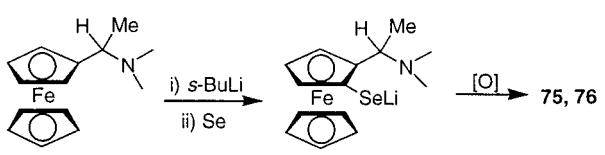
the selenium.[114] Addition of 4-methoxybenzenethiol to the solution of **84** produced a new selenenyl sulfide (**87**, Scheme 5), which was not the case with **80** (Scheme 4).[117]

Scheme 5



The Se···N interaction in the selenenyl sulfide state may not be very strong with GSH, due to the bulky nature of the glutathionyl moiety. A careful analysis of the crystal structure of GPx[112,122] and the molecular modeling of the intermediates[123] reveals that an interaction between sulfur and nitrogen instead of selenium and nitrogen in the selenenyl sulfide state may possibly enhance the regeneration of selenols.[124] The chiral ferrocenyl diselenides **75** and **76** could be synthesized by diastereoselective lithiation of commercial (*R*)- and (*S*)-[1-(dimethylamino)ethyl]ferrocene **(88**, **89)** with *s*-BuLi, followed by addition of elemental selenium and air oxidation. (Scheme 6).[121]

Scheme 6



88, (R) (+)
89, (S) (-)

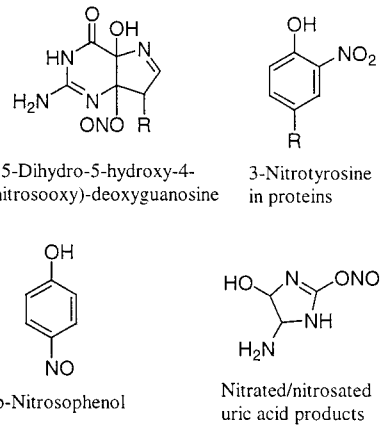


Figure 13.

B. Reduction of Peroxynitrite

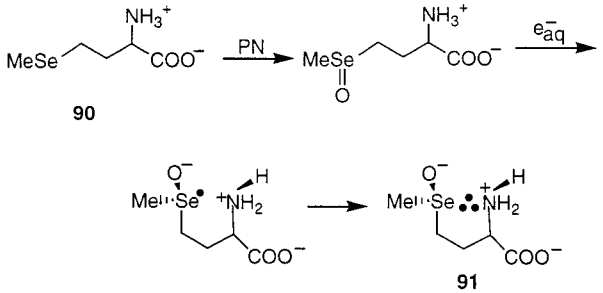
Beckman et al.[125] suggested that two relatively unreactive but biologically important free radicals, superoxide and nitric oxide, would combine under physiological conditions to form peroxynitrite (PN) (eq 1).[126,127]

$$O_2^{-\bullet} + {}^{\bullet}NO \rightarrow {}^{-}OON{=}O \qquad (1)$$

PN is considered a strong biological oxidant that induces DNA damage and initiates lipid peroxidation in biomembranes or low-density lipoproteins. PN inactivates a variety of enzymes by oxidation, nitration, and nitrosation reactions.[128] A few examples of products formed by oxidizing and nitrating/nitrosating reactions of PN are given in Figure 13.[129] Peroxynitrite also plays a role in activating signal transduction pathways capable of modulating gene expression.[130] Selenoproteins such as GPx[131] or selenoprotein P[132] have been reported to reduce PN. These protective effects rely upon reactions operating in a catalytic way.[129]

Selenomethionine (**90**) protects against PN more effectively than its sulfur analogue, methionine.[133] The oxidized selenomethionine (methionine selenoxide) is effectively and rapidly reduced to **90** by GSH, permitting a catalytic action by selenomethionyl residues in proteins.[134] The reduction of methionine selenoxide by GSH involves the formation of an Se∴N transient species **91** (Scheme 7), which plays a key role in the overall reduction process.[135]

Scheme 7



The inhibition of superoxide and nitric oxide release by ebselen in rat Kupffer cells sparked interest

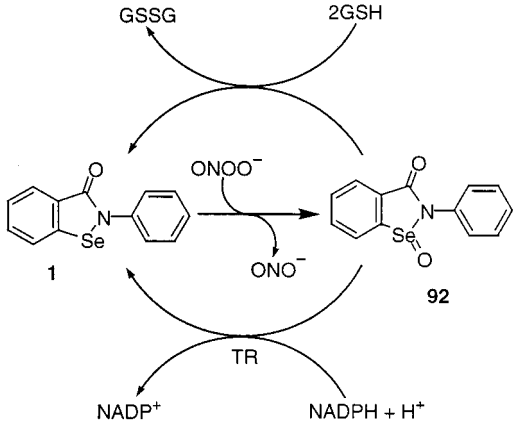


Figure 14.

in other synthetic organoselenium compounds as to their potential function in scavenging PN.[136] Ebselen protected DNA from single-strand break formation caused by peroxynitrite more effectively than its sulfur analogue.[137] Ebselen reacts with PN to produce ebselen selenoxide (**92**), and the rate of the reaction is about 3 orders of magnitude faster than that of biologically occurring small molecules, such as ascorbate, cysteine, and methionine.[138] Similar to the GPx reaction, redox shuttling of the selenium can be maintained with glutathione (GSH). However, in contrast to the GPx, other thiols such as DTT can be used as a thiol cofactor for the peroxynitrite reductase activity. The mammalian thioredoxin reductase (TR) has also been shown to reduce the selenoxide to ebselen (Figure 14).[139]

Compound **93**, which has been thought for long time to be an inert metabolite of ebselen, reacted with PN much faster than with H_2O_2.[140] Since radical scavengers and metal chelators do not affect the oxidation of **93**, metal and radical species are not involved in the reaction. In vitro and ex vivo studies on tyrosine nitration of prostacyclin (PGI_2) synthase by PN revealed that not only ebselen but also the selenol **3**, selenenyl sulfide **2**, and diselenide **94** may function as antioxidants in cells.[141] Similar to the reaction of selenocysteine, the selenol reacted with PN to produce selenenic acid, which could be reduced to selenol by GSH or by TR and NADPH.[139]

Phenylaminoethyl selenides (**95**−**101**, Figure 15) possessing antihypertensive activity in spontaneously hypertensive rats have been shown to play a protective role in the defense against PN.[142] As in the case of ebselen, these compounds are oxidized to the corresponding selenoxides (**102**−**108**) by PN, and catalytic cycles can be exerted by using ascorbate as reducing equivalents (Figure 15). While the substitution on the alkyl side chain had no effect on the rate of oxidation of **95**, the change in the para-substituent on the aromatic ring significantly affected the rate (Table 4). Compounds **99** and **100,** with electron-withdrawing substituents, were less active compared with **97** and **101,** having electron-donating substituents. This indicates that the reaction rate increases with the nucleophilic nature of the selenides. This substituent effect is consistent with a bimolecular nucleophilic displacement (S_N2) mechanism, which

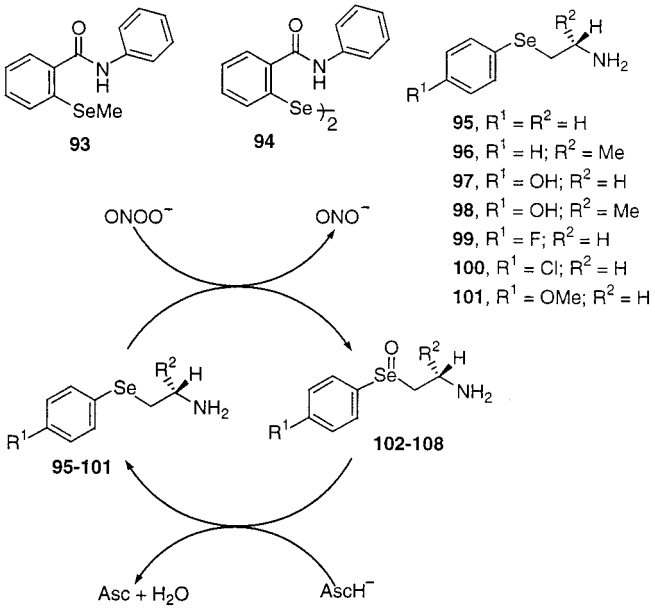


Figure 15.

Table 4. Second-Order Rate Constants for the PN-Mediated Oxidation of Selenium Compounds

compound	k_{ONOO^-} (M^{-1} s^{-1})	ref
ebselen (**1**)	$(2.0 \pm 0.1) \times 10^6$	138
90	$(2.4 \pm 0.1) \times 10^3$	140
93	2.7×10^3	143
95	$(1.8 \pm 0.01) \times 10^3$	142
96	$(1.6 \pm 0.01) \times 10^3$	142
97	$(3.0 \pm 0.02) \times 10^3$	142
98	$(3.0 \pm 0.02) \times 10^3$	142
99	$(1.1 \pm 0.01) \times 10^3$	142
100	$(0.9 \pm 0.01) \times 10^3$	142
101	$(2.3 \pm 0.02) \times 10^3$	142

involves an initial nucleophilic attack by the selenium atom on the oxygen of PN.[143]

In addition to these compounds, the water-soluble alkyl aryl selenide (**109**)[144] (Figure 16) and diaryl selenide (**33**)[145] also reduced PN, as proved by model studies on the PN-mediated nitration of 4-hydroxyphenyl acetate and oxidation of dihydrorhodamine 123 (**112**) (Figure 16). The mechanism of this reduction involves the oxidation of **33** and **109** to the corresponding selenoxides **110** and **111**, respectively.

The defensive effects of water-soluble compounds **113** and **114** against PN-mediated oxidation and nitration reactions of L-tyrosine have been reported (Figure 17).[146] These compounds effectively inhibited the formation of 3-nitro-L-tyrosine with IC_{50} values of 1.53 and 0.50 μM, respectively. These compounds also reduced the formation of 2,2′-dityrosine with IC_{50} values of 0.15 μM (**113**) and 0.37 μM (**114**).[146] Although compounds **113** and **114** inhibit both oxidation and nitration reactions, the mechanisms of their action may differ for these two reactions. It has been proposed that 2,2′-dityrosine (oxidation reaction) is formed from the dimerization of tyrosyl radicals derived by a caged radical like [ONO•···•OH], and that 3-nitrotyrosine (nitration reaction) is formed via a nonradical pathway, that is, electrophilic addition of nitronium cation, which may exist in a caged dipolar form such as [ONO^+···^-OH].[147]

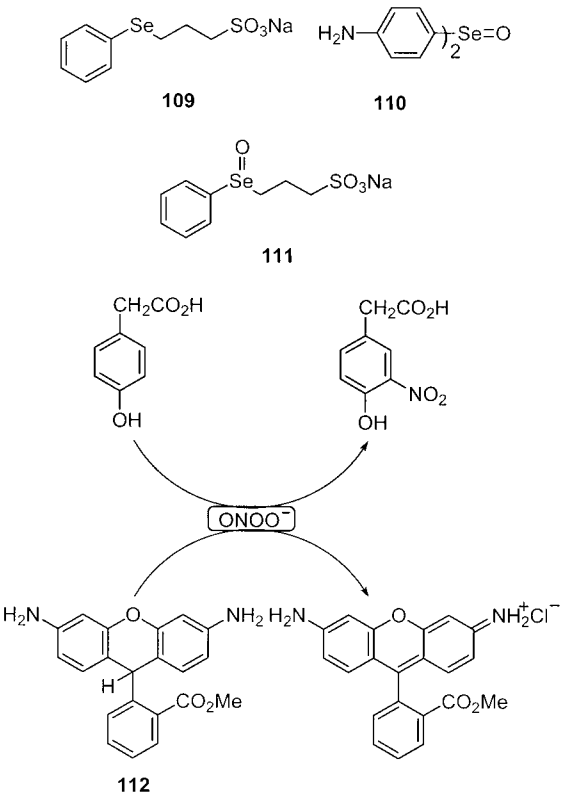


Figure 16.

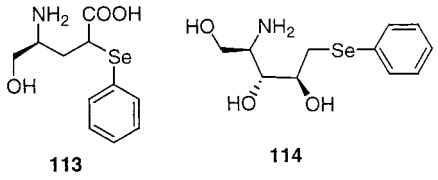


Figure 17.

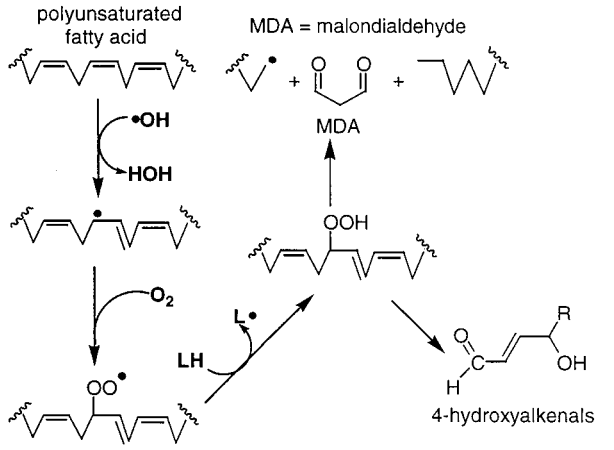


Figure 18.

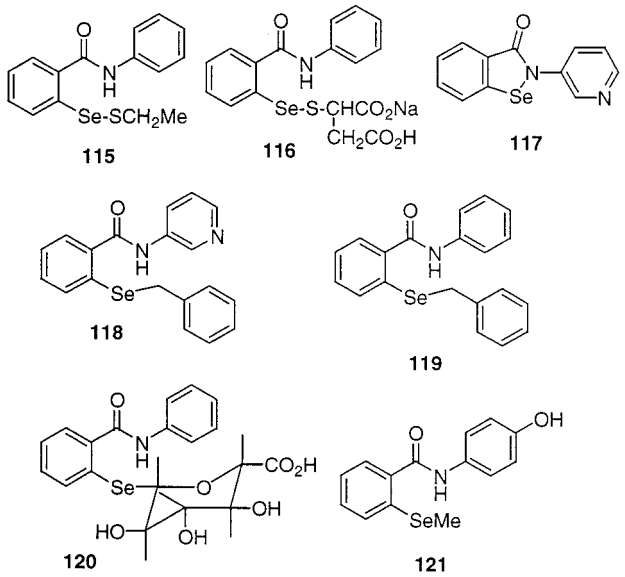


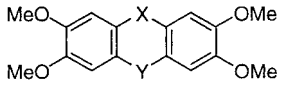
Figure 19.

C. Lipid Peroxidation

Reactive oxygen species can react with all biological macromolecules (lipids, proteins, nucleic acids, and carbohydrates). The initial reaction generates a second radical, which in turn can react with a second macromolecule to maintain a chain reaction. Polyunsaturated fatty acids are particularly susceptible targets. Abstraction of a hydrogen atom from a polyunsaturated fatty acid initiates the process of lipid peroxidation (Figure 18). In the third step of Figure 18, a hydrogen atom is abstracted from a second lipid, leading to a new ROS. Numerous lipid peroxidation products are formed that can react with sulfhydryl (cysteine) or basic amino acids (histidine, lysine).[58,148]

Ebselen and related derivatives have been shown to protect against lipid peroxidation induced by transition metals, e.g. iron/ADP-induced lipid peroxidation in microsomes[43] and by methyl linoleate.[149] This type of lipid peroxidation is brought about by a Fenton-type reaction of the metal ion with traces of hydroperoxides. In intact cells, the protective effect of ebselen depends on the presence of GSH, as the ebselen does not provide any protection in GSH-depleted hepatocytes.[150] It should be noted that under certain conditions ebselen may even stimulate lipid peroxidation.[151] On the other hand, the protection of certain forms of lipid peroxidation by ebselen does not depend on the presence of GSH, indicating that the hydroperoxide reducing action rather than the GPx-like activity is responsible for the protection. GSH is, however, required in such in vitro systems for the regeneration of ebselen from ebselen selenoxide.

Ebselen **1** and analogues **115**−**121** (Figure 19) have been studied for their protective effects against iron/ADP/ascorbate-induced lipid peroxidation.[152] It was observed that ebselen (**1**) has the highest antioxidant capacity by affording protection at very low concentration. Compounds **2**, **92**, and **115**−**117** exhibited protective activities comparable to that of ebselen. The activity of the sulfur analogue of ebselen was 15-fold lower than that of ebselen. The Se-benzylated form **118** was much less reactive than ebselen, but this compound was about 2-fold more reactive than the *N*-phenyl analogue (**119**). The Se-methylated derivative, methylselenobenzanilide, was practically inactive in preventing lipid peroxidation. However, substitution of a hydroxyl group in the para-position of the phenyl ring (**121**) enhanced the antioxidant activity. Compound **120**, 2-(glucuronylseleno)benzoic acid-*N*-phenylamine was also found to have substantial antioxidant activity.[152]



122, X = S; Y = O; **123**, X = Se; Y = O
124, X = S; Y = S; **125**, X = Se; Y = S
126, X = Se; Y = Se

Figure 20.

Table 5. A Comparison of the Half-Wave Oxidation Potentials with Their Antioxidant Activity[153]

compd	half-wave potential, $E_{1/2}$ (V)	*t*-BuOOH-induced lipid peroxidation in isolated hapatocytes	
		% inhibition	IC_{50} (μM)
122	0.87	74	12
123	0.89	51	48
124	0.98	36	>50
125	1.02	22	>50
126	1.06	20	>50

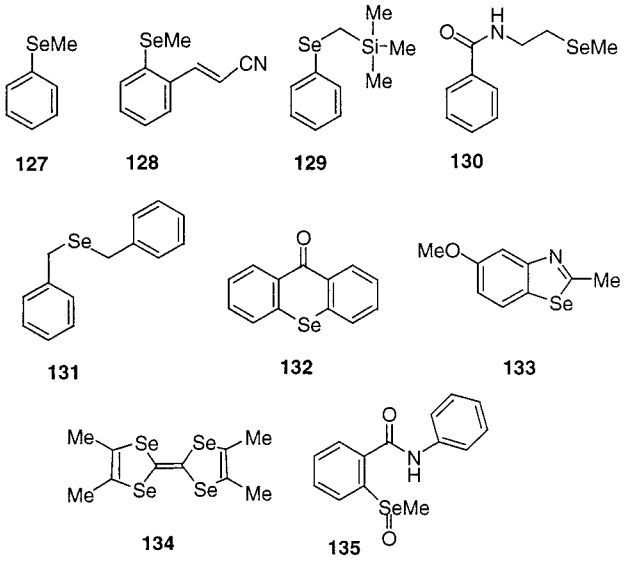


Figure 21.

The antioxidant activity of dibenzo[1,4]dichalcogenines (**122**−**126**, Figure 20) on free radical induced lipid peroxidation has been studied.[153] The antioxidant activity of this series of compounds in microsome and cell systems has been shown to correlate with their half-wave oxidation potential (Table 5). It is known that compounds with low redox potentials are good antioxidants. Generally, substitution by heavier chalcogens in the central ring leads to a lowering of the oxidation potential of the donor.[154] However, in the series of tetramethoxy-substituted tricyclic systems **122**−**126**, the lowest oxidation potential was found for an electron donor with oxygen substituents, i.e., compounds **122** and **123**. This has been explained by the increased planarity of the oxygen-containing heterocycles, allowing more efficient delocalization of the cation radical over the aromatic π electron system. It is evident from Table 5 that the compounds possessing lower potential are more potent antioxidants than those having higher oxidation potential.[154]

The oxidation of ebselen (**1**), **93**, and other organoselenium compounds such as **127**−**134** (Figure 21) catalyzed by liver microsomes and flavin-containing monooxygenase (FMO) has been extensively stud-

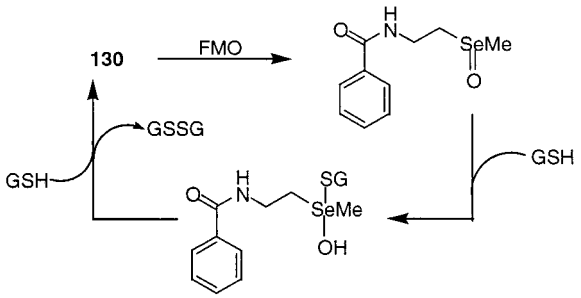


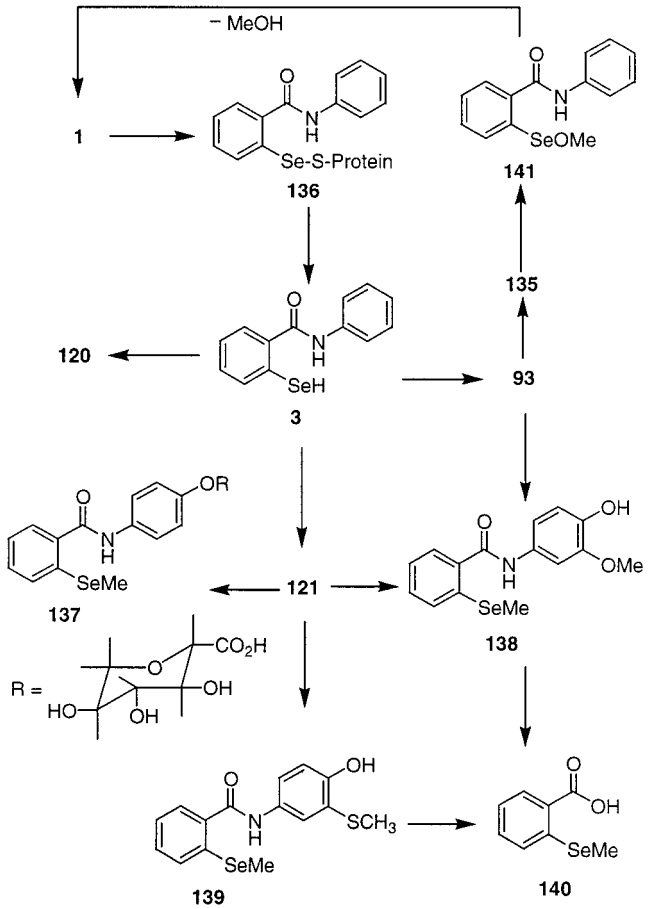
Figure 22.

ied.[74,75] In the case of ebselen, a facile ring opening of the heterocycle by GSH followed by oxidation of the resulting selenol to selenenic acid was observed.[74] Compound **93**, in which the Se−C bonds were stable under oxidative conditions, has been shown to afford the corresponding selenoxide (**135**).[75]

In both the cases, the oxidized species, i.e., the selenenic acid and selenoxide, could be reduced by GSH. While compounds **127**−**131** were readily oxidized to the corresponding selenoxides by FMO, none of the aromatic heterocyclic selenides **132**−**134** stimulated NADPH- and FMO-dependent oxygen uptake. The rapid oxidation of selenides catalyzed by microsomal monooxygenases could establish a cycle, illustrated in Figure 22, leading to the oxidation of GSH.[76]

The facile cleavage of the Se−N bond in ebselen by protein thiols not only leads to its oxidation to the selenenic acid but also to the formation of the methylated compound (**93**). The two major metabo-

Scheme 8



lites, selenol (**3**) and methylated derivative (**93**), further lead to a variety of metabolites. The metabolism of ebselen in intact rats, pigs, and man after oral administration has been studied in detail, and the biotransformation in rats is depicted in Scheme 8.[155,156] Another in vitro study on the metabolism of **93** by rat liver microsomes showed the formation of ebselen as the only major metabolite, and the mechanistic basis for the regeneration of ebselen from **93** has been shown to be an oxidative demethylation of **93** via selenoxide and selenenic ester **141**, as shown in Scheme 8.[157]

IV. Enzyme Inhibitors

Organoselenium compounds are known to inhibit a variety of enzymes such as nitric oxide synthase (NOS), inosine monophosphate dehydrogenase (IMPDH), lipoxygenases (LOX), uridine phosphorylase (UrdPase), thymidylate synthase (TMS), tyrosine kinase (TK), and iodothyronine deiodinase (ID). In addition to these enzymes, some other enzymes such as NADPH oxidase, protein kinase C (PKC), glutathione-S-transferase (GST), NADPH-cytochrome reductase, and papain are inhibited by ebselen and related derivatives. Some of these enzymes are implicated in inflammatory processes, and therefore, the inhibitory effects are expected to contribute to the antiinflammatory actions of the organoselenium compounds in vivo. It should be noted that ebselen blocks the activity of several enzymes by reacting with the critical −SH groups of the enzymes. However, when added to cells as the albumin complex it does not normally exhibit any inhibition.[158]

A. NOS Inhibitors

Nitric oxide synthases (NOSs) belong to the family of FAD- and FMN-containing cytochrome P-450-type hemoproteins that catalyze the biosynthesis of nitric oxide (•NO) from L-arginine (**142**, Figure 23).[159,160] The membrane-bound endothelial isoform (ecNOS), present in vascular endothelial cells[161] and brain,[162] are expressed constitutively, and their activity is calcium- and calmodulin-dependent. In contrast, the cytosolic, macrophage isoform is inducible (iNOS), i.e., it is expressed in cells only after their activation by cytokines and bacterial products.[163] NOS plays an important role in the regulation of vascular tone and activity of blood cells and mediates some of the cytotoxic effects of activated macrophages. However, an excessive amount of •NO produced by NOS mediates hypotension and hyporeactivity to vasoconstrictor agents in septic shock.[164]

Ebselen (**1**) and other related organoselenium compounds have been reported to be inhibitors of constitutive endothelial NOS (ecNOS). The inhibition

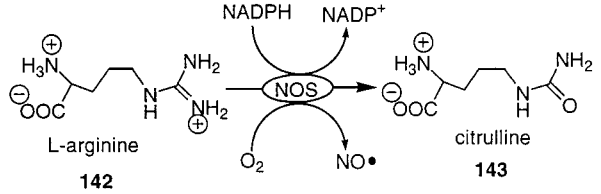


Figure 23.

Table 6. NOS Enzyme Inhibition Data for Ebselen (1)[166,167]

enzyme	species, tissue	IC_{50} (μM)
ecNOS	cell homogenate	8.5[a]
ecNOS	cell homogenate	13.0
iNOS	cytosol	2.5

[a] Inhibition prevented or reversed by glutathione.

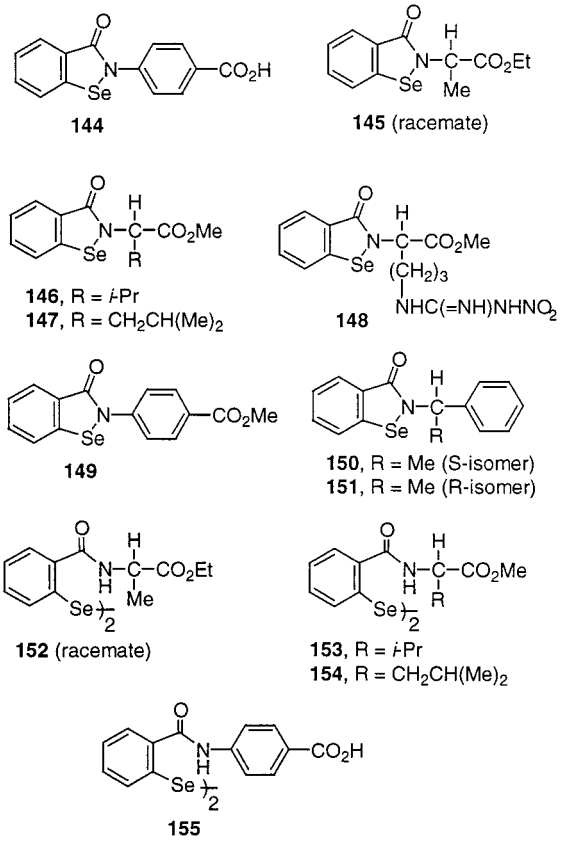


Figure 24.

of NO formation by ebselen was first observed on the cellular level in experiments with rat Kupffer cells.[136,165] In rings of rabbit aorta with intact endothelium, ebselen has been shown to block the vasorelaxant action of acetylcholine, which was dependent on endothelial generation of •NO.[166] Ebselen also blocked the vasorelaxant action of the calcium ionophore A23187, a receptor-independent activator of endothelial cells. In homogenates of bovine aortic endothelial cells, ebselen inhibited the activity of NOS with an IC_{50} of 8.5 μM (Table 6). The IC_{50} value of ebselen for the inhibition of iNOS obtained from spleens of LPS-treated rats was 250 μM.[166] Hattori et al.[167] reported that ebselen shows dual actions on the activities of both ecNOS and iNOS, depending upon the concentration. Ebselen enhanced the iNOS activity at a concentration of up to 1 μM and then inhibited the iNOS in a dose-dependent manner at concentrations greater than 2 μM. While the activity of iNOS was inhibited by 90% at 5 μM, the activity of ecNOS was slightly increased at the same concentration.

The carboxylated analogue of ebselen (**144**, Figure 24) has been reported to be more potent and more selective than ebselen in the inhibition of ecNOS.[168]

Table 7. Inhibition of ecNOS in Rabbit Aorta by 1 and 144−154[169]

compound	IC_{50} (mM)	compound	IC_{50} (μM)[a]
ebselen (**1**)	2.5	**149**	9.4
144	>100	**150**	6.7
145	22.0	**151**	12.4
146	13.5	**152**	3.5
147	14.2	**153**	7.6
148	>100	**154**	19.5

[a] Concentration of the compound causing 50% inhibition of L-citrulline-[2,3-^{3}H] formation.

The hydrophilic nature of the −COOH group, which is attached to the phenyl side chain at the para-position, is expected to increase the solubility of the compound in water, although this substituent may not contribute much to the redox status of the selenium atom. However, the inhibition of **144** in intact endothelial cells was relatively weak, which can be explained in terms of its lipophilicity. The relatively low lipophilicity of the molecule reduces its ability to diffuse through the cell membrane.

Further, as an extension to these studies, certain 2-carboxyalkyl- and aryl-1,2-benzisoselenazol-3(2*H*)-ones and related derivatives (**145**−**155**, Figure 24) have also been synthesized and evaluated for their inhibitory properties in rabbit aortic rings.[169] It has been recognized that changes on the side chain linked to the nitrogen atom of ebselen reduce the potency of the parent compound. The difference in the activity of two enantiomers **150** and **151** may be due to the difference in the stereospecific interaction between the inhibitor and the enzyme. Similar to the carboxy ebselen (**144**), compound **148**, bearing a polar substituent, was less active, which confirms that the transport through cell membranes plays an important role in the biological action of the NOS inhibitors.[168] In contrast to the diselenides **152**−**154**, compounds **94** and **155** were less potent inhibitors of ecNOS (Table 7). In addition to their inhibiting properties, compounds **145**−**154** have been shown as modest cytokine (TNF, INF) inducers in human peripheral blood leucocyte cultures (section IX.A).[169] Compounds **144**−**154** were synthesized from 2,2′-diselenobis(benzoic acid), as shown in Scheme 1. The dichloride **9** was prepared in high yield by reaction of **8** with $SOCl_2$ in the presence of DMF as a catalyst.[170] Reactions of **9** with amino acid esters afforded the 2-carboxyalkyl-1,2-benzisoselenazol-3(2*H*)-ones **144**−**146**. Bis[(2-carbamoyl)phenyl]diselenides **151**−**153** were prepared by treating the corresponding cyclic compounds (**144**−**146**) with hydrazine monohydrate.

Studies on the inhibition of ebselen on cytokine-induced NOS expression in insulin-producing cells show that ebselen prevents the increase in nitrite production by rat islets exposed to interleukin-1β (IL-1β). Similar effects have been observed in rat insulinoma (RIN) cells exposed to IL-1β (Table 8).[171] The reduction in NO$^\bullet$ production determined by ebselen in RIN cells has been associated with a decrease in iNOS mRNA expression but not with an inhibition of IL-1-induced NF-xB activation.

A few selenourea derivatives such as aminoethylisoselenourea (**156**, Figure 25), aminopropylisoselenourea (**157**), and 2-aminoselenazaline (**158**) have also been reported as potent inhibitors of the iNOS.[172] These derivatives effectively inhibited the conversion of L-arginine to L-citrulline by iNOS in lung homogenates when compared to the most commonly used inhibitor of NOS, N^G-methyl-L-arginine (L-NMA). These derivatives also inhibited the activity of NOS in immunostimulated J774 macrophages (Table 9).

Table 8. Effect of IL-1β and/or Ebselen on Nitrite Production[171]

ebselen concn (μM)	IL-1β (U/mL)	nitrite production: rat islets[a]	nitrite production: RIN cells[b]
0	0	1.12 ± 0.15	155 ± 36
0	25	2.16 ± 0.27	480 ± 19
20	0	1.12 ± 0.12	246 ± 14
20	25	1.32 ± 0.16	195 ± 25

[a] pmol/h × islet. [b] pmol/h × 10^6 cells.

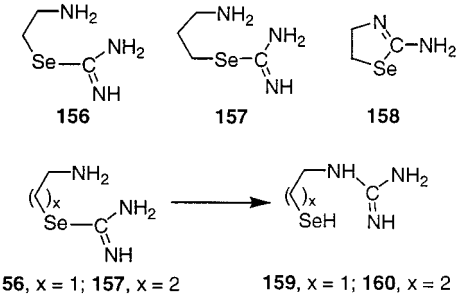


Figure 25.

Table 9. EC_{50} Values (μM) for the Inhibition of Various Isoforms of NOS by 156−158[172]

compd	homogenates[a]: iNOS	homogenates[a]: ecNOS	J774 cells iNOS
L-**NMA**	22	16	160
156	1.1	104	11
157	0.1	15	4
158	0.3	110	18

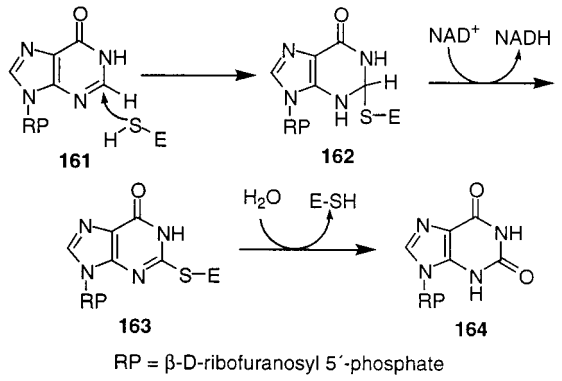
[a] The absolute values of NOS activities in the homogenates are 1.8 ± 0.1 pmol/mg/min for ecNOS and 1.2 ± 0.04 pmol/mg/min for iNOS.

In contrast, compounds **156** and **157** were less effective inhibitors of (ecNOS) activity in homogenates of bovine endothelial cells. Accordingly, in vivo studies on compound **156** showed only modest effects on blood pressure, suggesting only a small effect on ecNOS. On the other hand, compounds **157** and **158** showed pressure effects similar to those of L-NMA. These results suggest that aminoalkylisoselenoureas may have vascular actions unrelated to inhibition of NOS. Compounds **156** and **157** are unstable in aqueous solution at pH values above 6. Since **156** and **157** rearrange to form selenoalkylguanidines **159** and **160**, respectively (Figure 25), these selenols are likely to be the active NOS inhibitor species. The mechanism of the rearrangement is similar to the one reported for the corresponding sulfur analogues.[173,174] In both cases, the aminopropyl derivative is more stable than the aminoethyl counterpart and the selenium compounds rearrange at lower pH values compared with the sulfur analogues.

B. IMPDH Inhibitors

Inosine 5′-monophosphate dehydrogenase (EC 1.1.1.205) catalyzes the conversion of inosine 5′-monophosphate (IMP, **161**) to xanthosine 5′-monophosphate (XMP, **164**) utilizing NAD^+ as a hydrogen acceptor. According to the covalent mechanism (Scheme 9), the sulfur atom of the active site cysteine

Scheme 9



residue first reacts with IMP to form a tetrahedral intermediate (**162**). A hydride transfer to NAD^+ to produce **163** followed by hydrolysis yields XMP. The active site of IMPDH is a long cleft with a binding pocket for IMP and a binding groove for NAD. A stacking interaction between IMP and NAD facilitates the necessary hydride transfer and also preserves the stereochemistry of the mechanism. Since the activity of IMPDH increases significantly in proliferating cells,[175,176] the IMPDH inhibitors are expected to be promising antitumor and immunosuppressive agents. These inhibitors increase the intracellular concentration of IMP, which can serve as a phosphate donor for the phosphorylation of 2′,3′-dideoxynucleosides. Owing to this property, IMPHD inhibitors are considered as potentiators of the anti-HIV activity of retroviral drugs such as 2′,3′-dideoxyinosine (ddI).[177,178]

The commonly studied inhibitors of IMPDH are either IMP site-binding inhibitors or NAD site-binding inhibitors. While the IMP site-binding inhibitors have a common structural theme in that they are all ribonucleosides, inhibitors binding at the NAD site are structurally more heterogeneous. The observation that the oncolytic C-nucleosides that are analogues of NAD are converted to potent inhibitors of IMPDH generated interest in the synthesis of selenium-containing nucleosides and related derivatives.[179] Both tiazofurin (**165**, Figure 26) and selenazofurin (**166**) are metabolized in tumor cells to the corresponding dinucleotides and have pronounced antitumor activity in animals and broad spectrum antiviral as well as maturation-inducing activities.[180−182] It has been found that selenazofurin is 5−10 times more potent than tiazofurin in several antitumor screens and in vitro studies[183] and the antiproliferative and maturation-inducing effects of this nucleoside appear to be due to the inhibition of IMPDH.[184] Selenazofurin has been reported to be a potent inhibitor of phlebovirus infections, as this compound suppresses liver virus titers when admin-

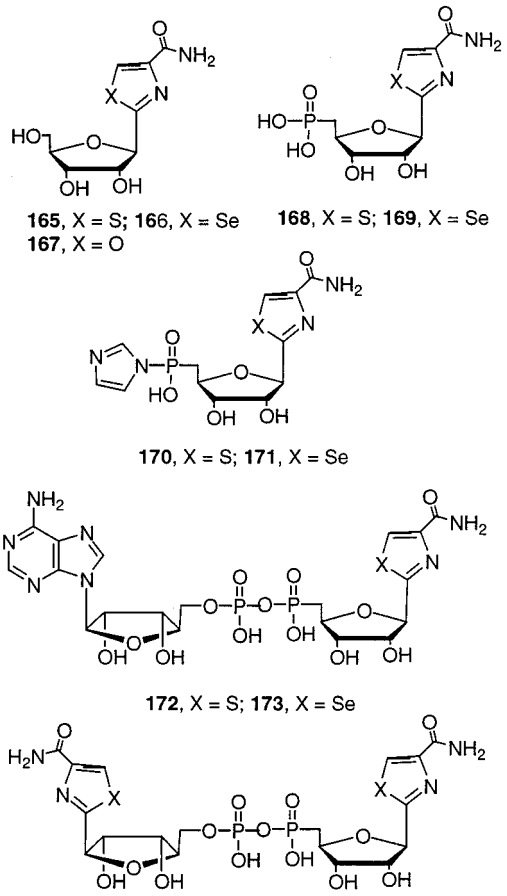


Figure 26.

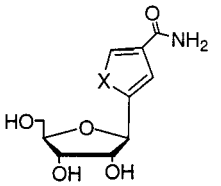
Table 10. IMPDH Inhibition and Cytotoxicity of 168, 169, and 172−175[179]

compd	IMPDH inhibition, av K_i (μM)[a]		cytotoxicity ID_{50} (μM)[a]
	IMP	NAD	
168	265	405	10
169	170	470	1.4
172	0.13	0.24	7.5
173	0.05	0.04	9.8
174	140	370	4.6
175	190	240	0.6

[a] Inhibitory effects and cytotoxicity of compounds against P388 cells in culture.

istrated orally (80−320 mg kg^{-1}) for 5 days.[185] In L1210 leukemia cells, selenazofurin inhibited the growth of cell culture in a dose-dependent manner with an IC_{50} value of 0.2 μM.[186] While the compound lowered the GTP/ATP ratio (5-fold), the inhibition resulted in an increase of IMP/AMP (9-fold), indicating the selective inhibition of guanylate synthesis from IMP caused by this drug. The inhibitory activity of the 5′-monophosphate and NAD derivatives of tiazofurin and selenazofurin has also been reported (Table 10).

From Table 10, it is evident that the dinucleotides **172**−**175** are more potent inhibitors than the monophosphate derivatives **168** and **169**. The inhibitory activity of the symmetric dinucleotides **174** and **175** is inferior to that of the adenine-containing dinucleotides **172** and **173**. In addition to the inhibitory effect, these studies show that tiazofurin and selenazofurin are the only ribonucleosides in this series



176, X = S; **177**, X = Se
178, X = O

Figure 27.

Table 11. Inhibition of IMPDH Activity by Compounds 165, 166, 176, and 177[188,189]

compd	IMPDH activity[a]	% inhibition
none	5.04 ± 0.14	0
165	2.07 ± 0.15	59
166	1.01 ± 0.12	80
176	2.62 ± 0.19	52
177	1.06 ± 0.28	76

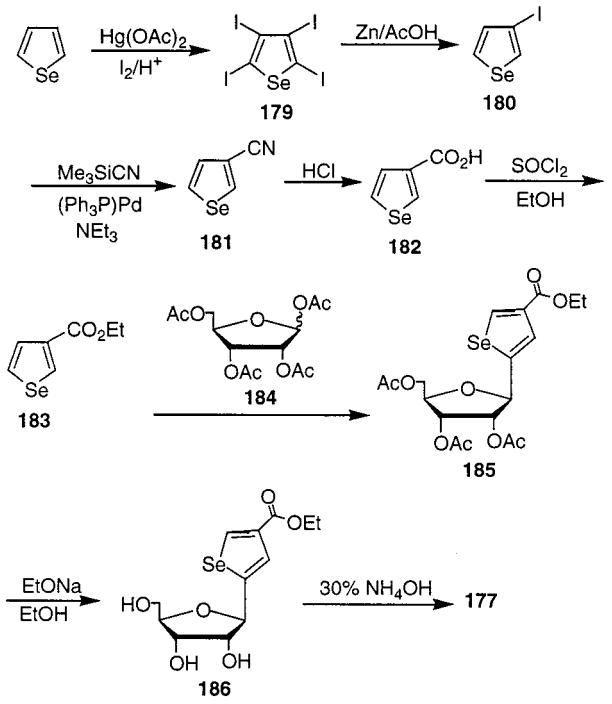
[a] Nanomoles of XMP formed per hour per milligram of protein on human myelogenous leukemia K562 cells in culture.

that are converted to dinucleotides in vivo. The superior inhibitory effects of **172** and **173** may not be due to the resemblance of these dinucleotides to NAD but rather to very stringent stereochemical and conformational requirements that are uniquely met by these compounds. Since the inhibition by some of the compounds is not competitive with respect to NAD, it is quite unlikely that all these derivatives bind to the NAD^+ catalytic site. A comparison of the inhibitory activity of selenazofurin and tiazofurin with benzamide riboside in human myelogenous leukemia K562 cells shows that selenazofurin is the most potent of the three drugs.[187] Replacement of selenium with oxygen in selenazofurin resulted in oxazofurin **167**. Oxazofurin lost the ability to inhibit the growth of P388 and L1210 murine leukemia and HL 60 human promylelocytic leukemia.[188]

Selenophenfurin (**177**, 5-β-D-ribofuranosylselenophene-3-carboxamide), a C-nucleoside isostere of selenazofurin, has also been shown to exhibit antiproliferative and IMPDH inhibition activities.[188,189] This compound inhibited the IMPDH activity in K562 cells with a potency equal to that of selenazofurin and greater than that of thiophenfurin (**176**) and tiazofurin (**165**) (Figure 27, Table 11). This indicates that the isosteric replacement of the nitrogen atom with a CH group retained the biological activity. Similar to the selenazofurin, the presence of some type of interactions between selenium and nearby oxygen atom appears to be the crucial factor for the IMPDH inhibitory activity of selenophenfurin since substitution of selenium with oxygen has been proved to abolish all potency, rendering furanfurin (**178**) inactive (see the section on X-ray crystallography).

The synthesis of selenophenfurin (**177**) can be achieved by direct C-glycosylation of ethyl selenophen-3-carboxylate (**183**) under Friedel−Crafts conditions (Scheme 10).[189] Compound **183** can be prepared by treating the 3-carboxylic acid **182** with $SOCl_2$ and EtOH. Although compound **182** can be synthesized in different ways,[190,191] its synthesis from tetraiodoselenophen (**179**), as shown in Scheme 10,

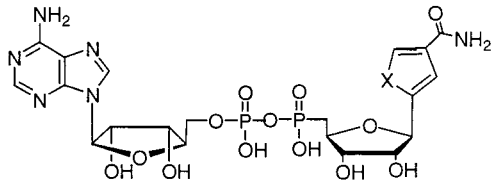
Scheme 10



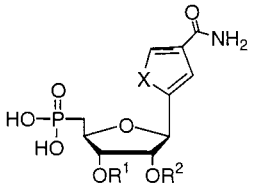
is found to be more convenient.[189] Zinc-induced deiodination of **179**, followed by cyanation with Me_3-SiCN and subsequent hydrolysis, gives the acid **182**. The reaction of **183** with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**184**) in the presence of $SnCl_4$ gives the β-anomer **185** along with other 2- and 5-glycosylated regioisomers. The mixture is treated with a catalytic amount of EtONa to give the deblocked ethyl esters, which are separated by column chromatography. The desired compound **177** is thus obtained by the treatment of **186** with NH_4OH.

Similar to the selenazofurin derivatives **172**−**175**, the isosteric analogues of NAD (**187**, **188**, Figure 28) derived from selenophenfurin have also been reported to be mammalian IMPDH inhibitors.[192] Compounds **187** and **188** exhibited an uncompetitive type of inhibition toward IMP and NAD substrates. The selenium compound **188** was slightly more potent than the sulfur analogue but less potent than **173**. The corresponding monophosphates of the respective NAD analogues (**189**, **190**) were weak inhibitors of IMPDH similar to the monophosphates (**168**−**171**) of the parent compounds, tiazofurin and selenazofurin.

The Se-containing NAD analogue **188** can be synthesized starting from acetonide-protected selenophenfurin **191** (Scheme 11).[192] Phosphorylation of **191** by following the Yoshikawa method[193] gives a mixture of acetonide-protected selenophenfurin 5′-monophosphate **190** and the corresponding nitrile **192**, which are separated as ammonium salts by chromatography on a silica gel column eluting with *i*-PrOH/NH_4OH/H_2O. Activation of **190** with carbonyldiimidazole and reaction of the imidazolide intermediate with AMP give the protected dinucleotide **193**. The desired NAD analogue **188** can be obtained by deisopropylidenation of **193** with Dowex 50W/H^+ resin in water.



187, X = S; **188**, X = Se

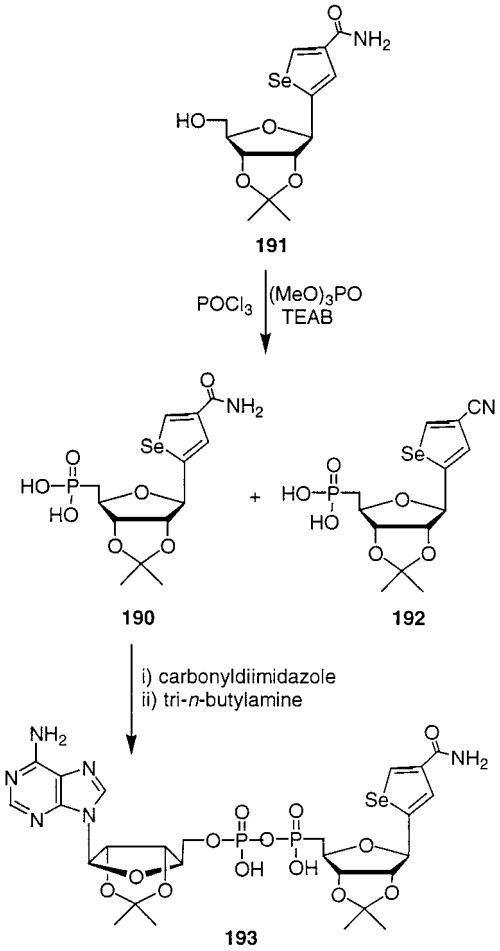


189, X = S; $R^1 = R^2 = H$

190, X = Se; R^1, $R^2 = >C(CH_3)_2$

Figure 28.

Scheme 11



C. LOX Inhibitors

Lipoxygenases (LOXs) are a family of structurally related enzymes, catalyzing the oxygenation of arachidonic acid or other polyenoic fatty acid.[194,195] LOX catalyzes the two initial steps in leukotriene biosynthesis from arachidonic acid. Leukotrienes such as leukotriene B_4 (LTB_4) are known as important mediators of asthma, allergy, arthritis, psoriasis, and inflammatory bowel disease. The active site of the LOX family of enzymes contains a nonheme iron that

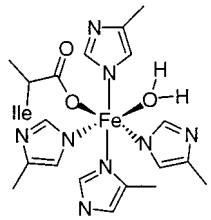


Figure 29.

is essential for the catalytic activity. The active site of mammalian LOX involving amino acid and water coordination to the iron metal center is shown in Figure 29. With respect to their positional specificity of arachidonic acid oxygenation, LOXs may be further classified as 5-, 8-, 11-, 12- and 15-LOXs. However, a more accurate classification of lipoxygenases that considers the genetic relationship has been recently reported.[195] 5-LOXs are involved in the biosynthesis of mediators of inflammatory and anaphylactic disease,[196] whereas 15-LOXs have been implicated in cell differentiation[197] and atherogenesis.[198] Several structural analogues of arachidonic acid have been reported as moderately potent inhibitors of 5-LOX. Inorganic selenium species such as selenite and selenodiglutathione (GSSeSG) are also known to block the 5-LOX pathway.

With a view to designing new drugs with an improved safety profile, certain selenazoles that are dual inhibitors of both cycloxygenase (COX) and 5-LOX are being studied as potential antiinflammatory agents. The beneficial effects of ebselen have been attributed to the inhibition of the enzyme 5-LOX, thereby preventing production of proinflammatory cysteinyl leukotrienes.[199] The ebselen inhibition of LOX may occur either directly by forming an enzyme−ebselen complex or indirectly by lowering the hydroperoxide tone.[158] The latter phenomenon is based on the fact that LOXs require a certain level of hydroperoxy fatty acids in the micromolar range to initiate their catalytic cycle. Therefore, organoselenium compounds that reduce hydroperoxides are generally capable of inhibiting LOX reactions. While the pure enzyme was strongly inhibited by an ebselen concentration as low as 0.1 μM,[200] higher concentrations (>20 μM) were required for the inhibition of the formation of 5-LOX products in polymorphonuclear leukocytes.[201] The nonredox type 5-LOX inhibitors such as methoxytetrahydropyran derivatives also require selenium species such as GPx for efficient inhibition,[202] which indicates that low hydroperoxide concentrations are important for efficient 5-LOX inhibition.

Galet et al. reported that benzoselenazolinones **194**−**204** and the corresponding diselenides **205**−**212** (Figure 30) dramatically decrease the formation of LTB_4.[203] From Table 12, it is evident that the open-chain diselenides are more potent than the cyclic selenazolinones. In the diselenide series, an increase of the lipophilic character by substitution on C-6 with benzoyl, *p*-chlorobenzoyl, phenylcarbinoyl, and benzyl substituents enhanced the inhibition of LTB_4 formation. In the benzoselenazoline series, electronic or steric parameters on the aromatic group seem to be important since a 6-substitution with a nicotinoyl or

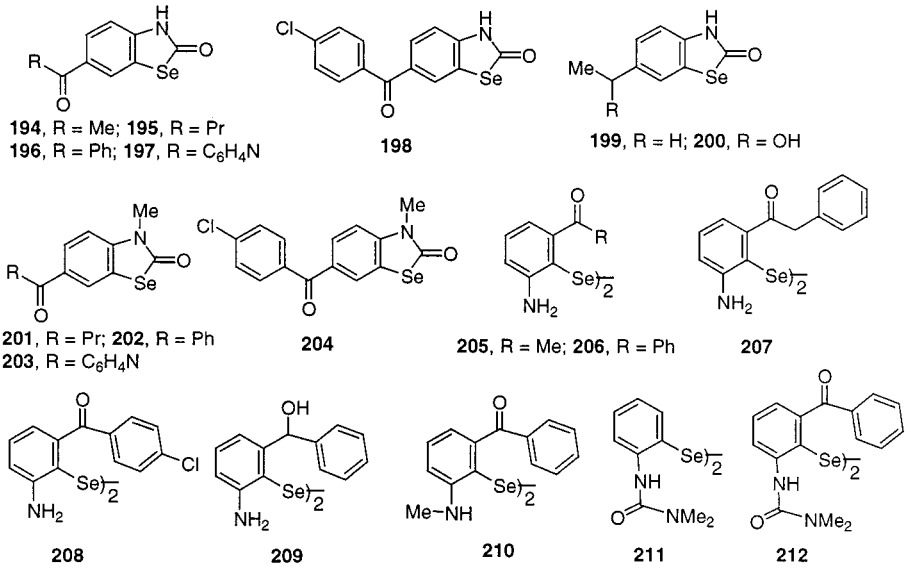


Figure 30.

Table 12. 5-LOX Inhibition by Benzoselenazolinones Derivatives[203]

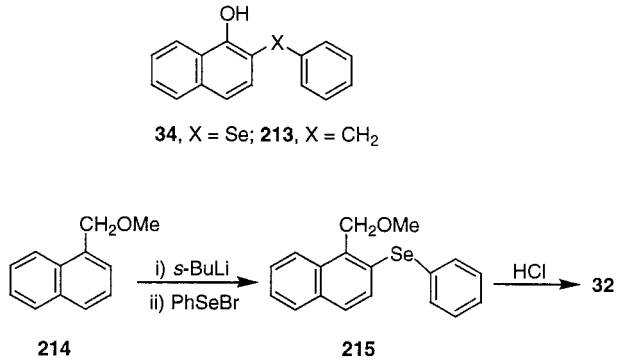
compd	LTB4 inhibn (%)[a]	compd	LTB4 inhibn (%)[a]
ebselen (1)	40	**203**	5
194	45	**204**	3
195	27	**205**	68
196	75	**206**	77
197	3	**207**	92
198	2	**208**	91
199	53	**209**	96
200	56	**210**	0
201	0	**211**	85
202	0	**212**	65

[a] The final concentration of the compounds was 10^{-5} M.

p-chlorobenzoyl group (**197**, **198**, **203**, **204**) decreased the inhibitory effects toward 5-LOX. These features may be correlated to a hydrophobic cavity on the enzyme with fixed size. This rule does not apply to the diselenides, as evidenced by the higher inhibitory properties of compounds **206**−**209**, **211**, and **212**. Although the nitrogen substitution in benzoselenazolinones and diselenides generally decreases the inhibition (e.g., **201**−**203**, **210**), nitrogen substitution with polar groups enhances the inhibition (e.g., **211**, **212**).

The diaryl selenide **34** has been reported to be a potent inhibitor of 5-LOX.[105] This compound inhibited

Scheme 12



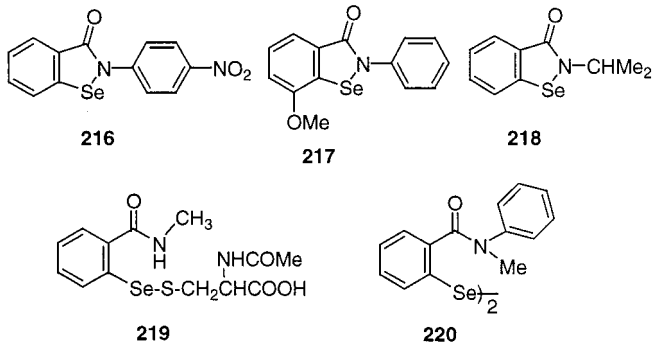


Figure 31.

Table 13. Inhibition Effects of Ebselen and Related Derivatives on Reticulocyte 15-LOX[200]

compd	IC^{50} (μM)	% inhibition at 1 μM	compd	IC^{50} (μM)	% inhibition at 1 μM
ebselen (**1**)	0.14	85	**121**	100−200	0
2	125	0	**216**	−[a]	0
11	0.35	71	**217**	2	36
92	−[a]	0	**218**	110	45
93	100	0	**219**	5	40
94	5	34	**220**	−[a]	0
117	0.3	72			

[a] The compounds do not inhibit at 100 μM concentration.

the production of LTB_4 in A 23187 activated human neutrophils with a potency (IC_{50} = 0.079 μM) greater than that of DuP 654 (**213**, IC_{50} = 0.40 μM), a compound which is considered as a topical antipsoriatic agent.[204] Compound **34** can be prepared in one pot by ortho-lithiation of 1-naphthyl methoxymethyl ether (**214**), followed by addition of phenylselenenyl bromide (Scheme 12). Deprotection of **215** affords the desired compound in 61% yield.[105]

The inhibitory effects of organoselenium compounds **216**−**220** (Figure 31) have also been demonstrated on 15-LOXs.[200] Ebselen (**1**) and some of its derivatives were found to be potent inhibitors of mammalian 15-LOX in the absence of thiols (Table 13). It should be noted that ebselen (**1**) is the most potent inhibitor among all 15-LOX inhibitors hitherto

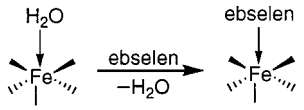


Figure 32.

known. Ebselen (**1**) can selectively block the extracellular actions of 15-LOX. However, its inhibitory potency is drastically decreased in the presence of GSH. This may be due to the ability of GSH to react with ebselen via opening the isoselenazol ring, thereby forming a selenenyl sulfide, which affects the 15-LOX only at higher concentrations (Table 13). In the extracellular space, GSH is virtually absent, and therefore, it may not interfere with LOX inhibition. Although, LOXs are intracellular enzymes, at the site of inflammation, where cell death may occur, the enzyme may be released into the extracellular space to initiate extracellular lipid peroxidation. The selective inhibition of LOX-induced extracellular lipid peroxidation without affecting the intracellular LOX activity may, therefore, be important for anti-inflammation.[201]

It could be inferred from these data that the size of the molecules plays a significant role in the ability of these compounds to bind with the enzyme. Introduction of a nitro group in ebselen completely abolished the inhibitory activity of the parent compound. On the other hand, the activity was not affected significantly by ring substitution (**117**) or ring expansion, indicating the retention of the basic structure. The open-chain compounds **94** and **219** also exhibited significant inhibition, which could be due to the fact that these compounds may regenerate ebselen in the presence of hydroperoxides and thiols. Similarly, the inactivity of compound **220** may well be due to the presence of a tertiary amino group that cannot be involved in a ring closure reaction.

The mechanism of 15-LOX inhibition has been studied by using both inorganic and organic selenium species. It is possible that ebselen may react with a nonessential cysteine residue located in the vicinity of the active site to form an ebselen−protein−selenosulfide adduct. However, the X-ray crystal structures[205−207] and spectral data[208−210] of 15-LOX and 15-LOX−inhibitor complexes reveal that the oxidation state of iron is changed during the inhibition. The inhibition studies on ebselen also show that ebselen alters the geometry of the iron ligand sphere by forming a enzyme−ebselen complex. It might be possible that the drug displaces a water molecule from the sixth iron ligand position.[211] Therefore, the iron-complexing action rather than reaction with free thiol groups is responsible for the inhibition of 15-LOX by ebselen (Figure 32). This is consistent with the observations that selenium compounds such as selenophenol are capable of complexing enzyme-bound iron.[212] Since ebselen is a rigid and "space-filling" molecule,[211] it may fit into the substrate binding pocket without major alterations of the three-dimensional enzyme structure. Molecular modeling of the enzyme−ebselen complex shows that there are no major steric constraints preventing ebselen from binding in the vicinity of the nonheme iron.

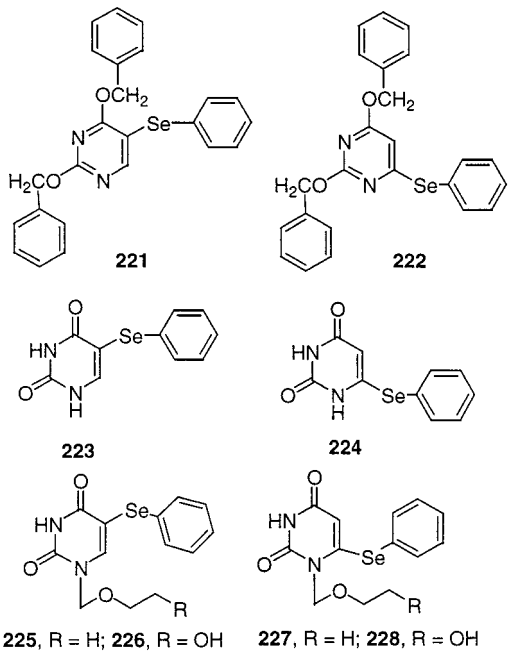


Figure 33.

D. UrdPase and TMS Inhibitors

Uridine phosphorylase (EC 2.4.2.3.) is a pyrimidine nucleoside phosphorylase responsible for the catabolism of 5-fluoro-2′-deoxyuridine (FdUrd) to 5-fluorouracil (FUra). Since FdUrd is used for the treatment of various human solid tumors, including hepatic metastases of advanced gastrointestinal ovarian cancer, advanced breast cancer, and squamous cell carcinoma of the head and neck, UrdPase inhibitors could be used in combination with FdUrd in cancer chemotherapy to prevent FdUrd cleavage in such tumors.[213,214] This combination enhances the selective toxicity of FdUrd against tumors. Several acyclouridine derivatives are shown to act as specific inhibitors of UrdPase, and these compounds significantly inhibit the cleavage of FdUrd in extracts of tumors.[215,216] The phenylseleno-substituted pyrimidines (**221**−**228**, Figure 33) are more lipophilic than the non-selenium-based inhibitors, and their effects are, therefore, directed mainly to the metabolism in the liver, which is the main site for pyrimidine metabolism in the body.[217]

Compound **223** exhibited significant inhibiting properties that could be enhanced by introducing an "acyclo tail." The most active compound, **226**, with a hydroxyl group at the end of "acyclo tail", inhibited UrdPase from mouse liver with an apparent K_i value of 3.8 μM. There was no toxicity observed when compound **226** was given intraperitoneally at doses up to 50 mg/kg/day for 5 days and monitored for 30 days. Moreover, **226** at 30 mg/kg raised the plasma uridine level and half-life by 3-fold. Therefore, compound **226** may be useful when combined with certain anticancer or anti-HIV agents, since the toxicity of anticancer (e.g. FUra) and anti-HIV drugs (e.g., 3′-azido-3′-deoxythymidine) can be prevented by

Table 14. Inhibition Constants and Cytotoxicity of Phenylseleno-substituted Pyrimidines[217]

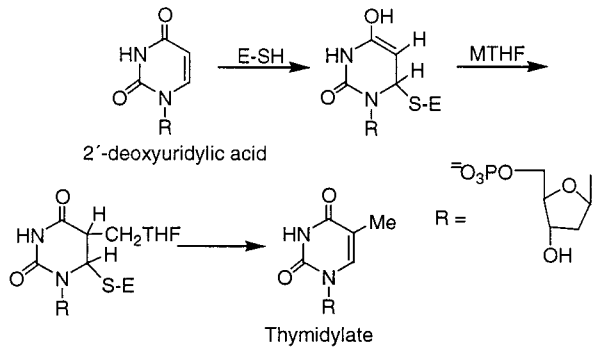
compd	inhibition of UrdPase (K_i, μM)	cytotoxicity, IC_{50} (μM) in PBM cells	in CEM cells	in Vero cells
221	—[a]	>100	>100	65.0
222	—[a]	>100	>100	27.4
223	205 ± 35	>100	>100	>100
224	—[a]	>100	>100	>100
225	313 ± 0.8	>100	>100	69.3
226	3.8 ± 0.8	>100	>100	>100
227	—[a]	—[b]	—[b]	—[b]
228	19.3 ± 1.5	—[b]	—[b]	—[b]

[a] No inhibition up to 1.0 mM. [b] Not tested.

elevating the levels of plasma uridine.[218] Further studies on the inhibiting effects of **226** show that this compound alone can increase the plasma uridine concentration and bioavailability in a dose-dependent manner.[219] As can be seen from Table 14, all the selenium derivatives exhibit no cytotoxicity in human PBM cells or in CEM cells. However, the anticancer efficacy of the combination of UrdPase inhibitors and FdUrd is not general and is dependent largely on the type of tumor under treatment and the mode of FdUrd metabolism in the tumor.

Several 5-phenylseleno derivatives of pyrimidine nucleosides have been reported to be inhibitors of TMS (EC 2.1.45),[220] an enzyme that undergoes conjugate addition with the 5,6-unsaturated portion of pyrimidine nucleosides (Scheme 13).[221] The 5-phenylseleno-substituted pyrimidine nucleosides (**229**−**234**) inhibit the TMS due to their structural resemblance to the 2′-deoxyuridylic acid. 5-Hydroseleno-2′-deoxyuridylate has also been reported to be a potent inhibitor of *Lactobacillus casei* TMS.[222] It has been proposed that the selenium compounds **229**−**234** may exert their inhibiting properties by an oxidative mechanism shown in Scheme 13. For compound **235**, the addition may take place by abstraction of a proton from the 1-position of 6-azauracil, followed by addition of the electrophile to the resulting 4,5-enolate.[220]

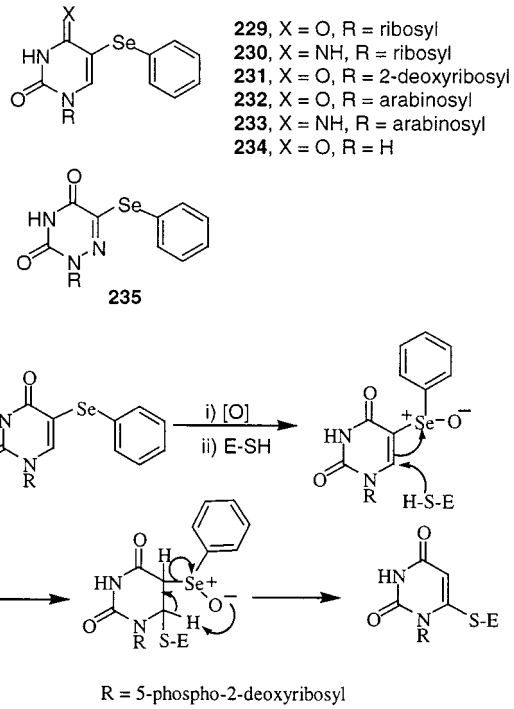
Scheme 13



E. TK and ID inhibitors

A series of N- and 3-substituted 2,2′-diselenobis (1*H*-indoles) (Figure 34) are known to inhibit TKs,[223] a family of enzymes playing a major role in the loss of growth control related to a number of diseases, including cancer, atherosclerosis, and psoriasis.[224,225] It is believed that the selective interruption of signal transduction by specific TK inhibitors could have therapeutic potential in the control of certain proliferative diseases. From Table 15, it is evident that the biological activity of selenium compounds is more affected by substitution at the 3-position than the overall nature of the indole substitution pattern. The replacement of the bridging −S−S− bond with the longer −Se−Se− bond demonstrates that the diselenides are generally more potent (up to 10-fold) than the disulfides. Compounds **237**−**242** showed

Scheme 14



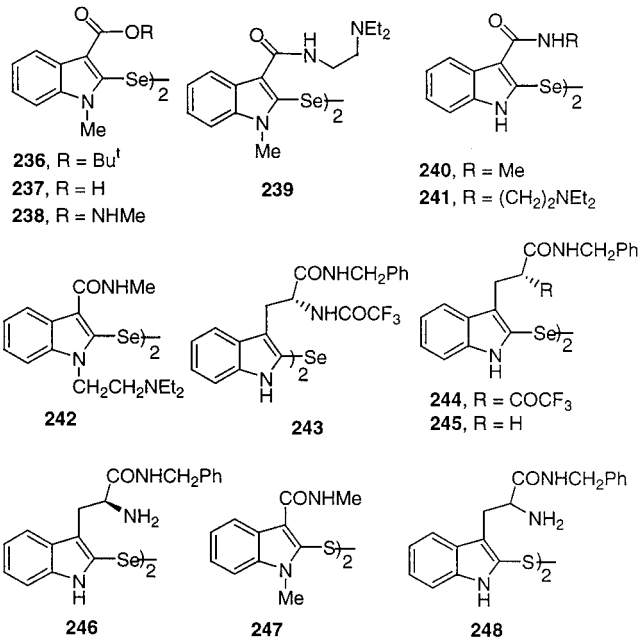


Figure 34.

Table 15. Inhibition of EGFR, PDGFR, and v-src TKs by Compounds 236−248[223]

compd	EGFR[a]	PDGFR[a]	v-src[a]	compd	EGFR[a]	PDGFR[a]	v-src[a]
236	>100	>31	1.5	**243**	>50	>50	>50
237	3.5	17.4	2.4	**244**	7.0	>50	6.2
238	6.1	4.7	0.4	**245**	0.9	14.1	2.0
239	4.7	3.4	1.8	**246**	1.3	25.5	3.8
240	13	8.0	2.8	**247**	6.9	28.1	6.7
241	4.6	12.8	3.6	**248**	7.4	−	1.5
242	6.9	50	1.7				

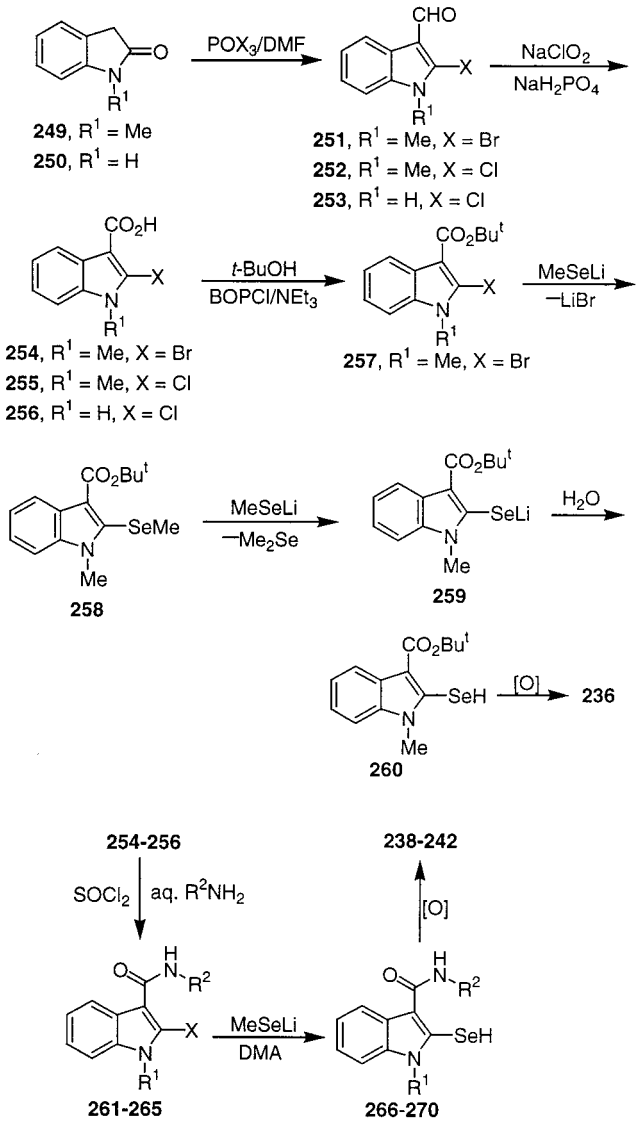
[a] IC_{50} values that represent the concentration of compounds required to inhibit various kinases.

comparable potency (IC_{50} = 3.5−6.1 μM) against the isolated epidermal growth factor receptor (EGFR). The (*R*)- and (*S*)-tryptophan derivatives **245** and **246**, respectively, with a basic amino side chain were highly potent inhibitors ($IC_{50} \approx 1$ μM). All the compounds were relatively less potent against platelet-derived growth factor receptor (PDGFR), and the greatest potency was associated with **238** and **239**, having amide functionally at C-3 and methyl substituents at N-1. In contrast to the EGFr assay, the tryptophan derivatives **245** and **246** were less potent in PDGFr experiments. However, the (*R*)-tryptophan derivative **245** displayed greater potency than its (*S*)-enantiomer **246** against PDGFR and v-src, which may be ascribed to a stereospecific interaction between the enzyme and the inhibitor.[223] The diaryl monoselenide **243** showed less potency in all three assays, indicating the requirement of a −Se−Se− bond.

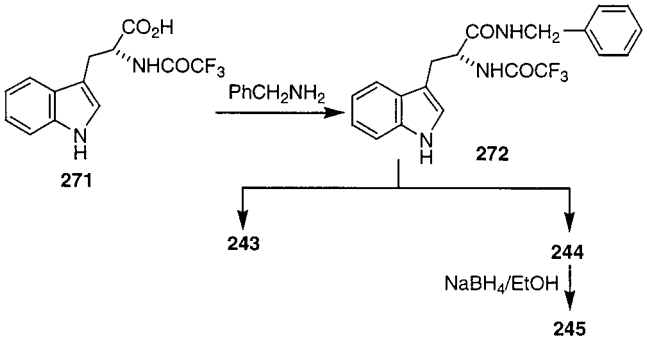
The common method of 2,2′-dithiobis(1*H*-indole) synthesis by direct thiation with P_2S_5 followed by oxidative dimerization[226] could not be used for the synthesis of corresponding selenium compounds. Alternatively, the 2,2′-diselenobis(1*H*-indoles) were synthesized from 2-halogeno-3-indolecarboxylic acid precursors (Scheme 15).[223] Starting from either 1-methyloxindole (**249**)[227] or oxindole (**250**), the synthesis of intermediates **251**−**253** was achieved under Vilsmeier conditions.[228] The carboxylic acids **254**−**256** were prepared by sodium chlorite oxidation, and the ester derivative **257** was prepared by using bis-(2-oxo-3-oxazolindinyl)phosphinic chloride (BOP chloride) as the condensing agent. Treatment of **257** with in situ generated lithium methylselenolate by using the Tiecco method,[229] followed by oxidative workup, afforded the bis-seleno ester **236**. Simple TFA hydrolysis of this compound then gave the diacid **237**. The carboxamide target compounds **238**−**242** were synthesized from Schotten−Bauman acylation of acids **254**−**256**, followed by nucleophilic displacement of the C-2 halogen of the resulting amides **261**−**265** with lithium methylselenolate.

Compounds **243**−**245** can be synthesized starting from either (*R*)- or (*S*)-tryptophan, as outlined in Scheme 16.[223] Trifluoroacetylation of (*R*)-tryptophan gives the trifluoroacetamide **271**, which is then coupled with benzylamine via DCC/HOBT condensation to provide **272**. Introduction of selenium into the 2-position is accomplished with Se_2Cl_2. The expected diselenide **244** is obtained along with the corresponding diaryl selenide **243**. The cleavage of the trifluoroacetate protecting group to give **245** is carried out

Scheme 15



Scheme 16



by $NaBH_4$ in refluxing ethanol. The same reaction sequence is carried out starting from (*S*)-tryptophan to provide the [*S*-(*R**,*R**)]-enantiomer. The main problem in the synthesis of **245** from **244** is the inversion of one of the chiral centers. To overcome this problem, a more easily cleavable amino protecting group such as t-BOC can be used instead of the $COCF_3$ group.[230]

The selenium analogues (**274**, **276**, **278**, Figure 35) of the antithyroid drugs 6-methyl-2-thiouracil (**273**,

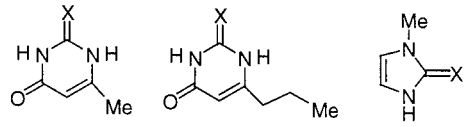


Figure 35.

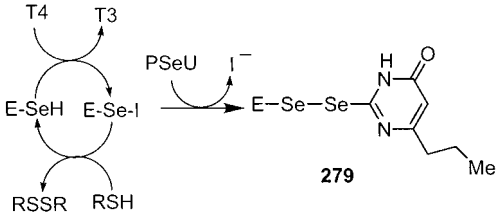


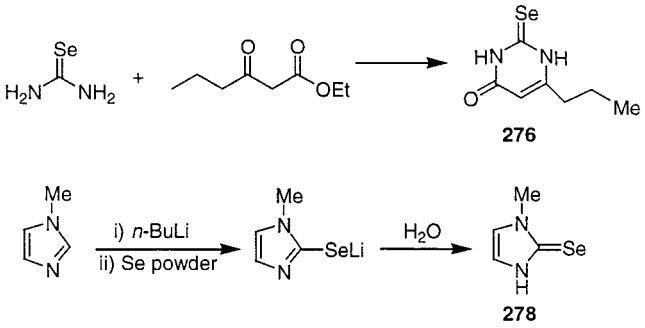
Figure 36.

MTU), 6-propyl-3-thiouracil (**275**, PTU), and methimazole (**277**, MMI) have been reported to be potent inhibitors of type I iodothyronine deiodinase (ID-1),[231−233] an enzyme that converts the prohormone thyroxine (T_4) to the biologically active hormone 3,5,3′-triiodothyronine (T_3) by monodeiodination.[234] The selenium derivatives are only slightly more potent than the sulfur analogues, and the thio and selenouracil derivatives (**275**, **276**) are better inhibitors than methimazole (**277**) and its selenium analogue (**278**).

These drugs are expected to react with the selenenyl iodide intermediate to form a stable selenenyl sulfide or diselenide adduct. Although the formation of a −Se−Se− bond with selenium drugs is expected to be more facile than the formation of a −Se−S− bond with sulfur drugs, the E−Se−Se−PTU (**279**, Figure 36) complex can be more easily reduced by thiols compared with the E−Se−S−PTU adduct. Therefore, the selenium analogues do not exhibit any strong inhibition compared with the sulfur analogues, because the inhibition may become reversible in the presence of a high concentration of GSH or DTT. As inhibitors of the catalytic effect of thyroid peroxidase (TPO), the selenium derivatives show an inhibitory effect similar to that of the sulfur analogues.[235]

The reaction sequence for the preparation of **276** and **278** is outlined in Scheme 17.[232,236] The PTU

Scheme 17



analogue **276** was synthesized by a direct condensation reaction of selenourea with ethyl 3-ketohexanoate. The methimazole analogue was synthesized by treating 1-*N*-methylimidazole in THF at −78 °C with *n*-BuLi (1.0 equiv), followed by selenium insertion and aqueous workup. The $^{13}C-^{77}Se$ NMR coupling constants of compound **278** showed that the compound exists in the selone form.[236]

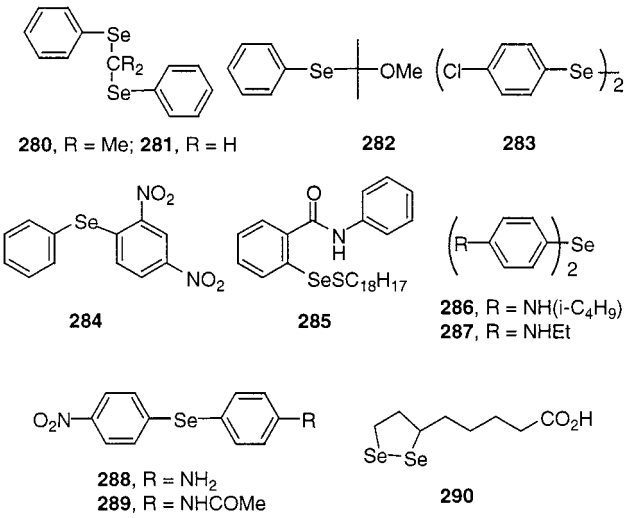


Figure 37.

Table 16. Inhibitory Effects of Ebselen on Enzymes

enzyme	species, tissue	IC_{50} (μM)	ref
NADPH oxidase	human granulocytes	0.5−1.0	239
proteine kinase C	human granulocytes	0.5	239
glutathione-S-transferase	rat liver	∼50	240
H^+/K^+-ATPase	pig stomach	0.15	241
NADPH-cytochrome P450 reductase	mouse liver	0.13	242
NADPH-cytochrome b5 reductase	rat liver	0.2−0.3	243
papin	papaya latex	−	240
prostaglandin H synthase 1	sheep vesicular glands	38	200

Table 17. Inhibition of Thioredoxin Reductase and Glutathione Reductase[244]

	inhibition IC_{50} (μM)			inhibition IC_{50} (μM)	
compd	TR	GR	compd	TR	GR
33	NA	NA	**286**	NA	NA
35	152.0	NA	**287**	NA	NA
36	60.1	24.5	**288**	NA	145.0
284	179.0	95.0	**289**	NA	145.0
285	NA	ND			

[a] NA = not active (<20% inhibition at the highest concentration tested). [b] ND = not determined.

F. Other Enzyme Inhibitors

Compounds **280**−**283** (Figure 37) inhibited δ-aminolevulinate dehydrate (δ-ALA-D),[237] an enzyme that catalyzes the condensation of two δ-aminolevulinic acid (ALA) molecules with the formation of porphobilinogen.[238] The inhibitory effect of **280** and **282** seems to be mediated by PhSeSePh formation. The *p*-chloro derivative **283** is slightly more potent than **280** and **282**. On the other hand, compound **281** does not inhibit δ-ALA-D. Ebselen has been reported to inhibit some other enzymes, which are summarized in Table 16.

A few selenides (**33**, **35**, **36**, **284**−**289**) have been reported to be inhibitors of thioredoxin reductase (TR) and glutathione reductase (GR) (Table 17).[244]

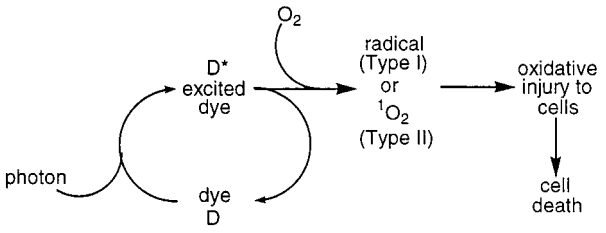


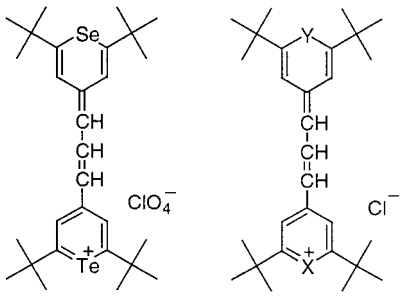
Figure 38.

Finally, the selenium analogue of lipoic acid (**290**) has been shown to be an inhibitor of mammalian pyruvate dehydrogenase complex (PDC).[245] While the sulfur compounds (*S*)-lipoic acid and (*R*)-lipoic acid markedly inhibited PDC activity, the selenium analogue displayed inhibition only at higher concentrations.

V. Photochemotherapeutic Agents

Several photoactive organoselenium compounds are being used as sensitizers in photodynamic therapy (PDT), which has regulatory approval in many countries for cancers of the lung, digestive tract, and genitourinary tract.[246−248] PDT is also used as a protocol for treating cancers of the head and neck region[249] and for treating pancreatic cancer.[250] The development of PDT involves various stages, which include (i) synthesis and initial evaluation of new photosensitizers, (ii) identification of subcellular targets involved in PDT cytotoxicity, (iii) evaluation and comparison of photosensitizer localization and cytotoxicity for normal and malignant cells, (iv) defining in vivo treatment parameters associated with PDT toxicity, (v) determining normal tissue responses following PDT, and (vi) documenting in vivo targets and systemic responses associated with PDT. As a therapy, PDT uses a light-activated sensitizer (dye) to produce a cytotoxic reagent or cytotoxic reaction in the tumor cell, typically via generation of singlet oxygen ($^1O_2$) or superoxide from molecular oxygen (Figure 38). An ideal sensitizer should absorb light strongly in the red region of the spectrum (700−900 nm), where the light has greater penetration into tissue. This photochemical phenomenon is highly efficient in destroying tumor cells. For a comprehensive background on various PDT topics, the reader may refer to a few reviews written by Rosenthal,[251] Gomer,[252] and Henderson et al.[253]

Although Photofrin (a mixture of porphyrins derived from hematoporphyrin) has received regulatory approval for use in PDT, this material is not an ideal sensitizer, since it has relatively weak absorption in the 700−900 nm spectral region. Therefore, cationic dyes such as rhodamine 123 have been used as sensitizers for PDT, as these dyes bind intracellularly.[254,255] However, rhodamine 123 is considered as relatively inefficient, because of its poor quantum yield for $^1O_2$ generation, even when immobilized in the lipophilic medium of the mitochondrial membrane. Interest in chalcogen (S, Se, Te) containing cationic dyes as photosensitizers started with the observations that the λ_{max} of these dyes can be modulated over 200 nm, by varying the chalcogen atom, to well above 800 nm.[256−258] A classical example



291

292, X = Se; Y = O
293, X = Se; Y = S
294, X = Y = Se
295, X = Te; Y = Se

Figure 39.

Table 18. Inhibition of Mitochondrial Cytochrome *c* Oxidase[262]

dye	% inhibition/J/cm^2	λ_{max}, nm (log ϵ)
292	2.55	665 (5.38)
293	4.05	708 (5.40)
294	4.20	730 (5.48)
295	3.00	770 (5.10)

[a] The inhibition values were established on the basis of constant absorbance from sample to sample.

of a photosensitizer is the lipophilic cationic dye **291** (Figure 39), which has been shown to possess an inherent ability to accumulate and concentrate in the electronegative environment of the mitochondrial membrane.[259]

Light activation of **291** significantly increased the mitochondrial-specific toxicity at low concentrations.[260,261] This suggests that compound **291** localizes to the mitochondrion and the photoactivation results in mitchondrial injury. These observations led to the development of chalcogenapyrylium dyes **292**−**295** as photochemotherapeutic agents. The common feature of compounds **292**−**295** is the presence of *tert*-butyl substituents, which impart greater kinetic stability toward the biological environment. Substitution of the O atom for heavier chalcogens induces sequential bathochromic shifts. On the other hand, the nature of the counterion does not affect the absorption spectra of the dyes. In vitro studies with these dyes suggest that these materials are targeted to mitochondria and that the activity of mitochondrial cytochrome *c* oxidase is inhibited upon exposure of dye-treated cells to light (Table 18).[262] While the addition of various scavengers, including catalase for H_2O_2, superoxide dismutase (SOD) for the superoxide anion, and mannitol for the hydroxy radical, did not affect the inhibition of the cytochrome *c* oxidase activity, the addition of a $^1O_2$ scavenger such as imidazole reduced the amount of inhibition, suggesting that $^1O_2$ is the active cytotoxic species. The large molar extinction coefficients of **292**−**295** are particularly important, since these values permit lower concentrations of sensitizers to be effective in treatment.

The effect of heavier chalcogens on triplet yields, quantum efficiencies of $^1O_2$ generation, rates of reaction with $^1O_2$, and emission quantum yields have been studied in solution by using compounds **296**−**303** (Figure 40).[263] The substitution of selenium and

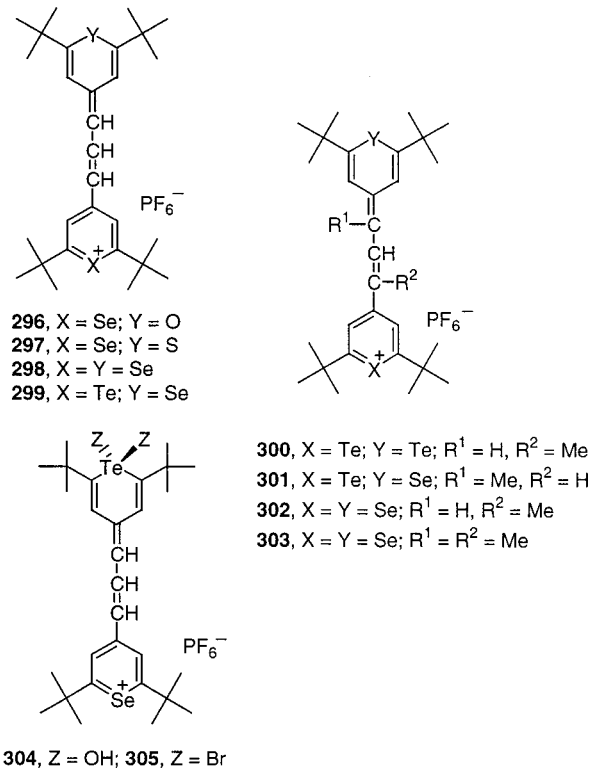
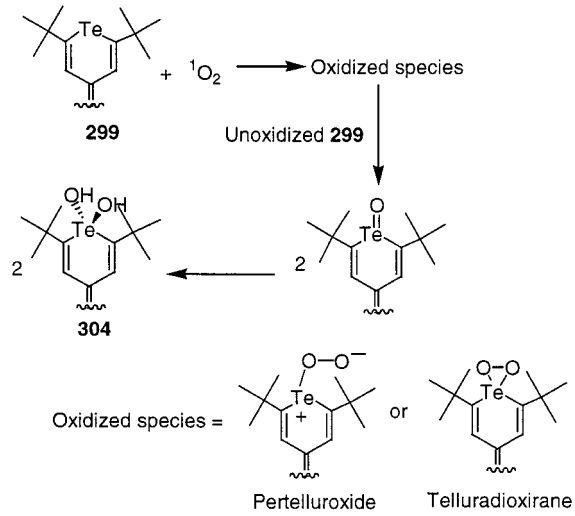


Figure 40.

tellurium atoms for oxygen and sulfur increases the quantum yields for triplet production and for $^1O_2$ production.[264] Compound **299**, having a Te atom, reacted with $^1O_2$ much more rapidly than compounds **296**−**298** to produce the oxidized dye **304**. The mechanism of its photooxidation has been reported to be similar to that of the oxidation of sulfides to sulfoxide by $^1O_2$.[265] The final photoproduct **304** is the hydrated form of telluroxide, resulting from rearrangement of an initially formed telluroperoxide or telluradioxirane intermediate (Scheme 18).[257,263,266]

Scheme 18

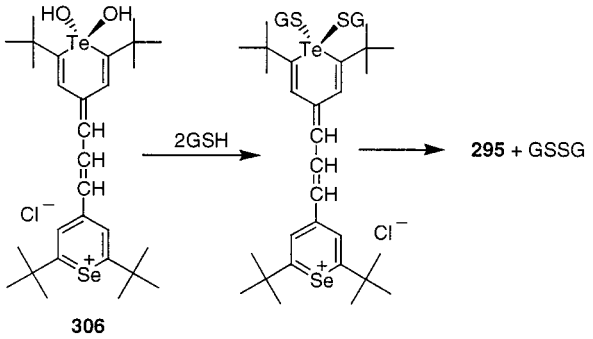


Similar types of oxidized derivatives have also been detected in vitro in cell cultures treated with tellurapyrylium dyes and light.[262]

Similar to the effect of chalcogen atoms, the substitution in the hydrocarbon backbone is also known to affect the quantum efficiency of $^1O_2$. It has been found that the increase in the steric hindrance reduces the quantum yields of $^1O_2$ in methyl-substituted dyes **300**−**303**.[263] Similar to the oxidation, oxidative bromination of **299** resulted in the addition of two bromine atoms to the Te center to yield **305**. The absorption spectrum of **305** exhibits a hypsochromic shift relative to the parent compound, reflecting loss of the Te $5p_z$ orbital.[264]

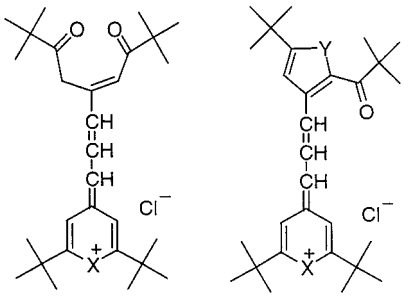
Electrochemical studies on **304** and its bromo analogue **305** show that the reduction of the dihydroxy compound **304** is at more negative potential than the corresponding dibromo derivative **305**.[264] In contrast to the effect of chalcogen atom on oxidation, the counteranions do not affect the reactions that occur at the Te center. Compound **295** oxidizes to the corresponding hydroxyl derivative **306**, which could be reduced back to **295** by GSH (Scheme 19). The

Scheme 19



reduction of **306** to **295** by GSH involves two discrete steps in which the hydroxyl derivative **306** first reacts with GSH to form a tellurium(IV) dithiolate, which then eliminates GSSG via a reductive process.[267] This suggests that the depletion of GSH levels in tissues treated with tellurapyrylium dyes via the dye-sensitized generation of $^1O_2$ should be possible through the intermediacy of Te(IV) derivatives **304** and **305**. If GSH depletion leads to impairment of the GSH−GPx repair cycle in transformed cells, more efficient treatment with $^1O_2$-generating photosensitizers would be possible.[267]

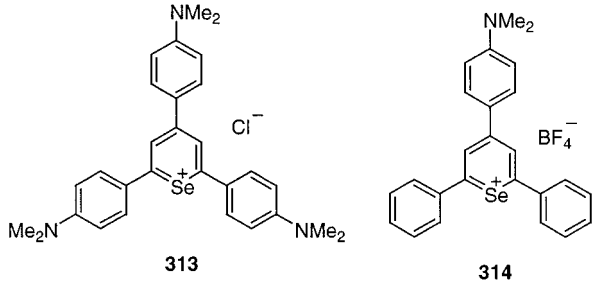
The hydrolysis of the dyes also has a major impact in PDT, as the kinetics of hydrolysis affect the circulating lifetime of the drug in vivo. Hydrolyses of compounds **292**−**295** give product distributions depending upon the nature of the heteroatom.[268,269] Compounds **293**−**295** gave hydrolysis products derived from addition of hydroxide to the 2-position of the selenapyrylium ring as well as to the central carbon of the trimethine backbone under both anaerobic and aerobic conditions. The resulting selenohemiketals ring-opened to the corresponding selenoketones, which were then hydrolyzed to the 2-pentene-1,5-diones **307**−**309** (Figure 41) from **293**−**295**, respectively. Under aerobic conditions, some oxidation of these selenohemiketals from **293** and **294** gave selenophenes **310** and **311**, respectively, and oxidation of the tellurohemiketal from **295** gave tellurophenes **312**. In chalcogenapyrylium compounds, increasing the size of the chalcogen atom decreases the effectiveness of orbital overlap in the π-framework.



307, X =S
308, X = Se
309, X =Te

310, X = S; Y = Se
311, X = Y = Se
312, X = Se, Y = Te

Figure 41.



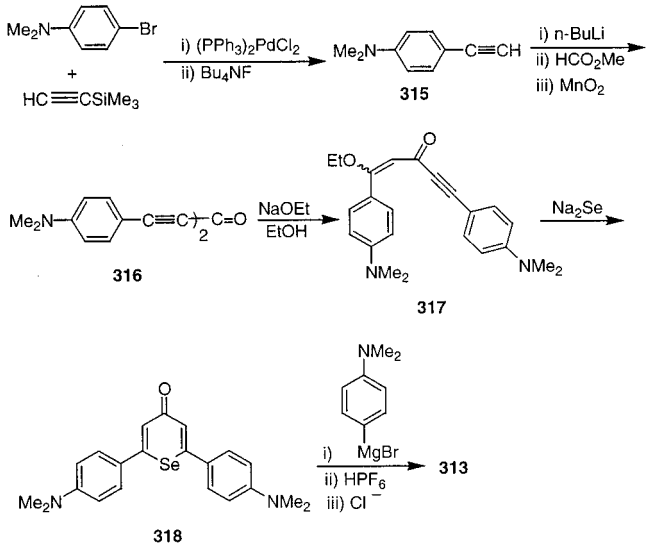
313 **314**

Figure 42.

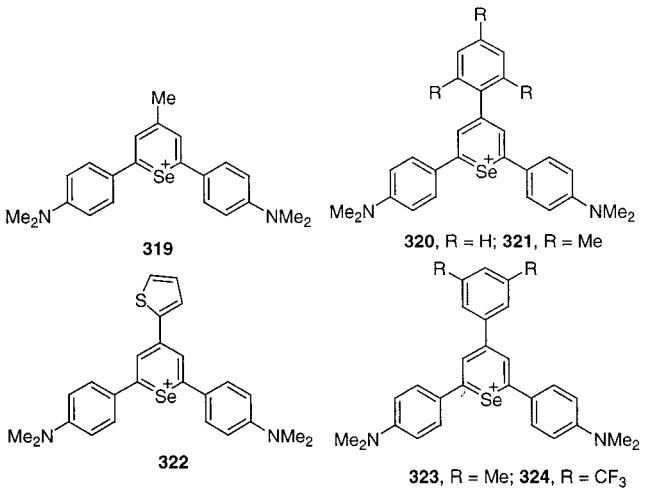
Therefore, the tellurapyrylium dyes are more active than the selenapyrylium and thiapyrylium dyes.[269]

Recently, selenapyrylium dye **313**, bearing 4-(dimethylamino)phenyl substituents at the 2-, 4-, and 6-positions (Figure 42), has been reported to be an in vitro sensitizer for PDT.[270] This dye displayed in vitro phototoxicity against R3230AC mammary adenocarcinoma cells and inhibited cytochrome *c* oxidase activity upon irradiation of isolated mitochondria. Initial in vivo acute toxicity studies suggest that compound **313** is not toxic at therapeutic PDT doses. Compound **313** gave an absorption maximum at 631 nm that was similar to the one observed for 2,6-diphenyl-4-(dimethylamino)phenylselenapyrylium dye **314** (630 nm). Compound **313** hydrolyzed slowly with a half-life of 680 min, and this compound was much more stable than telluraphyrylium dye **295**.[270] Therefore, compound **313** is expected to have an appropriately longer circulating lifetime in vivo.

Scheme 20



The synthesis of dye **313** is outlined in Scheme 20. Acetylene derivative **315** was prepared by Pd-catalyzed coupling of trimethylsilylacetylene and 4-bromo-*N*,*N*-dimethylaniline, followed by desilylation with tetrabutylammonium fluoride. *n*-BuLi was added to **315** to generate the corresponding lithium acetylide, which was then added to methyl formate followed by oxidation with MnO_2 to give ketone **316**, which was further converted to **317** by reaction with NaOEt. The addition of Na_2Se to a solution of **317** gave **318** as the only product. The addition of the Grignard reagent prepared from 4-bromo-*N*,*N*-dimethylaniline to **318** followed by dehydration of the intermediate alcohol with HPF_6 gave dye **313** as the one PF_6^- salt.[271] The PF_6^- anion can be exchanged for Cl^- ion with an ion-exchange resin to give **313**.[270] As an extension of this work, selenapyrylium dyes **319**−**326** (Figure 43) were synthesized and their photosensitizing properties studied. Among these symmetrical and unsymmetrical derivatives, compound **321**, the with highest quantum yields for $^1O_2$ generation, was found to be a promising photosensitizer in vitro against Colo-26 cells.[272] These compounds were synthesized by addition of various Grignard reagents to compound **318**. The unsymmetrical dyes **325** and **326** were synthesized from phenylpropargyl aldehyde as shown in Scheme 21.



319

320, R = H; **321**, R = Me

322

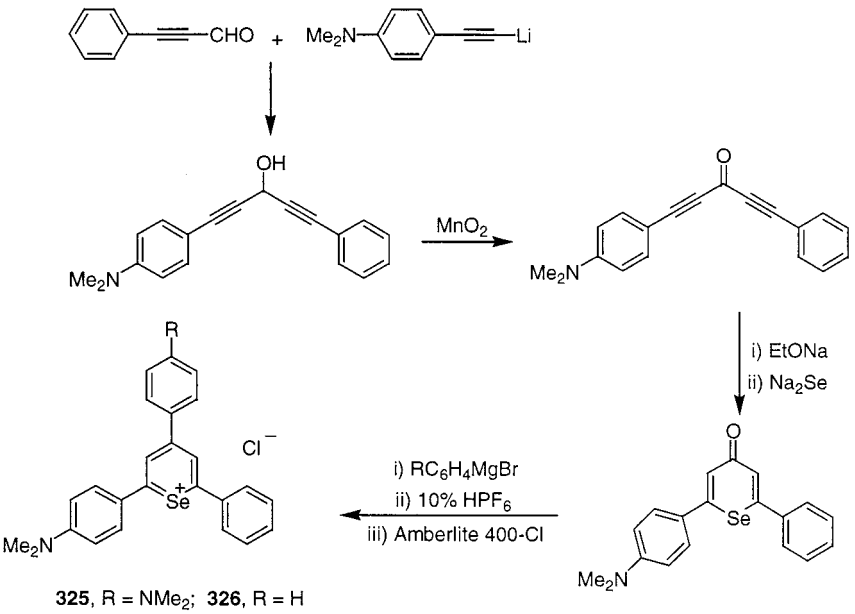
323, R = Me; **324**, R = CF_3

Figure 43.

The toxicity of **319**−**326** was evaluated in clonogenic assays of human carcinoma cell lines. Importantly, the substituents at the 2-, 4-, and 6-positions had a much greater impact on cytotoxicity. The IC_{50} values determined in the clonogenic assays did not correlate with chemical properties in the dye molecules such as reduction potential or lipophilicity. However, initial in vivo toxicity studies showed that compounds **319**−**324** are not toxic at dosages between 7.2 and 38 μmol/kg in BALB/C mice.[272]

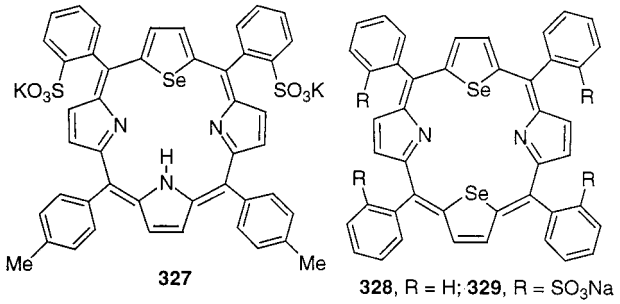
Modification of the core of porphyrins, by the introduction of selenium in place of one or two pyrrole NH groups, allows preparation of new heterocycles that could be used as sensitizing agents for PDT. In this regard, 5,20-bis(*p*-tolyl)-10,15-bis(*O*-sulfophenyl)-21-selenaporphyrin (**327**, Figure 44) has been synthesized and used successfully as a sensitizer for

Scheme 21



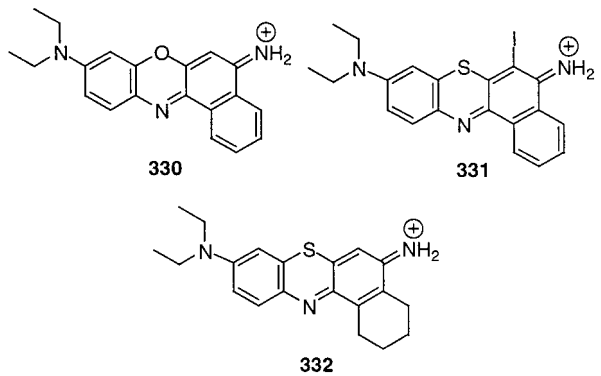
325, R = NMe_2; **326**, R = H

PDT.[273] Similar to the sulfur analogue, **327** absorbs light at the wavelength considered useful for PDT. The strong in vivo photodynamic activity of **327** and relatively lower activity in vitro studies indicate that compound **327** may act on tumor cells indirectly via destruction of nearly formed tumor neovasculature or proliferating endothelial cells. The absorption of light at 680 nm, chemical homogenity, and rather low cytotoxicity to human cancer cells lines in vitro are the important characteristics of compound **327**. The application of the porphyrin analogues in PDT can also be seen in a more recent study with water-soluble, substituted porphyrins **328** and **329**, which have been shown as longer wavelength-absorbing sensitizer for PDT.[274]



327 **328**, R = H; **329**, R = SO_3Na

Figure 44.



330 **331** **332**

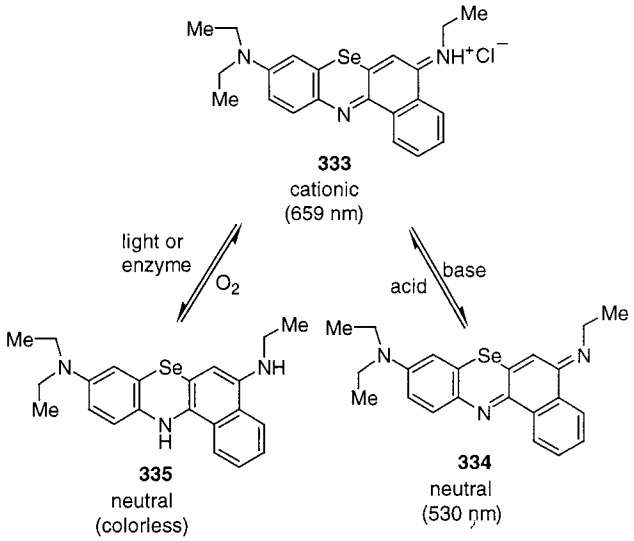
Figure 45.

Structural modifications of the benzophenoxazine dye Nile blue A (NBA, **330**, Figure 45) can yield derivatives with substantially improved $^1O_2$ quantum yields and photosensitizing properties. The combination of iodination, sulfur substitution (**331**), and ring saturation (**332**) has been shown to increase the $^1O_2$ yield from 0.5 to 82%.[275−277] These derivatives also showed up to 5000-fold enhancement in their ability to induce photokilling of tumor cells in vitro.[278,279]

The incorporation of selenium into the benzophenoxazine moiety resulted in a lipophilic, red-absorbing (659 nm) chromophore which showed significantly higher singlet oxygen yield (0.65) compared with the sulfur (0.025) and oxygen (0.005) analogues.[280] The higher phototoxicity of compound **333** compared with Photofrin greatly enhanced its photochemotherapeutic efficacy in EMT-6 cells. While the Se compound photoinactivates 97% of EMT-6 cells in culture, the S analogue only kills 5% of the cells. On the other hand, the O analogue is inactive under similar experimental conditions. The chromophore in **333** readily undergoes a protonation/deprotonation reaction that results in a neutral imino compound **334** (Scheme 22), and this behavior increases the rate of its entry into the cell. This neutral form is expected to be highly membrane permeable. In addition to the neutral species **334**, this process also gives a colorless compound (**335**) that does not absorb light in the "therapeutic window" and, therefore, is not phototoxic. However, this inactive species can be reoxidized to cationic form **333** by oxygen. It appears that both the species (**333**, **334**) contribute to the observed photosensitizing ability of compound **333**.[280] The effect of the subcellular redistribution of **333** on photodynamic O_2 consumption has also been reported.[281]

Other heterocyclic compounds such as psoralens have been shown to act as photochemotherapeutic agents. This naturally occurring class of aromatic compounds consists of a furan ring fused to a coumarin.[282] They are found predominantly in plants

Scheme 22



from the *Umbelliferacea*, *Rutacea*, and *Leguminosae* families, but have also been isolated from microorganisms including fungi.[283] The two most heavily studied psoralens, 8-methoxypsoralen (**336**) and 4,5′,8-trimethylpsoralen (**337**), are found as fungal metabolites.[283] Clinically, the psoralens are employed in the treatment of psoriasis, vitilago, and cutaneaous T-cell lymphoma.[284] They have also proven efficacious for the treatment of diseases associated with autoimmune disorders, organ rejections, and the AIDS-related complex.[285] Recent synthetic efforts have focused on generation of psoralens with enhanced light absorption properties, those being longer lived and having more easily accessed excited states.[286] In this regard, selenium analogues of psoralen (Figure 46) have been synthesized for their use as photoactivated DNA cross-linking agents.

The quantum yields of $^1O_2$ production by selenium compounds (**338**−**343**) are much higher than that by psoralen (**343**).[287] The introduction of selenium for oxygen is also expected to lead to a higher triplet quantum yield and a reduced lifetime of the electronically excited states due to the heavy atom effect[288] and a slightly different geometry. The photochemotherapeutic behavior of selenopsoralens explains their antiproliferative (treatment of psoriasis) activity. The antiproliferative activity of these compounds is mainly due to their ability to form photoaddition products (mono- and diadducts) with DNA. The cycloadduct (**344**, Figure 47) formed in the reaction between compound **339** and DNA confirms that the cycloaddition reaction occurs between the furan-side double bond of **339** and the 5,6-double bond of a thymine moiety of DNA.[289]

In addition to the cycloaddition reaction, the formation of interstrand cross-links has also been observed with some psoralen analogues.[290] Among the selenium-containing compounds, **338**, carrying selenium in the five-membered ring, is a strong cross-linker, while other compounds carrying selenium in the six-membered ring (**340**, **342**) photoinduce very few or no cross-links in DNA. Similarly, compound **341** shows a poor cross-link yield, while compound **339** causes the highest cross-link formation. The

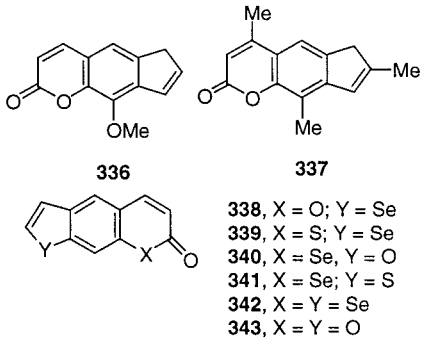


Figure 46.

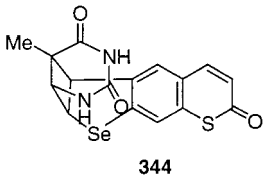
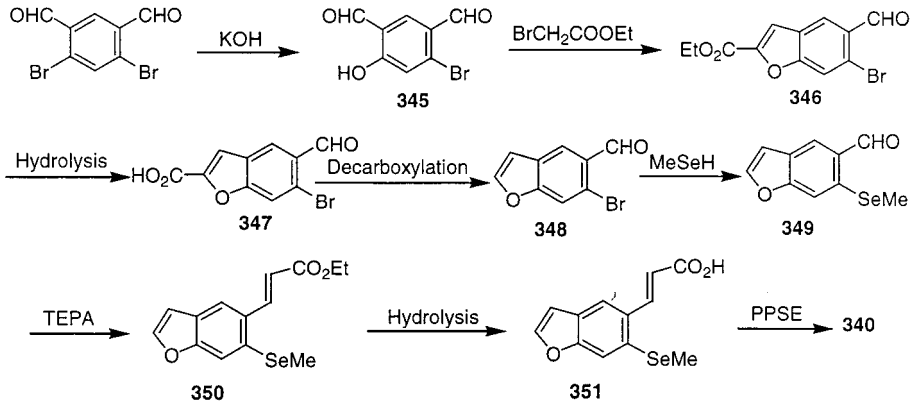


Figure 47.

enhanced DNA photobinding and DNA cross-linking ability of selenium-containing psoralens indicate that these compounds are potentially very active photochemotherapeutic agents.[289,290] Recently, a correlation between photophysical and photobiological behavior has been reported.[291] The high DNA-photobinding ability of **339** is due to the difference in the lifetime of the corresponding triplet state. The measured triplet-state lifetime in the absence of O_2 for **339** (27 μs) is longer than the corresponding lifetime for **341** (0.44 μs). In contrast, the triplet quantum yields for both selenium-containing compounds show that this heteroatom enhances the ISC process, leveling the quantum yields to unity. Moreover, quantum yields of $^1O_2$ generation for both **339** and **341** are 0.96 and 0.77, respectively. These observations strongly suggest that the [2 + 2] photocycloaddition reaction rate increases with the triplet-state lifetime of psoralen derivatives. Accordingly, compound **338**, with a triplet-state lifetime of 6 μs, has an intermediate photobinding ability with respect to **339** and **341**.[289] Further, studies on photosensitized generation of hydroxyl radical by the selenopsoralens showed that these compounds exhibit only weak propensity to generate hydroxyl radical.[292] Jakobs et al. described an efficient synthetic methodology for the monoselenium compounds **338** and **340** starting from substituted isophthalaldehyde.[293] The reaction sequence used for the synthesis of compound **340** is outlined in Scheme 23.

Merocyanine dyes such as MC 540 (**352**, Figure 48) have been used as a photosensitizer for the extracorporal photoinactivation of leukemia cells and enveloped viruses.[294] The biocidal activity of photoexcited MC 540 has been attributed to $^1O_2$, although the quantum yield for $^1O_2$ is low (ϕ = 0.007).[295] Replacement of the oxygen atom in the oxazole ring by a heavier chalcogen atom facilitates intersystem crossing and improves the $^1O_2$ yields. Substitution of Se for oxygen in the five-membered heterocyclic ring enhances the $^1O_2$ production.[296] The quantum efficiencies of dyes **355** and **358** were 5 and 7 times higher than that of **352** and **357**, respectively. In both

Scheme 23



the cases, the Se is conjugated with the chromophore and causes a substantial bathochromic shift in the absorption spectrum. Accordingly, compound **358** exhibited two times more biocidal activity compared with dye **352**.[297] The increase in the quantum efficiency is more pronounced on selenium substitution in the barbituric moiety. The quantum efficiencies of **353** and **354** were found to be 120 and 60 times higher than that of their sulfur analogues **352** and **356**, respectively.[297,298]

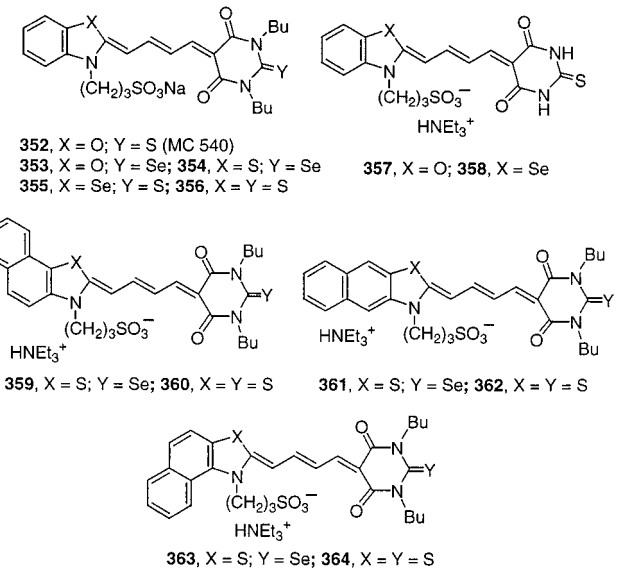


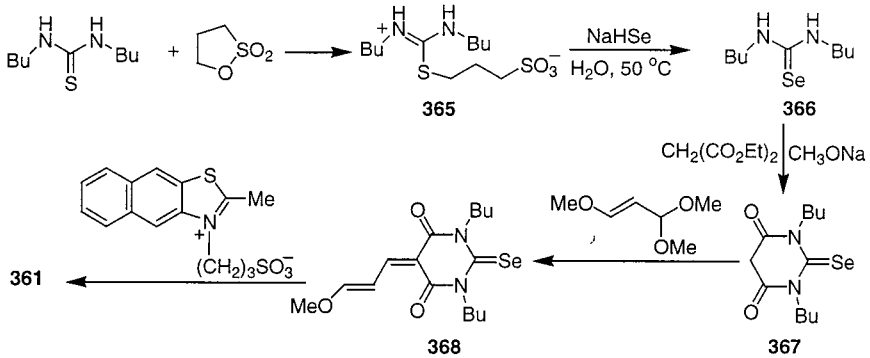
Figure 48.

Other structural modifications that have been explored to improve the biological efficiency of merocyanine dyes include addition of lipophilic substitutions on the electron-deficient barbituric moiety and modifications of the electron-donor heterocyclic moiety.[298] Such modifications increase the overall polarity, which reduces the phototoxicity toward mammalian cells and viruses. Replacement of the sulfopropyl group with sulfobutyl group did not give any benefits. On the other hand, introduction of a single methoxy group on the back ring improved the antileukemia action and provided a modest gain in the antiviral effect. A dramatic enhancement in photodynamic activity was observed by expanding the aromatic back ring from benzene to naphthalene.[297,298] The quantum efficiency of **359** was found to be 70 times higher than that of **360**. The selenium-containing naphthalene derivatives **359**, **361**, and **363** showed a multilog increase in their ability to activate tumor cells in vitro compared with their sulfur analogues **360**, **362**, and **364**.[297,299]

The synthesis of MC 540 analogues involves five major steps, as shown in Scheme 24.[297] The S-alkylation of 1,3-dibutyl-2-thiourea (**365**) using 1,3-propanesultone, followed by a nucleophilic displacement reaction with NaHSe, affords 1,3-dibutyl-2-selenourea (**366**). This compound reacts with diethyl melonate very slowly to give the cyclized product **367**, which is converted to 1,3-dibutyl-2-seleno-4,6-diketo-5-(3′-methoxypropenylidine)pyrimidine **368**. Further reaction of this derivative with 2-methyl-3-sulfopropyl salt affords the dye **361**.

In addition to these MC 540 analogues, a few selenium derivatives of carbocyanine and oxonol dyes (Figure 49) have been studied for their photodynamic properties.[296,300] The modified oxonol dyes have been

Scheme 24



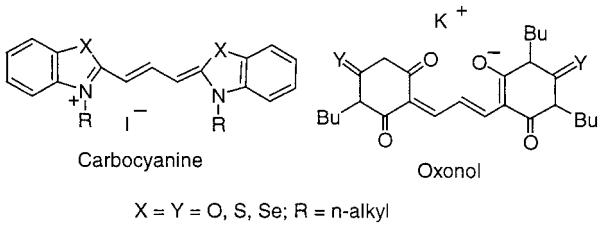


Figure 49.

shown to possess superior antineoplastic properties. A preclinical evaluation revealed that the selenium-containing dyes selectively induce photodynamic damage to the leukemia cells.[300] The selectivity of oxonol-induced photodamage observed in in vivo experiments simulating an autologous bone marrow transplantation leads to an assumption that oxonol dyes may be potentially useful for the purging of leukemia as well as breast cancer cells from autologous bone marrow grafts.[301]

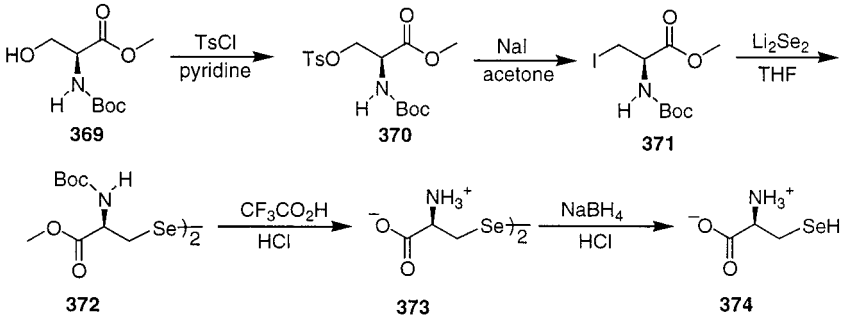
VI. Selenium Analogues of Amino Acids and Other Natural Products

The biosynthetic incorporation of selenium-containing amino acids into biomacromolecules has been used to produce both heavy-atom derivatives and NMR probes.[108,302−304] These selenium-based derivatives play an important role in the elucidation of both the local and global structures of many biomacromolecules. Particularly, replacement of active site cysteine residues by selenocysteine gives functional information based upon the differences in redox properties of the selenol and thiol groups.[111] Recently, the replacement of Cys residues with Sec has been reported to be an approach for studying conformational preferences of folding intermediates in peptides and proteins.[305] Despite the importance of the selenium analogues of amino acids, there are very few methodologies available for the synthesis of these compounds. Particularly, the synthesis of the widely used amino acid, selenocysteine, is complicated by the fact that it is readily oxidized in air to form selenocystine. A more convenient synthesis of selenocysteine and L-[^{77}Se]selenocysteine has been reported by using suitably protected β-haloalanines (Scheme 25).[306] In this synthetic route, the protected β-iodoalanine (**371**) was conveniently constructed in optically active form starting from the BOC-protected methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxypropionate (**369**). Reaction of the iodo compound with lithium diselenide followed by the removal of BOC protecting group afford the selenocystine. The ^{77}Se-enriched selenocystine can be reduced with $NaBH_4$ to obtain optically active selenocysteine (**374**).

A series of Se-substituted selenocysteine derivatives (Figure 50) has been synthesized and evaluated for their ability to act as potential kidney-selective prodrugs.[307] In an earlier study, Se-methyl selenocysteine was found to be an antitumor agent, and it has been shown that the β-elimination reaction is important for this activity.[308] It is now well-established that Cys-S conjugates can be used as kidney-selective prodrugs.[309−312] For example, *S*-(6-purinyl)-L-cysteine was bioactivated in the kidney by Cys-S conjugate β-lyases to the cytostatic agent 6-mercaptopurin.[309] The kidney selectivity of this compound is due to the fact that Cys-S conjugates are actively taken up by kidney cells.[313] On the basis of these reports, the kinetics of β-elimination reactions of Sec-Se conjugates **90** and **375**−**390** has been evaluated using rat renal cytosol (Table 19).

Although the Se and S conjugates exert their activities by β-elimination, the specific activities of β-elimination of selenium compounds are 50−140 times higher than that of their sulfur analogues. In the aliphatic series (**90** and **375**−**379**), the introduction of a $-CH_2-$ group into the *n*-alkyl substituent increased the turnover until Se-propyl-L-selenocysteine (**378**). Further expansion of the alkyl side chain by introducing one more $-CH_2-$ group (compound **379**) resulted in a considerable decrease in the activity. The isopropyl derivative (**378**) was the most active compound in this series, probably due to steric properties, resulting in a higher affinity to the β-lyases. The benzylic derivatives (**380**−**385**) were also found to be very good candidates for β-lyases. While the nature of the substituents on the benzene ring of the benzylic compounds does not affect the activity, the phenyl-based compounds **386**−**390** exhibited β-lyase activity, depending upon the substituents attached to the phenyl group. Compound **386**, without any bulky substituents in the aromatic ring, showed the highest activity of the series. The D-isomers were much less active compared with the corresponding L-isomers at low concentrations. Although the exact reason for this discrepancy is not yet known, the residual activity in cytosol can be explained by the fact that the D-isomers undergo oxidative deamination by renal D-amino acid oxidases followed by transamination by cytosolic transaminases to form the corresponding L-isomers, which finally can be β-eliminated by β-lyase.[314]

Scheme 25



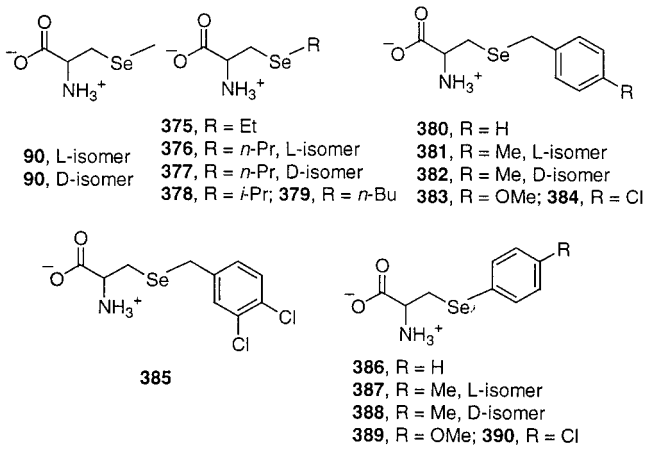


Figure 50.

Table 19. Specific Activities and Kinetic Parameters for the ss-Elimination by Rat Renal Cytosol[307,315]

compd	specific activity (nmol/min/mg)	compd	specific activity (nmol/min/mg)
90	7.1	**386**	13.8
90	4.0	**387**	3.8
375	11.0	**388**	0.1
376	8.0	**389**	4.3
377	3.3	**390**	5.2
378	14.9	**391**	10.3
379	0.9	**392**	17.8
380	9.8	**393**	13.8
381	13.1	**394**	9.2
382	4.9	**395**	7.4
383	10.2	**396**	8.8
384	11.6	**397**	15.5
385	12.3	**398**	11.8

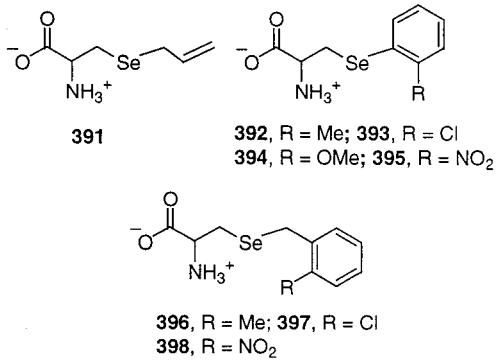


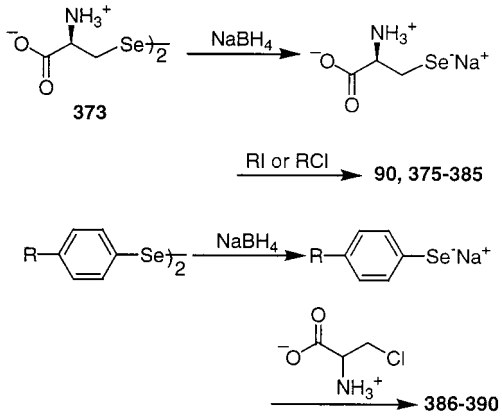
Figure 51.

The allyl derivative (**391**, Figure 51) and ortho-substituted phenyl derivatives (**392**−**398**) also showed good activity in rat renal cytosol.[315] Most of the compounds that were substrates for rat renal cytosol were also found to be good substrates for human cysteine conjugate β-lyase enzymes. However, rates of β-elimination in rat kidney cytosol were 22−877-fold higher than that observed in human kidney cytosol.[315] Further studies on the mechanism of β-elimination show that the enzyme β-lyase/glutamine transaminase K may play an important role in the reaction.[316] These data suggest that all these compounds are also expected to act as prodrugs of biologically active selenol compounds to the kidney, similar to compound **90**, which has been reported to have anticarcinogenic activity against dimethyl benzo[*a*]anthracene-induced tumors in rats.[308]

The cytotoxicity of some selenocysteine derivatives has also been studied with rat renal proximal tubular cells (RPTC).[317] The results showed that compounds **90**, **375**−**377**, and **379** did not cause significant cytotoxicity to RPTC up to concentrations of 500 μM, and no effect was observed on mitochondrial functioning. Compound **378**, however, was found to be cytotoxic, causing time- and dose-dependent cytotoxicity. Aminooxyacetic acid provided significant protection against cell death by **378**, indicating the involvement of cysteine conjugate β-lyase. Similar to the substituent effect on β-elimination activity, the cytotoxicity of the compounds are also known to be affected by various substituents. For example, the unsubstituted phenyl and benzyl-based compounds were nontoxic, whereas the substituted phenyl and benzylic compounds, particularly **383** and **390**, were toxic at a concentration of 200 μM. The overall results suggest that the nontoxic Se-alkyl compounds may be promising candidates for further evaluation for chemopreventive activities.

The synthetic pathways used for compounds **90** and **375**−**390** are summarized in Scheme 26.[307] The Se-

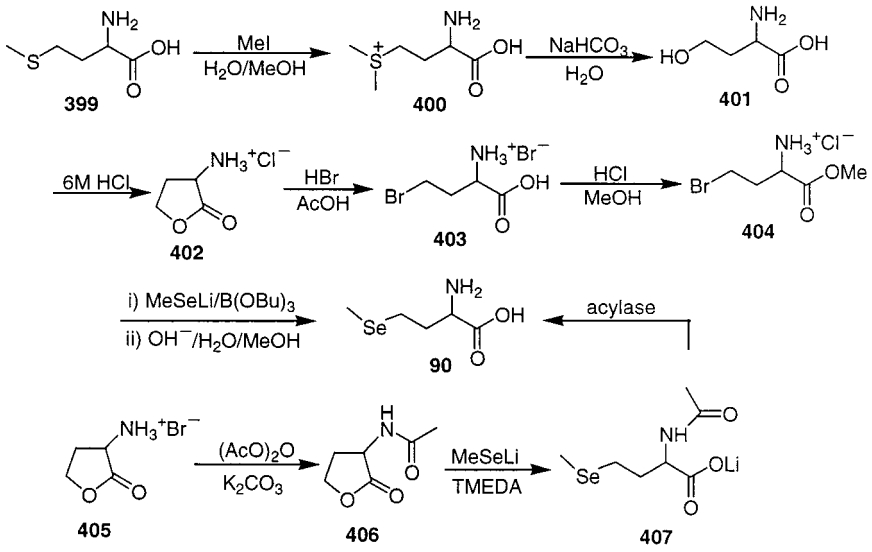
Scheme 26



substituted amino acids could be synthesized either by treating the selenolate form of selenocysteine with alkyl and aryl halides or by treating the aromatic selenolates with chloroalanine. The aliphatic and benzylic Se-substituents are introduced by reducing selenocystine to selenocysteine and subsequent reaction with the corresponding alkyl or benzyl halides (Scheme 26). The phenyl Se-substituted compounds are synthesized by reducing the appropriately substituted diphenyl diselenides to the corresponding selenolates and subsequent reaction with β-chloroalanine (Scheme 26).

Another unnatural amino acid that is employed more often in synthetic chemistry is the selenium analogue of methionine. While Sec substitution has been used in amino acids and proteins to alter the reactivity, the substitution of methionine residues with selenomethionine in proteins has been used to produce isomorphous variants as a new approach to solve the phase problem in protein crystallography.[302,318] Budisa et al. developed methods for specific and high-level incorporation of Sec as an isosteric analogue of methionine for structural investigations of human recombinant annexin V.[319,320] In addition

Scheme 27

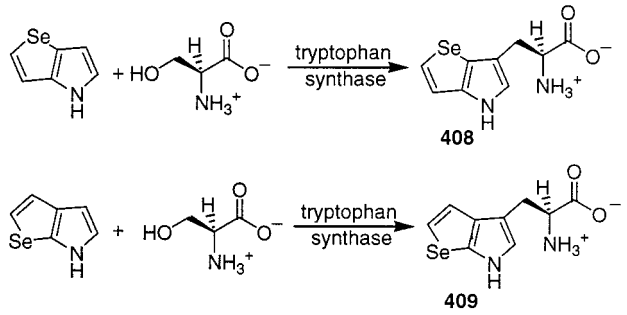


to the structural problems, other functions of the heavy atom analogues in protein engineering are being evaluated. It has been recently reported that substitution of methionine with selenomethionine enhances the stability of methionine-rich proteins.[321]

Although several methods have been reported for the synthesis of selenomethionine,[322−325] most of them afford racemic compounds. Initial efforts by Barton et al. led to the development of a photochemical method for the synthesis of L-(+)-selenomethionine (**90**).[323] A few years latter, Esaki et al. reported the stereospecific synthesis of selenomethionine by an enzymatic method.[325] Attempts to synthesize optically active SeMet in large scales by using these two methodologies met with limited success. As an extension of the synthetic work, Koch et al. showed that L-(+)-selenomethionine can also be prepared in large scale from L-(+)-methionine (**399**).[326] According to this methodology, the L-(+)-methionine was S-methylated with methyl iodide to generate **400**, which was subsequently hydrolyzed to yield L-(−)-homoserine **401**. The ring-closure reaction of **401** with 6 M HCl afforded L-(−)-α-amino-γ-butyrolactone hydrochloride **402**. The cyclic compound **402** was cleaved by HBr to form L-(+)-2-amino-4-bromobutanoic acid hydrobromide **403**, which was then converted to the corresponding methyl ester **404** and then treated with lithium selenolate (MeSeLi) to afford the expected selenium compound (**90**) (Scheme 27). The synthesis of L-SeMet has also been achieved via *N*-acetyl-(*R,S*)-2-amino-4-butyrolactone, as shown in Scheme 27.[327] This method is based on the ring opening of butyrolactone (**405**) by the soft nucleophile methyl selenolate via an S_N2 ester cleavage reaction at the soft sp^3 center.[328] The N-acylation also allows the enantioselective enzymatic decetylation by an amino-acylase-based procedure to generate the L-SeMet.

Other synthetic amino acids such as 6-(4*H*-selenolo[3,2-*b*]pyrrol)-L-alanine (**408**) and 4-(6*H*-selenolo-[2,3-*b*]pyrrolyl)-L-alanine (**409**) have been prepared by using tryptophan synthase, an enzyme from *Salmonella typhimurium* (Scheme 28).[329] It has been suggested that amino acids **408** and **409** can be incorporated into proteins as isomorphous replace-

Scheme 28



ments for L-tryptophan. Similar enzymatic synthesis of Se-substituted L-Sec with tryptophan synthase has been reported.[330] The incorporation of selenolopyrrole-alanine into proteins as isomorphous tryptophan analogue has been accomplished by Bae et al.[331] The incorporation has been achieved by fermentation and expression in a Trp-auxotrophic *E. coli* host strain using the selective pressure incorporation (SPI) method. Similar to the SeMet substitution, the bioincorporation of tryptophan surrogate into proteins is expected to be a useful method for X-ray crystallographic structure determination of proteins.[331,332]

Another good illustration of the selenium substitution for sulfur is observed in the 1,2-dithiolane ring of α-lipoic acid (**7**). Similar to the antioxidant properties of natural lipoic acid,[333,334] the mono and diselenium analogues of lipoic acid, **410** (Figure 52) and **290**, have also been reported to be potent antioxidants.[335,336] The diselenide **290** inhibited the formation of lipid peroxidation products in low-density lipoprotein after oxidation by copper. In contrast, α-lipoic acid did not inhibit the formation of lipid peroxidation products, which suggests that the selenium analogue could be a good antioxidant in lipid environments, whereas lipoic acid exerts its effects only in a hydrophilic environment. The monoselenolipoic acid **410** is also expected to be a versatile antioxidant with direct thioredoxin-like activity. It has been shown that compound **410** supports the growth of lipoate-dependent bacteria. These results suggest that **410** would be susceptible to reversible

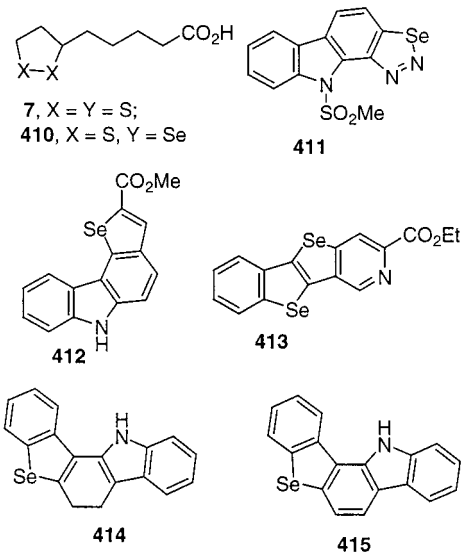


Figure 52.

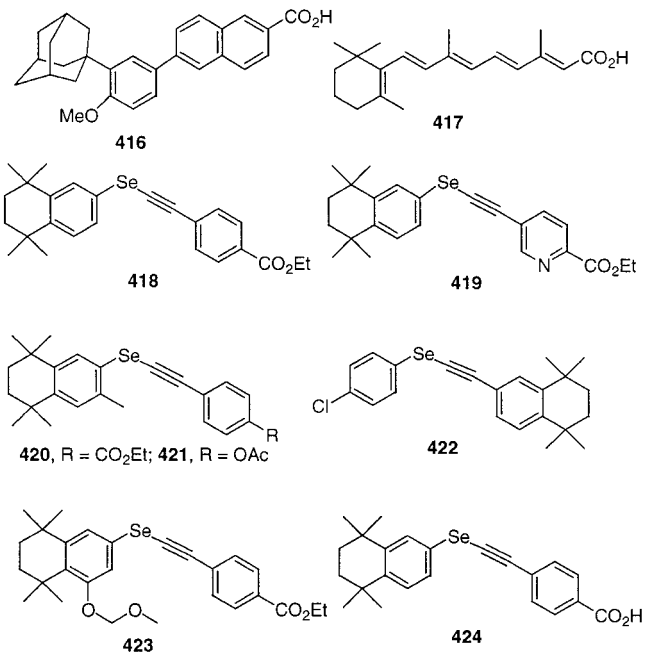


Figure 53.

reduction by one or more of the enzymes that reduces lipoic acid.[336] Recently, several selenium-containing heterocyclic compounds (**411**−**415**) were synthesized.[337] These compounds are considered as the selenium analogues of the antitumor alkaloids pyridocarbazoles and indolocarbazoles.

The application of the selenium incorporation into natural structures can also be seen in a more recent study with acetylenic retinoids, which were employed as agonistic probes of the pharmacophore of retinoic acid receptors (RAR).[338] Retinoids, both synthetic (Adapalene, **416**, Figure 53)[339] and natural analogues of *all-trans*-retinoic acid (**417**), exert marked effects on cell differentiation and proliferation[340] and their biological effects are mediated by interaction with specific nuclear receptor (RARs), which can induce transcriptional activation through response elements[341] and/or which affect the activity of the transcription factor AP-1.[342]

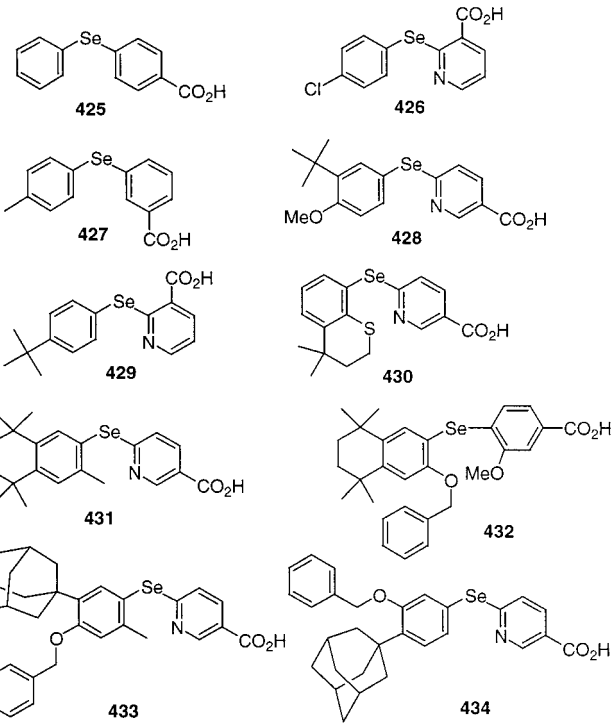


Figure 54.

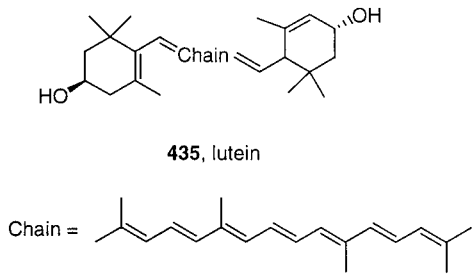


Figure 55.

Compounds **418**−**424** have been found to have significant RAR agonist activity. In particular, the carboxylic acid derivative **424** resulting from the saponification of ester **418** displayed a transcriptional activity as good as that of *all-trans*-retinoic acid. The potent RAR affinities of **424** led to the development of diaryl selenides such as **425**−**434** (Figure 54) possessing structural features of **416**.[343] These compounds are expected to act as RXR, one of the known types of retinoic acid receptors located in the cell nucleus. In the presence of a ligand, these receptors (RAR and RXR) form dimers that bind to DNA through distinct response elements. Compound **431** is found to be 10 times more potent as an RXR agonist than its sulfur analogue.[343]

Recently, selenium-containing carotenoids attracted considerable attention as natural analogues. The first natural carotenoid lutein (**435**, Figure 55) was discovered in 1837 by Berzelius,[344] who also discovered selenium in 1818.[1] The strong link between selenium and carotenoids was first realized when selenium and carotenoids were found together in certain plants[345−347] and algae.[348] Studies on biological functions and activities of natural carotenoids have been mainly performed in the fields of photosynthetic plants, algae, and bacteria, and two major functions have

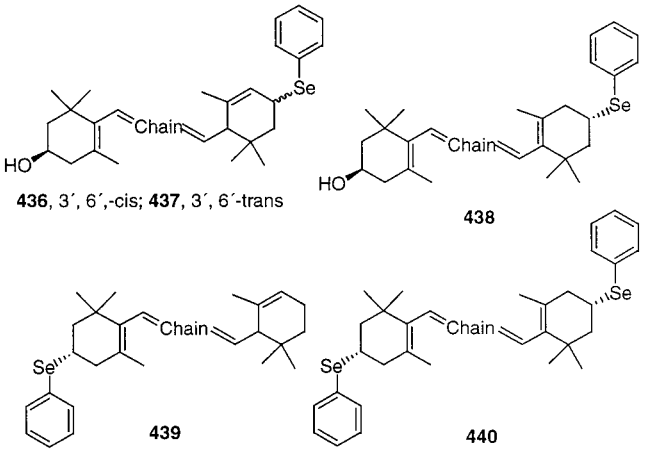


Figure 56.

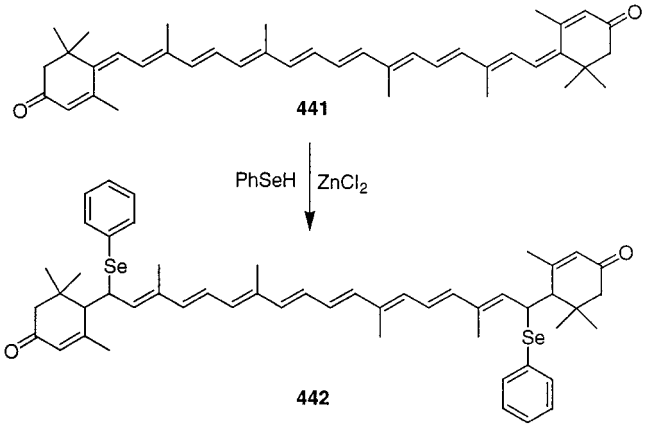
been revealed: (i) a light harvesting role in the antenna complexes of the chloroplast in photosynthesis and (ii) as protecting agents against the harmful photooxidative effects of bright light.[349] On the other hand, the well-established biological function of carotenoids in animals is as vitamin A precursors.[350] The synergistic effect of selenium and carotenoids has been shown to influence their action against biological oxidants[351] and cancer.[352]

It has been reported that the intake of inorganic selenium and β,β-carotene has an inhibitory effect on carcinogenesis in rats.[353] Synthetic selenocarotenoids are an important class of compounds because these are expected to be physiologically more active than their natural analogues. Selenocarotenoids (**436**−**440**, Figure 56) were synthesized from lutein and related derivatives. The two isomers **436** and **437** were obtained by a facile synthesis from lutein (**435**) with benzeneselenol.[354] Compounds **438**−**440** were synthesized by a reaction of (3*R*,3′*R*)-zeaxanthin with triphenylphosphine, diethyl azodicarboxylate, and benzeneselenol in the presence of triphenylphosphine and diethyl azodicarboxylate.[354] The aryl-substituted selenium derivatives **436**−**440** were found to be as stable as the corresponding carotenols.

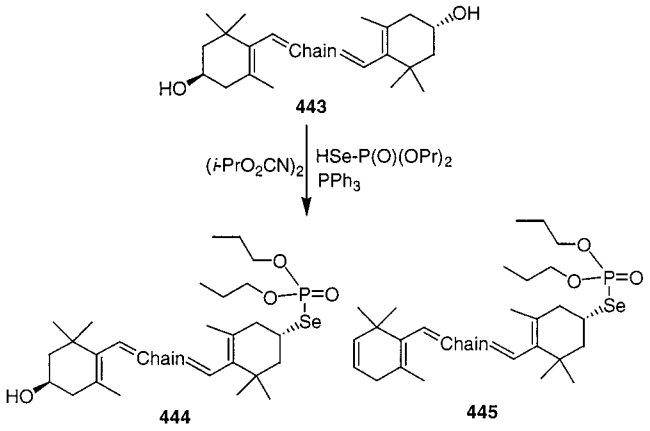
Some preliminary investigations on selenocarotenoids have been carried out with regard to their use as therapeutic agents.[355] For example, the phenylseleno derivative (**436**) exhibited better activity in the quenching of $^1O_2$ compared with its parent compound lutein.[355] The keto carotenoid rhodoxanthin (**441**) also reacted with benzeneselenol to form an addition product (**442**, Scheme 29).[356]

Because of steric hindrance at C(5),C(5′), the normally favored 1,4 (conjugate) addition of the selenide is difficult and therefore the 1,6-addition product **442** was formed preferentially. In contrast to **436**−**439**, compound **442** was found to be unstable and eliminated diphenyl diselenide to form the naturally occurring ϵ,ϵ-carotene-3,3′-dione. As an extension to the above group of selenocarotenoids, optically active carotenoid selenophosphates were synthesized from zeaxanthin.[357] Reaction of (3*R*,3′*R*)-zeaxanthin (**443**) with di-*O*,*O*-propyl-Se-hydrogen phosphate under Mitsunobu reaction conditions afforded selenophosphates **444** and **445** (Scheme 30). Compounds **444** and **445** were found to be as stable

Scheme 29



Scheme 30



as zeaxanthin. The circular dichroism (CD) studies on **436**−**445** show that the Se substituents destabilize the preferred conformer of the β-end group.[358]

Organoselenium-modified cyclodextrins are currently attaining a prominent position in supramolecular chemistry. Native cyclodextrins (**446**) are rigid molecules and offer limited utility in terms of size, shape, and availability of chemically useful functional groups. Chemical modifications of native cyclodextrins offer exquisite molecules that can be invaluable in investigations at the frontiers of chemistry ranging from enzyme-like catalytic activity to antibody-like binding.[359,360] In organoselenium-modified cyclodextrins, the longer and more flexible C−Se bond compared with C−C bond is conformationally favored for binding guest molecules.[361] The cyclodextrins possessing arylselenenyl moiety can recognize small differences between guest molecules based on their size, shape, rigidity, and chirality.[362,363] A few examples of selenocyclodextrin hosts are summarized in Figure 57.

Extensive studies on the molecular recognition by **447**−**450** showed that the substitution at phenyl ring (**448, 449**) or the introduction of a $-CH_{2-}$ group into the C−Se bond (**450**) lead to their tighter self-inclusion, which discourages the replacing inclusion of the guest molecules.[364−367] The bis(β-cyclodextrin)s **455**−**457** showed higher affinities toward guest molecules than native β-cyclodextrin.[368] Inclusion complexation of the naphthyl derivative **454** with aliphatic amino acids was too weak to be observed,

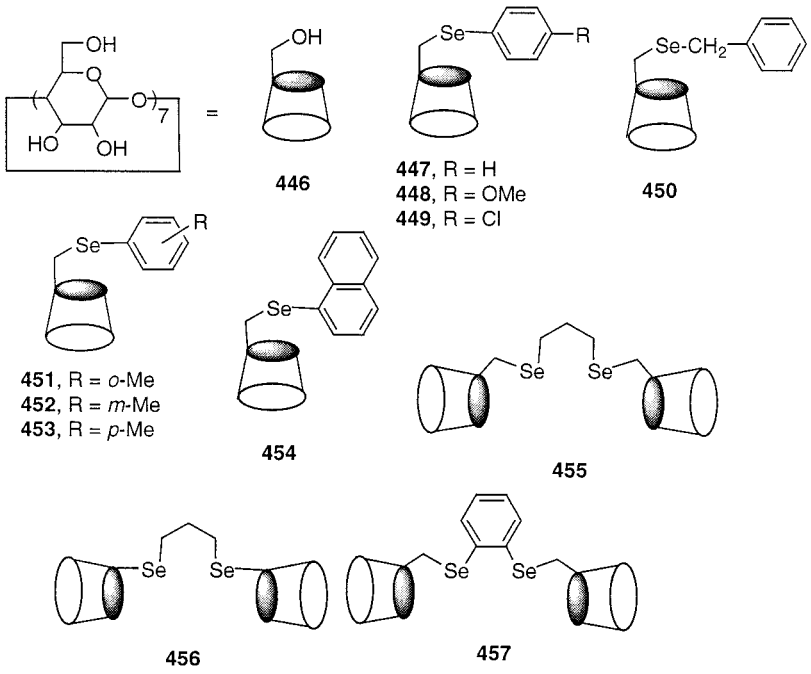


Figure 57.

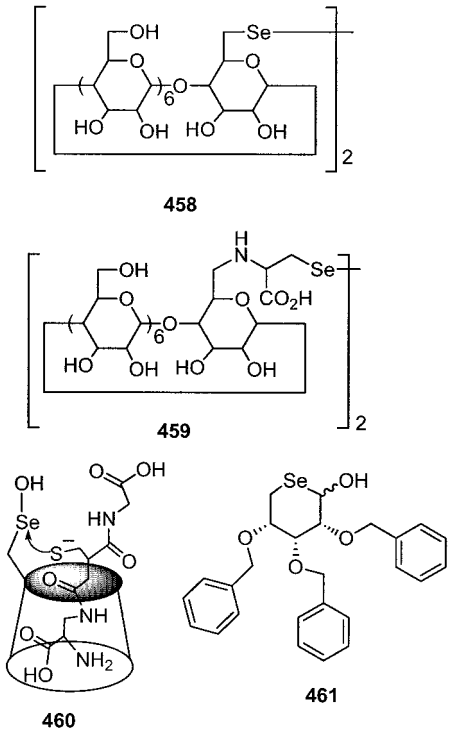


Figure 58.

which is attributable to the stronger self-inclusion of the naphthylseleno moiety attached to the primary side of cyclodextrin into the cavity. As in the case of native cyclodextrins, selenium derivatives also show preference toward the L-isomers during the molecular recognition.[364,369,370] The molecular recognition by organoselenium-modified cyclodextrins led to the development of enzyme mimics containing selenol or diselenide groups. Liu et al. reported the synthesis and GPx activity of 2- and 6-selenium-bridged β-cyclodextrins.[371−373] The GPx activity of the 6-selenium-bridged derivative (**458**, Figure 58) was found to be 4.3-fold higher than that of ebselen.

The higher activity of **458** compared with ebselen may be due to the presence of specific sites in the former case for GSH binding. As an extension of this study, introduction of selenocystine residues into the primary side of β-cyclodextrin through the two amino nitrogen groups of selenocystine also led to an efficient GPx mimic (**459**).[374] This selenium derivative catalyzes the reduction of a variety of hydroperoxides such as H_2O_2, *t*-BuOOH, and cumene hydroperoxides by using GSH. The GPx activity of this compound with H_2O_2 was 82 and 4.2 times higher than that of selenocysteine and ebselen, respectively. The enhancement in the reduction rate again indicates that the cyclodextrin moiety provides a hydrophobic cavity to bind the substrate GSH (**460**, Figure 58). The GPx activity of **459** was much higher with cumene hydroperoxide than with H_2O_2, suggesting the important role of the strict shape/size relationship.[374] Since cyclodextrin has a stronger ability to bind organic molecules, the cumene hydroperoxide may fit well into the cavity provided for substrate binding.[375]

Although a few isotope-labeled selenium compounds such as selenosteroids have been reported as adrenal scanning agents,[376−378] many other selenium-containing natural products have been used only as intermediates in organic synthesis. Since these compounds are beyond the scope of this review, such derivatives are not discussed here. One particular area worth mentioning here is the selenosugars. Schiesser et al. recently reported the synthesis of carbohydrate derivatives such as **461** containing selenium in the ring position.[379]

VII. Synthetic Peptides, Enzymes, and Catalytic Antibodies

Incorporation of unnatural amino acids or other structures into natural peptides and enzymes allows much greater diversity and precision in substrate binding. During the initial stages, chemical synthesis

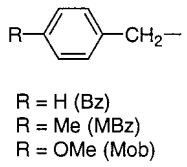
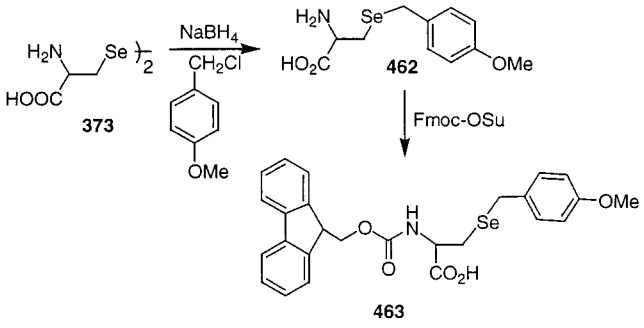


Figure 59.

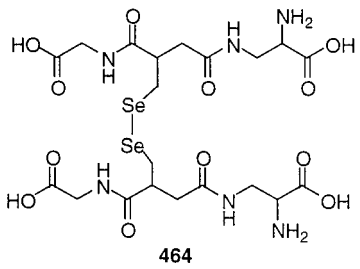
has largely been restricted to small peptides, because of the accumulation of side products that complicate product purification and decrease yields.[380,381] Synthetic peptides and enzymes containing selenocysteine, selenocystine, or selenomethionine are particularly important, since the incorporation of a selenium atom is expected to provide interesting chemical properties and biological activities.[382−384] Chemical methodologies have been successfully applied to synthesize metalloselenonein, in which all the cysteine residues in metallothionein were replaced by selenocysteine.[385] Besse and Moroder reported the synthesis of a series of octapeptides containing selenocysteine.[386] Recently, selenium-containing apamin analogues have been synthesized by replacing Cys residues with Sec and used for oxidative folding studies.[387,388] In contrast to the synthesis of selenium-containing amino acids, there is limited information available on the synthesis of selenopeptides due to the unavailability of suitable protecting groups. For example, the benzyl (Bz) group was initially introduced for the protection of the selenol group in the synthesis of selenium analogues of oxytocin[389] and somatostatin.[390] However, this method is not suitable for chemical manipulations, because the deprotection leads to side reactions. In recent syntheses of selenocysteine peptides, substituted benzyl derivatives such as *p*-methylbenzyl (MBz)[385] and *p*-methoxybenzyl (Mob, Figure 59)[391] have been used as protecting groups. The Mob group can also be used for Fmoc-based solid-phase peptide synthesis (Scheme 31).[392] In this case the Mob and

Scheme 31



Fmoc groups were used for the protection of selenol and amino groups, respectively. The selenocysteine derivative *N*-9-fluorenylmethoxycarbonyl-Se-4-methoxybenzyl selenocysteine (**463**) serves as a precursor for further coupling reactions.

The synthesis of selenopeptide **464**, the selenium analogue of glutathione disulfide (GSSG), has been reported by using the liquid-phase method.[391] The selenol group was protected by the Mob group, which was removed by acid hydrolysis with trifluoroacetic acid in the presence of thioanisol. The synthesis of



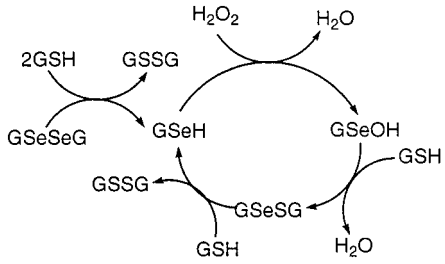


Figure 60.

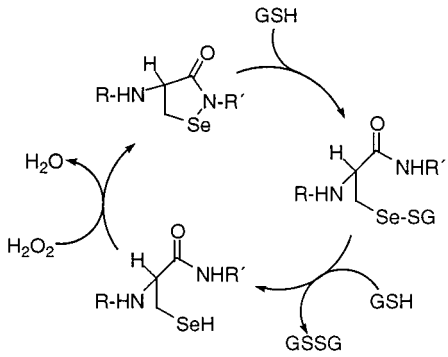
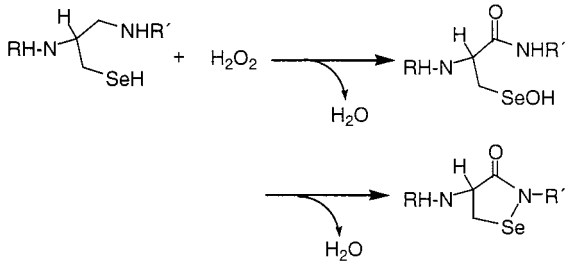


Figure 61.

four diastereomers of **464** by using a similar method has been reported.[393] All four diastereomers, i.e., LL-, DL-, LD-, and DD-isomers, exhibited significant GPx activity. The LL-isomer showed the highest activity of the series followed by the DL-, LD-, and DD-isomers. The ratio of activities of the LL-, DL-, LD-, and DD-isomers is 0.97:0.42:0.11:0.07 for various hydroperoxides. Although these isomers reduce H_2O_2, cumene hydroperoxide, and *t*-BuOOH, H_2O_2 is a better substrate than the organic peroxides. The mechanism involves the oxidation of GSeH by hydroperoxides to form GSeOH, which is reduced by GSH to regenerate GSeH through the glutathione−glutaselenone adduct (Figure 60). The difference in the GPx activity between the four diastereomers is probably due to a different mode of interaction between GSeOH and GSH. The stereospecific interactions between GSeSG and GSH may also contribute to the reactivity of GSeSG.

Chan et al. also reported that selenopeptides mimic the action of GPx.[394] Di- (Sec-Gly) and tetrapeptides (Sec-Gly-The-Thr) reduced H_2O_2 more effectively than ebselen using GSH as thiol cofactor. The proposed mechanism involves the oxidation of selenols to cyclic selenenamides, followed by ring opening by GSH to form selenenylsulfide intermediate (Figure 61). As in the case of ebselen, the formation of selenenic acid may be the basis for the selenenamides, as shown in Scheme 32. However, the forma-

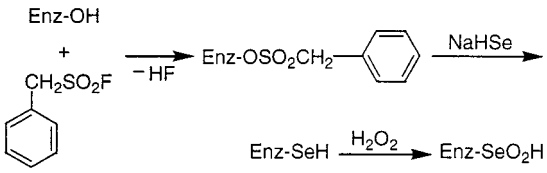
Scheme 32



tion of selenenic acid could not be observed during the catalytic cycle. In the synthesis of these di- and tetrapeptides, the selenol group is introduced at the last step by nucleophilic displacement of the *O*-tosyl group of a serine using $PhCH_2SeNa$ followed by deprotection of the benzyl group.[394] A further example of the selenopeptides is the synthesis of the selenium analogues of α-rat-atrial natriuretic peptide (α-ANP).[395] α-ANP peptide plays an important role in body fluid homeostasis and blood pressure control through its effect on a natriuresis/diuresis, vasorelaxation, and inhibition of aldosterone section.[396] The seleno-α-ANP peptides have been evaluated for receptor binding potencies in cultured rat vascular smooth muscle cells. The IC_{50} values for receptor binding suggest that the substitution (Sec for Cys) does not cause significant conformational change around the disulfide bridge and indicate that the Sec residues play a role similar to that of the Cys residues in exerting biological activity.

The successful chemical modification of the bacterial serine protease subtilisin to thiosubtilisin has generated interest in the chemistry of semisynthetic enzymes.[397,398] Following these reports, the first artificial selenoenzyme, selenosubtilisin, was synthesized by site-selective chemical modification of the catalytically important serine residue of the subtilisin Carlsberg. Introduction of the Sec residue into the binding pocket confers novel hydrolytic and redox properties to the original protease template. A simplified chemical conversion of subtilisin into the semisynthetic peroxidase selenosubtilisin is given in Scheme 33.[399] In subtilisin, three amino acid residues,

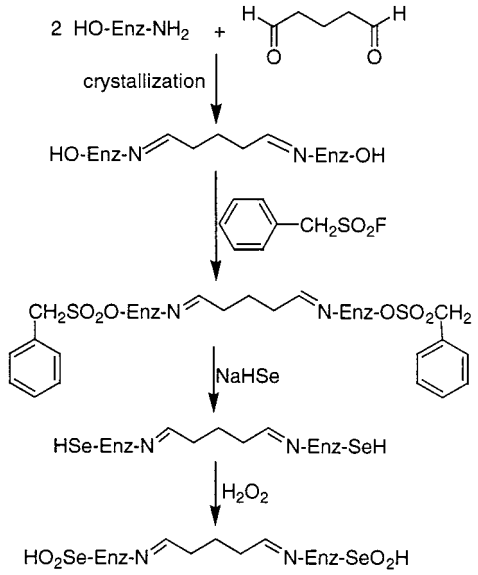
Scheme 33



Asp32, His64, and Ser221, form a "catalytic triad" that increases the activity and nucleophilic of the catalytically important hydroxyl group of Ser221.

The serine residue can therefore be activated by addition of phenylmethanesulfonyl fluoride (PMSF). Selenium is then introduced into the active site by reaction of the sulfonated species with hydrogen selenide. Addition of H_2O_2 to the resulting selenol affords the seleninic acid form of selenosubtilisin. As an extension of this work, Schreier et al. used several subtilisin preparations for an up-scaled synthesis of selenosubtilisn.[400,401] For example, the industrially produced Maxatase, an encapsulated detergent addition, was converted into selenosubtilisin as shown in Scheme 33. The stability of selenosubtilisin could be increased by cross-linking of the enzyme in its crystalline state. For this purpose, the subtilisin was chemically transformed into the cross-linked crystals (CLCs) of selenosubtilisin according to Scheme 34.[402,403]

Scheme 34



In this method, subtilisin was first subjected to batch crystallization with glutardialdehyde, yielding microcrystals of the cross-linked subtilisin. The catalytically active selenol group was then introduced into the crystals according to Scheme 33. The cross-linked selenosubtilisin represents an immobilized biocatalyst that can be easily recycled by filtration or centrifugation.[402] The cross-linked selenosubtilisin is much more stable than the non-cross-linked enzyme.

The amino acid residues Asp32 and His64, which are essential for the proteolytic activity of native subtilisin,[404,405] also contribute to the stability and reactivity of selenosubtilisin. The carboxylate side chain of Asp32 forms a hydrogen bond to His64, which orients the imidazole ring within the active site and allows it to serve as a general base in catalysis. The seleninic acid group is involved in hydrogen bonding and electrostatic interactions within the active site, particularly with the side chains of His64 and Asn155, as shown in Figure 62.[406] ^1H and ^{77}Se NMR data also suggest that the selenium exists predominantly in seleninic acid form and specific

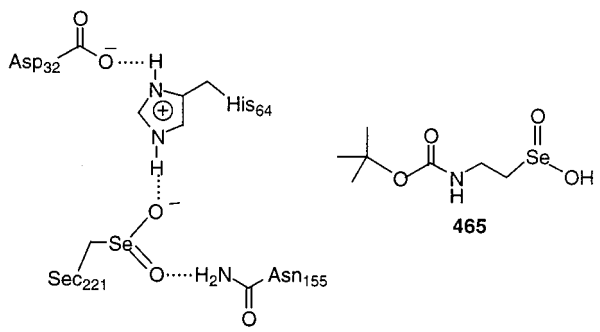
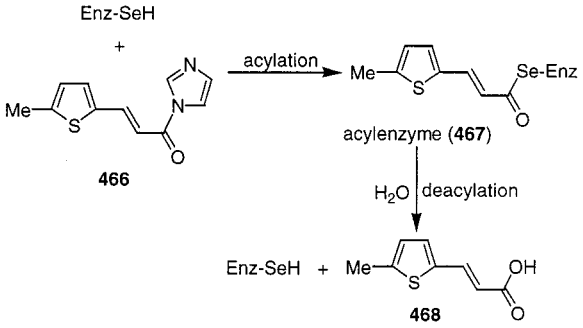


Figure 62.

hydrogen-bonding interactions between the seleninic acid and active-site residues stabilize this form ($EnzSeO_2^-$) of the prosthetic group.[407,408] The pK_a values of the seleninic acid form of the enzyme is at least 1.5 pH units lower than that of simple alkane seleninic acid such as *N*-(*tert*-butoxycarbonyl)selenohypotaurine (**465**). The seleninic acid group of the enzyme can be reduced by thiols. Treatment of the enzyme with excess DTT at neutral pH reduces the prosthetic group to selenol (EnzSeH). The large upfield shift of the ^{77}Se NMR chemical shift (−215 ppm) of the reduced enzyme suggests that the selenol is dissociated to selenolate by nearby amino acid residues.[408]

Selenosubtilisin acts as an acyl transferase, promoting the cleavage of activated acyl derivatives. For example, reduced selenosubtilisin has been shown to hydrolyze cinnamoylimidazole under anaerobic conditions via an acyl-enzyme adduct.[399] The aminolysis studies on the acyl-enzyme, cinnamoylated selenosubtilisin, showed that the rate of aminolysis of the cinnamoylselenosubtilisin by amines is much faster than that of cinnamoylated subtilisin and thiosubtilisin. For example, the transfer of the cinnamoyl group to butylamine rather than water is 14 000 times more efficient for selenosubtilisin than for native subtilisin and 20 times more efficient than for thiosubtilisin.[399] These observations are consistent with the fact that selenol esters normally undergo aminolysis considerably faster than esters and thiol esters.[409] Studies on the deacylation of (5-methylthienyl)acryloyl (5-MTA) bound selenosubtilisin (Scheme 35) by Raman spectroscopy[410] and molecular

Scheme 35



modeling[411] showed that the rate of the hydrolysis depends on the nature of interactions between the carbonyl group of the acyl enzyme (**467**) and the amino acid residues. According to the molecular mechanics model,[411] the carbonyl oxygen is hydrogen-bonded to the side chain of Asn155 and the thiophene ring stacks next to the ring of Tyr217. The conformation is s-trans about the ═C−C═O single bond with a dihedral angle of 168°. This indicates that all the atoms of the 5-MTA moiety and the carbons of the selenocysteine residue are more or less in the same plane. Because of this arrangement, 5-MTA-selenosubtilisin (**467**) exhibits no polarization. On the other hand, the replacement of Asn155 with a glycine residue (N155G) changes the orientation of 5-MTA moiety, and therefore, the acyl enzyme derived from N155G mutant experiences polarization. In the mutant acyl-enzyme, the carbonyl oxygen of the acyl group is hydrogen bonded to the backbone amides of Sec221 and Thr220, with the thiophene ring lying close to the side chain of Glu156, and the conformation is s-cis about the ═C−C═O single bond. The same explanation may hold true for the cinnamoylselenosubtilisin, where the N155G mutant deacylated approximately 1.5 times faster than the wild-type analogue,[411] and for the 5-MTA adducts with papain, cathepin B, and a number of its mutants.[412]

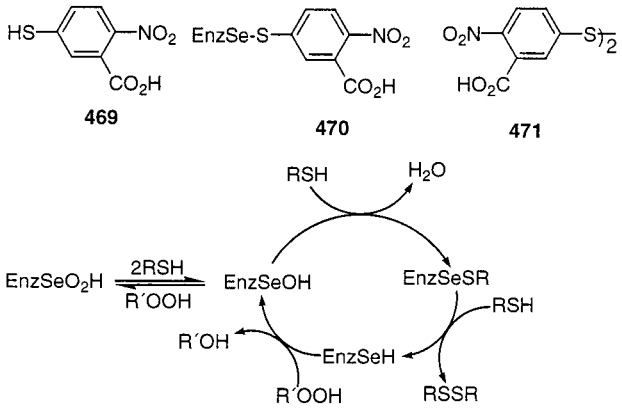


Figure 63.

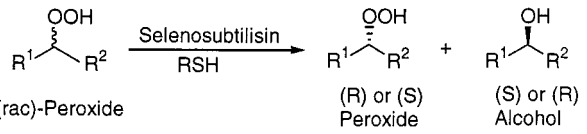
In addition to its hydrolytic properties, selenosubtilisin exhibits significant redox activity. This enzyme catalyzes the reduction of H_2O_2 and some alkyl hydroperoxides at the expense of thiols and thus mimics the action of GPx.[413] While the catalytic mechanism is similar to that of GPx involving selenenic acid, selenenyl sulfide, and selenolate forms of the enzyme, the choice of thiols is more restricted. For example, GPx uses GSH more efficiently than other thiols such as mercaptoethanol for the reduction H_2O_2, whereas selenosubtilisin preferentially uses aromatic thiols such as 3-carboxy-4-nitrobenzenethiol, and GSH or alkanethiols are poor substrates for catalysis. The seleninic acid form of selenosubtilisin is quite stable but readily reacts with 3 equiv of 3-carboxy-4-nitrobenzenethiol (**469**) to give the selenenyl sulfide form of the enzyme (**470**). Addition of an excess thiol produces the selenol form of the enzyme with the elimination of 5,5′-dithiobis-(2-nitrobenzoic acid) (**471**). The resulting selenol reacts with peroxides to form selenenic acid with the elimination of alcohol or water (Figure 63).[414] A direct comparison between the catalytic activities of GPx and selenosubtilisin could not be made since the pH−rate profiles of the two systems differ considerably. However, the GPx was approximately 10^5 times more active than the selenosubtilisin for the reduction of alkyl hydroperoxides.[414]

In addition to its acyl transferase properties, the mutant N155G also exhibited significant GPx activty.[415] Although some changes were observed in the reaction mechanisms between the wild-type and mutant, the kinetic parameters for both the enzyme showed that the stability of the key intermediates or transition state was not affected by the N155G substitution. Molecular dynamics simulations indicate that the side chain of Asn155 in the wild-type enzyme partially blocks the preferred trajectory for thiol attack on the selenenyl sulfide intermediate

(EnzSeSR).[406] The peroxidase activity of other mutants of selenosubtilisin has also been studied.[416] This study shows that the modification of several active site residues that are not directly involved in the redox chemistry may alter the kinetic mechanism. For example, replacement of the nonessential active site residues Glu156, Gly169, and Tyr217 (selenosubtilisin BPN′ sequence) with Ser156, Ala169, and Leu217 (selenosubtilisin Carlsberg sequence) changes the kinetic mechanism of selenosubtilisin BPN′ to that of Carlsberg enzyme.[416] Kinetic studies with various hydroperoxides revealed that the hydroperoxide-mediated oxidation of the selenolate is at least partially rate-limiting.[417]

The facile reduction of organic hydroperoxides by selenosubtilisin led to the development of synthetic methodologies for its use in enantioselective catalysis.[418−422] Selenosubtilisin efficiently catalyzes the kinetic resolution of racemic hydroperoxides (Scheme 36). In contrast to the kinetic resolution of

Scheme 36



racemic hydroperoxides by lipase or chloroperoxidase horseradish peroxidase, which are restricted to sterically unhindered substrates, selenosubtilisin can resolve large hydroperoxide substrates.[421,422] The enantioselectivities and the catalytic efficiencies (k_{cat}/K_m) observed for the selenosubtilisin catalysis are summarized in Table 20.

Schreier et al. developed a hypothetical model for the enantioselectivity of the reaction catalyzed by selenosubtilisin.[420] According to this model, the reason for the predictable enantioselectivity arises from the subtilisin template, which catalyzes the esterification or acylation of racemic alkyl aryl alcohols or amines, respectively.[423] The enantioselectivity of subtilisin for (*S*)-configured alkyl arylamines (**486**, **487**, Figure 64) or alcohols (**488**, **489**) has been associated with the arrangement of substrate-binding pockets S_1 and S_1'.[423−425]

In selenosubtilisin BPN′ enzyme, the residues Glu 156 and Gly169 are located in the S_1 pocket and the third residue, Tyr 217, is part of the S_1' pocket. While the S_1 pocket influences the substrate specificity, the relatively apolar nature of the S_1 sites may account for selenosubtilisin's preference for hydrophobic alkyl hydroperoxides.[414] Therefore, alkyl and aryl hydroperoxides fit well to the S_1 pocket compared with H_2O_2. The differences in the enantioselectivity between various alkyl and aryl hydroperoxides arise from their degree of interactions with the polar S_1' cleft. Because of this reason, α-hydroxy hydroperoxide (**474**) with polar −OH group near the S_1' pocket fits very well to these binding sites and exhibits high enantioselectivity.[422]

The influence of selenocysteine substitution has also been experienced in the case of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The replacement of the essential Cys149 in the active site of GAPDH by a Sec led to a selenoGAPDH that exhibited significant GPx activity.[426] Similar to the involvement of a histidine residue in selenosubtilisin, a histidine residue (His176) in GAPDH forms an efficient ion pair with the catalytically active selenocysteine (Sec149). The formation of this ion pair, which is different from the one proposed for the native GPx, has been assumed to account for the lower activity of selenosubtilisin and selenoGAPDH compared with the native GPx.[426] This difference may also arise from the fact that selenosubtilisin and selenoGAPDH do not have specific binding sites for the cofactor GSH. Therefore, these two enzymes have been shown to catalyze the reduction of hydroperoxide using 3-carboxy-4-nitrobenzenethiol instead of GSH.

To synthesize catalytic species with substrate binding sites, selenium-containing catalytic antibodies have been developed, since the antibodies that selectively bind almost any molecule of interest can be synthesized. Luo et al. developed a strategy for generating catalytic antibodies with GSH binding sites by using monoclonal antibody[427] or bioimprinting techniques.[428] In the monoclonal antibody technique, the reactive thiol group of GSH was first protected with 2,4-dinitrophenyl group to give the hapten (**490**) (Scheme 37).[429] The hapten was then cross-linked to BSA by using the bifunctional reagent glutaraldehyde. The resulting antigen (**491**) was immunized to give the monoclonal antibody (McAb) with serine residues that are converted to selenocysteine by chemical mutation as shown in Scheme 37.[427,430]

In the bioimprinting technique, the GSH derivative *N*,*S*-bis(2,4-dinitrophenyl)glutathione (**492**, Figure 65) was used as the imprinting molecule. The unstable thiol and amino groups of GSH were protected by 2,4-dinitrophenyl groups.[428] The protected GSH derivative **492** was then allowed to react with denatured egg albumin to form a new conformation via hydrogen bonds, ion pairing, and hydrophobic interactions. Cross-linking of the imprinted protein by using glutaraldehyde followed by dialysis to remove **492** yielded the protein with new binding sites. Similar to the monoclonal antibody technique, the reactive serine residues were converted to selenocysteine by chemical mutation.[428] The catalytic antibodies and printed protein synthesized by these two methods exhibited high GPx activity.[427,428,431]

VIII. Antitumor and Anti-Infective Drugs

A. Antitumor Drugs

The application of organoselenium compounds in cancer prevention and treatment is a fascinating field for selenium research. Selenium compounds have proved to be very potent anticarcinogenic agents in different models, with spontaneous, chemically induced, or transplanted tumors or in culture.[432−434] Several epidemiological studies confirmed the activity of selenium in the field of cancer prevention, and several intervention studies resulted in encouraging results.[432−434] Organoselenium compounds developed for antitumor activity at the initial stages were

Table 20. Enantioselectivities and Kinetic Parameters of the Selenosubtilisin-Catalyzed Kinetic Resolution of Hydroperoxides

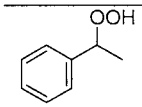
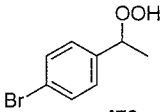
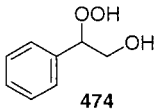
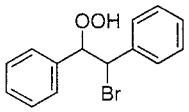
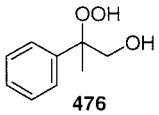
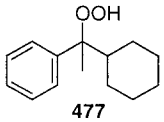
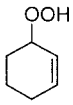
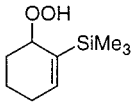
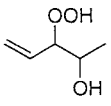
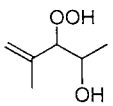
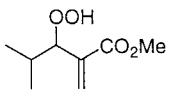
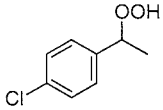
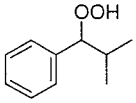
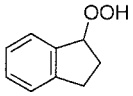
hydroperoxide	K_m [mM]	k_{cat} [min^{-1}]	k_{cat}/K_m [mM^{-1} min^{-1}]	Peroxide	Alcohol	Ref.
 **472**	15.7	2125	135	52(R)	60(S)	419
 **473**	4.3	592	138	34(R)	28(S)	419
 **474**	2.1	2443	1150	99(S)	99(R)	408, 419
 **475**-erythro	0.07	3322	47500	64	90	419
 **476**	9.3	905	96	-	-	419
 **477**	1.8	33	19	4	4-	419
 **478**	29.1	981	34	-	-	419
 **479**	8.5	820	97	80	96	419
 **480**-threo	5.3	449	84	14(S,S)	30(R,R)	419
 **481**-threo	12.2	643	53	22(R,R)	38(S,S)	419
 **482**	-	-	-	60	44	419

Table 20 (Continued)

hydroperoxide	K_m [mM]	k_{cat} [min^{-1}]	k_{cat}/K_m [mM^{-1} min^{-1}]	Peroxide	Alcohol	Ref.
 **483**	6.0	1723	287	74(R)	78(S)	418
 **484**	18.0	1745	97	70(S)	71(R)	418
 **485**	3.9	11	2800	53(R)	58(S)	422

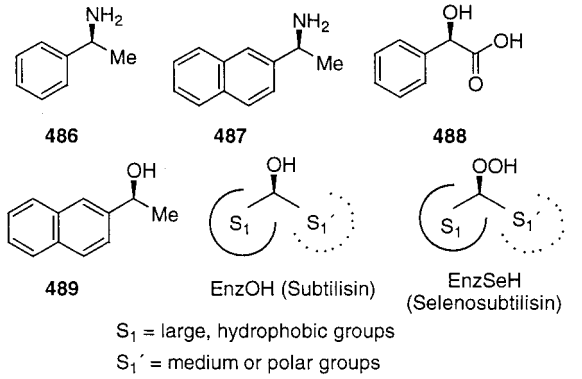
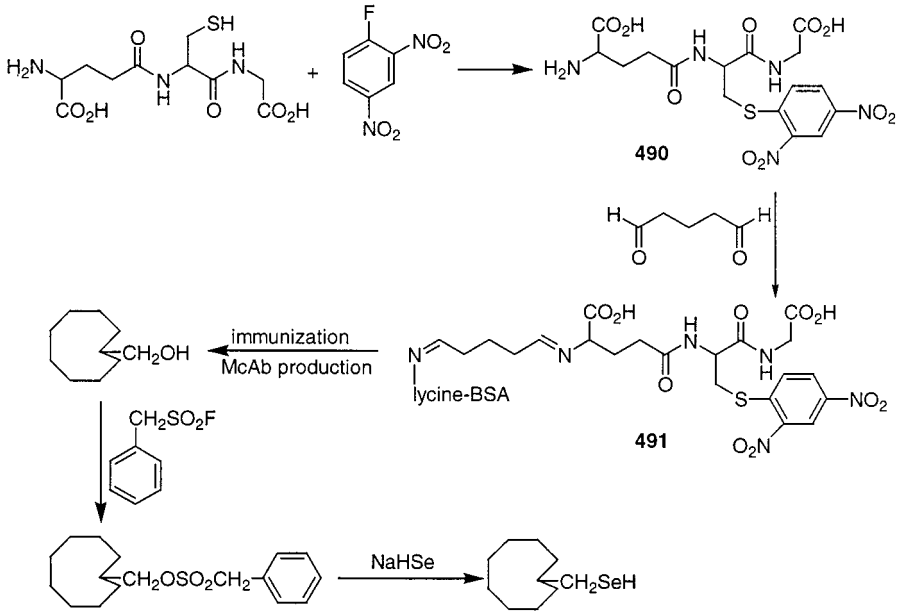


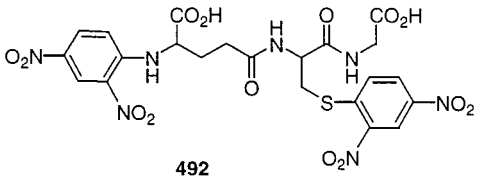
Figure 64.

mostly the selenium analogues of sulfur compounds with known activity. The interchanging of selenol for thiol can be considered as an important approach that has been used extensively in medicinal chemistry. This replacement is based on the ability of both these functional groups to be hydrogen-bond acceptors or donors. A classical illustration of this replacement is 6-thioguanine (**493**) and 6-selenoguanine (**494**) (Figure 66).[435]

The selenium analogues of 6-thioguanosine (**495**) and 6-mercaptopurine (**497**), which also belong to this category, have been studied for their antitumor activities.[436,437] Some aromatic α-benzylactones bearing a seleno substituent such as **499** and **500** (Figure 67) were found to be inhibitors of human colon 8r cell proliferation.[438] However, these selenium analogues offered no advantage in terms of efficacy or toxicity over the parent compounds. Therefore, significant development of this area of research was inhibited until the recent use of novel synthetic organoselenium compounds. One of the important classes of such compounds is the aromatic selenocyanates. It has been reported that benzylselenocyanate (**501**) effectively inhibits azoxymethane (AOM)-induced colon carcinogenesis in F344 rats.[439] Compound **501** also inhibited benzo[*a*]pyrene-induced forestomach tumors in CD-1 mice[440] and dimethylbenz[*a*]an-

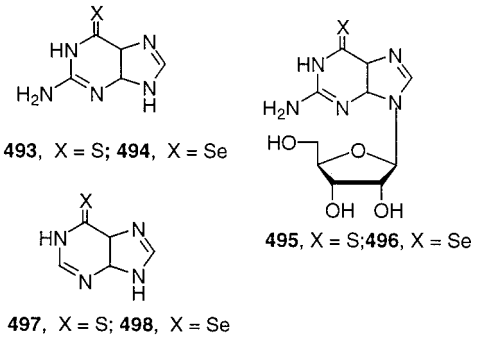
Scheme 37





492

Figure 65.

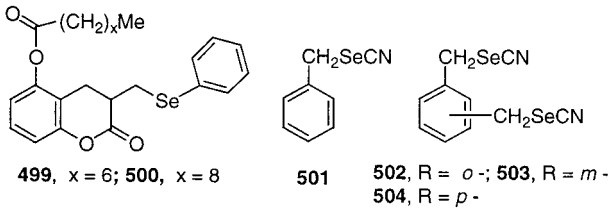


493, X = S; 494, X = Se

495, X = S; 496, X = Se

497, X = S; 498, X = Se

Figure 66.



499, x = 6; 500, x = 8

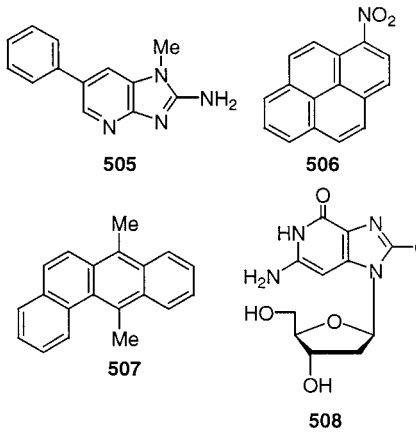
501

502, R = *o*-; 503, R = *m*-
504, R = *p*-

Figure 67.

thracene-induced mammary tumors in female Sprague−Dawley rats.[441] In these studies, the sulfur analogue of **501** had no tumor-inhibitory properties. In an attempt to enhance the tumor-inhibiting effects, 1,4-phenylenebis(methylene)selenocyanate **502** was synthesized.[442] Introduction of two selenocyanate groups resulted in enhanced potency with minimal toxicity compared with **501** and inorganic selenite.[443] Dietary administration of **502** was found to inhibit chemically induced mammary, lung, and colon carcinogenesis in laboratory animal models.[444−446]

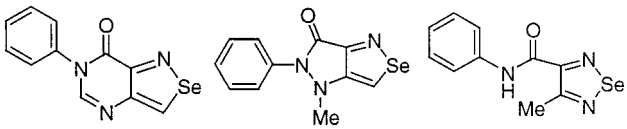
The chemopreventive effect of **504** against the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) has been studied.[447] The human lung cancer observed among cigarette smokers originates from NNK, since this nicotine-derived nitrosamine is present in tobacco and tobacco smoke.[448] Further studies on the antitumor activity of selenocyanates show that the ortho- and meta-analogues **503** and **502** also act against carcinogens. Compounds **501**−**504** inhibited activities of xenobiotic and procarcinogen oxidations catalyzed by human cytochrome P450 enzymes.[449] The bis-selenocyanates were found to be more potent than **501** and dibenzyldiselenide in inhibiting the xenobiotic and procarcinogen oxidation activities by P450 enzymes in human liver microsomes and by recombinant human P450 enzymes. More recently, the mechanism of the chemoprevention of colon cancer by compound **502** has been studied.[450] According to this study, compound **502** inhibits AOM-induced carcinogenesis by suppressing tyrosine protein kinase (TPK) and protein kinase C (PKC) activities and by up-regulating diacylglycerol kinase (DGK) activity. Particularly, the effect of



505 506

507 508

Figure 68.



509 510 511

Figure 69.

Table 21. Antitumor Effect of 509−511 on P388 Leukemia[454]

treatment (μg/day)	mean survival time (days)[a]	% increase above control
509	21.8 ± 2.6	165.9
510	16.9 ± 1.9	111.3
511	10.0 ± 0.9	11.9

[a] The mean survival time without test compounds was approximately 8 days.

organoselenium compounds against PKC activity has functional significance in the chemopreventive actions of selenium, since PKC serves as the receptor for tumor promoters and plays a crucial role in the events related to tumor promotion/progression.[451] Compound **504** has also been shown to modulate the effects of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine (**505**), 1-nitropyrene (**506**), and 7,12-dimethylbenz[*a*]anthracene (**507**) on 8-hydroxy-2′-deoxyguanosine (**508**) (Figure 68) levels in the rat mammary gland.[452] Upon metabolic activation, compounds **505**−**507** covalently bind to DNA, forming lesions that can induce the tumorigenesis in the rat mammary gland.[453]

The applicability of organoselenium compounds in tumor control has been demonstrated in five-membered ring systems. The search for novel antitumor agents resulted in the successful development of two clinically useful agents, 6-phenyl-7(6*H*)-isoselenazolo-[4,3-*d*]pyrimidone (**509**) and 4,5-dihydro-4-methyl-6-oxo-5-phenyl-6*H*-pyrazolo[4,5-*c*]isoselenazole (**510**) (Figure 69), that were tested against tumor growth in a mouse model.[454] Table 21 outlines the increase in life span over control after injecting the test compounds. Compound **511**, in which the selenium atom is bonded to two nitrogen atoms, has also been tested for comparison.

The organoselenium compounds **509** and **510** markedly inhibited the growth of P388 mouse leukemia at a dose of 100 μg/mouse/day without exhibiting any

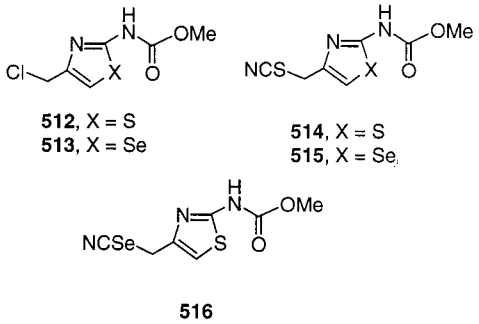


Figure 70.

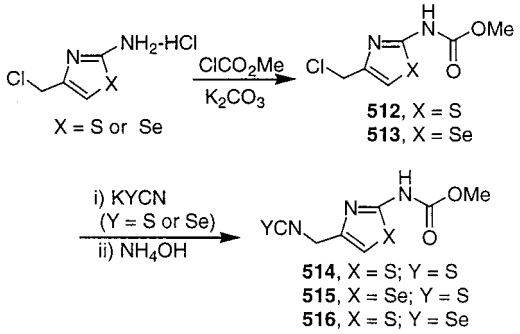
Table 22. Antiproliferative Activity of Thiazoles and Selenazoles against L1210 Cells in Vitro

compd	growth rate (% of control)	IC_{50} (μM)[b]	compd	growth rate (% of control)	IC_{50} (μM)[b]
512	96	–[c]	**520**	101	–
513	103	–	**521**	14	30
514	0	3.2	**522**	0	3.5
515	31	22	**523**	0	28
516	0	7.6	**524**	0	18
517	65	>100	**525**	0	21

[a] Concentration of the test compounds was 100 μM. [b] Concentration required to decrease growth rate to 50% of control. [c] No significant inhibition.

toxicity. The efficient tumor growth control by **509** and **510** and the relatively low activity of **511** suggest that at least one C–Se bond is necessary for antitumor activity of cyclic selenides. Some 2,4-disubstituted thiazoles and selenazoles (Figure 70) have also been evaluated for their antitumor activity.[455,456] The antitumor potential of these compounds was evaluated by determining their ability to inhibit proliferation of L1210 cells in vitro (Table 22). A structure–activity correlation showed that the presence of isothiocyanato or isoselenocyanato moiety at the 4-position is essential for antiproliferative activity. Compound **514**, bearing a isothiocyanato moiety, was the most potent, followed by the corresponding isoselenocyanato derivative **516**. The selenazole **515** was less potent compared with its sulfur analogue.[455] The synthetic route to compounds **512**–**516** is shown in Scheme 38.[455]

Scheme 38



In contrast to the effect of 4-substitution, replacement of the ester group in the 2-position by ketonic groups did not affect the antitumor activity of thiazoles and selenazoles.[456] However, a close structure–activity relationship was observed within the group of amido derivatives **517**–**522** (Figure 71). Similar

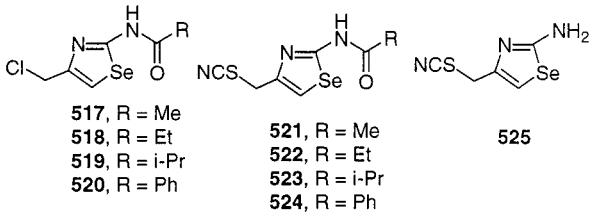


Figure 71.

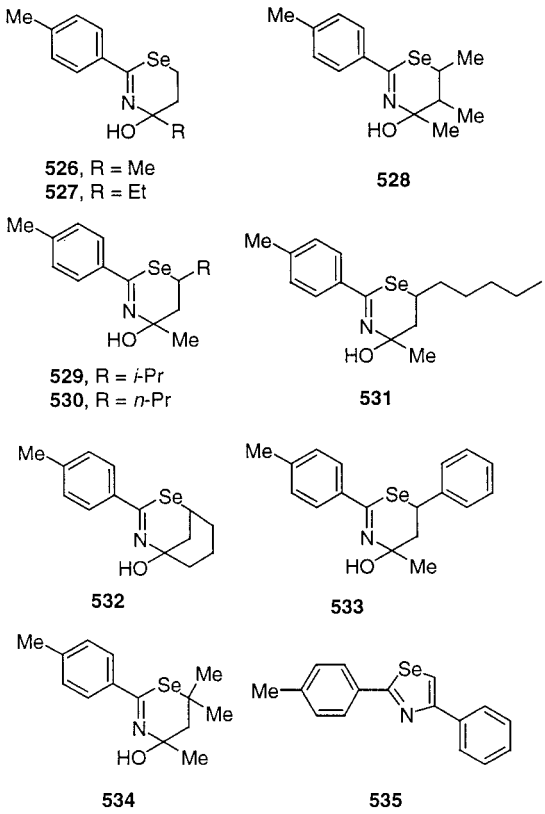


Figure 72.

Table 23. Inhibitory Effect of 526–535 on the Proliferation of Human Fibrosarcoma HT-1080 Cells[459]

compd	EC_{50} (μM)	compd	EC_{50} (μM)
526	18.3 ± 0.95	**531**	100 ± 12.52
527	7.76 ± 0.48	**532**	79.4 ± 7.12
528	80.1 ± 4.97	**533**	>100
529	36.9 ± 4.26	**534**	>100
530	8.40 ± 0.85	**535**	>100

to the carbamate derivatives **512**–**516**, the importance of the isothiocyanato substituents can also be seen in the amido compounds. The propionamido derivative **522** showed the highest activity of the series. Compound **525**, which lacks the amido group, was found to be less active.

Recently, a number of 1,3-selenazine and selenazole derivatives (**526**–**535**, Figure 72)[457,458] have been reported as antiproliferative agents.[458] The activities of these compounds against human fibrosarcoma HT-1080 cells are summarized in Table 23. It is evident from Table 23 that 4-ethyl-4-hydroxy-2-*p*-tolyl-5,6-dihydro-4*H*-1,3-selenazine (**527**) and 4-hydroxy-4-methyl-6-propyl-2-*p*-tolyl-5,6-dihydro-4*H*-1,3-selenazine (**530**) exhibit strong growth inhibition of tumor cells. The selenazole **535** and the sulfur

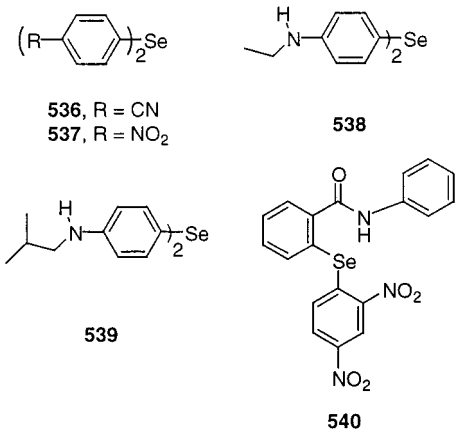


Figure 73.

analogues of most of the selenazines exhibited no inhibitory effects against tumor growth. Compounds with sterically bulky substituents (**531**−**534**) were less potent compared with **527** and **530**. Compounds **527** and **530** also induced nucleosome-size DNA fragmentation, a biochemical hallmark of apoptosis in the cells.[459]

Recent studies have shown that not only cyclic compounds but also alkyl aryl and diaryl selenides can act as antitumor agents.[460−462] Redox-active selenium compounds such as **1**, **2**, **33**, **35**, and **536**−**539** (Figure 73) inhibited tumor-promoter-induced downregulation of gap junctional intercellular communication (GJIC) between WB−F344 liver epithlial cells.[460] These compounds have been studied on the basis of the observations that many antioxidants exhibit antitumor promotive effects in in vivo systems.[463]

The activities of these compounds have been associated with their redox potential and GPx activity. Ebselen (**1**) and the glutathione derivative **2** exhibited equipotent activities. In contrast to the GPx activity, compound **35** was less potent against TPA-induced downregulation of GJIC compared with compounds **1** and **2**. Moreover, bis(4-aminophenyl) selenide (**33**) and related derivatives (**536**−**539**) that do not possess GPx activity demonstrated remedial activity against TPA-induced downregulation of GJIC. These compounds showed activity depending upon their oxidation potential. For example, compounds **536** and **538**, which possess poor antioxidant activity and a half-wave redox potential well above +1.0 V, did not affect TPA-induced effects on GJIC. Ebselen and compound **540** inhibited cancer cell growth human MCF-7 breast cancer with IC_{50} values of 121 and 19 μM, respectively.[462]

Chemoprevention of mammary cancer by diallyl selenide (**541**, Figure 74) and other lipophilic compounds **127** and **542**−**544** has also been evaluated.[461,464] The diallyl compound **541** exhibited several 100-fold more potency than its sulfur analogue against 7,12-dimethylbenz[*a*]anthrance (DMBA)-induced mammary carcinogenesis.[464] The anticancer activities of compounds **127** and **542**−**544** are summarized in Table 24.[461] Methylphenyl selenide **127** was the most effective, exhibiting 79% inhibition against methylnitrosourea-induced mammary tumor, followed by *p*-xylylbis(methylselenide), which exhib-

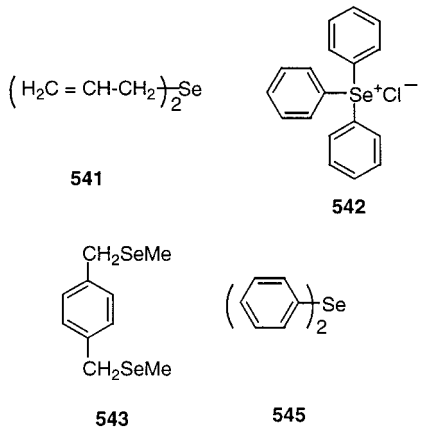


Figure 74.

Table 24. Mammary Cancer Prevention by Compounds 127 and 542−544[461]

compd	dietary selenium (ppm)	% inhibition
127	5.0	79
542	5.0	27
543	5.0	66
544	5.0	10

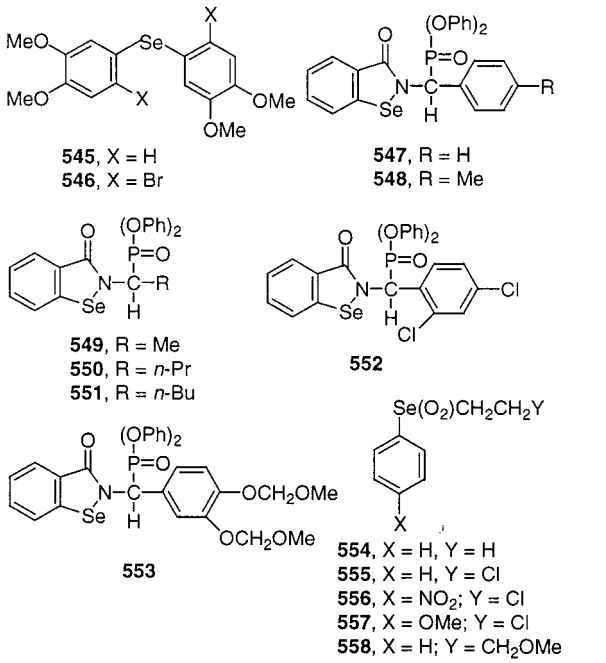


Figure 75.

ited 66% inhibition. The other two compounds, in which the selenium is bonded to aromatic rings, were found to be less potent inhibitors of tumor growth.

The cytotoxicity studies on substituted diphenyl selenides **545** and **546** show that these compounds are not themselves good candidates for antitumor agents but may be useful lead structures.[465] However, the in vivo potency of these two compounds remains to be determined. Further, certain 2-phosphonoalkyl-1,2-benzisoselenazol-3(2*H*)-ones (**547**−**553**, Figure 75) were synthesized and evaluated for their antitumor activities.[466] Although there was no strong structure−activity relationship observed in this series of compounds, some of them exhibited high inhibiting effects against human liver carcinoma

Table 25. Antitumor Activities of Compounds 547−553 against Human Carcinoma Cells in Vitro[466]

	IC_{50} (μM)	
compd	BEL-7402 cell	PG cell
547	35	50
548	20	50
549	50	40
550	150	–
551	90	30
552	75	180
553	20	100

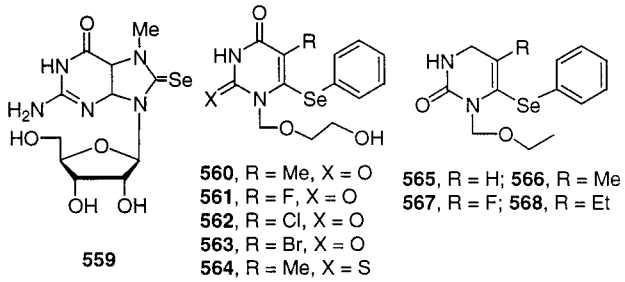


Figure 76.

(BEL-7420) cells and human lung carcinoma (PG) cells (Table 25). Finally, the alkylating organoselenones such as **554**−**558** have been shown to possess potent antiproliferative activities against L1210, L1210/L-PAM, and CCRF−CEM cell lines.[467]

B. Anti-infective Drugs

Studies of the anti-infective organoselenium compounds started as early as 1950, when the selenium analogues of sulfonamides were tested. The activities of many older compounds have been reviewed in detail by Klayman,[40] Shamberger,[41] and Parnham and Graf.[42]

1. Antiviral Drugs

As discussed in section IV.B, both tiazofurin (**165**) and selenazofurin (**166**) exhibit antiviral actions in addition to their antitumor activities. The antiviral activity of tiazofurin and selenazofurin against type I herpes simplex virus, type 3 parainfluenza virus, and type 13 rhinovirus was associated with inhibition of guanine nucleotide biosynthesis.[42] In contrast, selenazofurin did not show any antiviral activity against Pichinde virus (PCV) in infected hamsters.[468] Moreover, this compound was overtly toxic to uninfected animals. However, selenazofurin has been shown to have a broad spectrum of antiviral activity, being significantly more potent than tiazofurin and ribavirin against all virus families tested.[469]

Another good illustration of the use of this type of selenium derivatives is with the purine nucleoside analogue **559** (Figure 76) that was tested in vivo for antiviral activity against Semliki Forest virus (SFV) infection in a mouse model.[470−472] When administered at 50 mg/kg ip 24 and 18 h before virus infection, compound **559** provided 58% (7/12) survivors compared to no survivors in the control untreated mice.[470] The application of nucleoside analogues against human immunodeficiency viruses has been demonstrated in 6-(phenylselenenyl)pyrimidine systems.

Table 26. Antiviral Activity of Various Substituted Acyclic Pyrimidine Nucleosides[473]

	EC_{50} (μM)[a]			EC_{50} (μM)[a]	
compd	anti-HIV-1	anti-HIV-2	compd	anti-HIV-1	anti-HIV-2
228	13.0	9.6	**564**	2.8	5.8
560	0.96	25.6	**565**	36.0	14.8
561	2.0	9.1	**566**	0.64	27.1
562	3.1	7.9	**567**	18.2	8.5
563	3.7	2.0	**568**	0.017	14.6

[a] In human peripheral blood mononuclear cells.

The selenium-substituted acyclouridine derivatives **228** and **560**−**564** were evaluated in human peripheral blood mononuclear (PBM) cells infected with HIV-1 (strain LAV).[473] The median effective concentration (EC_{50}) for these compounds ranged from 0.96 to 13.0 μM (Table 26). The uracil analogue **228** was less effective than the 5-substituted compounds. When tested in human PBM cells infected with HIV-2 (strain ROD-2), compounds **228** and **560**−**564** were found to have activity similar to that obtained with HIV-1, with the exception of the thymine analogue **560**, which was about 25-fold less active compared with the other derivatives.

Substitution of the primary hydroxyl group for a hydrogen in the acyclic side chain afforded compounds **565**−**568** with similar spectrum of antiviral activities.[474] Compounds **566** and **568** exhibited selective in vitro activity against HIV-1 and HIV-2 in primary human lymphocytes. The most potent compound (**568**) exhibited antiviral activity with an EC_{50} value of 0.017 μM. This compound was also more potent than the hydroxyl analogues **560**−**564**. The ethyl analogue **568** was further studied for its antiviral activity against various HIV-1 mutants.[475] For this purpose, mutant proviruses encoding Thr-165 to Ile, Tyr-181 to Cys, or Tyr-188 to Cys were constructed and used to generate infectious virus by electroporation of proviral DNA into MT-2 cells. The Tyr-to-Cys substitution at residues 181 and 188 conferred at least 50- and 250-fold resistances, respectively. On the other hand, the Thr165-to-Ile substitution alone had no effect on viral susceptibility to compound **568**. The pharmacokinetics and toxicity studies on **568** indicate that this compound can act as effective antiviral agent at low concentrations without exhibiting toxicity.[476]

Recently several α- and β-anomers of oxaselenolane nucleosides (Figure 77) have been shown to act against HIV and hepatitis B viruses.[477,478] The racemic forms of cytosine and 5-fluorocytosine analogues (**569** and **570**) showed potent anti-HIV and anti-HBV activities with no toxicities up to 100 μM in various cell lines (PBM, CEM, and Vero). The racemic form of the α-isomer **571** also exhibited moderately potent antiviral activity against HIV with no toxicity up to 100 μM in all three above-mentioned cell lines.[477] The racemic α- and β-thymine, guanine, and adenine derivatives **573**−**578** also exhibited significant anti-HIV and anti-HBV activities.[478] The anti-HIV activity of the corresponding resolved α- and β-enantiomers has also been evaluated.[478] It was found that the (−)-enantiomers are more potent than their (+)-counterparts. The enantiomerically pure compounds showed

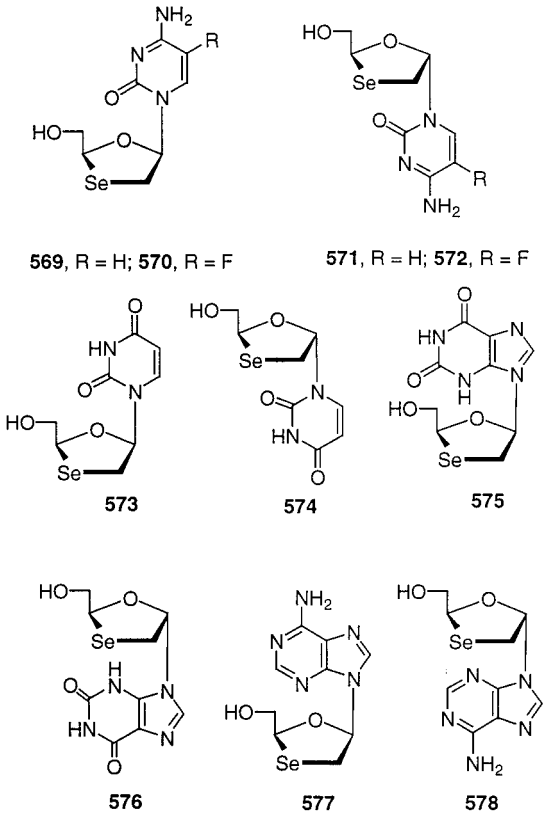


Figure 77.

much higher activities compared with the racemic compounds.

2. Antibacterial and Antifungal Drugs

Although many organoselenium compounds are more active as antibacterial and antifungal agents than their sulfur analogues, none have been developed successfully for the market, probably due to the assumption that selenium compounds are more toxic than their sulfur analogues.[42] Ebselen has been shown to possess antibacterial activity against *Staphylococcus aureus*,[479] and it has been postulated that the antibacterial activity of ebselen and other selenium compounds in vitro is due to their reactivity with an essential thiol group.[42]

Earlier studies on the antibacterial activity of selenium compounds have shown that cyclic compounds containing selenium in the ring position are suitable drugs. In this regard, certain selenacephems such as **579** (Figure 78) and dethia-1-selenapenems (**580**−**582**) have been synthesized and some of them have been evaluated for their antibacterial activity.[480] More recently, the azomethione ylide strategy has been developed for the synthesis of selenapenams.[481]

The antibacterial activities of several 4*H*-5,6-dihydro-1,3-selenazine (Figure 79) derivatives against *E. coli* as Gram-negative bacterium and *S. aureus* as Gram-positive bacterium have been reported.[482] In addition to their antitumor activity, the selenazine derivatives **526**−**535** also exhibit strong antibacterial activity. The inhibitory activities of these compounds along with some related derivatives (**583**−**588**) on *E. coli* and *S. aureus* are summarized in Table 27. As revealed by these studies, compounds **526**, **527**, and

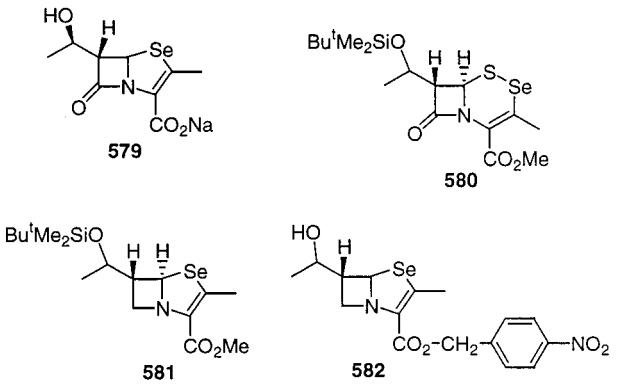


Figure 78.

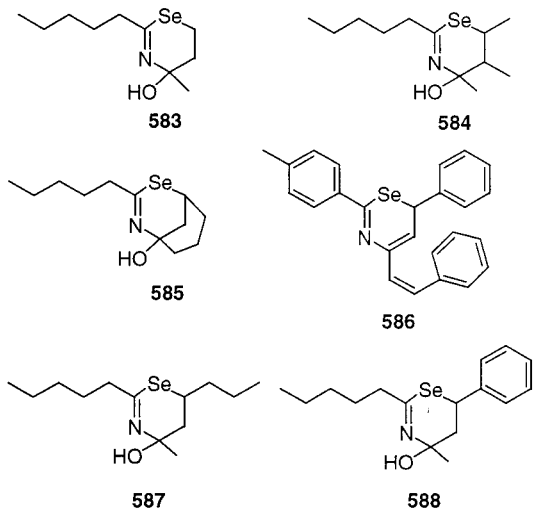


Figure 79.

Table 27. Antimicrobial Activity of 1,3-selenazine Derivatives against *E. coli* and *S. aureus*[482]

compd	E. coli	S. aureus	compd	E. coli	S. aureus
526	25 (4+)*a*	18 (2+)	**534**	13 (+)	13 (+)
527	21 (3+)	17 (2+)	**583**	18 (2+)	16 (2+)
528	–	11 (+)	**584**	–	9
529	–	11 (+)	**585**	–	11 (+)
530	–	9	**586**	–	–
531	11 (+)	13 (+)	**587**	11 (+)	16 (2+)
532	–	–	**588**	13 (+)	15 (+)
533	12 (+)	14 (+)			

a The growth inhibition zone (GIZ, mm) obtained by the paper disk method (disk diameter = 8 mm). The intensity of GIZ is shown as follows: 4+, GIZ ≥ 24 mm; 3+, 23 mm ≥ 20 mm; 2+, 19 mm ≥ GIZ ≥ 16 mm; and +, 15 mm ≥ GIZ ≥ 12 mm.

583 exhibited strong inhibitory activity against *E. coli*. These three compounds also showed strong inhibitory activity against *S. aureus*. In the aliphatic series, compounds **583** and **588** exhibited strong activity against both *E. coli* and *S. aureus*, whereas compounds **584** and **585** were found to be less active. As in the case of antitumor properties, the corresponding thiazine derivatives had no inhibitory activities against both bacteria.

From Table 27 it is evident that the 1,3-selenazine derivatives that do not possess substituents at the C5 and C6 positions of the six-membered ring might be good candidates for the antibacterial activity. Recently, certain organoselenium compounds (eb-

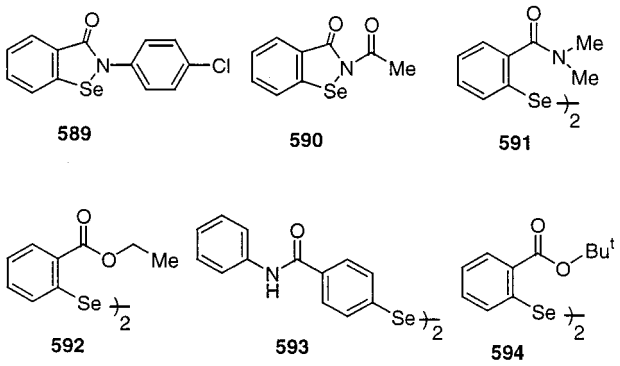


Figure 80.

selen, selenazoles **589**, **590**, and diselenides **591**−**594** (Figure 80) have been evaluated for their antibacterial and antifungal activities.[483]

Ebselen and the *p*-chloro analogue (**589**) exhibited strong inhibitory activity against the growth of *Saccharomyces cerevisiae* Σ127−8b strains. Compound **589** also inhibited the growth of *Candida albicans* 258 strains. On the other hand, the diaryl diselenides having carbamoyl (**591**, **593**) or carboxyalkyl (**592**, **594**) had no influence on fungi growth. The benzisoselenazolones also exhibited antibacterial activities. These compounds inhibited the growth of Gram-negative *E. coli* K-12 Row and Gram-positive *S. aureus* 209P bacteria strains.[483]

IX. Compounds with Other Biological Activities

A. Cytokine Inducers and Immunomodulators

It is known that selenium intake increases the inducibility of interleukin-2 receptor and that high-dose vitamin E and possibly chromium may counteract the down-regulatory effect of cAMP on interleukin-2 activity.[484] Selenium is known to assimilate in the alimentary canal, and the most favorable therapeutic effects are observed in combination with vitamins A, C, and E.[484] The main trends of the selenium studies concern their most suitable chemical forms, toxicity limits, and their effects on the immunological mechanisms.[485] The importance of selenium in AIDS-related viruses has been described.[486−488] Apart from certain selenosemicarbazides and other inorganic selenium species,[489] a number of organoselenium compounds have been described as potential immunostimulants and inducers of interferon γ (INF-γ) and other cytokines. Inglot et al. reported that ebselen and related compounds induce INF-γ and tumor necrosis factor (TNF) in human peripheral blood leukocytes (PBL).[490] When PBL was treated with compounds **1**, **67**, **94**, and **595** (Figure 81), the INF response was observed, depending upon the dosage and the structure of the compounds. Ebselen and compounds **94** and **595** with *o*-carbamoyl groups were more potent than the diselenide **67** having no substituent in the ortho-position.

From Table 28, it is evident that the INF- and TNF-inducing activities of the selenium compounds do not correlate with their cytotoxicity. The higher activity of the diselenide **94** compared with the cyclic

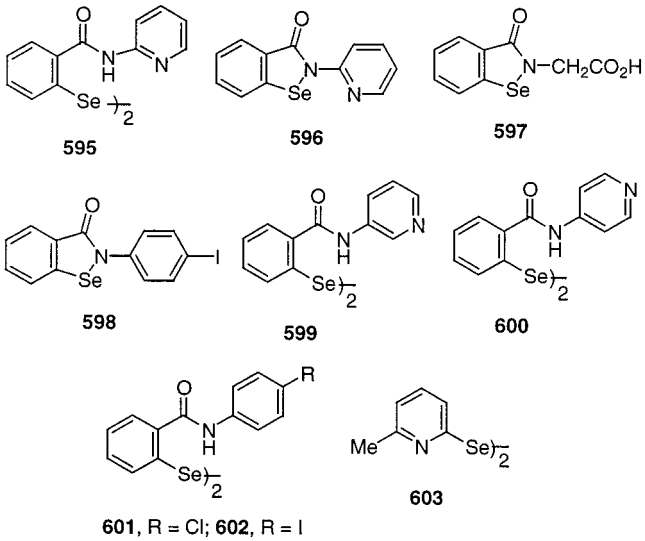


Figure 81.

Table 28. Cytokine-Inducing Activities of Compounds 1, 67, 94, and 595 in Human PBL[490]

compd	cytotoxicity (μg mL^{-1})[a]	maximum cytokine response (units mL^{-1}) INF	TNF
1	50	300	750
67	20	100	100
94	170	1000	2000
595	80	700	500

[a] Values represent the minimum cytotoxic concentration.

Table 29. Cytokine-Inducing Activities of Cyclic Se Compounds and Diselenides[491,492]

compd	cytotoxicity[a] CD_{50} (μg/mL)	cytokine yield (log units/mL) INF	TNF
117	100	0.61 ± 0.73	0.20 ± 0.50
144	20	0.73 ± 0.55	0.71 ± 0.79
155	100	0.56 ± 0.58	0.12 ± 0.32
589	100	1.42 ± 0.86	1.83 ± 0.92
596	200	0.91 ± 0.96	1.28 ± 0.94
598	50	1.21 ± 0.96	1.91 ± 0.48
599	25	0.81 ± 0.62	0.62 ± 0.73
600	15	0.62 ± 0.64	0.16 ± 0.36
601	100	0.94 ± 0.83	1.76 ± 0.74
602	100	0.80 ± 0.40	0.73 ± 0.92
603	10	1.44 ± 0.61	1.15 ± 1.18

[a] The cytotoxicity assays were performed in human lung carcinoma cell line A549.

analogue **1** indicates that the observed activity of ebselen is due to diselenide **94** being formed in the PBL culture by a metabolic pathway.[490] As an extension of this work, several substituted selenazoles and diselenides have been evaluated for their immunomodulating activities.[491−494] In addition to the above-mentioned compounds, other cyclic derivatives (**117**, **144**, **596**−**598**) and diselenides (**155**, **599**−**603**) have been shown to be potent immunomodulators. These compounds induced cytokines, such as TNF and INF-γ in human PBL (Table 29). The most potent activity was observed for bis(2-carbamoyl)phenyl diselenide bearing 4-chlorophenyl (**601**) and for the corresponding cyclic compound (**596**). The cytotoxicity of most of the compounds was low and no correlation was observed between the cytotoxicity and cytokine-

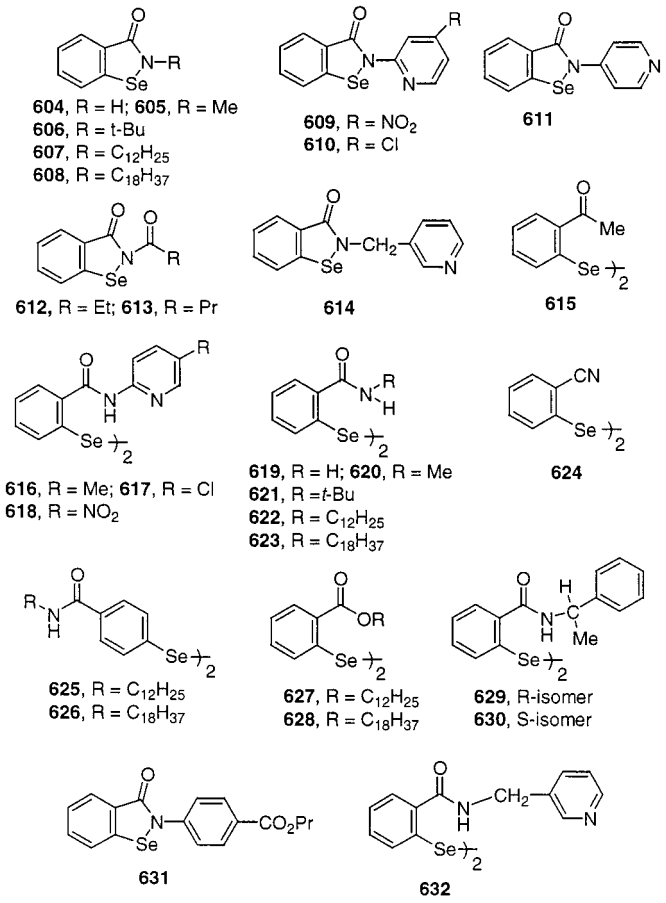


Figure 82.

inducing activity. Most of these compounds have also been studied for their immunopharmacological activities in mouse,[495] rat cell,[496] and chicken.[497] These studies suggest that the process of cytokine inducing by organoselenium compounds is species-specific. The drugs that were active in human PBL were found to be inactive in the mouse, rat, and bovine lymphoid cells.[496] In addition to the above-mentioned compounds, a number of other selenium compounds (**604**−**632**, Figure 82) have been shown to possess immunostimulating activities.[498]

A structure−activity correlation showed that the diselenides are more active than the cyclic compounds, and the relatively high efficacy of the cytokine induction was found to be associated with the presence of a phenyl or 2-pyridyl substituent with a halogen atom (Cl or I) at the para-position. On the other hand, highly lipophilic or hydrophilic substituents such as long hydrocarbon chains or 4-carboxyphenyl moiety showed negative effects.[498] It has been reported that the 2-pyridine derivative **596** can modulate the cytokine production in hyporeactive bronchoalveolar leukocytes of asthmatics, and therefore, this compound can be regarded as a potential therapeutic agent in asthma.[499] Other structural modifications of the basic unit in ebselen and the corresponding diselenide are associated with the replacement of the carboxamide group by a sulfonamide group.[500,501] Several examples of such derivatives (**633**−**650**) are shown in Figure 83. The synergistic effects of sulfur and selenium in these

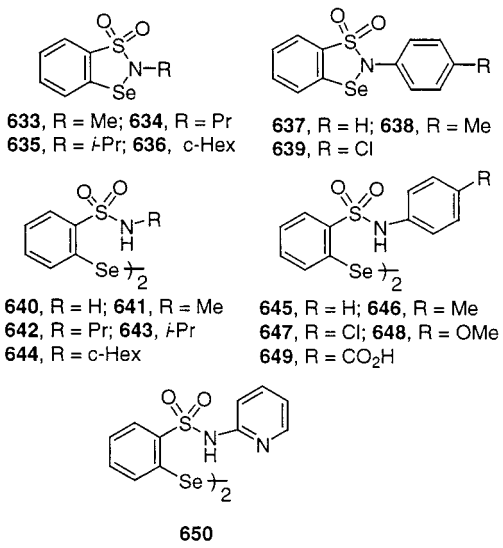


Figure 83.

compounds are expected to show potential immunostimulative activity with low toxicity.[501]

B. Antihypertensive and Cardiotonic Agents

In recent years, it became apparent that selenium exerts significant effects on the cardiovascular system. One of the important classes of organoselenium compounds acting on the cardiovascular system is the antihypertensive agents that are proposed to be potential alternate substrates for the key enzyme of catecholamine metabolism, dopamine-β-monooxygenase (DBM).[502] DBM is an attractive target point for modulation of peripheral adrenergic activity, and a number of DBM-directed inhibitors and pseudosubstrates have been shown to exhibit antihypertensive activity.[503,504] It has been reported that phenyl-2-aminoethyl selenide (**95**) is particularly an excellent substrate for DBM and that enzymatic oxygenation produces phenyl-2-aminoethyl selenoxide (**102**) via the normal ascorbate-dependent reductive oxygenation pathway of DBM catalysis (Figure 84).[505−508]

Other structurally related selenides such as **96**−**98** have also been shown to act as substrates for DBM.[508] Similar to the PN-mediated oxidation, all these compounds are oxidized to the corresponding selenoxides. The resulting selenoxides are nonenzymatically reduced back to the corresponding selenides with concomitant and stoichiometric oxidation of reduced ascorbate (Figure 84). In vivo pharmacological experiments on **95** showed that this compound exhibits dose-dependent antihypertensive activity when administered intraperitoneally to spontaneously hypertensive rats.[507] Compound **98**, having a *p*-hydroxyl group, exhibited restricted CNS permeability and oral antihypertensive activity.[508]

A few chalcogen analogues of bemoradan (**651**) and indolidan (**653**) (Figure 85) have been reported to be cardiotonic agents.[509,510] Replacement of an oxygen atom in bemoradan and a benzylic group in indolidan with selenium resulted in selenium derivatives **652** and **654**, respectively. While selenium substitution in indolidan resulted in retention of cardiotonic activity,[510] similar substitution in bemoradan lowered

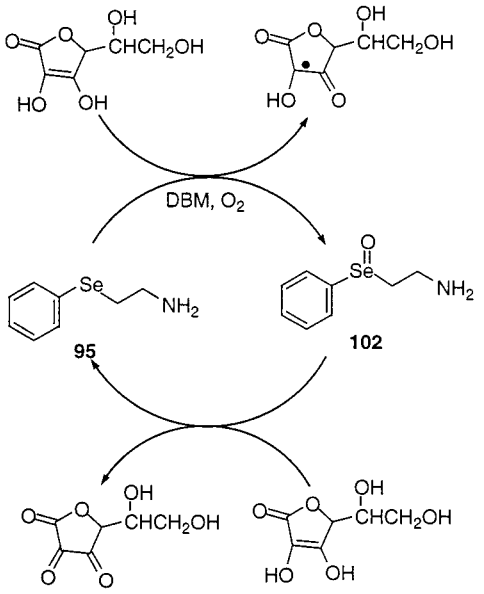


Figure 84.

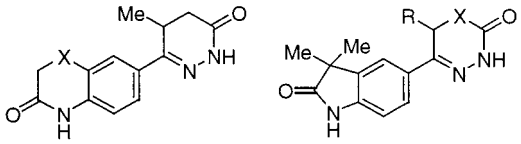


Figure 85.

Table 30. Biochemical Properties of Bemoradan and Indolidan Analogs[509,510]

compd	IC_{50} (μM)[a]	compd	IC_{50} (μM)[a]
651	0.3	**653**	0.24
652	2.0	**654**	0.54

[a] Concentration required to produce 50% inhibition of the cardiac phosphodiesterase.

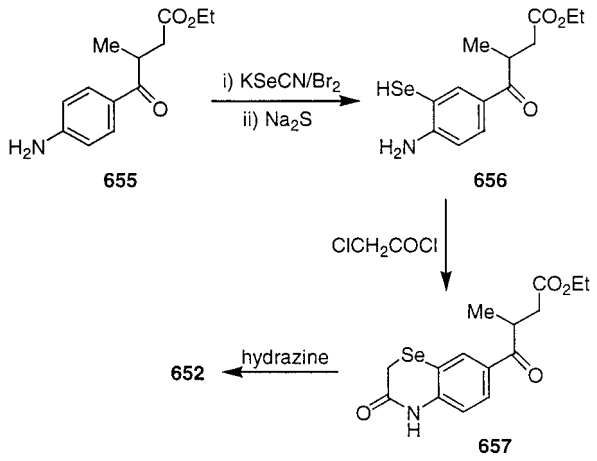
the activity of the parent compound (Table 30).[509] The reduced potency of the selenium compound **652** at the enzymic level was reflected in the poor in vivo activity of this compound. It is still unclear whether the oxidation of the selenide **652** to the corresponding selenoxide would contribute to the reduced potency of the drug.

The synthesis of compound **652** was carried out by the method outlined in Scheme 39. According to this method, ethyl 4-(4-aminophenyl)-3-methyl-4-oxobutyrate **655** was treated with KSeCN in acetic acid and bromine. The resultant cyanate was hydrolyzed with sodium sulfide to give the aminoselenol **656**, which was cyclized to the selenazine **657** with chloroacetyl chloride. Reaction of the selenazine with alcoholic hydrazine gave the desired pyridazinone.[509]

X. X-ray Crystallographic and Theoretical Studies

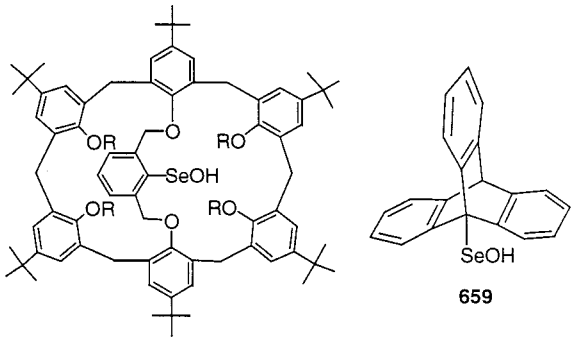
Selenium in organic compounds usually exists in a divalent state with two covalently bonded substituents and two lone pairs of valence electrons. However, the divalent selenium can further interact with nearby heteroatoms (O, N, S, Se, etc) in the solid state and in solution.[511−516] These noncovalent interactions play important roles in the conformations of biological macromolecules. The X-ray crystal structures of selenosubstilisin,[406] cGPx,[112] and pGPx[122] show that the selenium active site is involved in weak interactions with nearby amino acid residues that could stabilize the catalytically active intermediates. The diaryl diselenides **47**[517] and **51**[518] exhibit Se···N interactions that are known to facilitate the Se−Se bond cleavage in the presence of thiols. The stabilization of selenenic acid (ESeOH) is particularly important for GPx activity, since this species readily undergoes overoxidation to produce the seleninic acid ($ESeO_2H$) and selenonic acid ($ESeO_3H$) derivatives. The X-ray structural data on stable selenenic acid is still extremely rare. Okazaki et al. reported the crystal structure of an areneselenenic acid (**658**) (Figure 86) stabilized by calixarene macrocycle,[519,520] and Ishii et al. reported an alkaneselenenic acid (**659**) stabilized by a triptycyl group.[521] The X-ray crystal structures of these two compounds show that the −SeOH function resides in an environment apparently unfavorable for intermolecular processes leading to its decomposition.

Scheme 39



The intermolecular/intramolecular Se···O interactions in ebselen and some of the related derivatives are expected to modulate the biological activity of these compounds. Whereas compound **1** exhibits intermolecular Se···O interactions in the crystal lattice,[522] the selenium atom in compound **10** is involved in an intramolecular Se···O interaction [Se···O distance: 2.573(3) Å] with the nitro group (Figure 87).[523] Similar to the interactions in ebselen, compounds **605** and **612** exhibit intermolecular Se···O interactions to form infinite linear chains.[524,525] In compound **612**, the five-membered isoselenazolyl ring is severely strained at the Se atom, which would facilitate the Se−N bond cleavage.[525]

Interestingly, the carbonyl oxygen in ebselen interacts with selenium when the five-membered ring is opened by nucleophiles. For example, compounds **660** and **661** exhibit intramolecular Se···O interactions [2.829(2) Å (**660**), 2.636(4) Å (**661**)], and these interactions are expected to increase the electrophilic reactivity of selenium.[522,526] Other GPx mimics such as **36** and **37** also exhibit such interactions in the solid state, with Se···O distances of 2.834(4) and 2.84(4) Å, respectively.[527] Although compounds **36**



658 659

Figure 86.

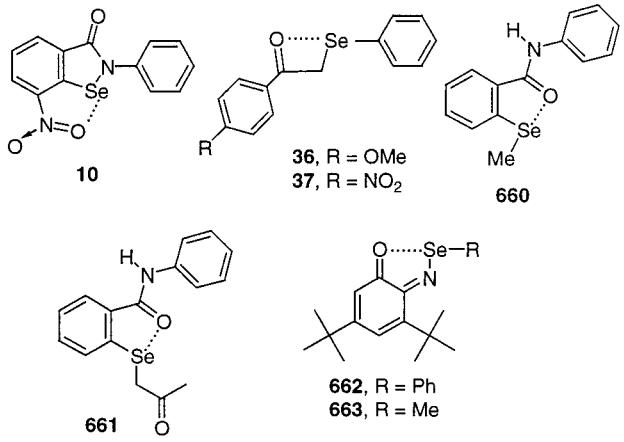


Figure 87.

and **37** exert their GPx activity by forming a common selenolate, the involvement of these interactions in the cleavage of the Se−C bond remains to be determined. Molecular modeling studies suggested that the electron-withdrawing nitro group in compound **12**, which stabilizes the ketone enolates formed in the reaction, increases the GPx activity.[527] The existence of attractive interactions between selenium and oxygen has also been reported for selenoiminoquinones **662** and **663**.[528] The X-ray data and ab initio calculations revealed that the magnitude of such interactions depends on the substituents on the selenium atom. These studies also suggested that the electronic structure around the selenium atom can be described as a three-center, four-electron (3c−4e) bond.

The modulation of the biological activity of selenium compounds by the Se···O interaction can be clearly seen in certain selenazole nucleosides. The crystal structures of selenazofurin and its α-anomer show selenium−oxygen contacts of 3.012 and 2.888 Å, respectively.[529] This hypothesis is supported by ^{77}Se NMR studies on selenazofurin[530] and crystal structures of other related derivatives.[531] The effective inhibition of IMPDH by selenazofurin and related derivatives has been attributed to the presence of Se···O interactions that restrict the rotation about the C-glycoside bond in the active anabolites TAD and SAD, influencing the binding of these dinucleotides inhibitors to the target enzyme.[532] Similarly, close Se···O contacts were found in analogues TAD and SAD bound to alcohol dehydrogenase.[533] The

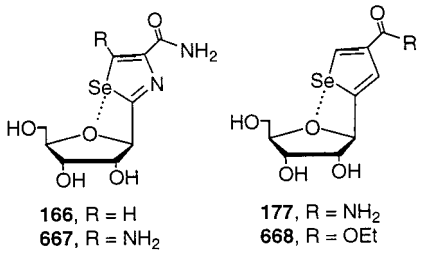


Figure 88.

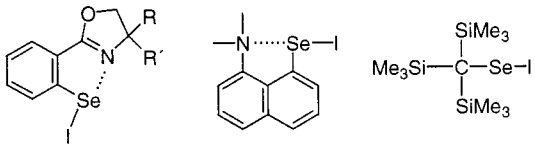


Figure 89.

strength of the Se···O interaction can be varied by substitution at the selenazole ring. Replacement of the H atom in **166** with the electron-donating amino group has been shown to increase the electron density on selenium.[534] This would decrease the net positive charge on selenium, with a resultant decrease in the electrostatic component of the Se···O interaction. The Se···O distance [3.314(4) Å] in compound **667** was found to be significantly greater than the corresponding distance [3.012(3) Å] observed in selenazofurin.

The IMPDH inhibitory activity of selenophenfurin (**177**) has also been attributed to close Se···O contacts, as seen in its carboxamide derivative (**668**, Figure 88). The inactivity of the oxygen analogues of selenazofurin and selenophenfurin has been proposed to be the result of the loss of favorable intramolecular interactions.[188,535] Computational studies on tiazofurin analogues also confirm the importance of sulfur or selenium in the heterocycle.[536] This study also suggests that any moiety replacing the sulfur or selenium needs to be large enough to impose a significant rotational barrier around the glycosylic bond. Recently, the mechanism of IMPDH inhibition by selenazofurin derivative has been proposed with the help of the crystal structure of human type II IMPDH.[537] The structure of a ternary complex between the enzyme and the substrate and cofactor analogues 6-ClIMP and SAD shows that the dinucleotide selenazole base is stacked against the 6-ClIMP purine ring in an orientation consistent with the B-side stereochemistry of hydride transfer. The stacking of SAD against 6-ClIMP is similar to the interaction between IMP and NAD in the IMPDH active site.[538] The stereochemistry of hydride transfer has been determined for IMPDH from murine lymphoblasts and *E. coli* and for IMPDH-hII and this shows that the stacking pattern facilitates this stereochemical mechanism.[539]

The areneselenenyl iodides **669**−**671** (Figure 89), which may be considered as models for the E-SeI intermediate of ID-1, have been studied by X-ray crystallography.[540−542] The stability of these compounds has been attributed to the existence of strong Se···N interactions. Another approach to stabilize the Se−I bond is the use of sterically hindered

substituents.[543−546] Among a few sterically hindered selenenyl iodides, compound **672** is a unique compound that does not undergo any disproportionation reaction in solution. Model studies on the inhibition of ID-1 by thiourea drugs (PTU and MTU) showed that the internally chelated compound **669** reacts with PTU and MTU faster than the selenenyl iodide **672** stabilized by steric protection.[547] This indicates that the Se···N interaction in compound **669** increases the electrophilic reactivity of selenium.

The phosphoalkyl derivative of ebselen (**548**) exhibits Se···O interactions,[466] which can diminish the electrostatic charge on the selenium atom and might be favorable to the pharmacological activity.[548] Molecular modeling of diaryl selenides **545** and **546** shows that the conformational changes between these two molecules affect their biological activity.[465] The dibromoselenide **546** has been shown to bind strongly to tubulin. The dissociation constant (4.6 μM) for binding is comparable to those of the antimitotic agents such as amphethinile and colchicine. The differences in the binding properties between **545** and **546** indicate that the sterically bulky bromine atoms in compound **546** increase the rigidity of the molecule. Therefore, the shape of the dibromo derivative may be a useful lead in the search for novel agents that inhibit mitosis. Molecular modeling of the 5-lipoxygenase inhibitor DuP654 and its selenium analogue revealed only minimal changes in the molecular conformation by selenium substitution.[105] The X-ray crystal structure of the alkylating organoselenone ethyl phenyl selenone **555** has been reported[549] As discussed in section VIIIA, this compound has been studied as a potential antitumor agent as a result of its tendency to undergo nucleophilic substitution by Se−C bond cleavage, probably related to the strong acidity of the seleninic acid leaving group.[467]

XI. Conclusion

It is now clear that many organoselenium compounds play important roles in biochemical processes ranging from antioxidants to anticancer and antiviral agents. The unique redox properties of selenium are influential in the catalytic activities of organoselenium compounds. It became apparent from the foregoing discussions that ebselen and related organoselenium compounds possess therapeutic potential against various diseases. In some cases, ebselen offers an advance over all other drugs. For example, ebselen can be used effectively for the treatment of cerebral ischaemia, for which no successful treatment is available.[550] Although the toxicity of many selenium compounds becomes the limiting factor of their use in pharmacology, recent evidence suggests that the toxicity could be considerably lowered by suitable substitution. In many cases, the replacement of sulfur by selenium without modifying the basic structure of the compound led to comparable activities with increased toxicity. Organoselenium compounds must, therefore, be designed and synthesized for a specific purpose rather than synthesizing analogues of sulfur compounds. For example, when constructing enzyme mimics, the compounds should be designed by incorporating active site features including substrate binding sites of the particular enzyme, and when synthesizing inhibitors, the conformational requirements must be considered. We envisage that the progress and perspective described in this review will stimulate further efforts from researchers all across the organoselenium community.

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XIII. Glossary

cGPx	cytosolic glutathione peroxidase
Cys	cysteine
COX	cyclooxygenase
DTT	dithiothreitol
Ebselen	2-phenyl-1,2-benzisoselenazol-3(2*H*)-one
EC	E. coli
FMO	flavin-containing monooxygenase
FdUrd	5-fluoro-2′-deoxyuridine
FUra	5-fluorouracil
GSH	glutathione
GPx	glutathione peroxidase
GR	glutathione reductase
GST	glutathione-S-transferase
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
giGPx	gastrointestinal glutathione peroxidase
IMPDH	ionosine monophosphate dehydrogenase
ID	iodothyronine deiodinase
IMP	inosine 5′-monophosphate
LOX	lipoxygenase
MTU	6-methyl-2-thiouracil
Methimazole	2-mercapto-1-methylimidazole
NOS	nitric oxide synthase
PDT	photodynamic therapy
PHGPx	phospholipid hydroperoxide glutathione peroxidase
pGPx	plasma glutathione peroxidase
PN	peroxynitrite
PKC	protein kinase C
PTU	6-*n*-propyl-2-thiouracil
PMSF	phenylmethane sulfonyl fluoride
ROS	reactive oxygen species
SOD	superoxide dismutase
Sec	selenocysteine
SeMet	selenomethionine
TR	thioredoxin reductase
Trx	thioredoxin
TMS	thymidylate synthase
TK	tyrosine kinase
TFA	trifluoroacetic acid

XIV. References

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