

# Selenium-Functionalized Molecules (SeFMs) as Potential Drugs and Nutritional Supplements

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**Abstract** Selenium (Se) is an essential element to humans and animals. Since its diverse functions were discovered, its importance has been more and more revealed. After the discovery of selenium as a key component of many proteins, significant research efforts have been focused on its therapeutic and nutritional potentials and possible impacts on human and animal health. To date, 25 selenoprotein genes have been identified in humans and animals, where selenium exists in the form of an amino acid, selenocysteine (Sec). Sec incorporation into selenoproteins occurs through a unique process by recognizing UGA stop codon via the Sec-charged tRNA for delivering Sec into proteins. Most selenoproteins are antioxidant proteins and play critical roles in maintaining redox balance in living systems by removing reactive oxygen species (ROS) and/or reactive nitrogen species (RNS). These reactive species are major threats to bio-macromolecules, such as DNAs, RNAs, proteins, lipids, and carbohydrates. They may cause molecular oxidation and DNA damage/mutation, alter gene expression patterns, weaken the immune system, and even lead to neurological disorders and other pathological conditions. As potential drugs and/or nutritional supplements, selenium-functionalized molecules (SeFMs) have shown benefits in preventing and/or treating many diseases, such as cancer, cardiovascular diseases, thyroid diseases, diabetes, AIDS, rheumatoid arthritis (RA), and neurodegenerative diseases. These promising results and therapeutic potentials of SeFMs have attracted tremendous attention from academia and pharmaceutical and biotech industries. However, further fundamental research and investigation are required to discover the ideal chemical, biochemical, and biological forms of selenium, appropriate dosages, and mechanisms of the selenium biological actions, since the tolerance window of selenium in humans may be limited.

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## 1 Introduction

Selenium is an essential trace element for humans and animals. It was discovered in 1817 by Jöns Jakob Berzelius (a Swedish physician and chemist), when he was trying to uncover the etiology of a mysterious disease among workers at a  $\text{H}_2\text{SO}_4$  plant in Gripsholm (Sweden). The reason that selenium research has become an intense field of research is because this trace element with nutritional importance has unique sets of biological activities. During early years of the twentieth century, selenium was reported as a toxic element that was manifested by feeding livestock forage grown in seleniferous soil. The first evidence of selenium essentiality in living systems appeared in the middle of the twentieth century. Selenium was reported as a nutritional factor in the formation of formic dehydrogenase in *E. coli* and *Aerobacter aerogenes* bacteria [1] and in prevention of exudative diathesis in chicks [2]. Around the same time, Klaus Schwarz and Calvin M. Foltz reported that selenium could prevent liver necrosis in rats fed with torula yeast grown in a selenium-rich medium [3]. Soon thereafter, selenium deficiency was implicated as a factor for the development of several diseases, such as white muscle disease of calves, lambs and foals, and exudative diathesis of poultry. In the late 1960s, epidemiological surveys in different parts of the United States suggested an inverse correlation between the incidences of certain cancers (particularly breast

cancer) and the selenium content of the plants growing in different regions. It took about 15 years after the establishment of selenium essentiality in human health to determine one biological role of selenium. The real excitement appeared in 1973 when selenium was found as a natural part of a redox enzyme, glutathione peroxidase (GPx) [4]. Subsequently, two other selenium-containing redox enzymes, formate dehydrogenase [5] and glycine reductase of *Clostridium thermoaceticum* (belonging to the glutathione peroxidase family), were identified [6]. Analysis of the glycine-reductase complex labeled with  $^{75}\text{Se}$  found an oxygen-labile chromophore, which implied the ionized -SeH group of the selenocysteine (Sec) residue [7]. Later, many other studies confirmed the existence of selenium in selenoproteins in the chemical form of selenocysteine [8, 9]. Sec is a cysteine (Cys) analog that differs from Cys by only one atom (selenium versus sulfur), and yet this switch dramatically influences the important aspects of the enzyme reactivity [10]. As Sec is invariably recognized by a codon and has its unique Sec-tRNA and other translation factors, Sec is referred as the 21st amino acid. Besides the glutathione peroxidase family, type 1 iodothyronine deiodinase (a Sec-containing enzyme), which catalyzes the hepatic conversion of thyroxine (T4) to triiodothyronine, was reported in the late 1980s [11, 12].

The puzzle of how selenium incorporates into the amino acid Sec and how Sec is stably co-translated into selenoprotein became clear in 1986. The mRNA sequences of glutathione peroxidase (*gpx*) of mouse [13] and formate dehydrogenase (*fdhF*) of *E. coli* [14] revealed an in-frame UGA codon (termination codon), which encodes for Sec. Simultaneously, another *cis*-acting element called Sec-insertion sequence (SECIS) element was identified at the 3'-untranslated region of mRNA, which forms the secondary structure and helps Sec-tRNA<sup>Sec</sup> to get position at the UGA codon [15, 16]. To date, 25 selenoproteins (Table 1) have been identified and characterized with various functions, such as hormone metabolism, selenium transport, antioxidant/redox properties, anti-inflammatory properties, etc. In every selenoprotein, the Sec residue invariably occupies the core position of the active site and plays very diverse but crucial role in enzymatic catalysis. Besides Sec, another selenium-containing amino acid (selenomethionine, Se-Met) also presents in proteins of all genera. But the incorporation of Se-Met into proteins is not regulated as Sec. Se-Met is randomly incorporated into protein in place of Met, and its incorporation rate depends on the availability of Se-Met during protein synthesis. Se-Met replaces methionine (Met) by charging with the same methionine tRNA (tRNA<sup>Met</sup>), which is efficiently recognized by all translational factors [54, 55]. The stably incorporated Se-Met does not significantly alter the protein structure, but may influence the activity of enzymes, if Se-Met replaces Met in the vicinity of the active site.

The natural occurrence of selenium is also reported in certain tRNAs from bacteria, archaea, and eukaryotes. The modified 5-methylaminomethyl-2-selenouridine (mnm5Se2U) has been reported to commonly occupy at the wobble position of the anticodons of tRNA<sup>Glu</sup>, tRNA<sup>Gln</sup>, and tRNA<sup>Lys</sup> [56–59]. It is thought to play a critical role in fine-tuning of codon–anticodon base pairing by positioning the Se-modified nucleotide at the first position of the anticodon. The proposed

**Table 1** Lists of 25 human selenoproteins identified to date

Entry	Selenoprotein	Short name	Main function	Subcellular or body localization
1	Deiodinase type 1	DIO1	To transform the prohormone thyroxine ( $T_4$ , 3,5,3',5'-tetraiodothyronine) to the active hormone ( $T_3$ , 3,3',5-triiodothyronine), convert $T_4$ to 3,3',5'-triiodothyronine ( $rT_3$ ), convert $T_3$ to 3,3',5'-diiodothyronine ( $T_2$ ), and convert $rT_3$ to $T_2$ . It can deiodinate both rings	ER (endoplasmic reticulum) membrane, plasma membrane [17, 18]
2	Deiodinase type 2	DIO2	To transform $T_4$ to $T_3$ and convert $rT_3$ to $T_2$ . It can only deiodinate the outer ring	Brain and other tissues [19]
3	Deiodinase type 3	DIO3	To transform $T_4$ to $rT_3$ and convert $T_3$ to $T_2$ . It can only deiodinate the inner ring	Cell and endosome membrane [20]
4	Glutathione peroxidase 1	GPx1/cGPx	To provide antioxidant environment (detoxification of hydrogen peroxide)	Cytoplasm and nucleus [21]
5	Glutathione peroxidase 2	GPx2/GI (gastrointestinal)-GPx2	To provide antioxidant environment (detoxification of hydrogen peroxide)	Cytoplasm [22, 23]
6	Glutathione peroxidase 3	GPx3	Detoxification of hydrogen peroxide	Skeletal muscle other tissues [24]
7	Glutathione peroxidase 4	GPx4/PH (phospholipid hydroperoxidase)-GPx	To provide antioxidant environment and protect against lipid peroxidation, redox signaling, and regulatory processes, such as inhibiting lipoxigenases and apoptosis	Cytoplasm, mitochondria, nucleus and cell membranes [25, 26]
8	Glutathione peroxidase 6	GPx6	Detoxification of hydrogen peroxide	Secreted, found only in olfactory epithelium and embryonic tissues [27, 28]
9	Thioredoxin reductase 1	TRxR1	Ubiquitous cytoplasmatic housekeeping enzyme, reduction of thioredoxin and other substrates	Cytoplasm, mitochondria, nucleus [29]
10	Thioredoxin reductase 2	TRxR2	Reduction of thioredoxin, disulfide bond isomerization, thioredoxin/glutaredoxin/glutathione reductase	Mitochondrial [30, 31]
11	Thioredoxin reductase 3	TRxR3	Reduction of thioredoxin, defense against oxidative stress	Mitochondrial, nucleus cytoplasm [32]

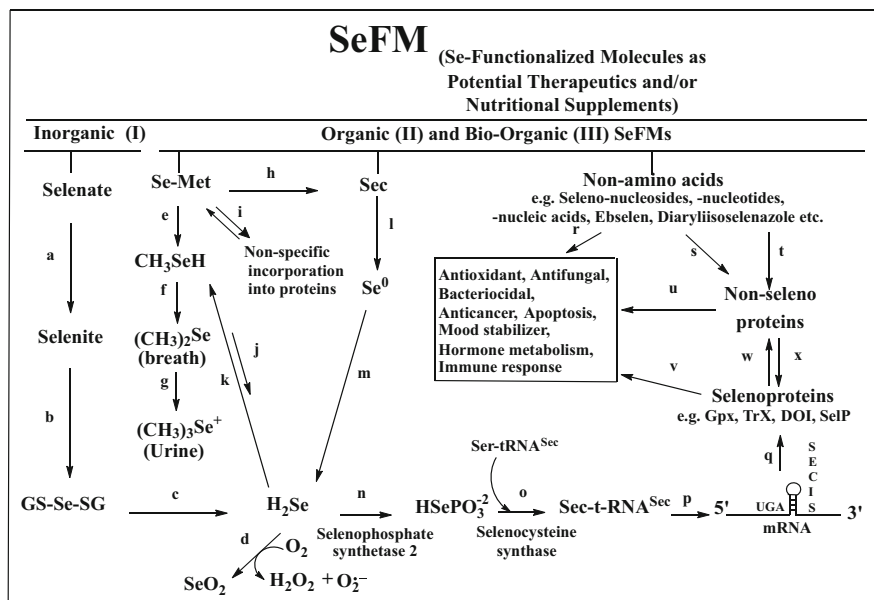
12	Selenoprotein H	SeH	Redox-sensing DNA-binding protein, antioxidant defense, and phase II detoxification	Overexpressed in brain, specific location unknown [33]
13	Selenoprotein I	SeI	Unknown	An integral membrane protein [34]
14	Selenoprotein K	SeK	Antioxidant defense, calcium regulation, ER-associated protein degradation	Membrane ER [35, 36]
15	Selenoprotein M	SeM	Thiol disulfide oxidoreductase, formation of disulfide bonds, calcium responses	ER, highly expressed in the brain [37, 38]
16	Selenoprotein N	SeN	Essential for muscle regeneration and satellite cell maintenance	ER membrane [39]
17	Selenoprotein O	SeO	Redox-dependent activity/specific function unknown	Unknown [40]
18	Selenoprotein P	SePP	Se transport and delivery, modulate metal ion-mediated A $\beta$ aggregation, reactive oxygen species (ROS) production, and neurotoxicity	Secreted [41–43]
19	Selenoprotein S	SePS/SeS	Inflammatory response, regulation cytokine production, protection against ER-stress-induced apoptosis	Plasma and ER membrane protein [44, 45]
20	Selenoprotein T	SeT	Intracellular Ca <sup>2+</sup> mobilization and neuroendocrine secretion	Plasma membrane/Golgi/ER [46]
21	Selenoprotein V	SeV	Redox-related function	Unknown/seminiferous tubules of the testes [34]
22	Selenoprotein W	SeW	Antioxidant, redox metabolism	Cytoplasm, cell membrane [47, 48]
23	Selenophosphate synthetase 2	SPS2	Conversion selenide to selenophosphate	Unknown [49, 50]
24	15 kDa	SeP15	Form complex with UDP-glucose:glycoprotein glucosyltransferase 1, prevent transport of improperly folded glycoproteins out of the ER	ER [51, 52]
25	Thioredoxin glutathione reductase	TGR	Testis-specific enzyme; it can reduce glutathione disulfide, while its specific function unknown	ER [53]

selenium function at the wobble position is supported by the recent research from Zhen Huang and co-workers [60–62]. In addition, the selenium introduction as a heavy atom via Se-Met into protein provides a magnificent tool to solve the phase problem in X-ray crystal structure determination of proteins. In the structure determination, selenium acts as a suitable resonance center for anomalous dispersion in MAD and SAD experiments, which require synchrotron radiation with precise beam wavelength control [63–68]. For nucleic acid structure studies, Zhen Huang and co-workers have developed selenium nucleic acids [61, 62, 68–72] to derivatize nucleic acids and protein–nucleic acid complexes for nucleic acid–protein X-ray crystallography [68].

The selenoproteins have a wide range of pleiotropic effects, ranging from antioxidant and anti-inflammatory properties to the production of active thyroid hormone. In the past 10 years, a large number of research projects have been dedicated to discover the disease-associated polymorphism in selenoprotein genes. Selenium is an essential microelement because of its participation in many important enzymes, and its deficiency hampers or reduces the activities of many enzymes essential for proper physiological functions. The two most notable classes of selenoproteins are antioxidants and deiodinases. The roles of antioxidant proteins are to scavenge or neutralize reactive oxygen species (ROS) and reactive nitrogen species (RNS) from the body, where they are continuously generated during normal metabolic and respiratory processes. These ROS and RNS are the major oxidation threats to bio-macromolecules, and they or their by-products may also cause alkylation of DNA, RNA, proteins, lipids, and/or many other biomolecules, which are potentially linked with many diseases, such as cardiovascular diseases, cancers, AIDS, neurodegenerative diseases, thyroid diseases, rheumatoid arthritis, etc. Selenoproteins have been reported to prevent these diseases by removing ROS and RNS. Selenium supplementation may help to maintain a proper level of selenoproteins, thereby reducing the threat of ROS and/or RNS in plasma and preventing many deadly diseases. Selenoproteins have a number of biological functions in oxidoreductions, redox signaling, antioxidant defense, thyroid hormone metabolism, and immune responses. This review focuses on the recent advancement and a few pathological conditions and/or diseased states that may be prevented by or even cured with selenium-functionalized molecules (SeFM; Fig. 1).

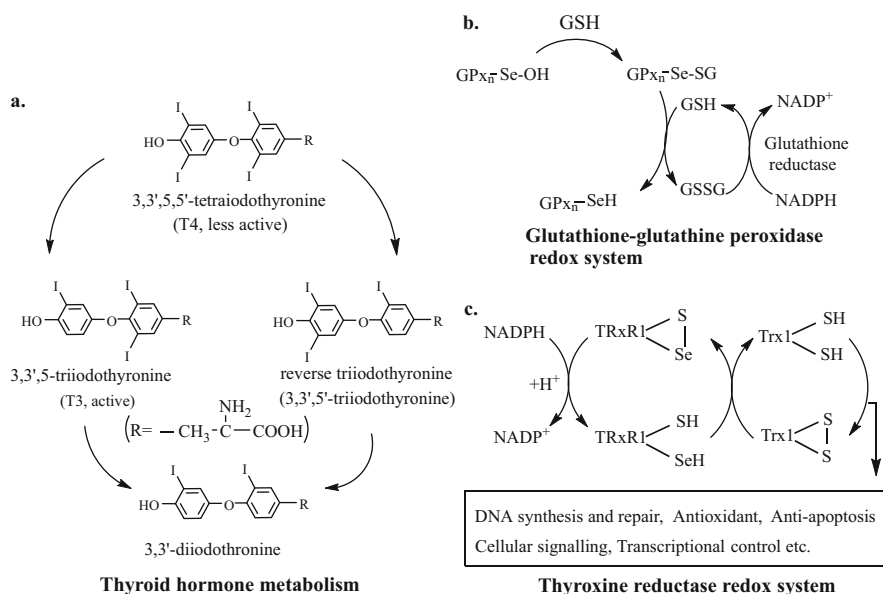
## 2 Naturally Occurring Selenium Proteins

Twenty-five selenoproteins have been identified in humans with different functions in different subcellular locations (Table 1). Selenoproteins are mostly enzymes that constitute two major metabolic pathways (Fig. 2), namely, redox metabolism and thyroid hormone metabolism (discussed later). The selenoproteins with redox activities are ubiquitous and help to maintain redox balance along with other pathways in the body. The selenoprotein GPx operates with a small ubiquitous



**Fig. 1** Potential metabolic mechanisms of Se-functionalized molecules (SeFMs) in the human body. SeFM enters the body in inorganic (I), organic (II), and/or bio-organic (III) forms. Selenium inorganic forms (selenate and selenite taken as supplements) are converted into hydrogen selenide (less reactive) through several reductions (*a–c*). The hydrogen selenide is phosphorylated and converted into selenophosphate by selenophosphate synthetase enzyme in an ATP-driven process (*n*). The highly active selenium donor (selenophosphate) donates selenium functionality to the serine-charged tRNA<sup>Sec</sup>, catalyzed by selenocysteine synthase, and produces Sec-tRNA<sup>Sec</sup>. The Sec-tRNA<sup>Sec</sup> is positioned on an in-frame UGA codon of an mRNA containing SECIS secondary hairpin structure during translation process (*q*), generating selenoproteins. The most common forms of the organic seleno-compounds are Sec and Se-Met, which are derived from the hydrolysis of proteins ingested as diet. Se-Met can be readily incorporated into proteins nonspecifically (*i*). Sec, which can be formed from Se-Met by the *trans*-selenization process (*h*) and derived from hydrolysis of selenoproteins, undergoes several chemical transformations and forms hydrogen selenide via elemental selenium (*l* and *m*). Methylation of the hydrogen selenide could readily occur and produces methyl selenol (CH<sub>3</sub>SeH) (*k*), which can also be formed from Se-Met (*e*). Further methylation of methyl selenol (CH<sub>3</sub>SeH) produces dimethyl selenide [(CH<sub>3</sub>)<sub>2</sub>Se] and trimethyl selenonium ion [(CH<sub>3</sub>)<sub>3</sub>Se<sup>+</sup>], which are excreted from the body through the breath and urine, respectively. The non-amino acid selenium compounds (including organic and bio-organic SeFMs) may also act as potential drugs and/or nutritional supplements [73, 74] in order to supply selenium for maintaining the proper level of selenoproteins and producing beneficial physiological effects [75, 76]

peptide called glutathione (GSH) and forms one of the major pathways to neutralize or reduce reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are generated continuously during metabolic and respiratory processes. In the GSH–GPx redox system, the sulfur-containing peptide glutathione (GSH) is a cofactor and acts as a reducing substrate in the peroxidase reaction. During the GPx catalytic cycle, the selenenic acid form (GPx–Se–OH) reacts with GSH and



**Fig. 2** General catalytic mechanisms of human selenoproteins. Selenoproteins constitute two major classes of enzymes: deiodinases (**a**) and antioxidants (**b** and **c**). Deiodinases are responsible for converting the less-active thyroid hormones T<sub>4</sub> into active form T<sub>3</sub>. Among the several redox systems in humans, glutathione–glutathione peroxidase (**b**;  $n = 1, 2, 3, 4,$  or  $6$ ) and thioredoxin–thioredoxin reductase (**c**) redox systems are very important for detoxification of ROS in the body. In the glutathione–glutathione peroxidase redox system, glutathione is a cofactor and acts as a reducing substrate. During the catalytic cycle, oxidized di-glutathione is reduced by the enzyme, glutathione reductase. Thioredoxin–thioredoxin reductase (**c**) system can also detoxify ROS, but this system has broader substrate specificity

generates GPx–selenenyl-sulfide adduct (GPx–Se-SG). Subsequently, the adduct reacts with an additional GSH to regenerate the active selenol (GPx–SeH), which reduces a peroxide molecule (Fig. 2) [77, 78]. Another selenoprotein family, involved in redox defense system, consists of thioredoxin, thioredoxin reductase, and peroxiredoxin. This is also a major redox pathway and plays a diverse role in metabolism with broader substrate specificity. This powerful system is involved in many central intracellular and extracellular processes, including cell proliferation, the redox regulation of gene expression and signal transduction, protection against oxidative stress, anti-apoptotic functions, growth factor and co-cytokine effects, and regulation of the redox state of the extracellular environment [79, 80] (Fig. 2).

Selenium in proteins exists as amino acid selenocysteine (Sec) and selenomethionine (Se-Met). The presence of selenium atom in proteins confers many advantages in protein functionality. Selenium belongs to the same elemental family (VI A) as oxygen and sulfur in the periodic table. Although the selenium atom (atomic radius, 1.16 Å) is bigger than oxygen (atomic radius, 0.73 Å) and sulfur (atomic radius, 1.02 Å) atoms, their chemical and electronic properties are closely related. As a result of this functional similarity with oxygen and sulfur, but



bigger atomic size on the other hand, incorporation of selenium into biomolecules offers many unique and important advantages. Due to the larger electron density, one of the major advantages is that selenium can exist in multiple oxidation states, such as elemental selenium (0), selenide ( $-2$ ), selenite ( $+4$ ), and selenate ( $+6$ ), which largely depend on the chemical properties of the environment. The reactivity of a selenoprotein arises from the selenol ( $-SeH$ ) side chain of the Sec residue. This SeH functionality provides unique properties to selenoprotein over other residues in the protein. The  $pK_a$  value of the SeH group in Sec is lower ( $pK_a$  5.2), compared to the SH group in Cys ( $pK_a$  8.5). As a result of lower  $pK_a$ , the SeH side chain is more acidic and remains deprotonated at physiological pH, which makes the SeH a more powerful reductant and stronger nucleophile. Most of the selenoproteins are enzymes, and their Sec residues are conveniently located in or near an active site in the close proximity with Cys residue. The catalytic function of a selenoprotein enzyme is dependent on the interplay or shuttling of electrons among SeH, SH groups, and cofactors (if any).

On the other hand, selenium in Se-Met exists as  $-Se-CH_3$  side chain, and proteins containing Se-Met do not completely depend on the Se function for their activity. Se-Met is an essential amino acid, and it cannot be synthesized *de novo* in humans. It must be ingested in the form of Se-Met or protein containing Se-Met. Plants and microorganisms can introduce selenium in place of sulfur during Met biosynthesis. In eukaryotes, if Se-Met is available, it can be charged onto  $tRNA^{Met}$ , which is recognized by all other translational components and stably incorporated into proteins in place of Met. Se-Met incorporation into protein is random, and it depends on the availability of Se-Met [81–83]. Se-Met does not significantly alter the protein structure, but may influence the activity of enzymes, if Se-Met replaces Met in the vicinity of the active site. In contrast, Sec may remain in the free form and cannot be introduced into protein readily. It is incorporated into protein via a specific translational control process, which is unique in relation to the standard protein translation [84]. The Sec incorporation into selenoproteins is discovered in all three domains of life: bacteria, archaea, and eukarya [85]. The pathway for selenocysteine incorporation into protein in archaea and eukarya is very similar to *E. coli*. Selenoprotein synthesis in *E. coli* begins with the acylation of the 3'-end of a special  $tRNA^{Sec}$  (encoded by *selC* gene) with L-serine by seryl-tRNA ligase, thus producing seryl-tRNA<sup>Sec</sup> (Ser-tRNA<sup>Sec</sup>) [86]. The  $tRNA^{Sec}$  has many unique and unusual features, such as formation of *O*-phosphoseryl-tRNA [87], its anticodon (UCA) suppressing UGA termination codon [88], longer in size (90 nucleotides in eukaryotes), relatively fewer base modifications [89, 90], and the unique cloverleaf model with a long extra arm [91]. The serine residue on the Ser-tRNA<sup>Sec</sup> is converted into Sec and forms Sec-tRNA<sup>Sec</sup> in two-step reactions by the pyridoxal 5'-phosphate-dependent enzyme, selenocysteine synthase (product of *selA* gene, EC 2.9.1.1). In the first step, a Schiff base is formed between  $\alpha$ -amino group of serine and carbonyl of pyridoxal 5'-phosphate, followed by 2,3-elimination of a water molecule to yield enzyme-bound dehydroalanyl-tRNA<sup>Sec</sup> intermediate. In the second step, nucleophilic addition of monoselenophosphate to the double bond of the dehydroalanyl residue produces selenocystyl-tRNA<sup>Sec</sup> [92–95]. The highly

active Se donor (monoselenophosphate) is formed by transferring  $\gamma$ -phosphate of ATP to hydrogen selenide, which is catalyzed by selenophosphate synthetase (product of *selD*) [96]. Once Sec-tRNA<sup>Sec</sup> is formed, the Sec-tRNA<sup>Sec</sup> is recognized by a *cis*-acting element, Sec-insertion sequence (SECIS), a secondary structure on the selenoproteins mRNA that helps in docking Sec-tRNA<sup>Sec</sup> at UGA codon and directs selenocysteine incorporation into protein [97–99]. A Sec-specific elongation factor (product of *selB* gene) binds to Sec-tRNA<sup>Sec</sup> and SECIS and guides the incorporation of Sec onto nascent selenoprotein [100–102]. In archaea and eukarya, *O*-phosphoseryl-tRNA<sup>Sec</sup> kinase phosphorylates the hydroxyl group of serine on seryl-tRNA<sup>Sec</sup> [103, 104], and the resulting *O*-phosphoseryl-tRNA<sup>Sec</sup> is subsequently converted to selenocysteinyl-tRNA<sup>Sec</sup> by Sec synthetase.

### 3 Naturally Occurring Selenium Nucleic Acids

The natural occurrence of selenium in certain tRNAs was also reported in bacteria [59, 105], archaea [58, 106], mammals [107], and plants [108]. The discovery of the presence of naturally occurring selenium in tRNAs further provides the evidence at the molecular level for the nutritional role of selenium in life. It is not clear whether selenium can be directly incorporated into nucleosides naturally. Nonetheless, the presence of seleno-modified uridine residue (5-methylaminomethyl-2-selenouridine, mmm<sup>5</sup>Se<sup>2</sup>U) in tRNAs was reported, and mmm<sup>5</sup>Se<sup>2</sup>U occupies the wobble (first) position of the anticodon of the tRNAs for glutamate (tRNA<sup>Glu</sup>), glycine (tRNA<sup>Gln</sup>), and lysine (tRNA<sup>Lys</sup>) in bacteria [56, 59, 109]. This modified nucleoside is thought to play a critical role in the fine-tuning of codon–anticodon base pairing by positioning the Se modification at the first position of the anticodon. In the anaerobic microorganism, *Clostridium sticklandii*, the most prominent selenium-containing tRNA was identified as the major glutamate-accepting species in the bulk tRNA preparation. It has been proposed that the presence of selenium in this tRNA<sup>Glu</sup> is essential for its aminoacylation activity [58, 106, 110].

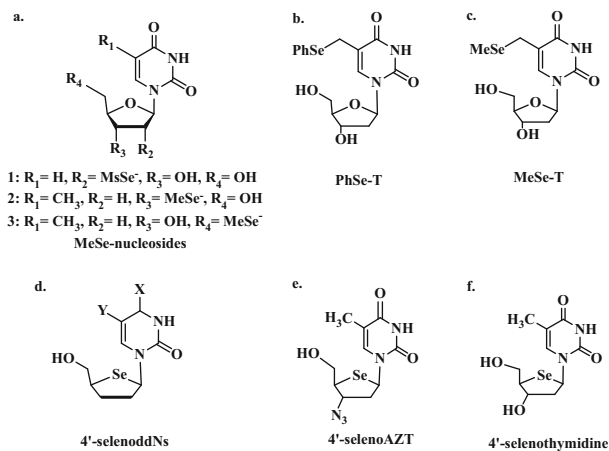
### 4 Artificially Seleno-Modified Nucleosides and Nucleotides and Selenium Nucleic Acids (SeNA)

The presence of the Se modification in natural tRNA has encouraged Huang and co-workers to pioneer and incorporate the selenium functionalities at various selected positions of RNA and DNA, namely, selenium-derivatized nucleic acids (SeNA) [68–71, 111–115]. Their experimental data have demonstrated that nucleic acid oxygen in various positions can be stably replaced with selenium for structure and function studies, especially the facilitation of phase solution, crystallization, and high-resolution structure determination in X-ray crystallography. Their studies

imply that selenium functionalities on nucleobases might improve the accuracy and efficiency of translation, RNA transcription, and even DNA replication by enhancing base-pair interaction, selectivity, and fidelity. For instance, the biochemical study of RNase H with the Se-modified DNA/RNA duplexes showed that the Se-modified nucleobase functionality can enhance the enzyme turnover rate by manyfold [68]. The supporting evidence from X-ray crystallographic structures has revealed that as a larger atom, the selenium functionality causes local unwinding of DNA/RNA substrate duplex. The Se-functionalized DNA acts as a guiding sequence shifting the scissile phosphate of the RNA substrate closer to the active site, therefore further activating the attacking nucleophilic water molecule [68]. Previously, it has been reported that the selenium-modified nucleic acids can resist nuclease digestion [116–118]. Thus, this Se-atom-specific mutagenesis (SAM) has opened a new research area to further explore and design nucleic acid-based novel therapeutics as well as nutritional supplements.

Therapeutic nucleosides, nucleotides, and nucleic acids can be used to block or interfere with specific pathological processes or pathways. The most common targets of these therapeutics are cancers and viruses. The potential oligonucleotide therapeutics, such as small interference RNAs (siRNA), antisense DNAs, or CRISPR-RNA (crRNA) [119, 120], are based on sequence complementarity to the target RNAs (such as mRNAs) or DNAs (such as genomic DNA). This is an intensely advanced area, because the short oligonucleotides can target a specific RNA very precisely and hydrolyze the RNA by Dicer–Argonaute system [121–125] or target a specific DNA by crRNA and Cas system [119, 120, 126]. Potential application of the Se functionalities in short oligonucleotides could offer additional benefits to the oligonucleotides by improving the base-pair stability, selectivity, and fidelity, by enhancing the catalytic rate, and by conferring resistance to nuclease digestion. However, extensive *in vivo* research is needed in order to take full advantage of all Se modifications, especially the Se-nucleobase functionalities [61, 68, 72, 127].

As we know, nucleoside analogs are a major class of chemotherapeutic agents that disrupt DNA and/or RNA synthesis (or repair) in targeting malignant cancer cells or viruses. Because the proliferative status of a malignant cell is higher than that of a normal cell, the malignant cell always maintains an elevated concentration of nucleosides. Thus, the malignant cells are generally more sensitive to modified nucleoside agents disrupting nucleic acid synthesis. However, research on the Se-modified therapeutic nucleosides is limited; few Se-modified nucleosides have been investigated to target cancers and viruses. Recently, after pioneering and developing selenium nucleic acids (SeNA) for the function and X-ray crystal structure studies [68–71, 111–115], Huang and co-workers have also investigated several Se-modified nucleosides for anticancer activity against various tumor cell lines. They reported that the MeSe nucleosides have relatively fine solubility in aqueous solution, have anticancer effects, and can generally inhibit cancer cell growth (Fig. 3). Among them, 5'-Se-thymidine (**3** in Fig. 3a) is more active than the other Se nucleosides against these prostate cancer cell lines [128]. Recently, Kim and co-workers [129] reported the synthesis of two Se-modified nucleosides:



**Fig. 3** Chemical structures of selenium-modified nucleosides used as anticancer and antiviral therapy. (a) Among these three MeSe nucleosides, compound 3 was more effective as cytotoxic agent. (b) and (c) PhSe-T and MeSe-T mediate apoptosis induced by p38 pathway. (d) 4'-SelenodN, (e) 4'-seleno-AZT, and (f) 4'-selenothymidine; they are inhibitors of reverse transcriptase

5-phenylselenenyl-methyl-2'-deoxyuridine (5-PhSe-T) and 5-methylselenenyl-methyl-2'-deoxyuridine (5-MeSe-T). They have been evaluated for the cytotoxic effect on human cancer cells (Fig. 3b and c). Their caspase activity study suggested that these nucleoside derivatives induced apoptosis by interfering with caspase-2 and caspase-3 pathways and, to a lesser extent, caspase-8 pathway. Another study on HL-60 cells by the same group suggested that the apoptosis induced by 5-PhSe-T and 5-MeSe-T is attributed to p38 pathway. This p38 pathway served as a link between ROS generation and DNA damage/caspase activation [129]. Moreover, several other Se-modified nucleosides have been investigated against HIV as potent inhibitors of reverse transcriptase (Fig. 3d-f) [130].

## 5 Selenium Deficiency and Diseases

### 5.1 Thyroid Health

The role of selenium in thyroid function has been established for a long time. In thyroid gland, the amount of selenium per gram of the tissue is the highest among all organs in human body [131, 132]. As an essential micronutrient, selenium is as important as iodine for the production of thyroid hormone thyroxine (or triiodothyronine,  $T_3$ ) and the maintenance of thyroid hormone homeostasis [133]. Thyroid peroxidase (TPO) is the major enzyme produced by the thyroid gland [134, 135], and it is a heme-containing oxidoreductase that catalyzes stepwise

iodination of the tyrosine residue on thyroglobulin to produce thyroxine or 3,3',5,5'-tetraiodothyronine (T4). The selenoenzyme iodothyronine deiodinase converts inactive thyroxine (T4) to its more active 3,5,3'-triiodothyronine form (T3) by removing one iodine atom [136, 137]. Three isozymes of the selenoproteins, iodothyronine deiodinase, types 1, 2, and 3 (DIO1, DIO2, and DIO3), have been characterized and cloned [20, 138, 139]. The expression and function of these isozymes are tightly regulated in a tissue-specific manner and represent a new family of eukaryotic selenoproteins. Two other seleno-enzymes, the antioxidant enzyme (such as glutathione peroxidase) and the redox enzyme (such as thioredoxin reductase), are present in thyroid glands in order to maintain proper environment for thyroid activity [140]. The role of glutathione peroxidase in the gland is to protect the thyroid cells from oxidative damage by catalyzing the reduction of  $H_2O_2$  with the help of thiol co-substrates, such as glutathione (GSH). The human TRxR also plays important roles in detoxifying ROS in the thyroid gland [132, 141, 142]. Therefore, adequate supply of both iodine and selenium is necessary for thyroid homeostasis and thyroid health. One of the most common thyroid diseases is hyperthyroidism, also called Graves' disease, often referred to as an overactive thyroid. Hyperthyroidism is a condition where the thyroid gland produces and secretes excessive amounts of thyroid hormones that circulate in the blood [143]. A number of studies have demonstrated an increase in oxidative stress and generation of ROS in Graves' disease and also reported an increased production of malondialdehyde found in urine [144].

In Graves' disease, the balance between intracellular and extracellular oxidants and antioxidants appears to be disturbed, and overall GPx activity has been reported to be diminished [145]. In treating hyperthyroidism, methimazole (MMI), propylthiouracil (PTU), and methyl thiouracil (MTU) are the commonly prescribed drugs for adult and pediatric patients. Recently, the FDA has issued a warning that use of PTU is associated with a higher risk for clinically serious or fatal liver injury compared to MMI in both adult and pediatric patients. There are also clinical study reports on the resistance of MMI in patients with hyperthyroidism in spite of good compliance [146]. In recent years there have been renewed interest is grown in developing selenium analogs of antithyroid drugs, such as, MMI (MSeI), PTU (PSeU), and MTU (MSeU) (Fig. 6). Besides toxicity and drug resistance, there are several other initial assumption was made for the selection of the selenium analog over thiol containing compounds. Selenium is a better nucleophile than sulfur; therefore, the seleno-compound may exhibit higher inhibitory effect toward ID-1, compared to the sulfur drug. As these compounds are anticipated to react with the selenenyl iodide intermediate of ID-1, the formation of Se-Se bond may occur more readily than the formation of Se-S bond [147]. In addition, the seleno-compounds may have a significant effect in neutralizing  $H_2O_2$ . There are a few other low-molecular-weight seleno-compounds listed in Fig. 4 that were designed and synthesized by several different groups to mimic Gpx activity as ebselen. These compounds are generated either by modifying the basic structure of ebselen or by incorporating some structural features of the native enzyme. The synthetic GPX mimics reported in the literature include benzoselenazolinones, selenenamide,



diaryl selenide, various diselenides, hydroxyalkyl selenides, a selenocysteine derivative, and a selenenate ester (Fig. 4).

Selenium deficiency is implicated in several other thyroid diseases. Chronic lymphocytic thyroiditis is the most common thyroid autoimmune disease where iodine supply is insufficient. Administration of selenium in the form of selenomethionine or sodium selenite along with levothyroxine substitution therapy improves the pathogenic conditions [148, 149]. The thyroid disease myxoedematous cretinism is also found to associate with selenium deficiency. Myxoedematous cretinism is characterized by the persistence of hypothyroidism despite of supplement with iodine. It has been reported that supplementation with iodine alone has no effect, but when together with selenium, it improves the pathogenic condition of myxoedematous cretinism [150].

## 5.2 Cardiovascular Disease

Cardiovascular disease (also called heart disease) is the number one disease causing death and disability worldwide [151, 152]. There are diverse causes for cardiovascular disease, but the main causes are arteriosclerosis and hypertension [153]. The manifestation and progression of arteriosclerosis involve the triad: hyperlipidemia, oxidative stress, and inflammation [154]. Hyperlipidemia is the condition of elevated level of low-density lipoprotein (LDL) in the blood, which is eventually deposited in adipose tissues. Lipids are the primary targets for oxidative modification (oxidative stress consequence) enzymatically or by free radicals, because they are the main repository of oxidizable olefinic or double bond [155]. Subsequently, these oxidized lipids activate an NF $\kappa$ B-like transcription factor and induce the expression of genes containing NF $\kappa$ B binding sites, which initiate the production of various cytokines [156, 157] involved in inflammation. The inflammatory response leads to the development of the fatty streak and atherosclerotic plaque. Many studies have clearly demonstrated that selenium can greatly reduce cardiovascular risks (such as arteriosclerosis, myocardial hypertrophy, etc.) and help to maintain cardiac health by enhancing antioxidant role of selenoproteins, especially glutathione peroxidases. These cardioprotective effects are thought to confer through the antioxidant actions of selenoprotein enzymes, which directly limit the levels of ROS (such as hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>) and reverse the oxidative damages to lipids and proteins [158–160]. It is generally accepted that the GPx level is decreased by the instability of GPx mRNA at low selenium intakes [161, 162].

In the glutathione–GPx redox system, the sulfur-containing peptide glutathione (GSH) is a cofactor and acts as a reducing substrate in the reduction of peroxidases. During the GPx catalytic cycle, the selenenic acid of GPx reacts with GSH to generate GPx–selenenyl-sulfide adduct (GPx–Se–SG). Subsequently, the adduct reacts with an additional GSH to generate the active selenol (GPx–SeH), which in turn reduces peroxide [77, 78]. Another selenoprotein family, involved in redox

defense system, consists of thioredoxin, thioredoxin reductase (TRxR), and peroxiredoxin, which play a pivotal role in both hematopoiesis and heart function. Among three types of TRxR in humans, the cytosolic TRxR1 and mitochondrial TRxR2 are required for protecting heart from ROS or free radical-mediated damages [163, 164]. The primary function of the TRxR system in normal cell is to keep thioredoxin (Trx) in reduced state, serve as a redox regulator for cell signaling, and contribute to the antioxidant defense to cells [165, 166].

SeFMs as potential therapeutics and nutritional supplements have been reported to prevent cardiovascular risks and/or to treat cardiovascular diseases. Some SeFMs (Fig. 5) have been studied for preventing or treating cardiovascular diseases by many research laboratories. Though the exact mechanisms of actions for these compounds are not clear, it has been proposed that the cardioprotective effects may be attributed to the antioxidant defense [167]. One particular organo-seleno-compound, diphenyl diselenide (DPDS), has been reported to inhibit human LDL oxidation, and this phenomenon was related to the thiol-peroxidase activity [168]. Another SeFM, ebselen (2-phenyl-1,2-benzisoselesazol-3(2*H*)-one), has been investigated for a long time and is a nontoxic organo-seleno-compound that has anti-inflammatory, anti-atherosclerotic, and cytoprotective properties. Ebselen acts by mimicking the active site of GPx, and it is also an excellent scavenger of peroxynitrite. Moreover, it inhibits activities of cyclooxygenase and lipoxygenases at the micromolar concentrations [169, 170].

### 5.3 *SeFMs for Diabetes Treatment*

Diabetes mellitus (DM) affects over 170 million people worldwide, with more than 90% of the patients suffering from type 2 diabetes (T2D) [171]. T2D is a heterogeneous metabolic disorder characterized by increased blood sugar level resulting from the resistance of liver, skeletal muscle, and fat tissue to insulin. These conditions cause dyslipidemia, hyperglycemia, and an increase in insulin secretion by pancreatic beta cells for compensation of the poor insulin response by major target tissues [172–175]. T2D is a major risk factor for the incidence of cardiovascular disease characterized by arteriosclerosis and hypertension [153, 176, 177]. In the United States, 12.9% of the adults older than 20 have T2D, of which 39.8% remain undiagnosed [178, 179]. The relationship between glucose metabolism and selenium status in the body is conflicting [180]. In spite of the antioxidant effect of many selenoproteins in maintaining redox balance in the cellular system, some studies indicate a deleterious effect of selenium in T2D. A cross-sectional study on almost 9,000 American adults, along with other analysis, showed a positive link between high selenium levels and T2D [181, 182]. A similar study performed on 7,182 women from Northern Italy has indicated that the increased dietary selenium intake is associated with an increased risk of T2DM [183]. It has also been suggested that selenium impairs hepatic insulin sensitivity through opposite regulation of ROS [184].

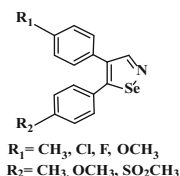


In the insulin signaling pathway, binding of insulin to its receptors initiates a signaling cascade with a mild oxidative burst, where  $H_2O_2$  acts as secondary messenger [180]. In turn,  $H_2O_2$  oxidizes redox-regulated Cys residues, therefore leading to the deactivation of tyrosine phosphatase 1B (PTP-1-B) and tensin homolog protein (PTEN). PTP-1B deactivates insulin receptor substrate (IRS), whereas PTEN inhibits phosphatidylinositol 3-kinase (PI3K). This results in overall stimulation of the signaling pathway for glucose uptake [185–187]. The selenoproteins (such as glutathione peroxidase 1) reduce  $H_2O_2$ ; therefore, the presence of antioxidant proteins produces an inhibitory action on the signaling cascade [188, 189]. However, it has been observed that insulin combined with selenium significantly decreased blood glucose levels and maintained the expression of insulin receptor substrate (IRS-1), phosphatidylinositol-3 kinase (PI3K), and glucose transporter 4 (GLUT4) in skeletal muscles of diabetic rats [190]. This experimental result indicates that the relation between insulin and selenium still remains a challenging mystery, which requires more investigation.

#### 5.4 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune condition with unknown etiology. It is characterized by chronic inflammatory polyarthritis with progressive erosion of tissues within and surrounding the joints. It affects 1% of the population and is associated with significant morbidity and increased mortality [191]. Recent studies indicate a possible role of selenium on the etiology and disease progression of RA. Clinical data shows that patients with RA have a lower level of selenium in serum, compared to healthy controls [192, 193]. The exact mechanism is unknown, but it is assumed that selenium deficiency affects the signaling pathway of nuclear factor kappa-B (NF- $\kappa$ B), which is correlated with interleukin-6 and TNF- $\alpha$  production [194, 195]. Recently, diaryl isoselenazole compounds (4,5-diaryl isoselenazoles) have been reported (Fig. 6) as multiple target nonsteroidal anti-inflammatory drugs (MTNSAIDs) for the inhibition of inflammatory processes [196]. This SeFM could be a safe alternative as a nonsteroidal anti-inflammatory drug (NSAID), which is associated with serious side effects, such as hypertension, gastrointestinal hemorrhage, ulceration, and kidney failure [197]. The mechanism of NSAIDs is to inhibit cyclooxygenase enzymes (COX-1 and COX-2), which catalyze the biotransformation of arachidonic acid to the prostaglandins [198, 199]. But the new class MTNSAIDs (4,5-diaryl isoselenazoles) have been

**Fig. 6** Diaryl isoselenazole, a new class of SeFMs with dual COX/LO inhibition property



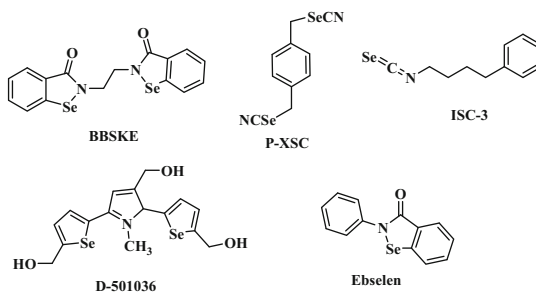
described as dual COX/LO inhibitors with potential radical scavenging by the selenium moiety. This compound is related to the well-known SeFM ebselen with a variety of beneficial properties, such as antioxidant function, radical inactivation, and lipid peroxidase inhibition.

## 5.5 *SeFMs for Cancer Prevention and Treatment*

Cancer is becoming an increasingly deadly disease, and there are seven million deaths occurring around the globe each year. According to World Cancer Research Fund and American Institute for Cancer Research, 40% of all cancer can be prevented by the combination of appropriate diets and physical activities. Therefore, cancer management becomes an ideal approach for reduction of cancer mortality by preventing, delaying, or even reversing cancer incidence. Since the initial discovery of selenium as an essential element in humans, the nutritional aspect of selenium has become an intense field of research for connecting the possible role of selenium in cancer prevention. Extensive epidemiological and specific studies have started after the identification of selenoproteins decades ago. Since then, many selenol compounds have been examined, and some have demonstrated potential clinical and molecular role in cancer prevention. A few selenol compounds have been found very effective against many types of cancers, such as lung cancer [200], bladder cancer [201], colorectal cancer [202], liver cancer [203], esophageal cancer [204], gastric–cardiac cancer [205], thyroid cancer [206], and prostate cancers [202, 207–210]. The pivotal role of selenium in cancer prevention is the antioxidant defense, which protects the cells against ROS. Both inorganic forms (such as sodium selenite:  $\text{Na}_2\text{SeO}_3$ ) and organic forms of selenium (such as selenocysteine or selenomethionine) have shown effectiveness in antioxidant defense [76]. Several mechanisms have been proposed for the selenium-mediated anticancer effects. The major mechanisms are (1) the reduction of DNA damage [211, 212], oxidative stress [213–218], and inflammation [219], (2) induction of phase II conjugating enzymes that detoxify carcinogens [220, 221], (3) Se incorporation into selenoproteins, (4) alteration in DNA methylation status of tumor suppressor genes [222–224], and (5) induction of apoptosis and kinase modulation [225–227].

In addition to chemopreventive and anticarcinogenic properties, several seleno-compounds have been reported to halt progression and metastasis of various types of cancers [228–230]. Among them, prostate cancer has been studied most extensively with SeFMs. Administration of oral capsules of sodium selenate to patients with prostate cancer significantly boosts the activity of the protein PP2A phosphatase and impedes tumor neovascularization and angiogenesis [231]. Selenium effect has also been examined in ovine pulmonary adenocarcinoma (OPA), as an animal model for studying lung cancer, by treating animals with sodium selenate [232]. Selenium at higher concentration suppresses expression of TrxR in tissues with lung cancer and causes rapid cell death [233]. The possible mechanism is due

**Fig. 7** Examples of seleno-compounds with cytotoxic effect on cancer cells. The Se nucleoside analogs for cancer chemotherapy are in Fig. 3



to an impaired function of SBP2 (SECIS binding protein 2), therefore reducing TrxR production and activity [234]. Methylselenol, a selenium metabolite, has been reported to induce cell cycle arrest in G1 phase and mediate apoptosis via inhibiting extracellular-regulated kinase 1/2 (ERK1/2) pathway and c-Myc expression [235].

More SeFMs are listed in Fig. 7, and they have been examined as cytotoxic agents with anticancer properties. A clinical trial report revealed that the administration of 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC) to 40 patients with end-stage cancers has increased predicted survival time in 76% of these cases [236]. The cytotoxic effect of this compound is thought to function by modulating TrxR activity through mitochondrial dysfunction [237]. Another selenol derivative cytotoxic compound acting by Akt3 signaling modulation is isoselenocyanate-4 (ISC-4, similar to ISC-3 in Fig. 7) with a four-carbon alkyl chain. Topical application of ISC-4 on xenografted melanoma mice resulted in reducing tumor cell expansion and development by 80% and increasing apoptosis rates by threefold [238]. Another heterocyclic selenium compound, 1,2-[bis(1,2-benzisoxazolone-3-(2H)-ketone)]ethane (BBSKE), has been reported to induce apoptosis of the tongue cancer Tca8113 cells by activating caspase-3 pathway. BBSKE is structurally related to ebselen, a thioredoxin reductase inhibitor (Fig. 7) [239].

## 5.6 SeFMs for HIV Treatment

HIV belongs to the retrovirus family. It carries a single copy of genomic RNA and RNA genes required for reproduction and pathogenesis. The HIV infection process begins with attachment of viral protein gp120 to the specific type of CD4 receptor and a co-receptor on the surface of the CD4 cell. Upon its entry into cells, the viral RNA genome is reversely transcribed into DNA (cDNA) by reverse transcriptase (viral protein), followed by integration of the double-stranded DNA into the host genome. The picture of the HIV-infected population is dire. According to the recent estimates from UNAIDS, there were 35.3 million people living with HIV in 2012, which was up from 29.4 million in 2001. HIV is a leading cause of death worldwide, and the number one cause of death by disease in Africa. Sub-Saharan Africa is most heavily affected by HIV and AIDS than any other regions in the world. An

estimated 22.9 million people live with HIV in this region, which is approximately two-thirds of the total number. In 2012, 1.6 million deaths were caused by AIDS worldwide. Though enormous research has been performed on many aspects of HIV/AIDS, critical research on nutritional aspect (especially micronutrient) on HIV epidemic has not been done thoroughly. There are growing concerns on why HIV infection, disease progression, and mortality rates are much higher in the underdeveloped areas in the world. Recently, a randomized clinical trial on the effect of micronutrient supplementation on HIV-infected ART (antiviral treatment) has been reported. It has been found that the combined supplementation with selenium and vitamins B, C and E largely improved the CD4 cell counts of the HIV-infected patients. A supplement containing multivitamins and selenium was found to be safe. The supplement significantly reduced the risk of immune decline and morbidity, if the supplement treatment was started in the early stages of HIV infection [240].

The HIV-infected individuals are immunocompromised. The mechanisms on why vitamins and selenium exert immune stimulatory effects are not fully elucidated, but the role of each micronutrient has been documented. The role of selenium in HIV infection, activation, and disease progression has been investigated in human and animal models extensively [241]. Like many other pathological conditions, oxidative stress is also a potent inducer of HIV activation and DNA damage to the infected cells [242]. The antioxidant properties of selenoproteins are assumed to play an important regulatory role in immune response [243, 244]. Since HIV infection causes the inflammatory response, many inflammatory cytokines are elevated during HIV infection [245], which include tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), interferon- $\alpha$ , and various interleukins (IL-1, IL-4, IL-6, IL-10) [246]. Elevation of TNF $\alpha$  was related with viral activation, a high level of free radicals, and DNA damage [242].

Oxidative stress is also reported to upregulate the activation of viral replication through the actions of nuclear factor kappa-B (NF $\kappa$ B) [247, 248]. All of these free radical productions lead to body-wide inflammation and depletion of primary antioxidants that cells use to protect themselves against free radicals. In addition, various free radicals generated from inflammatory response cause hypermutation of the viral genome, which lead to the generation of HIV with new pathogenesis. Several identified selenoproteins can decrease the viral replication by mitigating the oxidative stress. It has been found that the levels of selenoproteins that are expressed in T cells, including thioredoxin reductase, glutathione peroxidase, and phospholipid hydroperoxide glutathione peroxidases, are increased in the presence of selenium supplements [249–253]. Unlike some Se-containing compounds used as potential antioxidant supplements, several Se-modified nucleosides have been reported as potential antiviral agents. The 2',3'-dideoxy-4'-selenonucleosides [254] and 4'-seleno-AZT [255], which are bioisosteric analogs of 2',3'-dideoxynucleosides (ddNs) and AZT, have been reported as potential inhibitors of HIV reverse transcriptase (Fig. 3).

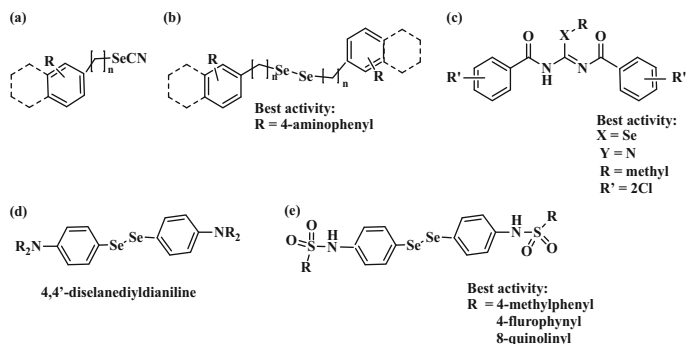
## 5.7 SeFMs for Tropical Disease Treatment

Tropical diseases refer to diseases that occur solely or principally in the tropics, such as tuberculosis, malaria, leishmaniasis, schistosomiasis, onchocerciasis, lymphatic filariasis, Chagas disease, African trypanosomiasis, and dengue [256, 257]. As a result of selenium functionality being a natural component of antioxidants and redox proteins in mammals, many experiments have been designed to observe the effect of selenium on tropical diseases as a preventive agent and cure. Among the different diseases that constitute tropical disease, only tuberculosis (TB) and leishmaniasis are discussed here in relation to potential selenium drugs.

Leishmaniasis is a tropical disease, prevalent in the Southeast Asia and East Africa. It occurs all over the world, but the form of leishmaniasis is different from one region to another [258, 259]. Leishmaniasis is caused by several species of flagellated protozoa belonging to the genus *Leishmania* in the *Trypanosomatidae* family, whose members are characterized by the presence of the kinetoplast, a unique form of mitochondrial DNA [260]. Leishmaniasis can be caused by over 20 *Leishmania* species, and it is transmitted to humans through the bite of infected female phlebotomine sand flies [261, 262]. The World Health Organization (WHO) estimates that leishmaniasis results in two million new cases a year and threatens 350 million people in 88 countries. There are 12 million people currently infected worldwide [261, 263]. Recently, the trace element selenium has been identified as a protective agent against oxidative damage caused by *Leishmania* infection [264, 265]. The biochemical pathways that constitute the cellular targets for selenium are still under investigation.

A class of compounds, which were originally developed as anticancer drugs, has been found very effective as antiprotozoal agents (Fig. 8) [265–268]. These seleno-compounds were synthesized and reported as potential antileishmanial drugs by Carmen Sanmartín's laboratory. It has also been reported that between the selenocyanide (Fig. 8a) and diselenide (Fig. 8b) derivatives, the diselenide compound with aryl ring substitution (especially with 4-aminophenyl) showed better cytotoxic effects, compared to selenocyanide [265]. Another class of imidothiocarbamate and imidoselenocarbamate compounds (Fig. 8c) has been synthesized and tested against leishmaniasis. Among various substitutions, the imidoselenocarbamate compound (especially with X = Se, Y = N, R = methyl, and R' = 2Cl groups) was very effective as an antileishmaniasis agent [264]. In 2013, the same laboratory has synthesized and tested a new class of compounds for the treatment of leishmaniasis. The new compound was designed based on the 4,4'-diselanediyldianiline (Fig. 8d) with sulfonamide moieties bound to different rings (Fig. 8e) [269]. Their data suggest that compounds with R group, such as 4-methylphenyl, 4-fluorophenyl, or 8-quinoliny, exhibit high leishmanicidal activity and low cytotoxicity, which are required for antileishmanial drugs.

Tuberculosis (TB) is a highly infectious disease. The etiological agent of TB is *Mycobacterium tuberculosis*. The notoriety of this highly infectious disease is rested on the fact that the *Mycobacterium tuberculosis* could develop multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains within



**Fig. 8** Selenium containing antiprotozoal agents. (a) Selenocyanide, (b) Diselenide derivatives (best cytotoxic effect with aryl ring substitution with 4-aminophenyl), (c) Imidothiocarbamate and imidoselenocarbamate compounds (among various substitutions, X = Se, Y = N, R = methyl, R' = 2Cl was shown very effective), (d) 4,4'-diselanediyldianiline (e) 4,4'-diselanediyldianiline with sulfonamide moieties bound to different rings

the long treatment regimen [270]. The bacteria have evolved drug resistance mechanisms through a persistent phenotype and mutations in drug target genes [271]. Recently, a new target, the antigen 85 (Ag85) protein family consisting of Ag85-A, Ag85-B, and Ag85-C, has been identified. Ag85 has mycoloyl transferase activity, which produces an envelope lipid called trehalose dimycolate (TDM) and cell wall with arabinogalactan-linked mycolic acids, and it is required for cell wall synthesis [272–274]. The organoselenium compound ebselen has been reported to inactivate the Ag85 activity by covalently linking with a cysteine residue located near the mycoloyl transferase active site. Mutational and crystal structure studies indicate that linking ebselen to the cysteine residue disrupts the hydrogen bonding network within the active site that is essential for enzymatic activity [275]. Tuberculosis often coincides with nutritional deficiencies. The effect of selenium on tuberculosis has also been explored in the nutritional aspect. It has been reported that a 2-month intervention with vitamin E and selenium supplementation reduced oxidative stress and improved the outcome in patients undergoing TB chemotherapy [276, 277].

## 5.8 SeFMs for Neurodegenerative Disease Treatment

Free radicals or, more generally, reactive oxygen species are generated continuously during the process of normal oxidative metabolism. There is growing evidence that free radicals play an important role in onset and progression of several neurodegenerative diseases. Those diseases include cerebral ischemia, Parkinson's disease, Huntington's disease, epilepsy, amyotrophic lateral sclerosis, Down's syndrome, and Alzheimer's disease (AD) [278–281]. Among these neurodegenerative diseases, Alzheimer's disease (AD) is among the most pressing health

concerns. The pathology of AD is characterized by the formation of large insoluble plaques made of misfolded amyloid beta ( $A\beta$ ) protein. Amyloid beta ( $A\beta$ ) protein is a short peptide that is a proteolytic by-product of the transmembrane protein amyloid precursor protein (APP), whose function is unclear, but thought to be involved in neuronal development [282, 283]. The experimental data suggests that beta-amyloid aggregation and toxicity are probably caused by the cellular oxidative stress, which plays a key role in the conversion of soluble to insoluble beta-amyloid, suggesting that the oxidative stress is primary to the beta-amyloid cascade [284, 285].

The effect of selenium on neurodegenerative diseases has been investigated. It has been found that the brain is enriched with selenium and its level declines with age, which may be linked to cognitive impairment and AD development. Administration of selenium in a rat model of acute dementia is found to reduce tau phosphorylation and improve memory loss, which implies the neuroprotective role of selenium [37, 286]. It has also been reported that selenium could attenuate  $A\beta$ -induced neuronal death by reducing amyloidogenic  $\beta$ - and  $\gamma$ -secretase activities [286]. Gene knockdown experiments have revealed a potent effect of glutathione peroxidase 4, which protects cells against lipid hydroperoxide damage, the characteristic feature of neurodegenerative disease and AD [287]. A number of studies suggest that GPx4 is an antioxidant enzyme possessing broader substrate specificity than other glutathione peroxidases. It accepts many reductant substrates in addition to glutathione and reacts with a wide array of organic and inorganic peroxides [25, 288, 289]. The neuroprotective role of SelP protein has also been investigated. SelP protects neuronal cells from  $A\beta$ -induced toxicity, suggesting a neuroprotective role for SelP in preventing neurodegenerative disorders in AD [290, 291]. Consistently, several studies have demonstrated the significantly lower Se levels (micronutrient status) in plasma, erythrocytes, and nails in AD patients [292]. The therapeutic effect of *p,p'*-methoxyl-diphenyl diselenide [(MeOPhSe)<sub>2</sub>], a SeFM, against streptozotocin (STZ)-induced sporadic dementia of Alzheimer's type (SDAT) in rats has been evaluated [293]. Several interesting observations from (MeOPhSe)<sub>2</sub> supplementation on SDAT rats have been reported. The results indicate that dietary supplementation with (MeOPhSe)<sub>2</sub> reverts STZ-induced memory impairment in rats and also reverts oxidative stress in the STZ group by decreasing reactive species and tyrosine nitration levels and enhancing nonprotein thiol levels [294]. The dietary supplementation with (MeOPhSe)<sub>2</sub> has also shown to normalize acetylcholinesterase (AChE) activity, which was reported to enhance by STZ injection, but did not revert the deficit in cerebral energy metabolism caused by STZ [293, 295].

Huntington's disease (HD) is also a fatal neurodegenerative disorder caused by CAG-repeat expansion encoding a polyglutamine tract in the huntingtin (Htt) protein characterized by motor and psychiatric disturbances and cognitive decline leading to dementia [296, 297]. In HD neurodegeneration, an increase in intracellular reactive oxygen species (ROS) and caspase-3 activity has been reported. Oxidative stress from ROS is primarily implicated to the impaired activity of the major endogenous glutathione redox system. This impaired system causes oxidative damage and formation of 8-hydroxydeoxyguanosine (8-OH-dG) [298, 299] as

a DNA oxidative damage marker, malondialdehyde (MDA) [300] as a lipid peroxidation marker, and protein oxidation [301]. The role of antioxidant defense by glutathione peroxidases in HD has been demonstrated via genome-wide screening, two suppressors of Htt encode glutathione peroxidases, and their overexpression could ameliorate the toxicity of a mutant Htt fragment in yeast [302]. Moreover, studies indicated that mGPx1 overexpression and ebselen treatment mimicking GPx activity can lower the ROS production and significantly reduce caspase-3/caspase-7 activation.

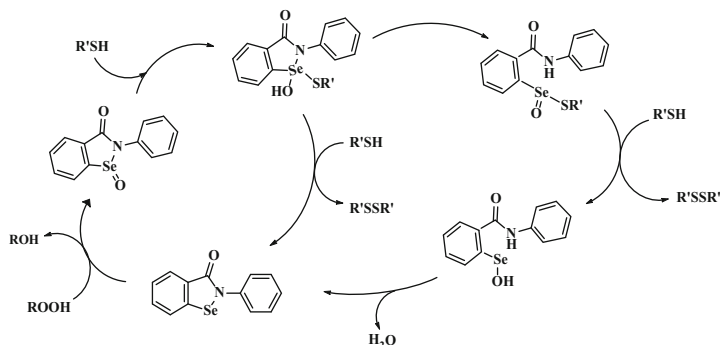
Selenium supplementation and therapeutics have also been examined in treating bipolar disease. Recently, ebselen has been examined as a potential drug for bipolar disease. They reported ebselen as an effective drug candidate inhibiting inositol monophosphatase (IMPase) activity and acting as a lithium mimetic in mouse models of bipolar disorder [303]. Lithium has been used as the most effective mood stabilizer for the treatment of bipolar disorder, but it is toxic at only twice the therapeutic dosage and has many undesirable side effects [304, 305]. The selenium moiety of ebselen irreversibly inhibits IMPase by forming a selenylsulfide (–Se–S–) bond with the cysteine residue, which is conserved in both human and mouse isoforms. It has also been demonstrated that ebselen permeates blood–brain barrier and inhibits endogenous inositol monophosphatase in mouse brain with no selenium toxicity at pharmacological dosage.

Epilepsy, which affects about 2–3% of the general population, is a common chronic neurological disorder with various etiological factors [306]. Several causes have been suggested to be responsible for idiopathic epilepsy, such as imbalances in trace elements [307] and genetic reasons, including mutations in ion and non-ion channel genes [308, 309] and mutations in genes involved in the antioxidant system [310]. The impaired antioxidant system, which generates oxidative stress by producing free radicals, has been implicated as a leading cause to seizures or the risk of their recurrence in idiopathic epilepsy [311, 312]. A control case study suggests that the Se and Zn levels are significantly decreased in patients with epilepsy and there are no significant differences in gender or age, which implies the possible cause for impaired antioxidant system in epilepsy patients [313].

## 6 SeFMs in Clinical Trials

After discovery of several selenocysteine-containing key enzymes in humans and vertebrates, tremendous attention has been paid to evaluate synthetic Se compounds for pharmaceutical applications. Among many Se compounds that have been studied, ebselen is studied and tested most extensively for its therapeutic value in many pathological conditions. Ebselen [2-phenyl-1,2-benzoisoselenazol-3(2*H*)-one] was first synthesized in 1924. In 1984, it was first reported that ebselen has the properties of thiol peroxidase and antioxidant and acts by mimicking glutathione peroxidase (GPx; Fig. 9) [315, 316]. Since then, a large number of research articles have been published: approximately 1000 ebselen-related publications may be found in the NCBI database. Due to its properties as antioxidant [317, 318] and





**Fig. 9** Mechanism of glutathione peroxidase-like activity of ebselen. R = polyunsaturated phospholipids, cholesterol esters, cholesterol, or any other organic hydroperoxide or hydrogen. R' = glutathione, dihydrolipoate, *N*-acetylcysteine, or thiol protein residue [314]

anti-inflammatory [319, 320] and its low toxicity in vivo [321], ebselen was first clinically tested for using in the stroke treatment by Daiichi Pharmaceuticals, Japan, in 1997 [322]. Unfortunately, it was not authorized (as harmokisane) for registration by MHLW, the Japanese regulatory authority in therapeutics [323]. Ebselen has a broad range of biological activities due to the GPx-like activity, which catalyzes the reduction of hydrogen peroxide and other hydroperoxides by utilizing glutathione and a variety of other thiols [324]. Ebselen can also reduce the peroxynitrite ( $\text{ONOO}^-$ ) to nitrite, thereby diminishing harmful effect of peroxynitrite to cellular constituents in host, when the concentration of peroxynitrite is too high [325]. Ebselen is in the National Institutes of Health Clinical Collection, a chemical library of bioavailable drugs considered clinically safe but without proven use.

Ebselen was studied clinically for treating the late stages of development of stroke. The pathological condition of stroke involves a variety of destructive mechanisms, which include oxygen radical generation [326], lipid peroxidation [326], calcium overload [327, 328], and the generation of inflammatory mediators [329–331]. In vitro and animal studies showed that Ebselen can mimic glutathione peroxidase, react with peroxynitrite, and inhibit acute ischemic stroke-related enzymes, such as cyclooxygenase [332], lipoxygenases [169], NO synthases [333, 334], NADPH oxidase [335–337], and H(+)/K(+)-ATPase [317].

Ebselen is the most characterized for its high bioavailability. It can readily cross the blood–brain barrier and also produce promising results in clinical trials for noise-induced hearing loss (NIHL) along with stroke. NIHL is manifested by the acute exposure to loud noise, which affects several structural elements in auditory hair cells, including cell membrane and intracellular biochemical pathways [338]. Loud noise can reduce GSH and increase the level of oxidized glutathione in the inner ear, therefore leaving it prone to mediated cell damages, which also overwhelm resident detoxification and other antioxidant mechanisms [339, 340]. Intriguingly, GPx activity, which regenerates reduced GSH, has been

reported to decrease following noise exposure. Therefore, administration of ebselen improves NIHL conditions by mimicking GPx activity [341–343].

## 7 Conclusion and Perspective

Because it is a natural part of proteins and tRNAs, selenium is an essential trace element for living systems. Many selenoproteins (such as GPx, TRxR, and Sel proteins) modulate the redox status and systems. Therefore, selenium deficiency will lead to insufficient and/or nonfunctional selenoprotein production, which disrupts the balance of cellular redox systems, thereby resulting in pathological conditions, such as cardiovascular disease, thyroid disease, cancer, neurological disease, depression, diabetes, pancreatitis, and tropical diseases. Furthermore, the deiodinase selenoprotein family is responsible for maintaining a circulating level of thyroid hormone. As an atypical element, selenium has been successfully incorporated into numerous potential therapeutic agents. Approximately 30,000 research articles on selenium have been published and deposited into the NCBI database, indicating the importance of selenium in human health. However, the tolerance range of elemental selenium in humans is narrow: from 55 µg/day (the adequate intake level) to 400 µg/day (the tolerable upper intake level). Epidemiological and pharmacological studies and randomized clinical trials should be conducted to further investigate the link between Se supplement intake and health benefits.

Clearly, selenium function in living systems and its effects on human health are essential. As selenium naturally occurs in protein and tRNA via replacement of oxygen or sulfur atom(s), we predict that the selenium functionality may also present in genomic DNA to assist genome functions, including DNA condensation and accuracy and efficiency of DNA replication and RNA transcription. Thorough research is required to discover and fully understand the selenium functionalities in living organisms. Moreover, selenium-atom-specific modification (SAM) will transform the design of potential oligonucleotide therapeutics via antisense DNA, siRNA, miRNA, and crRNA strategies, by improving the molecular specificity and substrate cleavage efficiency [68].

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