CHARLES UNIVERSITY
FACULTY OF PHARMACY IN HRADEC KRALOVE

Department of Pharmaceutical Chemistry and Drug Analysis

SYNTHESIS OF NEW ORGANIC COMPOUNDS CONTAINING CHALCOGENS

SYNTÉZA NOVÝCH ORGANICKÝCH LÁTEK OBSAHUJÍCÍCH CHALKOGENY

Diploma Thesis

Saarland University
Bioorganic Chemistry
Saarbrücken, Germany 2017 **Lucie Miltová**

This Diploma Thesis was processed as part of the Erasmus+ programme 2016/2017 at the Saarland University at the department of Bioorganic Chemistry under the supervision of Prof. Dr. Claus Jacob and Wesam Ali.

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V Hradci Králové dne:

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Lucie Miltová

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1. ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Kralove

Department of	Pharmaceutical Chemistry and Drug Analysis
Candidate	Lucie Miltová
Supervisor	doc. PharmDr. Radim Kučera, Ph.D.
Consultant	Prof. Dr. Claus Jacob
Title of Diploma Thesis	Synthesis of new organic compounds containing chalcogens

A new triazole containing selenium was prepared by Huisgen 1,3-dipolar cycloaddition of alkyne and azide, known as the click reaction. Another two tetrazoles were synthesized by click reaction. Both compounds have the same structure, except the first compound is tetrazole bound to selenium, in the second compound to sulfur. Additionally, two more compounds contain the SeCN fragment and sulfur at the aromatic ring was prepared.

The focus of this thesis is on the synthesis of new organic compounds containing chalcogens, specifically sulfur and selenium. Due to the presence of chalcogens in the structure, they are expected to show antioxidant, anticancer, antifungal or antibacterial effects or a combination thereof.

A new triazole was synthesized and subsequently characterised by TLC, ^{1}H-NMR, ^{13}C-NMR and MS. It is suitable for future testing of biological activity. Tetrazole contains sulfur was prepared. The final product is, however, unstable and prone to rapid degradation. Using the same reaction condition, tetrazole containing sulfur was not synthesized. Two compounds with SeCN fragment and sulfur at the aromatic ring were prepared. The final products were partially hydrolysed during purification and are therefore unsuitable for further biological testing.

Keywords:

Click chemistry, selenium, sulfur, cycloaddition, selenocyanate, copper, organoselenium compounds

2. ABSTRAKT

Univerzita Karlova, Farmaceutická fakulta v Hradci Králové	
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Název diplomové práce	Syntéza nových organických látek obsahujících chalkogeny

Triazol obsahující selen byl připraven pomocí Huisgenovy 1,3-dipolární cykloadice alkenu a azidu, známé jako klik reakce. Dva další tetrazoly byly syntetizovány klik reakcí. Obě látky mají stejnou strukturu, kromě navázání selenu na tetrazol u první sloučeniny a síry u druhé sloučeniny. Připraveny byly další dvě sloučeniny obsahující SeCN skupinu a síru navázané na aromatickém cyklu.

Tato práce je zaměřena na syntézu nových organických sloučenin obsahujících chalkogeny, konkrétně síru a selen. Vzhledem k přítomnosti chalkogenů se očekává, že látky budou vykazovat antioxidační, protirakovinné, antimykotické nebo antibakteriální účinky nebo jejich kombinaci.

Dosud nepopsaný triazol byl syntetizován a následně charakterizován pomocí TLC, ^{1}H-NMR, ^{13}C-NMR a MS. Je vhodný pro budoucí testování biologické aktivity. Tetrazol obsahující selen byl připraven, ale konečný produkt je nestabilní a náchylný k rychlé degradaci. Při využití stejných reakčních podmínek se nepovedlo připravit tetrazol obsahující síru. Podařilo se syntetizovat dvě látky obsahující SeCN skupinu a síru navázané na aromatickém cyklu. Bohužel, tyto produkty byly během čištění částečně hydrolyzovány a nejsou proto vhodné pro další biologické testování.

Klíčová slova:

klik chemie, selen, síra, cykloadice, selenokyanatan, měď, organické sloučeniny selenu

3. LIST OF ABBREVIATIONS

AOM	azoxymethane
$CDCl_3$	deuterochloroform
DCM	dichlormethan
DIPEA	*N,N*-diisopropylethylamine
DMBA	7,12-dimethylbenzanthracene
DMSO	dimethylsulfoxide
DMSO-d_6	hexadeuterodimethyl sulfoxide
GPx	glutathione peroxidase
GSH	glutathione
LC	liquid chromatography
MS	mass spectrometry
Mw	molecular weight
NMR	nuclear magnetic resonance spectroscopy
NNK	4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone
p-XSC	*p*-xylylselenocyanate
R_f	retardation factor
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RT	retention time
SEMCYS	methylselenocysteine
SEMET	selenomethionine
TBTA	*tris*(benzyltriazolylmethyl)amine
TCEP	*tris*(2-carboxyethyl)phosphine
THPTA	*tris*(3-hydroxypropyltriazolylmethyl)amine
TLC	thin layer chromatography
TMEDA	tetramethylethylenediamine

4. INTRODUCTION

An intensive research into chalcogens, especially of selenium, has been carried out at the Department of Bioorganic Chemistry at the Saarland University in Germany for several years now.

Organic selenium, as well as selenium salts and elementary selenium are considered to be antioxidants, which have the ability to scavenge free radicals. These selenium compounds can be used in chemoprotection or chemotherapy, they can modulate intracellular redox homeostasis and possess inflammatory, antibacterial and antifungal effects.[1] Nowadays the main focus is on selective cytotoxic properties of selenium compounds which allow them to selectively kill cancer cells or macrophages during inflammation and microbes during infections.[2]

This work describes compounds, which contain selenium and sulfur or both. New compounds are prepared mostly by click reaction, which was introduced as a method for obtaining new chemical compounds. Primary, secondary, tertiary or aromatic azides and terminal alkynes can provide Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of alkynes and azides to gain 1,2,3-triazoles. If the alkyne contains a CN group at the end, it is possible to obtain tetrazole via this reaction.

5. THE AIM OF THE WORK

This Diploma Thesis concerns itself with the synthesis of new organic compounds containing chalcogens. These compounds are expected to have antibacterial, antifungal, antioxidant, chemoprotective, chemotherapeutic effects or a combination thereof.

The main tasks were:

- synthesis of new compounds containing sulfur and selenium or both;
- characterization of new compounds by TLC, ^{1}H-NMR, ^{13}C-NMR and MS.

6. THEORETICAL PART

6.1 Selenium

Selenium was discovered by Berzelius in 1817 as a sulfur oxidation residue on the wall of a lead chamber. It is a member of the group 16, known as the chalcogen group. Selenium had only been considered as a toxic element for a long time, especially after the discovery of selenosis (chronic selenium toxicity) by Marco Polo in horses in the Nan Shan and the Tien Shan mountains of Turkestan, a place with plenty of selenium in the soil. In the 1950s, however, vitamin E was replaced by selenium in experimental animals and the results showed no adverse effects. The revelation that selenium is an essential component of glutathione peroxidase in the early 1970s is considered a breakthrough for the expansion and development of organoselenium chemistry.[3, 4]

Selenium occurs in biological systems as a part of various proteins and enzymes. The most famous ones are selenocysteine and selenomethionine.[2]

Since selenium is an essential trace element in human body it needs to be present in the human diet. The dosage is crucial.[1, 5, 6] Large amounts of selenium are toxic to humans, animals and aquatic life.[4] The results of a Chinese study showed that dietary intake of 100-200 μg Se/d (selenium per day) inhibits genetic damage and prevents cancer in humans. A dose of 600 μg Se/d is the maximal safe intake.[6] Mild adverse effects were reported in doses of 900-1600 μg Se/d while doses of 5000 μg Se/d caused brittle and fragile nails or hair loss.[7]

In contrast, selenium deficiency can cause malnutrition, lack of mental alertness, impotence, Keshan disease (type of endemic cardiomyopathy) and Khasin-Beck disease (osteoarthritis) or cancer (thyroid, prostate, lung, colon, gastric).[2, 7, 8] Chinese results indicate that 11 μg Se/d or less can cause these deficiency symptoms. 40 μg Se/d is the minimum requirement.[7]

The current legislation of the Czech Republic[9] states that 55 μg Se/d is the recommended dietary allowance and corresponds with those of the Food and Nutrition Board, whilst the tolerable upper limit of selenium intake is 400 μg Se/d.[10]

<u>Selenium compounds in plants:</u>

Inorganic selenate (SeO_4^{2-}) and selenite (SeO_3^{-}) ions from the soil are transformed to hydrogen selenide (H_2Se), which reacts with *O*-acetylserine and forms

selenocysteine. This selenium amino acid creates organic selenium compounds such as selenomethionine (SEMET), methylselenocysteine (SEMCYS), dimethylselenide ($(CH_3)_2Se$), selenoneine or trimethylselenium.[2, 7]

Selenium compounds in animals and humans:

Organic selenium such as SEMET or inorganic selenium can be transformed to common intermediate hydrogen selenide. In contrast to plants, there are no known pathways for creating SEMET from inorganic selenium.

The SEMET catabolism in animals and humans runs through two routes, the first is transamination and decarboxylation (**figure 1**). This way 90 % of SEMET is metabolised. The second route is transsulfuration through selenocystathione to selenocysteine, which is present in all eukaryotic selenoproteins. It even has its own triple code (UGA) and is considered to be the twenty-first genetically coded amino acid. Selenocysteine is thereafter converted to hydrogen selenide by the enzyme β-lyase.[2, 7]

As is shown in the **figure 1**, SEMCYS can occur in the form of γ-glutamyl-SEMCYS or SEMCYS. In garlic the predominant form is γ-glutamyl-SEMCYS. This particular form is hydrolysed in the intestinal tract to SEMCYS and then transformed to methylselenol by the enzyme β-lyase at the tissue level. Methylselenol is the active form against tumor formation. SEMCYS is directly converted to methylsenol by only one way. Therefore, SEMCYS is more effective in prevention of cancer than SEMET, which is incorporated into proteins rather than into compounds that are active against tumor tissue.[7]

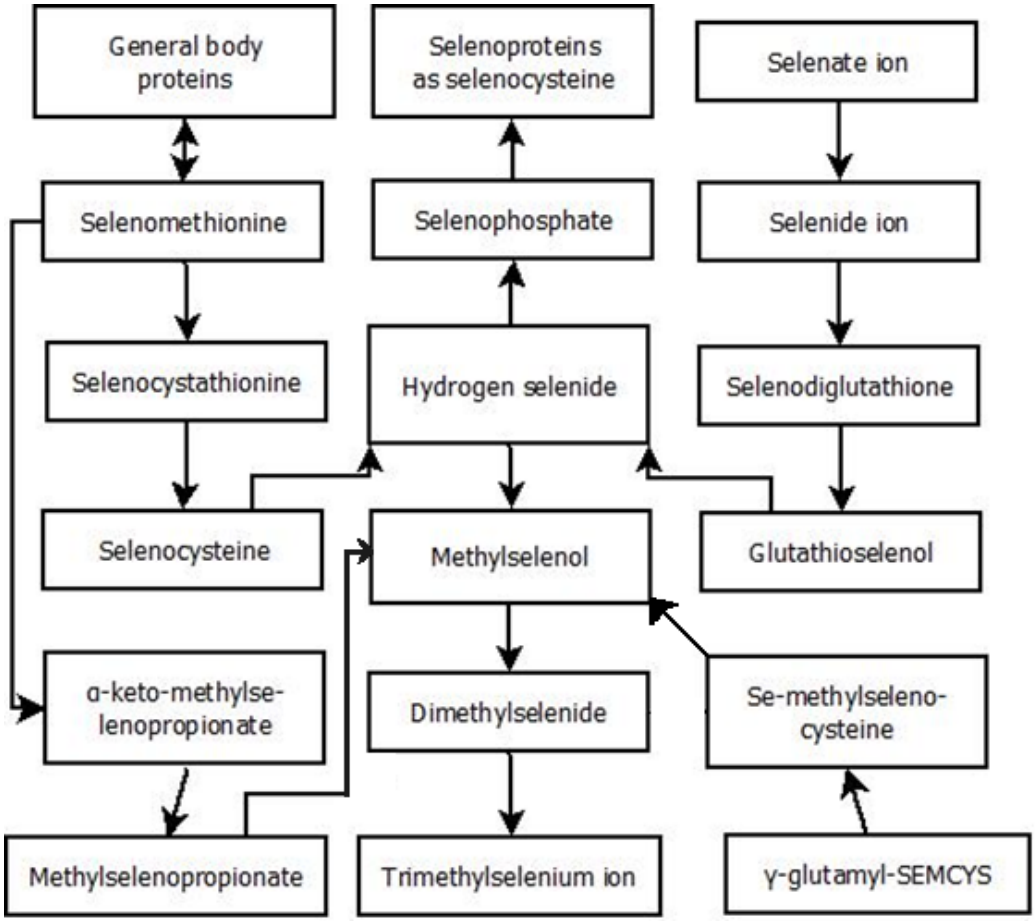


Figure 1: Schematic overview of selenium metabolism in animals and humans (adopted and modified from[7]).

6.1.1 Organoselenium compounds as antioxidant agents

Oxidative stress can be described as disturbance in the balance between the production of reactive oxygen species (ROS) or reactive nitrogen species (RNS) and antioxidants. Many diseases, such as atherosclerosis, diabetes mellitus, cancer, rheumatoid arthritis, cardiovascular diseases, chronic inflammation, stroke, are associated with oxidative stress.[11] ROS and RNS cause cell death through oxidation of membranes, proteins and DNA.[5, 12, 13] Antioxidants reduce those oxidative stressors.

Unfortunately, high amounts of antioxidants are necessary, which creates a barrier in the therapy development. However, antioxidant catalyst, e. g. glutathione peroxidase (GPx) can increase the effect of naturally occurring antioxidants. These compounds are located inside the cells during oxidative stress. Furthermore, they are not consumed

during the reaction and can be reused in the next reaction cycle. Consequently, it is not necessary to consume or produce them in high quantities.[13]

The human enzyme glutathione peroxidase (GPx) prevents oxidative stress in biological systems. It is located in the cytosol and mitochondria and is composed of four identical subunits, each containing one selenium atom in the active site.

GPx catalyzes the reduction of toxic hydrogen peroxide. The mechanism is shown in the **figure 2**. Hydrogen peroxides and organic peroxides are reduced to the corresponding alcohols using selenol. Selenol is oxidized to unstable selenenic acid, which immediately reacts with the reduced form of glutathione and creates a selenosulfide adduct and water. Reaction with the second glutathione forms an oxidized form of glutathione and recycles selenol.[5, 13]

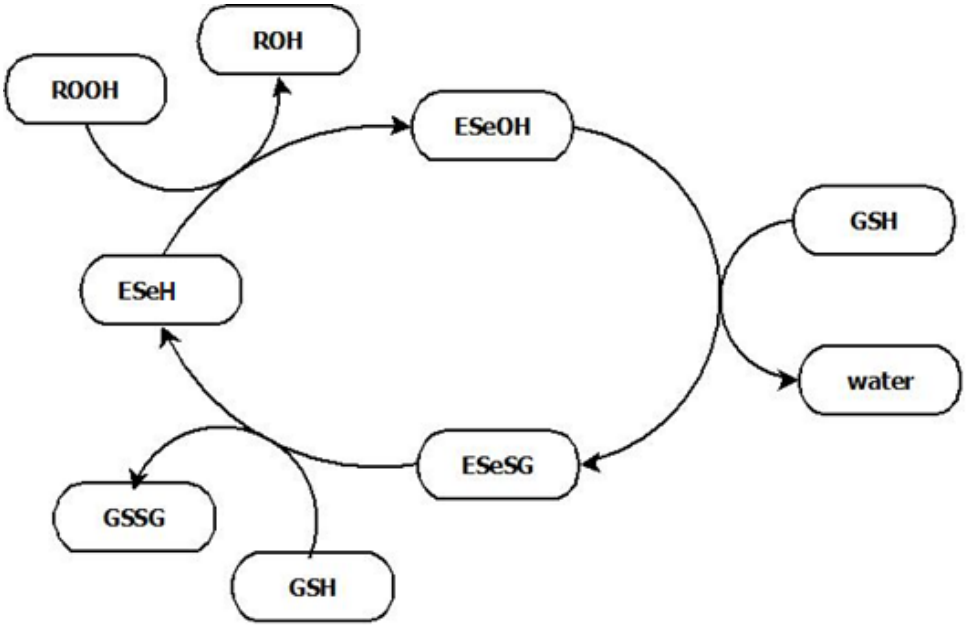


Figure 2: Catalytic mechanism of GPx. ESeH = selenol, ESeOH = selenenic acid, ESeSG = selenosulfide adduct (selenenyl sulfide), GSH = glutathione (reduced form), GSSG = glutathione (oxidized form), ROH = alcohol, ROOH = hydrogen peroxide or organic peroxide (adopted and modified from[5]).

Compounds that imitates GPx activity, even with broader substrate specificity, can be created by covalent incorporation of a selenium atom into an inorganic framework.[13]

The first example of this synthetic antioxidant catalyst is ebselen (compound 1 = 2-phenyl-1,2-benzisoselenazol-3(2*H*)-one), that causes destruction of peroxides. In the **figure 3** ebselen (compound 1) reacts with reduced form of glutathione to yield selenodisulfide (compound 2), which creates selenol (compound 3) with second equivalent of GSH. Compound 3 reacts with hydrogen peroxide to form water and ebselen seleninic acid (compound 4), which produces water and turns by the reaction with GSH into ebselen (compound 1).[5, 13, 14]

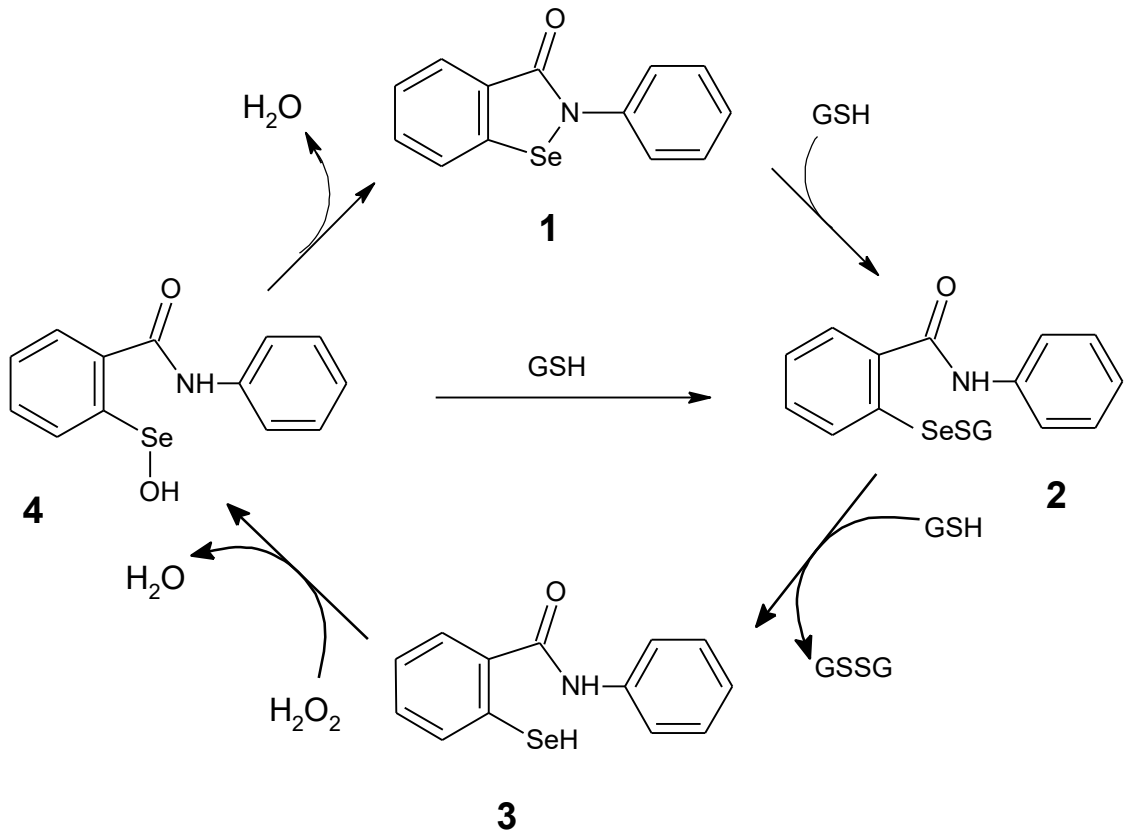
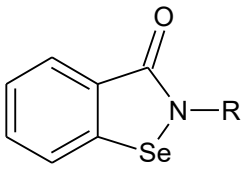


Figure 3: Catalytic reduction of hydrogen peroxide by ebselen. GSH = glutathione (reduced form), GSSG = glutathione (disulfide form), H_2O_2 = hydrogen peroxide, SeSG = selenosulfide adduct (adopted and modified from[5, 14]).

Chang et al. prepared cyclic derivatives of ebselen (compound 5a-5c) and acyclic derivatives of ebselen (compound 6a-6c). Both of them show activity against

the 1,1-diphenyl-2-pycryl-hydrazyl (DPPH) radical and peroxynitrite, even higher than ebselen. Compound 6c is five times more active than ebselen.[15]



R:

a) (*p*)-CH_3-Ph-
b) (*m*)-CH_3-Ph-
c) (*o*)-CH_3-Ph-

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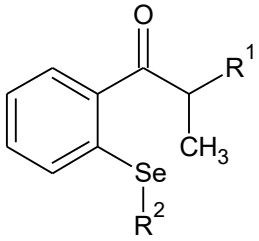
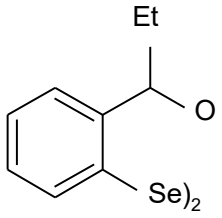


a) R^1=Ph, R^2=CH_3
b) R^1=(*p*)-CH_3-Ph, R^2=CH_3
c) R^1=(*m*)-CH_3-Ph, R^2=CH_2=CH-CH_2

6

Diselenides (compound 7, 8, 9) with an oxygen atom near the selenium show GPs-like activity.[16]



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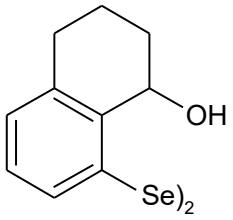

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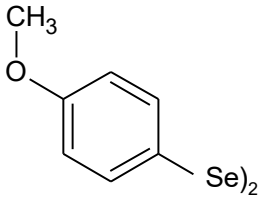

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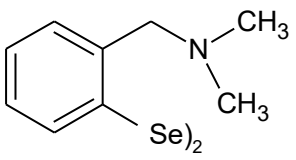
Diselenides with a nitrogen atom in proximity to selenium (compound 10, 11) show GPx-like activity as well. The amino group activates oxidative cleavage of the Se-Se bond and stabilizes the selenenic acid against further oxidation.[17]



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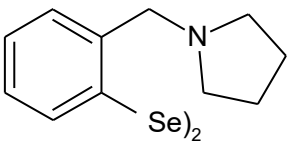

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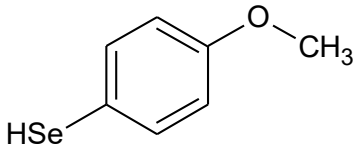
6.1.2 Organoselenium compounds as antitumor agents

There are numerous hypotheses on how selenium compounds may reduce tumor formation. It is most likely caused by a combination of some of the following mechanisms: the increase of apoptosis, the raise in frequency of DNA repairs, anti-angiogenic activity and through selenoenzymes.[7]

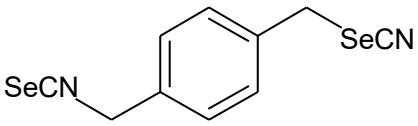
The above-mentioned ebselen (compound 1) causes apoptosis in human hepatoma cell line HepG2 and inhibits breast and colon cancer cell growth in humans.[5]

Another organoselenium compounds which shows anti-tumor activity is *p*-methoxybenzeneselenol (compound 12).[5] This compound inhibits azoxymethane (AOM), which induces fulminant hepatic failure in mice[18] and benzo[*a*]pyrene, which causes tumors in mice, rats, ducks and monkeys.[19]



12

p-xylylselenocyanate = *p*-XSC (compound 13) is active against AOM-induced rat colon, DMBA-induced rat mammary and NNK-induced mouse lung tumor models. *P*-XSC shows liver and hematopoietic system toxicity in rats.[20, 21]



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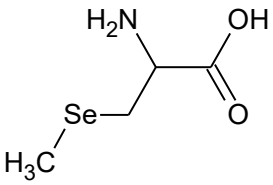
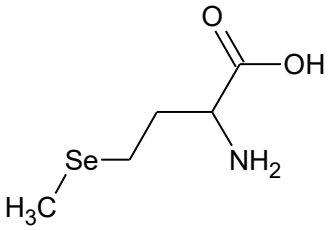
SEMCYS (compound 14) showed some level of activity against mammary cancer and is currently being tested for anti-tumor activity in humans with prostate cancer.[7]

It has been noted, that SEMET (compound 15) is less effective in the treatment of cancer. SEMET acts more as a co-treatment with the enzyme methioninase in the prevention of prostate cancer. Together, they increase levels of phosphorylated p53 and p53 translocation into the mitochondria.[22]



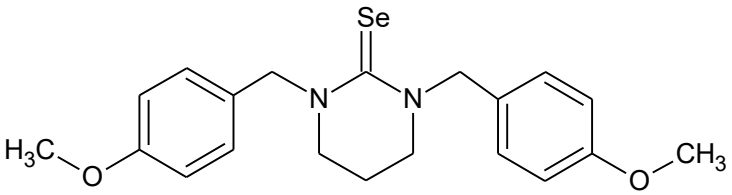


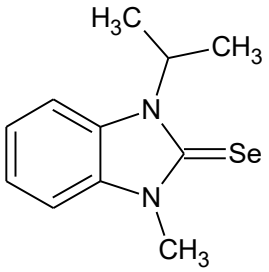
14 **15**

6.1.3 Organoselenium compounds as antifungal and antibacterial agents

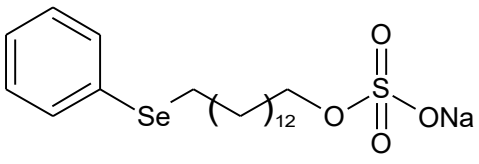
Ebselen (compound 1) is one of the most effective selenium compounds in this field. It has antiinflammatory, antiatherosclerotic, cytoprotective and antimicrobial activity against *Staphylococcus aureus*.[5]

Compounds 16 and 17 show antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa, Escherichia coli*, *Candida albicans* and *Candida tropicalis*.[23] Compound 18 exhibit activity against *Candida guilliermondii.*[24]





16 **17**



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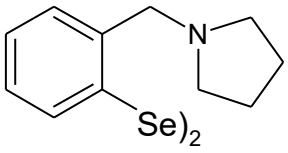
Diaryl diselenide (compound 10) and its derivate (compound 19) provide activity against the growth of *Saccharomyces cerevisiae*. The latter compound also inhibits the growth of *Candida albicans*.[5]



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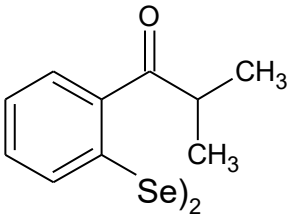

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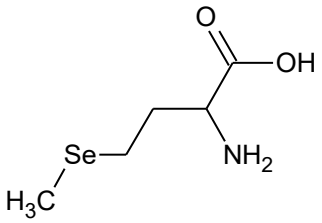
6.2 Sulfur

Sulfur and selenium are both in the group of chalcogens. They have similar chemical and physical properties, such as valence-shell electronic configurations, atomic size, bond energies ionization potential or electron affinities.

The main difference is their metabolic pathway in biological systems. Selenium is metabolized to reduced states and sulfur to more oxidized states. Another major difference is in the hydrides of these elements. H_2Se is much more acidic than is H_2S. The difference may be shown in the dissociation of the selenohydryl groups in selenocysteine and sulfhydryl groups in cysteine. Thiols are mainly protonated at physiological pH, whereas selenols are mainly dissociated at physiological pH. Due to these two main differences, selenium compounds are usually 600 times more active against cancer than their analogues containing sulfur.[7, 24]

To introduce an example, SEMET (compound 15) shows a higher level of protection against peroxynitrite and is more effective than its sulfur analogue - methionine (compound 20).[25]



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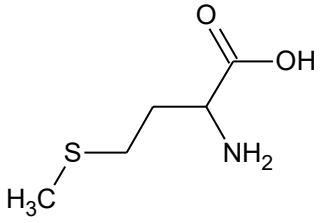

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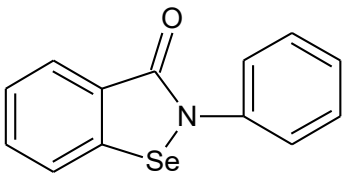
Ebselen (compound 1) protect DNA from single-strand break formation more frequently than its sulfur counterpart (compound 21). Ebselen is also more active against peroxynitrite than compound 21.[26, 27]



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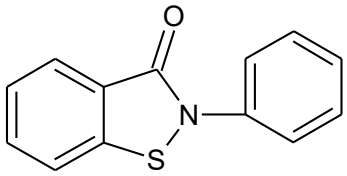

21

6.3 Click chemistry

Click chemistry was introduced by Prof. Barry Sharpless's group at the 217th American Chemical Society annual meeting in 1999.[27] The idea behind this approach was quickly, reliably and easily develop new substances by joining small units together with heteroatomes (C-X-C).[29]

Click chemistry was introduced as a group of chemical reactions, which should meet several criteria.[29]

Such a chemical reaction must be quick, wide in scope, modular, stereospecific and provide a high yield. A high thermodynamic driving force (mostly greater than 20 kcal mol^{-1}) turns the process faster and more selective. Reaction should produce minimum amounts of by-products, which are non-toxic, inoffensive and readily removed by non-chromatographic methods. The reaction must be simple to perform. Reaction conditions must be insensitive to oxygen and water, starting material and reagents have to be easily accessible. Product isolation and purification should be carried out by distillation or crystallization, without requiring chromatographic methods. Products have to be stable under physiological conditions. The most successful click reactions require water as the reaction medium. Reaction without water can be dangerous on a large scale since click reactions are highly exothermic and water is the best heat-sink for processing the enormous heat output. Another function of water is the prevention of interference with protic functional groups (alcohols, amides).[28, 29, 30, 31]

Reactions will not damage biological and fragile structures in the body, because they are performed under mild conditions. Water in the human body provides good conditions for applications of click reactions.[28]

6.3.1 Applications of click chemistry

Click chemistry was introduced as an easier method for obtaining new chemical compounds. Nowadays, it has extensive applications, especially in polymer chemistry. It allows preparation of a wide range of functional polymers and complex macromolecules.

Linear polymers have been synthesized by Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition, connected with dendrimers, dendritic polymers or nanoparticles.[28, 32]

In bioconjugation 1,2,3-triazoles are used as linkers, because of their solubility in water and stability in biological conditions. These linkers are extremely rigid and do not allow two linked substances to aggregate or interact with each other.

Click chemistry can be used in radiolabelling, where a radionuclide is attached to the drug. One example of this is the process of attaching ^{99m}Tc to organic molecules. It is usually achieved by creation of an organometallic complex which is then linked to a ligand already attached to the molecule. This ligand is usually histidine that must be synthesized in few steps and is not easily incorporated into the molecule of the drug. 1,2,3-triazoles can replace histidine, because they have similar electronic properties to the imidazole in histidine and are synthesized in one step. Another use of bioconjugation can be in tagging the *Escherichia coli* or labeling DNA.[28, 33]

6.3.2 Major classifications of click chemistry reactions

- **Figure 4** summarises the reactions which match the click chemistry criteria.
  - Cycloaddition of two unsaturated species, especially 1,3-dipolar cycloaddition and also the hetero-Diels-Alder reactions.
  - Nucleophilic ring-opening reactions of strained heterocyclic electrophiles such as epoxides, aziridines, cyclic sulfates, cyclic sulfamidates, aziridinium and episulfonium ions.
  - Carbonyl chemistry non-aldol type, where oxime ethers can be formed, hydrazones, amides, aromatic heterocycles, ureas or thioureas. Reaction with aldol are not classified as click reaction, because of their low thermodynamic driving forces, which cause long reaction times and significant amounts of by-products.
  - Additions to carbon-carbon multiple bond are primarily oxidation reactions, for example epoxidation, dihydroxylation, nitrosyl or sulfenyl halide addition, aziridination or Michael addition of Nu-H reactants.[30, 31]

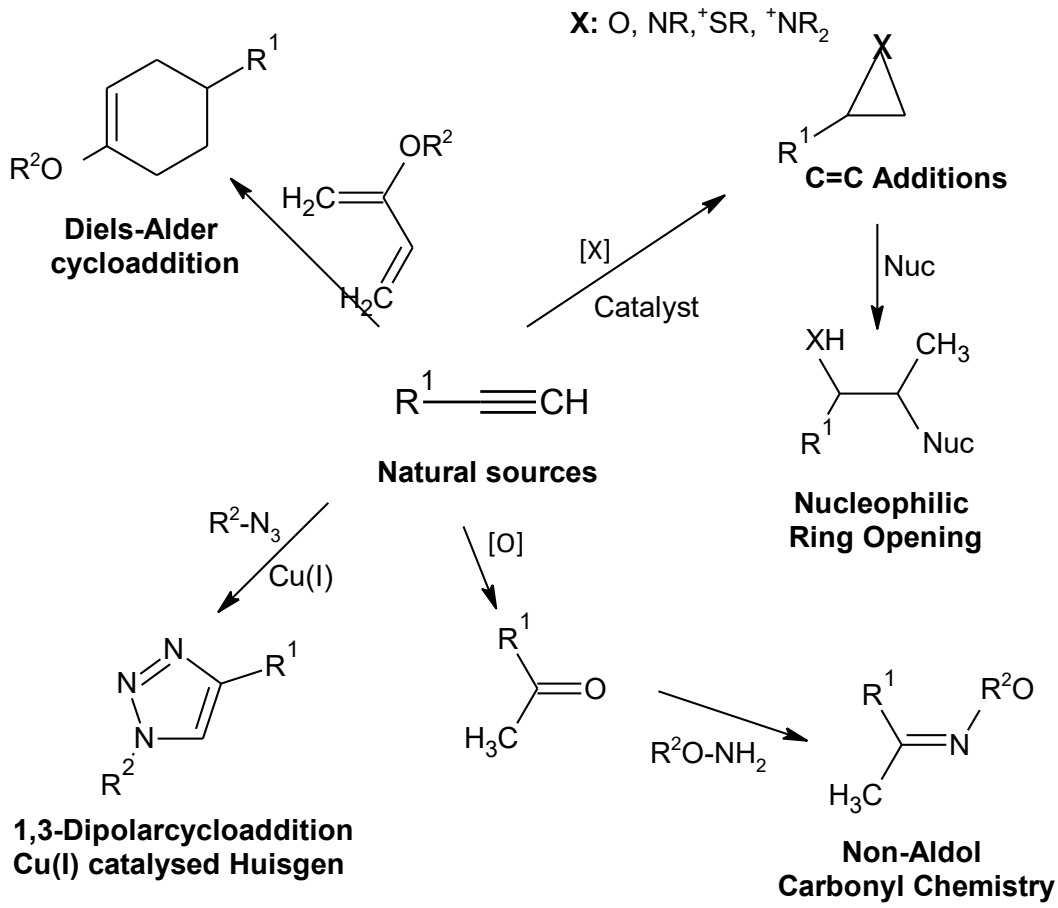

Figure 4: Classifications of click chemistry reactions. Nuc = nucleophile (adopted and modified from[30, 31]).

6.3.3 Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of alkyne and azide

Primary, secondary, tertiary or aromatic azides and terminal alkynes can provide Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of alkynes and azides to gain 1,2,3-triazoles (**figure 5**). It has become a premier example of click reaction, because it is highly reliable, azides with alkynes are easy to install and it is 10^7 times faster than uncatalyzed reaction. It can be performed in various solvents, over a wide range of pH (5-12) or temperature (0-160 °C). Purification of product is generally not required. This reaction does not require protective groups, because it is selective for 1,4-disubstituted 1,2,3-triazole.[28, 29, 30, 31, 32]

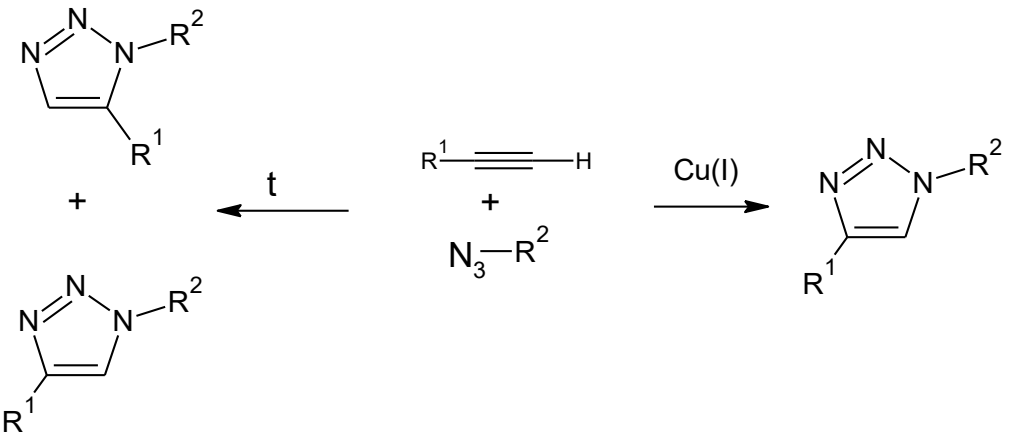

Figure 5: 1,4-disubstituted 1,2,3-triazole. Huisgen click reaction catalysed by Cu(I) is selective for 1,4-disubstituted 1,2,3-triazole. The thermally induced Huisgen cycloaddition results in an approximately 1:1 mixture of 1,4 and 1,5-disubstituted 1,2,3-triazole stereoisomers (adopted and modified from[31]).

6.3.4 Mechanism of Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition

It has been previously suggested that the reaction proceeds between mononuclear Cu(I) acetylide and an organic azide. Kinetic experiments, theoretical studies and computational investigation, however, indicated a participation of *bis*(copper) complex (**figure 6**). Both copper complexes could lead to the triazole under stoichiometric conditions, but kinetically favoured is the *bis*(copper) acetylide path. Cu(I) creates the Π,σ-*bis*(copper) acetylide complex with triple bond of alkyne. By reaction with azide 3,5-*bis*(metallated) triazole is created and thereafter triazole.[35, 36]

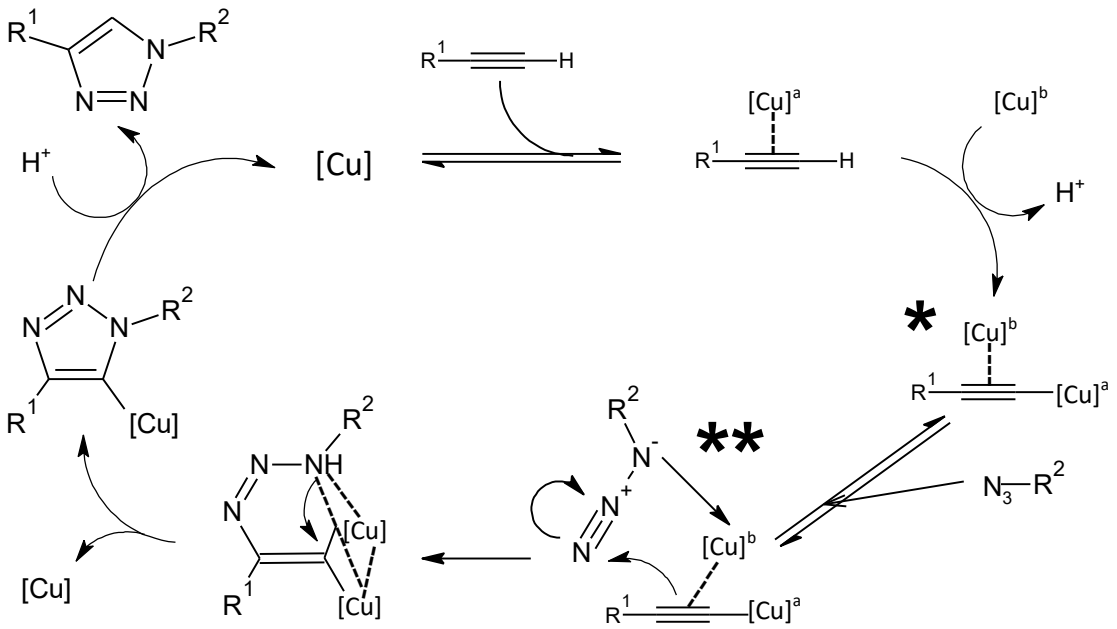

Figure 6: Proposed catalytic model for the Cu(I)-catalyzed azide-alkyne cycloaddition with two copper atoms. * = Π,σ-*bis*(copper) acetylide complex, ** = 3,5-*bis*(metallated) triazole (adopted and modified from[35]).

Cu(I) saturation is rare, but can occur when the Π,σ-*bis*(copper) acetylide complex is surrounded by terminal alkynes, which can chelate together and cause saturation. Consequently, for azide it is not possible to reach the complex.

Alkyne homocoupling is caused by reaction of two alkynes, instead of reaction with azide. Small amines, pyridine or tetramethylethylenediamine (TMEDA) cause stabilization of intermediates of the homocoupling reaction and increase the yield of the reaction. In contrast, sterically bulky base slows down this side reaction.[28, 37]

6.3.5 Catalysts

Cu(I) salts:

A broad range of acetylene and azides can react with Cu(I) salts such as CuI, CuBr, $Cu(CH_3COO)_2$, $[Cu(CH_3CN)_4]PF_6$ or $[CuBr \bullet SMe_2]_2$. It is usually required to add acetonitrile as a co-solvent with one equivalent of nitrogen base such as triethylamine, 2,6-lutidine, *N,N*-diisopropylethylamine (DIPEA) or pyridine for deprotonation.[37] Although, undesired by-products (primarily diacetylenes, *bis*-triazoles or 5-hydroxytriazoles) can sometimes be observed. Eventually, 2,6-lutidine and DIPEA together with an inert gas condition provide less by-products. They prevent the oxidation and formation of unreactive polynuclear Cu(I) aggregates.[28, 32, 34, 37, 38, 39]

Reduced Cu(II) salts:

The use of reduced Cu(II) salts is more reliable, simple, often more pure and less expensive than the direct use of Cu(I) salts. Cu(II) salts, such as $CuSO_4 \bullet 5H_2O$ are reduced *in situ* by sodium ascorbate, used in a 3- to 10-fold excess over the copper catalyst.[35] Another reducing agent is hydrazine, which is used in the presence of limited amounts of air with decreased catalyst concentration.[37] *Tris*(2-carboxyethyl)phosphine (TCEP) is also used in biological samples, because it prevents oxidative coupling of cysteine residues. TCEP should be applied in small amounts, because it binds to copper ions and can inhibit the reaction.[28, 34, 38]

These types of reactions do not require inert gas conditions, because they are compatible with oxygen and water. Reactions proceed in between 6 to 36 h at the room temperature in various solvents including water without organic co-solvent.[34, 38]

A disadvantage to using Cu(II) salts is the possibility of reduction of Cu(I) to Cu(0) or re-oxidation to Cu(II) by air. This can be solved by adding a copper stabilizing agent such as *tris*(3-hydroxypropyltriazolylmethyl)amine (THPTA) or *tris*(benzyltriazolylmethyl)amine (TBTA), which acts as a tetradentate ligand and prevents the attack of oxidants by blocking each coordination site at the metal centre.[38]

Oxidizing copper metal with an amine salt:

Cu(0) nanosized powder or clusters are oxidized to Cu(I) by amine salts (mostly amine hydrochloride). This method provides a good yield, but has several disadvantages. It is necessary to use larger amounts of copper, the reaction takes a long time and is more expensive. Cu(0) nanosize powder is seven times more expensive than other sources of copper. Cu(0) nanosize clusters are not commercially available. Furthermore, reactants with acidic groups in the structure can be destroyed, because the reaction requires a slightly acidic environment to dissolve the metal (approximately pH = 5). These disadvantages limit the application of this method in industry and research.[28, 34, 37]

7. EXPERIMENTAL PART

Experimental procedures have been included into Attachment due to confidential reasons.

8. RESULTS AND DISCUSSION

Results and discussion have been included into Attachment due to confidential reasons.

9. CONCLUSIONS

This work describes the synthesis of five compounds containing selenium, sulfur, or both. For all compounds their synthesis, optimal conditions for preparation were defined and described. Compounds were characterised by TLC, ^{1}H-NMR, ^{13}C-NMR or MS.

Compound 25 was unstable and prone to rapid degradation. Compound 27 proceed under the same condition as the click reaction of compound 25. Unfortunately, the synthesis of compound 27 was not formed in these condition.

Compounds 34, 40 and 41 were successfully synthesized, but compound 40 and 41 became partially hydrolysed during purification.

Testing for biological activity of compound 34 is going to be performed in the future in order to determine anticancer, antimicrobial, antifungal and antioxidant effects. Its synthesis was described for possible re-synthesis and its structure had been confirmed.

Due to the content of impurities in the prepared compounds 25, 40, 41 the biological tests are postponed till compounds of better quality will be obtained. Less polar Al_2O_3 or nonpolar poly(dimethyl siloxane) stationary phases will be investigated.

10. REFERENCES

1. ESTEVAM, Ethiene, et al. Aspects of a Distinct Cytotoxicity of Selenium Salts and Organic Selenides in Living Cells with Possible Implications for Drug Design. *Molecules,* **2015**, *20*(8), 13894–13912.

2. MÁNIKOVÁ, Dominika, et al. Intracellular Diagnostics: Hunting for the Mode of Action of Redox-Modulating Selenium Compounds in Selected Model Systems. *Molecules*, **2014**, *19*(8), 12258–12279.

3. NOGUEIRA, Cristina W. and João B. T. ROCHA. Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. *Archives of Toxicology,* **2011**, *85*(11), 1313–1359.

4. BARCELOUX, Donald G. and Donald BARCELOUX. Selenium. *Journal of Toxicology: Clinical Toxicology*, **1999**, *37*(2), 145–172.

5. KARVEKAR, MD, AK DAS and C NARAJJI. Biological importance of organoselenium compounds. *Indian J. Pharm. Sci.,* **2007**, *69*(3), 344–351.

6. JAIN, Rohan, et al. Extracellular Polymeric Substances Govern the Surface Charge of Biogenic Elemental Selenium Nanoparticles. *Environ. Sci. Technol.,* **2015**, *49*, 1713–1720.

7. WHANGER, P. D. Selenium and its relationship to cancer: an update. *Br. J. Nutr.,* **2004**, *91*(01), 11–28.

8. HUANG, Yang, et al. Daily Dietary Selenium Intake in a High Selenium Area of Enshi, China. *Nutrients.,* **2013**, *5*, 700–710.

9. Vyhláška č. 225/2008 Sb., ze dne 17. června 2008, kterou se stanoví požadavky na doplňky stravy a na obohacování potravin, in: Sbírka zákonů České republiky, 2008, částka 71, 3230–3244, ISSN 1211–1244.

10. Food and Nutrition Board USA Institute of Medicine. In Dietary References Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids; *National Academy Press: Washington, DC*, USA, **2000**, 284–324.

11. UTTARA, Bayani, Ajay SINGH, Paolo ZAMBONI and R. MAHAJAN. Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. *Current Neuropharmacology,* **2009**, *7*(1), 65–74.

12. BETTERIDGE, DJ. What is oxidative stress? *Metabolism,* **2000**, *49*, No 2, Suppl 1), 3–8.

13. GILES, Gregory I., et al. Evaluation of sulfur, selenium and tellurium catalysts with antioxidant potential. *Org. Biomol. Chem.,* **2003**, *1*, 4317–4322.

14. NOGUEIRA, C. W., Gilson ZENI and J. B. T. ROCHA. Organoselenium and organotellurium compounds: toxicology and pharmacology. *Chem. Rev.*, **2004**, *104*(12), 6255-6285.

15. CHANG, Tsu-Chung, et al. Synthesis and biological evaluation of ebselen and its acyclic derivatives. *Chem. Pharm. Bull.,* **2003**, *51*(12), 1413–1416.

16. WIRTH, Thomas. Glutathione Peroxidase-like Activities of Oxygen-Containing Diselenides. *Molecules,* **1998**, *3*(7), 164–166.

17. WILSON, Stephen R., Paul A. ZUCKER, Ruey Ruey C. HUANG and Abraham SPECTOR. Development of synthetic compounds with glutathione peroxidase activity. *J. Am. Chem. Soc.,* **1989**, *111*(15), 5936–5939.

18. MATKOWSKYJ, Kristina A., et al. Azoxymethane-induced fulminant hepatic failure in C57BL/6J mice: characterization of a new animal model. *Am. J Physiol*., **1999**, *277*(2), G455–G462.

19. IARC WORKING GROUP ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS. *A review of human carcinogens*. International Agency for Research on Cancer, France, Lyon 2012. ISBN 9789283213239.

20. KELLOFF, Gary. J., et al. New agents for cancer chemoprevention. *J. Cell. Biochem.,* **1996**, *26*, 1–28.

21. EL-BAYOUMY, Karam and Raghu SINHA. Mechanisms of mammary cancer chemoprevention by organoselenium compounds. *Mutation Research.,* **2004**, *551*(1-2), 181–197.

22. ZHAO, R., F. E. DOMANN and W. ZHONG. Apoptosis induced by selenomethionine and methioninase is superoxide mediated and p53 dependent in human prostate cancer cells. *Mol. Cancer Ther.,* **2006**, *5*(12), 3275–3284.

23. DI LEO, Iris, et al. Organoselenium compounds, an overview on the biological activities beyond antioxidant properties**.** *Proceedings of The 20th International Electronic Conference on Synthetic Organic Chemistry*. Basel, Switzerland: MDPI, **2016**, b018–b032.

24. VISWANATHAN, Uma Maheswari. Study of antimicrobial, biochemical and nanotechnological aspects of novel sulfur, selenium and tellurium compounds. Dissertation work. Saarland University. Department of Bioorganic Chemistry, Saarbrücken 2014.

25. BRIVIBA, Karlis, Ivan ROUSSYN, Victor S. SHAROV and Helmut SIES. Attenuation of oxidation and nitration reactions of peroxynitrite by selenomethionine, selenocystine and ebselen. *Biochem. J.,* **1996**, *319*, 13–15.

26. MASUMOTO, Hiroshi and Helmut SIES. The Reaction of Ebselen with Peroxynitrite. *Chem. Res. Toxicol.,* **1996**, *9*(1), 262–267.

27. MUGESH, Govindasamy, Wolf-Walther DU MONT and Helmut SIES. Chemistry of Biologically Important Synthetic Organoselenium Compounds. *Chemical Reviews.,* **2001**, *101*(7), 2125–2180.

28. HEIN, Christopher D., Xin-Ming LIU and Dong WANG. Click Chemistry, A Powerful Tool for Pharmaceutical Sciences. *Pharmaceutical Research*, **2008**, *25*(10), 2216–2230.

29. KOLB, Hartmuth C., M. G. FINN and K. Barry SHARPLESS. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angew. Chem., Int. Ed.,* **2001**, *40*, 2004–2021.

30. KOLB, Hartmuth C. and K. Barry SHARPLESS. The growing impact of click chemistry on drug discovery. *Drug Discov Today*, **2003**, *8*(24), 1128–37.

31. MOSES, John E. and Adam D. MOORHOUSE. The growing applications of click chemistry. *Chem. Soc. Rev.,* **2007**, *36*(8), 1249–1262.

32. LIANG, Liyuan and Didier ASTRUC. The copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) "click" reaction and its applications. An overview. *Coord. Chem. Rev.,* **2011**, *255*(23-24), 2933–2945.

33. LINK, A. James and David A. TIRRELL. Cell Surface Labeling of *Escherichia coli* via Copper(I)-Catalyzed [3+2] Cycloaddition. *J. Am. Chem. Soc.,* **2003**, *125*(37), 11164–11165.

34. ROSTOVTSEV, Vsevolod V., Luke G. GREEN, Valery V FOKIN and K. Barry SHARPLESS. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective Ligation of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed.,* **2002**, *41*(14), 2596–2599.

35. WORRELL, B. T., J. A. MALIK and V. V. FOKIN. Direct evidence of a dinuclear copper intermediate in Cu(I)-catalyzed azide-alkyne cycloadditions. *Science,* **2013**, *340*(6131), 457–460.

36. AHLQUIST, Mårten and Valery V. FOKIN. Enhanced Reactivity of Dinuclear Copper(I) Acetylides in Dipolar Cycloadditions. *Organometallics,* **2007**, *26*(18), 4389–4391.

37. BOCK, Victoria D., Henk HIEMSTRA and Jan H. VAN MAARSEVEEN. CuI-Catalyzed Alkyne-Azide "Click" Cycloadditions from a Mechanistic and Synthetic Perspective. *Europ. J. Org. Chem.,* **2006**, *1*, 51–68.

38. BERG, Regina and Bernd F. STRAUB. Advancements in the mechanistic understanding of the copper-catalyzed azide-alkyne cycloaddition. *Beilstein J. Org. Chem.,* **2013**, *9*, 2715–2750.

39. GOLAS, Patricia L., Nicolay V. TSAREVSKY, Brent S. SUMERLIN and Krzysztof MATYJASZEWSKI. Catalyst Performance in "Click" Coupling Reactions of Polymers Prepared by ATRP: Ligand and Metal Effects. *Macromolecules,* **2006**, *39*(19), 6451–6457.

11. THE LIST OF FIGURES