REVIEW ARTICLE

Organodiselenides: Organic Catalysis and Drug Design Learning from Glutathione Peroxidase

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Abstract: Organodiselenides are an important class of compounds characterized by the presence of two adjacent covalently bonded selenium nuclei. Among them, diaryldiselenides and their parent compound diphenyl diselenide attract continuing interest in chemistry as well as in close disciplines like medicinal chemistry, pharmacology and biochemistry. A search in SCOPUS database has revealed that in the last three years 105 papers have been published on the archetypal diphenyl diselenide and its use in organic catalysis and drug tests. The reactivity of the Se-Se bond and the redox properties of selenium make diselenides efficient catalysts for numerous organic reactions, such as Bayer-Villiger oxidations of aldehydes/ketones, epoxidations of alkenes, oxidations of alcohols and nitrogen containing compounds. In addition, organodiselenides might find application as mimics of glutathione peroxidase (GPx), a family of enzymes, which, besides performing other functions, regulate the peroxide tone in the cells and control the oxidative stress level. In this review, the essential synthetic and reactivity aspects of organoselenolides are collected and rationalized using the results of accurate computational studies, which have been carried out mainly in the last two decades. The results obtained in silico provide a clear explanation of the anti-oxidant activity of organodiselenides and more in general of their ability to reduce hydroperoxides. At the same time, they are useful to gain insight into some aspects of the enzymatic activity of the GPx, inspiring novel elements for rational catalyst and drug design.

Keywords: Organodiselenolides, diphenyl diselenide, glutathione peroxidase, antioxidants, DFT calculations, reaction mechanism, bioinspired catalysis.

1. INTRODUCTION

Organic selenides are well-known mimics of Glutathione Peroxidase (GPx), the family of ubiquitous selenoproteins playing a key role in mitigating the oxidative stress in the cell [1-3]. In fact, these enzymes catalyze the reduction of H₂O₂ and organic hydroperoxides to water/alcohol, consuming two equivalents of glutathione, according to the chemical equation:

(1) ROOH + 2GSH → ROH + GSSG

The GPx mechanism is sketched in Fig. (1).

A pivotal role is played by a selenocysteine residue (Sec46 in rat cytosolic GPx4) in the catalytic pocket, which is identified as the active center [3-5]. The role of the chalcogen as well as the importance of the surrounding tetrad of conserved residues (Sec46, Gln81, Trp136 and Asn137) has been discussed in several studies [6-8]. Nevertheless, the role of selenium is still under debate in the scientific community: the sulfur mutant of GPx has a much lower catalytic activity, while Te semi-natural enzymes, recently obtained by inserting a tellurocysteine (Tec) in subtilisin [9] and replacing

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Fig. (1). Enzymatic mechanism of GPx. The selenol E-Se-H reduces the hydroperoxide ROOH and is oxidized to selenenic acid (E-Se-OH) (oxidative step); E-Se-OH reacts with one equivalent of glutathione (GSH) to form a selenenylsulfide intermediate (E-Se-S-G, first reductive step). Finally, E-Se-S-G reacts with another equivalent of GSH to generate the initial E-Se-H and the oxidized glutathione (GSSG, second reductive step).
Ser9 in glutathione transferase from *Lucilia cuprina* to form telluro-LuGST-1 [10], display significant peroxidase activity, which, nevertheless, remains lower than that of GPx. In addition, while in GPx the presence of selenium results in an enhanced enzymatic activity, in other proteins no drop of activity is observed when replacing it with sulfur [11]. This points out that a general explanation of the biological role of selenium, which is present in 40 proteins in vertebrates, has not been found yet, two centuries after the discovery of this element. Different communities are involved in this challenging research field, among which chemists, biologists and pharmacologists.

Since few decades, much effort has been dedicated to the synthesis and reactivity properties of organochalcogenides as mimics of GPx for their potential anti-oxidant activity and possible use as drugs [12, 13]. Organoselenides are undoubtedly the most studied, although also organotellurides have shown promising results *in vitro* as well as *in vivo* [12, 14-16]. From a biochemical point of view, the most remarkable differences between sulfur and selenium are the larger polarizability of Se and the lower pKa of SeH [11]. In contrast, tellurium, which has no biological role, is nearly different being actually a metalloid. Some of us have recently assessed that indeed the presence of Tec in the GPx implies a different mechanism for the reduction of a hydroperoxide [17]. The study of small organochalcogenides is fundamental because they might have direct application in pharmacology. According to Mugesh and Bhattachar [18], these compounds can be conveniently grouped into three classes: selenenylamides, dichalcogenides and monochalcogenides. This review focuses on the dichalcogenides, because they appear to be particularly promising. In fact, (i) their synthesis is rather straightforward; (ii) their reactivity is higher than that of selenenylamides; (iii) they have two chalcogens, that is two catalytic centers acting separately after chalcogen bond cleavage, which occurs easily upon oxidation; (iv) substituents can be added to the rings to finely tune their reactivity.

Diorganoyldiselenides are largely employed in organic catalysis too, recently also in water [19-22]. In this case, the catalytic role of the selenide is to reduce the hydroperoxide, triggering an autocatalytic mechanism and generating *in situ* a powerful oxidant agent which is much more efficient than the initial hydroperoxide in oxidizing organic substrates [23].

In this review, we present an overview of the synthesis and the experimental testing of the catalytic activity of low molecular weight diselenides with direct Se-Se bond, integrated with information collected from selected computational works which provide a rationale for their reactivity. The text is organized as follows: at the beginning, a brief excursion of the experimental protocols used for the preparation of these compounds is presented, together with some details concerning the setup of GPx activity tests; then, the experimentally observed effects on the catalytic performance are discussed in terms of steric hindrance, aromatic and aliphatic substituents and nature and position of heteroatoms introduced in the scaffold of aliphatic and aromatic diselenides; finally, those molecular features, which have been identified in literature as general elements for a rational design of these catalysts, are reported, based on data produced *in silico* for the oxidation of diselenides by H2O2 and for the reaction of diselenides with a thiol/thiolate.

### 2. DIPHENYL DISELENIIDE

The preparation of diphenyl diselenide 1 is historically dated to the end of XIX century. As reported by Bradt and Green [24], in 1888 Chabrie described the products of the reaction between SeCl4 and benzene in the presence of anhydrous AlCl3, i.e. chlorobenzene, diphenyl selenide (C6H5)2Se, a third compound with the formula (C6H5)2(C6H4Cl)Se2 and phenyl selenol C6H5SeH. In 1894, Kraft and Lyons, while repeating the reactions of Chabrie and carrying out some precipitation assays for comparison using phenyl selenol, postulated that Chabrie’s phenyl selenol was actually diphenyl diselenide [24].

Diphenyl diselenide is a stable compound, currently employed in organic synthesis as catalyst. In combined experimental and theoretical studies, Back and Wirth have extensively explored its catalytic properties and those of its derivatives in numerous reactions [20, 25-34], while Santi and co-workers have mainly focused on the eco-friendly aspects of the use of diphenyl diselenide in green organic and medicinal chemistry [19-22, 34-50].

The phenyl groups of diphenyl diselenide are arranged at approximately 90° with respect to the chalcogen-chalcogen bond, and may be found in two different conformations corresponding to different values of the C-C-Se-Se dihedral (Φ), i.e. close to 0° and close to 90°, respectively (Scheme 1). These structures easily interconvert in solution [51], being the rotation of the phenyl rings a process with a very small energy barrier [52].

![Scheme 1](image1)

**Scheme 1.** Conformations of diphenyl diselenide.

Diphenyl diselenide 1 is oxidized by H2O2 to selenoxide 2. This implies a nucleophilic attack of one peroxide oxygen to one Se nucleus with concomitant transfer of the proton to the second peroxide oxygen and O-O bond breaking (Scheme 2) [23].

![Scheme 2](image2)

**Scheme 2.** Oxidation mechanism of diphenyl diselenide by H2O2.

This oxidation has an activation energy of 19.1 kcal mol⁻¹ *in vacuo* (level of theory: ZORA-OLYP/TZ2P-sc). *In silico*, it is possible to perform a second oxidation with analogous mechanism leading to the diselenoxide 3 with an energy barrier of 18.9 kcal mol⁻¹ (Scheme 2). In presence of excess H2O2, the experimentally detectable product is the seleninic acid 4. To explain this evidence,
one may postulate that the Se-Se bond of 3 is easily broken via hydrolysis (the reaction energy is 1.2 kcal mol\(^{-1}\); Scheme 3) [23].

Alternatively, the mono-oxidized 2 can react with one equivalent of H\(_2\)O\(_2\) to form the hydroxy perhydroxy species 6 with an activation energy of 14.7 kcal mol\(^{-1}\) (Scheme 4) [23]. This last intermediate has never been observed.

Notably, NMR kinetics measurements reveal that the mechanism is autocatalytic, but the possibility that the hydroxy perhydroxy species 6 oxidizes the mono-oxidized derivative 2 (Scheme 5) is energetically less favored than the oxidation performed by the benzeneperoxyseleninic acid 7 obtained via oxidation of the seleninic product by H\(_2\)O\(_2\) (activation energy of 11.8 kcal mol\(^{-1}\), Scheme 6) [23]. Indeed, benzeneperoxyseleninic acid 7 is the real oxidizing agent when organic compounds are treated with H\(_2\)O\(_2\) in presence of a catalytic amount of diphenyl diselenide.

Scheme 3. Hydrolysis of diphenyl diselenoxide.

Scheme 4. Oxidation of diphenyl selenoxide to the corresponding hydroxy perhydroxy species.

Scheme 5. Oxidation of diphenyl selenoxide by a hydroxy perhydroxy species.

Scheme 6. Autocatalytic mechanism of oxidation of diphenyl diselenide by H\(_2\)O\(_2\).

Scheme 7. Ebselen.

diphenyl diselenide 1 is also widely tested as anti-oxidant drug, because it mimics the catalytic activity of GPx [53, 54]. For example, this compound has been shown to prevent oxidative stress at the cardiovascular level in animal models [55, 56]. Diphenyl diselenide 1 has higher thiol peroxidase activity than ebselen (2-phenyl-1,2-benziselenazol-3(2H)-one) 8 [57-59], which is the most popular GPx mimic (Scheme 7). On the other hand, it has been demonstrated that diphenyl diselenide 1 may interfere with the synthesis of heme-containing proteins via ALA-D inhibition [60]. Braga, Rocha, Nogueira and co-workers have been studying exten-
sively the applications of diphenyl diselenide and its derivatives in biochemistry, pharmacology and toxicology, with a particular focus on the synthetic aspects [12, 15, 35, 37, 38, 50, 53-55, 60-142]. In presence of hydroperoxides and thiols, nucleophilic attack of sulfur at a selenium center occurs first, promoting Se-Se bond cleavage [143]. A second nucleophilic attack of thiol occurs at sulfur of the selenenylsulfide intermediate 9 leading to the formation of the sele

do species 10 which can react with the hydroperoxide. These mechanistic considerations (Scheme 8) are derived from the scheme proposed by Iwaoka and Tomoda (see next paragraphs), but so far, to the best of our knowledge, the reactions of diphenyl diselenide 1 with thiols have not been thoroughly investigated in silico. By comparing the energy barrier of a thiolate attack to a sulfide attack, the presence of a Se-Se bond in the diphenyl diselenide substrate (0.8 kcal mol$^{-1}$ in vacuo, level of theory: ZORA-OLYP TZ2P-sc) with the energy barrier of the oxidation of diphenyl diselenide 1 by H$_2$O$_2$ (19.1 kcal mol$^{-1}$), we can assert that the mechanism by Iwaoka is still valid [144].

**3. LOW MOLECULAR WEIGHT GPX MIMICS: EXPERIMENTAL MODELS**

Several human diseases are connected to corrupted radical scavenging activities of the organism: cancer, cardiovascular diseases, neuroinflammation, immune disorders and neurodegeneration represent only some of the most popular examples [145, 146]. In fact, an impaired ability in inactivating ROS has been recognized as one of the main causes of such pathologies. Consequently, the medicinal chemists are more and more attracted by the opportunity of designing small molecules capable of counteracting an important cause of the onset of these disorders. In the last three decades, organic chemists have prepared many Se-based low molecular weight compounds showing GPX-like activity, have investigated their performance as catalysts and have provided details of the molecular mechanism. Ebselen and selenenylamides, selenides, selenolates, diselenides, selenoxides and more complex artificial Se-based artificial enzymes represent the GPx mimics investigated so far [18]. A limited number of tellurium analogs have also been synthesized and studied [12]. But while the literature on selenies and diselenides is very prolific, ditellurides have not received the same attention so far. This is likely due to the less accessible synthetic procedures required to prepare and isolate Te organic compounds, which often undergo Te-Te and Te-C bond cleavage under normal reaction conditions. After few pioneering studies [147, 148], some examples of ditellurides behaving like GPX-mimics have been recently reported by Rocha and coworkers [15, 16, 70, 71, 106, 149]. Lin and Liu studied the protective effect of 2-telluriur-bridged $\beta$-cyclodextrin (2-TeCD) against oxidative stress, and highlighted that this macromolecular ditelluride was more efficient in catalyzing the reduction of H$_2$O$_2$ by GSH than the corresponding diselenide analog and ebselen [150]. More recently, Vernekar and Mugesh studied the GPX-like mechanism of an ortho-alkylaminostibuted diphenyl ditelluride [151]. High GPX-like activity has also been reported for a cyclodextrin-derived tellurium compound [152].

Focusing on Se-based low molecular weight molecules, in 2013 Santi et al. reviewed the GPx activity of several classes of Se derivatives with the aim of assessing their suitability as drugs [39].

More recently, the goal of the synthetic approaches has pointed towards the efficient and stereoselective preparation of Se-based peptide-like derivatives [96]. In this review, we have collected the relevant and latest reports about diselenides as GPx molecular mimics. Diphenyl diselenide 1, the “traditional” lead and reference compound, was found by Wilson et al. to be two times more active
The efficiency of the catalysts designed to mimic the essential structural elements found in the catalytic pocket of GPx. The quantification of the GPx catalytic cycle, selenium is first oxidized and then compared to ebselen 8 in GPx-like catalytic cycles [153]. After all, a diselenide is a key intermediate in the catalytic cycle proposed for ebselen [18, 154, 155]. Throughout the years, diselenides, and in particular diphenyl diselenide 1, were synthetically decorated to optimize their catalytic performance. In part, these molecules were designed to mimic the essential structural elements found in the catalytic pocket of GPx.

In the GPx catalytic cycle, selenium is first oxidized and then reduced as shown in Fig. (1). In these catalytic steps the selenium nucleus, in its different oxidation states, is either the nucleophile, the central atom or the leaving group [156]. When compared to sulfur, selenium has rate-accelerating effect in the catalysis. To be more specific, it is generally accepted that selenol/diselenide exchange is faster than the analogous thiol/disulfide reaction because of the higher polarizability and weaker Se-Se bond strength, which makes selenium a better nucleophile as well as a better leaving group [156-159]. Because of these attractive features and the biological relevance of the biochemical antioxidant activity, organic and medicinal chemists aim at further enhancing this natural feature of selenium, in order to boost its catalytic performance. Diselenides have been modified and substituted with amino and hydroxyl group coordinating the selenium atom to tune the molecular antioxidant properties. Electron donor or withdrawing groups have been also added to the diselenide scaffold and their effects evaluated [160].

Hodage et al. have clearly summarized the strategies for the design of efficient Se-based catalysts: i) organoselenium compounds containing -OH, -NH2 and -COOH groups are active; ii) diselenides are more active than the corresponding monoselenides; iii) diselenides showing weak secondary intramolecular interactions (Se•••X with X=O or N) show good activity; iv) diselenides containing heterocyclic rings are better catalysts than compounds bearing simple aryl groups [161].

4. TESTING THE GPX-LIKE ACTIVITY OF DISELENIDES: EXPERIMENTAL MODELS

The quantification of the GPx-like activity is carried out, as described in literature, following a small set of experimental procedures based on routine analytical techniques. Despite some differences in the experimental protocol, these methods, generally, share the same principle: the catalytic performance of the GPx mimics are measured in terms of the time required by a molecule (in this case, the Se-based catalyst) to catalyze the oxidation of a substrate by a peroxide. The efficiency of the catalysts is usually expressed and compared in terms of half-life (t_{1/2}: the time required for the 50% conversion of the thiolate to disulfide). This value is obtained by measuring the concentration of the species in solution during the reaction by using analytical UV- or NMR-based techniques. Wilson et al. reported a spectrophotometrical method based on the quantification of NADPH, used as a cofactor by glutathione reductase to transform GSSG to GSH [154]. Another widely used method involves thiobenzene (PhSH) as substrate and H2O2 as the oxidant [145, 161]. The reaction between the thiol and H2O2 is easily followed using UV, HPLC and/or NMR, and several modifications to the experimental protocol, e.g. the kind of employed thiol and peroxide or the catalyst concentration, have been later introduced [148, 162]. The use of tert-butyl hydroperoxide (TBHP) as oxidant and benzylthiol as substrate (BnSH) turned out to be particularly practical and thus became a widely adopted experimental setup (Scheme 9) [163].

Clearly, the evidence obtained from these experimental procedures not provide detailed mechanistic insights and information about the intermediates involved in the catalysis, but just represents a tool for assaying the suitability of diselenides, or chalcogen-containing catalysts. This is the most widely adopted approach, in some works the single reaction steps can be found. For example, one of the pursued strategies is to separately treat the organoselenium with the peroxide or the thiol, using different concentrations of these reactants. These studies will be discussed in the next paragraphs.

5. STRUCTURE-ACTIVITY RELATION IN GPX-LIKE CATALYSIS: CLASSES OF SUBSTITUTED DISELENIDES

5.1. Amino-substituted Diselenides: Three Roles for the Price of One

The class of amino nitrogen containing diselenides represents the most traditional and, at least until recently, by far the most populated class of derivatives of diaryl and dialkyl diselenides. These compounds have a structural connection with the GPx enzyme, which they should mimic, and which inspired the insertion of amino groups in the scaffold of model diselenides. To be more specific, it has been observed that in the protein structure, characterized by X-ray diffraction, two amino groups of Glu and Trp, which are conserved residues, are located at 3.3 and 3.4 Å from the selenium atom of selenocysteine, respectively, [164] suggesting the possible involvement of these basic groups in close proximity of the catalytic site. Iwaoka and Tomoda investigated the roles of the non-bonded interaction between selenium and amino nitrogens by synthesizing ortho-alkylamino substituted diphenyl diselenides 11 [144]. The compounds were prepared, following a canonical procedure, by reaction of 2,2′-diselenobis( benzyl chloride) with the corresponding secondary amine. The GPx-like activity was assayed using the reaction between PhSH and H2O2 and monitoring its evolution by 77Se NMR.

Iwaoka and Tomoda let the selenide react separately with the peroxide and the thiol to shed some light on the catalytic mechanism (Scheme 10). The authors observed a very slow reaction between the diselenide and H2O2 in absence of thiol, while the diselenide reacted promptly with the thiol in absence of H2O2, suggesting that the formation of the selenenylsulfide 12 and selenolate may represent the first step of the catalytic cycle. Diphenyl diselenides

1 It has to be clarified that experimental works generally refer to the thiol as the ‘substrate’ of the oxidation, while in biology this term is usually attributed to the hydroperoxide which is inactivated by the enzyme. In addition, in this context the general term ‘peroxide’ will be used for simplicity to denote both hydroperoxides (R-O-O-H) and peroxides (R-O-O-R) to comprehensively include all the oxidant species which may find application in the functional tests here described, while GPx is able to reduce only hydroperoxides.
modified with tertiary amines were found to be 50 times more efficient (in terms of initial reduction rate) in catalyzing the reaction when compared to diphenyl diselenide 1. On the basis of these results, the authors highlighted in the same work the three potential roles of amino groups in improving the catalytic activity: (i) the amino nitrogen, acting as a base, can activate the selenol intermediate into the kinetically more reactive selenolate ion; (ii) the direct interaction of the amino nitrogen with selenium in the selenenic acid intermediate may prevent its overoxidation; (iii) the Se-N interaction in the selenenyl sulfide intermediate prevents the nucleophilic attack of another thiol to selenium and directs the reactivity towards sulfur due to electronic effects [144].

Similar compounds had been investigated, some years before, by Wilson et al. [153]. In this case, the synthetic procedure was carried out through ortho-lithiation of N,N-dimethylbenzylamine followed by reaction with metallic selenium and then by oxidation. In the same paper, other compounds were prepared following a similar procedure, i.e. starting from 2-bromobenzyl bromide and performing a substitution with cyclic amines such as pyrrolidine. The products were assayed through a test which evaluated NADPH consumption. Diphenyl diselenide derivatives substituted with tertiary amines were found to be 5 times and 2 orders of magnitude more efficient than diphenyl diselenide 1 and ebselen 8, respectively.

A more advanced study was undertaken in recent years by Mugesh et al. [165], providing better insight on the role of the strength of this amply discussed Se–N interaction. Depending on the strength of this non-covalent bond, the authors distinguish between diselenides with strong and weak Se–N interactions. Both classes of compounds, which were prepared through the classical ortho-lithiation method, were anyway found to be more efficient than the parent diphenyl diselenide 1. The mechanism of the catalytic activity, which was measured by NMR using the reaction of PhSH with H₂O₂, was also studied in detail by making the compounds react separately with the thiol and the peroxide. According to the experimental observations, two different mechanisms can be postulated. Firstly, diselenides having strong Se–N interactions (in the reported study they are represented by oxazoline-based diphenyl diselenide 15) were found to be less active in terms of GPx-like catalytic performance than N,N-dimethylaminoethyl-ferrrocene derivatives characterized by weak Se–N bonding. To be more specific, the thiol peroxidase activity seems to depend on the strength of the bond in the intermediates produced in the catalytic process; the amino nitrogen “stabilizes” the selenol and the selenenic acid in more active compounds (weak bonding), while it is involved in interactions with all three intermediates, namely the selenol, the selenenic acid and the selenenylsulfide, in less active compounds (strong bonding). In the case of more efficient catalysts (weak bonding), the diselenide reacts with PhSH producing selenol and selenenylsulfide. Selenol is then oxidized by H₂O₂ to selenenic acid, which, due to the Se–N interaction, reacts with PhSH to produce the selenenylsulfide. At this stage, the Se–N bonding is weaker and further reaction with PhSH leads to the regeneration of the selenol. This catalytic cycle is analogous to the mechanism proposed by Iwaoaka and Tomoda (Scheme 10). In the case of less active compounds, the diselenide 15 reacts with PhSH and selenol 17 and selenenylsulfide 19 form. The selenol 17 is then oxidized by H₂O₂ to the selenenic acid 18 and the subsequent reaction with PhSH leads to the selenenylsulfide 19. Due to the strong Se–N interaction, this intermediate is promptly oxidized by H₂O₂ and the selenenic acid 18 is regenerated (Scheme 11) [165].

Mugesh and Bhabak provided further insight on the role of basic nitrogens in enhancing the activity of GPx mimics with a diselenide scaffold [166]. Diselenides were synthesized by ortho-lithiation from N,N-dialkylbenzylamine derivatives (N-methyl, -ethyl and -propyl). The authors confirmed that the presence of strong Se–N bonding and subsequent stabilization of Se-S bond in the selenenylsulfide allow a longer half-life of this intermediate in solution. Consequently, H₂O₂ cleaves the Se-S bond producing selenenic acid, which can be further oxidized and converted to seleninic acid which leaves the catalytic cycle. On the basis of this evidence, the authors reformulated the three key roles played by basic nitrogen in improving GPx-like activity: the tertiary amino substituents i) should not be involved in any Se–N interactions in

Scheme 10. Catalytic mechanism proposed by Iwaoaka and Tomoda for H₂O₂ reduction catalyzed by ortho-alkylamino-substituted diselenides.

PhSH

PhSSPh

13

H₂O₂

H₂O

PhSH

14

11

12

10

11

12

13

14
the selenols, but should be sufficiently basic to deprotonate the selenols to produce more reactive selenolates; ii) should not participate in strong interactions with selenium in the selenenyl sulfide intermediates; iii) should exhibit some non-covalent interactions with selenium in the selenenic acid intermediates to increase the electrophilic reactivity of selenium [166]. In the same study, the authors discussed also the effects of the 6-OCH$_3$ group as substituent on the diselenide scaffold. Further details on the relevance of Se•••O interactions will be given in the dedicated section of the review.

More recently, Bhowmick and Mugesh prepared and studied a set of more complex ortho-substituted, amino-based diphenyl diselenide derivatives (20-24) [167], because the introduction of an additional secondary or tertiary amino moiety or an oxygen atom on
models to screen the catalytic performance and diselenobisnenylnenylamides prepared in the position of the heteroatoms (O and N) in the catalytic site of the enzyme. In particular, the ortho-CH$_2$-substituted sec-amino-diselenide 22 exhibits the highest activity in catalyzing the oxidation of aromatic thiols. Regarding the mechanism of the catalytic cycle, the stability or reactivity of the selenenylsulfide and selenenic acid intermediates is not affected much by the additional amino group, while the selenol intermediate further interacts with the protonated additional amino moiety through hydrogen bonding. Moreover, sec-amino-based diselenides showed higher catalytic activity than tert-amino-based diselenides; in contrast, a lower catalytic activity was observed when the amino moiety was substituted by an alcohol, further confirming the important role of the nitrogen in that position [167].

5.2. Amide-based Mimics: Getting Closer and Closer to the Structure of the Catalytic Site

In the 2000s, amide-based ortho-substituted diphenyl diselenide derivatives were synthesized as second-generation GPx mimics. These compounds were designed to resemble even more closely the position of the heteroatoms (O and N) in the catalytic site of the enzyme.

Bhabak and Mugesh prepared a set of ortho sec- and tert-amide diphenyl diselenides (26a and 26b, Schemes 14, 15) [168]. The sec-amide-based diselenides were synthesized by treating cyclic selenenylamides with triphenyl phosphine, while tert-amides were prepared from the appropriate secondary amine and 2,2'-diselenobis(benzoyl chloride). The reactions between PhSH and H$_2$O$_2$, tBuOOH or Cum-OOH, followed by HPLC, were used as models to screen the catalytic performance and tert-amide-based compounds were found to be almost 20 times more efficient than analogous sec-amide derivatives. Curiously, their catalytic activity was found to be dependent on the nature of the peroxide; in contrast sec-amide-based catalysts exhibit almost identical performance when changing the peroxide. On the basis of the experimental evidence, the authors have drawn some interesting mechanistic conclusions: (i) Differently from the tertiary amine-based diselenides discussed above, the reactivity of tert-amides towards thiols was found to be poor and this indicates an increased relevance of the chemical nature of the oxidant which is involved in the reaction; (ii) The tert-amide moiety prevents, or anyway inhibits, the thiol exchange reaction that occurs at the selenium of the selenenylsulfide intermediate 33 by reducing the strength of Se•••O non-bonding interactions. In conclusion, the catalytic cycles described by the authors were found to be similar for sec- and tert-amide-based diphenyl diselenides (26a and 26b), but selenenic acids with sec-amide substituents 28 can undergo a cyclization reaction producing selenenylsulfide 29 [168]. As pointed out by Santi et al., the GPx-like activity of amide-based selenides should be ascribed to their direct reaction with the peroxide [39].

In 2011, Selvakumar et al. reported the synthesis of some diaryl diselenides bearing amino acid functions, intramolecularly coordinating the selenium atom [169]. These compounds were prepared by reacting 2,2'-diselenediyldibenzoic acid with C-protected amino acids using DCC coupling procedures. The best compounds of this group were identified among those bearing an alanine residue, in terms of both coupled reductase and thiophenol assay method. Interestingly, the results for L- and D- derivatives were very similar, suggesting that, at least in this kind of experimental model, the chirality does not play a relevant role on catalysis. The authors also described the preparation of more hindered diselenides which anyway had low solubility issues and were thus discarded [169].

Scheme 14. Mechanism of H$_2$O$_2$ reduction catalyzed by ortho-sec-amide based diselenides proposed by Bhabak et al.
Nascimento et al. carried out a more comprehensive study on amide-based aromatic and aliphatic diselenides, attempting to build a preliminary Structure-Activity Relationship (SAR) [170]. They prepared a set of diphenyl diselenide derivatives starting from carboxylic acid diselenide, which was obtained by diazotization from anthranilic acid. Aliphatic diselenides, bearing spacers with different length, were synthesized by reaction of bromo-carboxylic acids with Na₂Se₂. Differently from the procedure reported by Selvakumar et al. [169], EDC-mediated peptidic coupling was preferred. GPx-like activity was measured using the PhSH/H₂O₂ reaction followed with UV spectroscopy. Through their more pharmaceutical-oriented approach, Nascimento et al. found that the compounds were active in inhibiting the production of Thiobarbituric Acid Reactive Substances (TBARS) in the brain, which means that the compounds are active against lipid peroxidation, probably reacting as selenols towards thiol groups in brain homogenates [170]. In general, the reported compounds showed higher GPx-like activity than ebselen 8, and aromatic diselenides were found to be more active as GPx mimics. This result, according to the authors, can be ascribed to the presence of a non-covalent Se•••N interaction and, in particular, to the fact that “in the aromatic derivative the structural constraint forces such interactions to occur and enhances the GPx-like activity” [170].

A similar synthetic strategy was adopted by Rafique et al. to prepare a small set of aliphatic and aromatic diselenide derivatives of 2-picolyamide with spacers of different length between the Se atoms and the heteroatoms of the amides [171]. The aim of the authors was to optimize the spacer for an optimal reproduction of the distances measured in the catalytic site of GPx. The synthetic approach for the preparation of aliphatic diselenides consisted in the reaction of the bromocarboxylic acid with Na₂Se₂, followed by the coupling with 2-picolyamine using DCC/DMAP. The authors excluded other coupling agents such as DIC/HOBt and EDC or BOP to avoid the production of byproducts. Aromatic derivatives were prepared from carboxylic acid diselenide. In agreement with the results obtained by Nascimento et al. [170], the diphenyl diselenide derivatives were found to be more active in the thiol peroxidase and TBARS tests (PhSH/H₂O₂).

Very recently Pacula et al. reported the preparation of diphenyl diselenide derivatives as novel GPx mimics [172]. The preparation of these compounds was original and consisted in the synthesis of opportune N-substituted benzisoselenazolones 35a-d as intermediates and their subsequent conversion into the corresponding diselenides 36a-d after reaction with NaBH₄ (Scheme 16). These N-substituted benzisoselenazolones were prepared from an orthiodobenzamide and lithium diselenide or from anthranilic acid, Na₂Se₂ and SOCl₂. The reaction between dithiothreitol (DTT) and H₂O₂ was used as a model, in the presence of 10% Se-based catalyst; N-allyl diselenide was found to be the most active catalyst. In general, all the prepared N-alkyl diselenides were found to be better catalysts than ebselen 8. Moreover, the compounds from this study were also tested for their antiproliferative activity on breast carcinoma MCF-7 cell line and non-cancerous epithelial PNT1A cell line. Interestingly, the most promising compounds, which induced cell death in cancerous cells with a certain degree of selectivity, were those with low antioxidant activity [172].

### 5.3. Oxygen-containing Diselenides

Pursuing the strategy of more and more closely mimicking the GPx enzymatic core, the role of other heteroatoms, in particular of oxygen, in giving non-covalent interactions with selenium, was investigated.

Wirth studied the GPx-like activity of some oxygen-containing derivatives of a series of diphenyl diselenides (Scheme 17) [160]. The most active compounds according to the NADPH model reac-

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**Scheme 15.** Mechanism of H₂O₂ reduction catalyzed by ortho tert-amide-based diselenides proposed by Bhabak et al.
tion, were found to be those bearing two benzyl alcohol moieties 37a-d, with the selenium atom in the *ortho* position. All bis-*ortho* substituted derivatives showed lower activity due to steric hindrance. As above discussed, the importance of the distance between selenium and the heteroatom was confirmed by the observation that in those compounds in which the oxygen is located further from the chalcogen, the catalytic performance decreases. Moreover, *para*-OCH₃ diphenyl diselenide 41 was found to be very active, likely due to the electron donor effect of the substituent. On the other hand, an electron withdrawing group (−CF₃) 37c caused a drop of the catalytic performance [160].

Bhabak and Mugesh studied *ortho*-OCH₃ diphenyl diselenides 43b,e,f as GPx mimics, to assess the influence of the methoxy substituent on the catalytic activity and mechanism [166]. The authors highlighted that the *ortho*-OCH₃ group is efficient in inhibiting the thiol exchange on the selenenylsulfide stage of the catalytic cycle due to the onset of Se•••O non-bonding interaction: as a result, the GPx-like activity is enhanced. In general, diphenyl diselenides containing methoxy or carbonyl substituents in *ortho* position show higher activity than analogous derivatives without oxygen atoms close to chalcogen nucleus [166, 169]. In greater detail, Bhabak and Mugesh demonstrated that the catalytic activity of these derivatives is almost one order of magnitude higher than that of the corresponding compound not bearing the methoxy group in the PhSH/peroxide test (Scheme 15) [166]. In this case, as in the above section dedicated to compounds bearing Se•••N interactions, also in this case, the strength of the Se•••O non-covalent bonding seems to have a role. In particular, strong Se•••O interactions were observed to reduce the catalytic activity in the selenenylsulfide intermediate [169]. In fact, a conformationally restricted peridiselenide bearing two OCH₃ substituents in *ortho* positions showed good catalytic performances, like the Te-based analog. These results were reported by Press and Back [27].

Scheme 16. Preparation of the substituted diphenyl diselenides studied by Pacula et al.

Scheme 17. Oxygen-containing diphenyl diselenides studied by Wirth et al and by Bhabak et al.
5.4. Other Classes of Diselenides: Towards Macromolecular GPx Mimics

Back and Moussa synthesized and studied some alkyl diselenides with a peptidomimic structure [26]. The GPx-like activity was tested by reacting TBHP with BnSH. The authors explain that they have studied alkyl selenides because they undergo facile oxidation, “providing rapid entry in the catalytic manifold”. Moreover, another advantage of alkyl selenides is the possibility of accommodating substituent on the alkyl chain to tune their activity. The diselenides, obtained by the treatment of the corresponding halides with Na2Se2, were found to be very modest catalysts.

Kawasoko et al. reported the synthesis of non-natural L-chalcogen and L-diselenide amino acids from L-glutamic acid with stereochemistry retention [96].

Moreover, Iwasaka et al. recently described the stereoselective preparation of selenocystine derivatives using Woolins’ reagent [173]. These compounds are particularly interesting for application in organic catalysis: Santi et al. reported the asymmetric dihydroxylation and hydroxymethylation of alkenes catalyzed by L-selenocystine [174]. The reactivity of selenocystine and its role in the BnSH/peroxide catalysis mechanism had also been previously studied by Back and Moussa [26].

In the field of macromolecular GPx mimics, the recent trend is represented by the development of artificial enzyme macrostructures to overcome the endow enzyme instability, the antigenicity and the poor availability of the natural protein.

Li-Wu et al. recently studied the use of Cyclodextrins (CDs) as host systems for the artificial catalytic core [152]. Cyclodextrins are cyclic oligosaccharides of at least six α-1,4-linked α-D-glucopyranoside units, with a peculiar internal toroidal shape with a cavity able to recognize and host several molecules. In the past, CDs had already been used to build enzyme models [175], and Li-Wu et al. used this strategy to study Se- and Te-based artificial enzymes, highlighting a higher efficiency of Te-based CDs in catalyzing the model reaction (NADPH), especially when the chalcogens are inserted in the 2-position rather than in the 6-position. Moreover, the activity of dual-bridged enzymatic mimics was found to be higher than that of single-bridged ones. The relevance of these results goes beyond this isolated study: building a macromolecular structure around the artificial catalytic core paves the route to the development of mid- or high-molecular weight enzyme mimics which can selectively recognize substrates according to molecular shape, size, and non-covalent interactions within the cavity [152].

6. COMPUTATIONAL MECHANISTIC STUDIES OF DI-CHALCOGENIDES

The computational mechanistic investigation on chalcogenides and dichalcogenides chemistry began thirty years ago with the pioneering works by Pappas [176] and by Aida and Nagata [177]. Theoretical and experimental studies are significantly different: in theory, multi-step mechanisms and enzyme-like activities are necessarily split and catalysts and substrate are often simplified molecules. In addition, reactions are studied in the gas phase or solvent is taken into account as a dielectric continuum, or, in more sophisticated models, few water molecules are considered and involved in the mechanism. Nevertheless, this is not to be considered a limitation: the use of simplified models allows to unravel intimate features of the reactions and discern specific contributions to the experimental observable, such as the role of the chalcogen, the importance of steric hindrance, the effect of the substituents and so on.

One of the first pioneering benchmark to test the levels of theories suitable to describe organoselenium compounds, partially extended to homo and hetero-dichalcogenides, was presented in 2010 by Heverly-Coulson and Boyd [178]. They demonstrated the efficiency of B3PW91 exchange-correlation (XC) functional combined with Pople double/triple ζ Gaussian-Type Orbital (GTO) basis set, like 6-31G(d,p) or 6-311G(2df,p). A more recent DFT benchmark, dedicated exclusively to diaryldichalcogenides, was published in 2016 [52]. In this study, twenty-three density functionals have been tested in combination with Slater-Type Orbital (STO) basis sets of increasing size (TZP, TZ2P, QZ4P). Both frozen core and all-electron descriptions have been considered and ZORA scalar approximation has been used to account for relativistic effects. Molecular geometry parameters of several differently substituted diphenyl dichalcogenides have been compared to the crystallographic data of structures extracted from CSD [179] and six XC functionals showed good performances. For the geometry optimizations, the authors recommend plain GGA like OPBE or dispersion corrected functionals such as BP86-D3(BJ) and BLYP-D3(BJ), in combination with TZ2P-se (small frozen core). In contrast, ZORA-OLYP/TZ2P-se gave optimal predictions for energetics, reproducing the correct decreasing bond strength from Se-Se to Te-Te. In this case, caloriometric and stopped-flow measurements were used to estimate bond dissociation energies. In this study, it was confirmed that the barrier associated to the rotation of the phenyl rings is almost negligible (lower than 1 kcal mol-1 for both Ph2Se2 and Ph2Te2), implying free rotation in solution. Thus, in order to facilitate the calculation of observables like 77Se NMR chemical shift of 1, which is widely employed in organic synthesis, the general AMBER force field (GAFF) for a series of differently substituted diphenyl diselenides and diphenyl ditellurides has been parameterized from scratch revisiting the computational protocol [180].

The computed values obtained averaging on numerous conformations derived from a MD simulation were found more accurate than the value computed for the single optimized geometry, i.e. 507 ppm versus 644 ppm and 462 ppm calculated for the two conformers shown in Scheme 1; the experimental value in chloroform is 463 ppm. Notably, these force fields are amenable to be employed in hybrid quantum mechanics/molecular mechanics (QM/MM) calculations, [181] which are the mandatory approach when testing these molecules as drugs in a biological environment.

The computational studies on diselenides can be conveniently grouped referring to the studied reaction, which is typically a step encountered in the GPx mechanism, i.e. oxidation by H2O2 (or hydroperoxide) and nucleophilic attack of a thiol/thiolate. In both cases, the reaction outcome is the Se-Se bond breaking. Thus, a complete mechanistic investigation of the GPx like activity of a diselenide includes necessarily reaction steps involving monoselenide fragments.

6.1. Oxidation of Organodiselenides by H2O2

Kice and Chiou first conceived a possible oxidation path of diselenides by hydrogen peroxide or hydroperoxides to selenoxides, producing water or the corresponding alcohol, respectively [182].

This initially implies a nucleophilic attack of one peroxide oxygen to one Se nucleus with concomitant transfer of the proton to the second peroxide oxygen and O-O bond breaking, as shown in Scheme 2 for 1. This oxidation weakens the Se-Se bond, which elongates (Scheme 18). The selenoxide 44a-c can isomerize to the
anhydride 45a-c, in which the two selenium nuclei are bridged by an oxygen atom (Scheme 16). The existence of this product was discussed for conformationally restricted naphthalene peri-diselenides 47 (Scheme 19) [27] and in silico, calculations on diphenyl diselenide 1 have shown that the activation energy associated to this conversion is rather high and the product destabilized [183].

\[ \text{H}_2\text{O}_2 \xrightarrow{\text{H}_2\text{O}} \frac{R}{R} \text{Se} \equiv \text{Se} \frac{R}{R} \]

Scheme 18. Formation of selenoxide by oxidation of a diselenide by H2O2 and isomerization to the corresponding anhydride.

The oxidation of a diselenide is also postulated in the catalytic cycle of ebselen 8 [18, 154, 155, 185]. In silico, the oxidation of ebselen diselenide, modeled as dimethyl-diselenide 44b, has been investigated using DFT approach, i.e. B3PW91/6-311G(2df, p) for geometry optimization and B3PW91/6-311++(3df, 3pd) for energy calculation, in the gas phase as well as in solvent (CPCM) [186]. An activation energy of 35.3 kcal mol\(^{-1}\) was computed, which lowered to 29.6 kcal mol\(^{-1}\) in condensed phase (water). The isomerization of the selenoxide 45b to anhydride 46b was excluded because the energy of the former is 12 kcal mol\(^{-1}\) lower than the energy of the latter and so, if it occurred, the process could not be fast.

Few years later, Anthony and Bayse accurately investigated the GPx-like mechanism of ebselen 8 [187]. The authors made the hypothesis that the homodiselenide 51 can form by reaction of the selenol species 49 with one equivalent of selenenylsulfide 48 or by reaction of ebselen 8 in the selenenylamide cyclic form with one equivalent of selenenylsulfide 48, or by slow disproportion of two equivalents of selenenylsulfide 48 (Scheme 20). Bayse excluded that this homodiselenide forms in vivo, due to (i) the high concentration of nucleophiles, (ii) the slow rate of disproportion of the selenenylsulfide, which can be isolated, (iii) the fact that an intermediate formed in a bimolecular reaction of two equivalents of catalyst has a rate of formation much slower than steps that are first-order in [Se].
In 2010, Heverly-Coulson and Boyd computed the barriers of a single step oxidation of the N,N-dimethylbenzylamine diselenide (DMBS 53, Scheme 21), its selenol analog and the charged analogs of the two (level of theory: B3LYP/6-311++G(3df,3pd)/CPCM//B3LYP/6-31G(d,p)) [188].

Scheme 21. DMBS.

If the reaction occurs via a one-step mechanism, the selenium-oxygen bond forms and simultaneously a proton is transferred to the second oxygen of the peroxide. Upon oxidation, the Se-Se bond elongates from 2.387 Å (in the reactant complex) to 2.510 Å (in the product complex). In this case, the barrier is higher than the Gibbs free activation energy computed by Pearson and Boyd for the oxidation of ebselen diselenide, i.e. 41.5 kcal mol\(^{-1}\) versus 38.4 kcal mol\(^{-1}\) [189]. Since the reaction takes place in a neutral or slightly basic environment, the amine groups near the active region may be protonated. When only one of the amines is protonated, the Se-Se bond breaks. When both amines are protonated, the Gibbs free activation energy drops by 16.7 kcal mol\(^{-1}\), but the Se-Se bridge elongates (2.447 Å in the reactant complex) and the accessible reaction paths are only those in which the amine acts as a proton shuttle. Following the idea of the mechanism proposed by Iwaoka and Tomoda (Scheme 10) [144], Pearson and Boyd considered that the active species might be in a zwitterion form and found that the activation energy lowers to 19.8 kcal mol\(^{-1}\).

6.2. Nucleophilic Attack of a Thiol/thiolate Anion at Diselenides

Both the second and the third steps in GPx mechanism, which constitute the reductive phase of the enzyme, are nucleophilic attacks of a thiol (glutathione) to a dichalcogenide substrate (Fig. 1). In particular, in the former one, the selenenic intermediate E-Se-OH is converted into the selenenylsulfide E-Se-SG form with elimination of a water molecule. An extensive computational work has been done to establish the nature of the entering nucleophile, i.e. thiol or a deprotonated thiolate, in the selenenic derivative of N,N-dimethylbenzylamine-2-selenol (DMBS 53), in different solvents [190]. The applied level of theory was chosen referring to the previous benchmark by the same authors, [178] that is B3PW91/6-31+G(d,p)/CPCM-B3PW91/6-311+G(2df,p). Intuitively, the most favorable path is expected using the thiolate for its higher nucleophilicity. However, this implies that the leaving group is the hydroxyl anion. Conversely, the authors demonstrated that the most likely is the thiol that attacks the substrate and its proton is transferred to the –OH groups of the selenenic acid so that the leaving group is water. Explicit solvent molecules were used as proton shuttles: the activation energy calculated in water with thiolate as nucleophile is around 42 kcal mol\(^{-1}\) and decreases to around 10 kcal mol\(^{-1}\) in presence of two water molecules.

The second reductive step of GPx is the attack of a thiol/thiolate to a selenenylsulfide (disulfide if the sulfur mutant is considered) [191]. The first mechanistic hypothesis is the S\(_{2}\)2 synchronous process characterized by the nucleophile and the leaving group acting at the same time in the transition state [192]. Alternatively, one can postulate an addition-elimination mechanism, in which a three-center intermediate is identified with both entering and leaving group bonded to the central chalcogen [193, 194] (Scheme 22).

Scheme 22. Mechanisms of the nucleophilic attack of a chalcogenolate to a dichalcogenide: S\(_{2}\)2 and addition-elimination (A-E).

In the potential energy landscape, these mechanisms correspond to a double well and a triple well profile, respectively (Fig. 2).

Fig. (2). Energy profiles for a symmetric S\(_{2}\)2 (top) and an addition-elimination (bottom) reaction in gas-phase.

In both cases, in the gas phase, first a stabilized reactant complex \(I_{in}\) forms from the initial reactants (Fig. 2) [195]. In analogous manner, an energetically stabilized product complex \(I_{out}\) precedes the formation of the free products. An archetypal and widely studied process is the thiolate-disulfide reaction, which mimics the second reductive step of the Cys-GPx. In the past, it has been classified several times as S\(_{2}\)2: one of the pioneering computational works was done in 1984 by Aida and Nagata, who employed HF/6-31G level of theory [178]. Notably, in his old study Pappas [177] reported the formation of a three-center intermediate when using F as nucleophile, and this supported the idea that a similar complex might also form with thiolate, by analogy with plausible formation of G\(_{2}\)S\(_{2}\) in the mechanism of the oxidative phosphorylation in which GSH is oxidized by cytochrome c in the presence of GSSG [196]. Pappas used HF approximation and prompted for further studies. In 1996, Bachrach et al. [197] described a two-step addition-elimination mechanism found only when using an accurate level of theory (combination of HF/6-31+G* for structure guess, MP2/6-31+G* for final geometries and MP4SDTQ/6-31+G* and CCSD(T)(fc)/6-31+G* for energy evaluation). In fact, the potential energy surface (PES) of this reaction is very sensitive to electron correlation and the bimolecular nucleophilic substitution reaction proceeds via S\(_{2}\)2 when plain HF is used. However, when explicitly correlated methods are employed, the energy profile changes and the reaction proceeds along an addition-elimination pathway. An analogous work at MP2(full)/6-31+G* and MP4SDTQ(fcc)/6-311+G*/MP2(full)/6-31G* levels of theory describes the attack of a thiolate to trisulfides (Scheme 23), which are model systems for the drug calicheamicin [198].

Theoretically, the nucleophilic attack may occur at the central or at a terminal sulfur, producing either a sulfide and a trisulfide or a disulfide and a thiosulfenate as leaving group, respectively. The major outcomes of this study are: (i) the mechanism in the gas
phase is addition-elimination, also when the lower level of theory is used, i.e. MP2(full)/6-31+G*; (ii) attack at a terminal sulfur is slightly favored both kinetically (2-4 kcal mol⁻¹) and thermodynamically, since the thiosulfenate is a better leaving group than thiolate; (iii) steric hindrance prevents attack at the central sulfur, which thus may be tuned. The effect of the solvent, included as a continuum within the PCM approach, on the reaction of nucleophilic attack of a thiolate to di- and trisulfides was also investigated by Bachrach et al. [199] with ab initio as well as DFT methods. The main conclusions are that (i) nucleophilic substitution occurs via addition-elimination mechanism in the gas phase as well as in solution when S bears a hydrogen; (ii) when sulfur bears a bulkier substituent, the mechanism, i.e. addition-elimination versus S₂, depends on the phase; (iii) results obtained with the explicitly correlated method MP2 are qualitatively in nice agreement with results obtained with the hybrid functional B3LYP. The thiolate-disulfide exchange reaction was then investigated by Bachrach and co-workers [200] on ring strained systems at B3LYP/aug-cc-pVDZ and MP2/6-31+G*. The reaction was the attack of the nucleophile HS⁻ to small cyclic disulfides (Scheme 24); the reactions were studied in the gas phase.

Scheme 24. Nucleophilic attacks of a thiolate to cyclic disulfide substrates.

The main results of this theoretical study are (i) the mechanism depends on the size of the ring, being S₂ in small strained systems (three and four-membered rings 54 and 57) and addition-elimination in larger systems 59 and 61, in agreement with the findings reported for linear systems. In the former couple of compounds, strain prevents the formation of the three-center intermediate.

Addition-elimination was the mechanism found in the gas phase also for the nucleophilic substitution of thiolate at the disulfide bridge of cyclo-L-cystine [201], a model system well representing the disulfide bond in a protein environment.

More recently, nucleophilic substitutions at cyclic diselenides and selenenylsulfides have also been studied [159]. Both at B3LYP/6-31+G* and at MP2/6-31+G* level of theory, the S₂ mechanism well describes the reaction with highly strained three or four-membered rings, while in presence of five and six membered disulfides and diselenides the reaction proceeds via addition-elimination. In this study, the evidence of preferential attack at selenium rather than at sulfur has been reported.

A systematic study on the reaction shown in Scheme 25 has been performed by Bortoli et al. in 2016 at (COSMO)-ZORA-OLYP/TZ2P sc level of theory [203].

\[
\text{CH}_3X + \text{CH}_3X'S'\text{CH}_3 \rightarrow \text{CH}_3XX'\text{CH}_3 + \text{CH}_3X^- \\
X,X',X'' = S, Se, Te
\]

Scheme 25. Reaction scheme for a nucleophilic attack of a methylchalcogenolate to a dimethyl dichalcogenide.

Eighteen substitution reactions have been considered, which correspond to different combinations of S, Se and Te in the methylchalcogenolate and dimethylchalcogenide reactants. The authors, in agreement with Bachrach and co-workers, showed that the reaction proceeds via an addition-elimination path, except when a ditelluride is used as substrate or a tellurolate is used as nucleophile and attacks a Te atom. The authors have investigated the mechanism of these reactions also in water. S₂ mechanism is confirmed when a S atom of the substrate is attacked by all the tested chalcogenolates. Conversely, when the nucleophilic attack occurs at Te, a single well PES is present, that is the process is a barrierless addition-elimination. A transitional regime is predicted for the remaining situations, when selenium is involved, with a central plateau at positive energies in which at least two minima and a transition state might be located, but the peculiar flatness of the PES makes an accurate interpretation lengthy and numerically challenging (Fig. 3).

Other significant outcomes of this study are: (i) the S² + SSe and S¹ + S² cases mimic the second reductive step of Sec-GPx and Cys-GPx, respectively. In the gas phase, the mechanism is addition-elimination, implying the formation of a three-center intermediate which is not detected in the enzymatic pocket (and probably cannot form also for obvious steric reasons). In water, both reactions become S₂; (ii) by comparing the S² + SSe and S¹ + SeS cases, the authors provided insight on the competition between the process of GPx selenol form regeneration and undesired thiol scrambling process. While in the gas phase both reactions occur via addition-

\[\text{S} + \text{SeS} \rightarrow \text{S} + \text{SeS} + \text{H}_2\text{O}\]

This notation is used: The reaction is indicated using the couple of reactants, and the first atom of the substrate represents the site of nucleophilic attack; both in the nucleophile and in the substrate methyl substituents are omitted for clarity. For example, S¹ + SSe indicates the reaction between methylthiolate CH₃S and CH₃SSeCH₃ giving as products CH₃SSCH₃ and CH₃Se.
elimination mechanism and attack at sulfur is energetically favored, in water the former proceeds via $\text{S}_2\text{Se}$ and in the latter a three-center intermediate is located on the PES at +3.7 kcal mol$^{-1}$. This suggests that in the GPx catalytic site, selenium is protected by the surrounding residues which prevent the attack of the second GSH as well as the formation of a bulky three-center intermediate; (iii) notably, when comparing $\text{S}^2+\text{SSe}$ and $\text{S}^2+\text{STe}$, both proceeding via $\text{S}_2\text{Se}$ mechanism in water, the activation energy slightly increases by around 1.5 kcal mol$^{-1}$, a result which is not promising for the design and employment of Tec-GPx. Other disadvantages of the Te mutant in the oxidative step of the GPx cycle have been very recently reported [17].

In this context, it is worth to mention the computational study by Bayse about the GPx-like mechanism of phenylselenol [203]. In fact, this is one of the rare works which describes both the oxidative and the reductive steps; the computational approach, based on the combination of mPW1PW91 functional and TZVP with relativistic core potential basis sets for the chalcogens, includes solvent as three explicit water molecules, while bulk effects are treated with a continuum model. Notably, the water network allowed to obtain $\text{S}_2\text{Se}$ barriers consistent with a catalysis at physiological temperature.

As above outlined, the attack of a thiolate at a mixed Se/S substrate occurs in principle at sulfur and this aspect deserves particular attention when designing GPx mimics. Sarma and Mughesh [205, 206] have worked on this topic by combining experimental and computational analyses and highlighted that the presence of strong S···N or S···O interactions can turn on or enhance the process of interest, i.e. the attack of a nucleophile (thiol) at sulfur in selenenylsulfides, lowering the activation energy for the generation of the selenol. Bayse and Pavlou [143] investigated the tuning of the GPx like activity of diarylselenides through intramolecular S···N,O interactions incorporating the solvent effects through SAPE approach\textsuperscript{3}. These conclusions can be drawn from their study: (i) in the first reductive step, ortho substituents block the attack of the thiol and the selenenic acid reduction barrier increases and (ii) in the selenenylsulfide intermediate, weak interactions between Se and N, O drive the attack of the second thiol to sulfur by reducing its partial charge. Other mechanisms might operate to reduce the selenenylsulfides, which otherwise represent a sort of trap in the GPx like mechanism of diselenides, since the reaction with thiols that gives these intermediates consumes half of the available selenium equivalents.

Another consideration is that the strength of the nucleophile is as much important as the leaving group ability. This topic has been rigorously investigated by Bachrach and co-workers [208] who investigated the mechanism of a series of nucleophilic substitution reactions involving different species as nucleophiles and leaving groups in methylsulfenyl derivatives (level of theory: B3LYP/aug-cc-pVDZ) and found in all cases an addition-elimination mechanism.

An important contribution on the same topic is the combined experimental and computational work (B3LYP/6-31G(d)) by Bhabak and Mughesh [207] on a series of selenium compounds analogous to ebselen 8. The GPx-like activity was tested with different peroxides and different thiols. While the nature of the former reactants has little effect on the catalysis, the nature of the thiol is crucial. In particular, aromatic thiols such as PhSH and its para-CH$_3$ derivative lead to poor reactivity, which is greatly enhanced when GSH is used. The presence of aromatic thiols leads to the unwanted scrambling reaction occurring at the selenium center which is ‘activated’ by a non-covalent interaction with a close oxygen atom.

Finally, it is worth to mention also some analyses focused on the chalcogen-chalcogen bond nature, carried out on symmetric and asymmetric disulfides and diselenides of general formula $R_3\text{XXR}_3$ ($X=S$, Se; $R_1$, $R_2=\text{CH}_3$, OH, F, NH$_2$) [208]. Yanez, Boyd and co-workers investigated the nucleophilic attacks of F and CN$\textsuperscript{-}$ at the dichalcogenide substrates (level of theory: B3PW91/6-311+G(2df,p)). Despite these reactions are not directly related to a GPx-like mechanism, the authors described how strongly electro-negative substituents in the substrate can lead to breaking of the chalcogen-X bond rather than to the commonly expected chalcogen-chalcogen bond cleavage.

CONCLUSIONS

Especially in the last two decades, much effort has been dedicated to the synthesis and reactivity properties of organo diselenides, in particular diaryl diselenides. Their use in many organic chemistry reactions is consolidated, but their pharmacological activity is still debated. Furthermore, despite their GPx like antioxidant efficiency is superior to that of the well-known ebselen, and this is known since almost thirty years, these molecules present several problems mainly related to their toxicity, and these prob-

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\textsuperscript{3}In the mechanistic computational works by Bayse and co-workers the water molecules are treated explicitly and few of them are included in the model so that a hydrogen bonded network establishes. In the oxidative step the proton is shuttled via this network and this helps reducing the strain in the transition state, thus lowering the activation energy of the process. This approach is called SAPE, that is Solvent Assisted Proton Exchange.
lems become even more critical when analogous ditellurides are employed.

From a purely chemical perspective, the activity of diselenides consists in nucleophilic substitution reactions with peroxides and thiols. All the results collected so far, from the experiments as well as from the computational investigation, have confirmed the great potential of these compounds as anti-oxidant drugs and their role as efficient catalysts for oxidation processes in organic synthesis, have disclosed the mechanistic details of their action and have highlighted important structural features able to tune their reactivity. Undoubtedly, their catalytic mechanism reproduces the steps of the cycle of glutathione peroxidase, which is a clear biological example of the advantageous presence of selenium rather than sulfur. Focusing on drug design, the efficiency of GPx remains nevertheless superior to that of diselenides and probably the main reason has been glimpsed. Specific intermolecular interactions occurring in the catalytic pocket are missing in molecular mimics, whose reactivity can be increased by adding specific functional groups so that analogous intramolecular interactions are established. To this purpose, diselenides can be functionalized with larger and larger groups, paving the route to replace the molecular mimics with bioinspired semi-natural systems or de novo designed enzymes. Indeed, there is still a lot to learn from GPx!

LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AMBER</td>
<td>Assisted Model Building with Energy Refinement</td>
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<tr>
<td>B3LYP</td>
<td>X: Becke, Three-parameter, C: Lee-Yang-Parr (functional)</td>
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<td>B3PW91</td>
<td>X: Becke, Three-Parameter, C: Perdew-Wang (functional)</td>
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<tr>
<td>BLYP</td>
<td>X: Becke, C: Lee-Yang-Parr (Functional)</td>
</tr>
<tr>
<td>GSSH</td>
<td>Glutathione Disulfide</td>
</tr>
<tr>
<td>BnSH</td>
<td>Benzyl thiol</td>
</tr>
<tr>
<td>BOP</td>
<td>(benzotriazol-1-yl-oxy-tris(Dimethylamino)Phosphonium Hexafluorophosphate)</td>
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<tr>
<td>BP86</td>
<td>X: Becke, C: Perdew (Functional)</td>
</tr>
<tr>
<td>CCSD(T)</td>
<td>Coupled Cluster (full treatment: singles, doubles; estimated: triples)</td>
</tr>
<tr>
<td>CD</td>
<td>β-cyclodextrin</td>
</tr>
<tr>
<td>COSMO</td>
<td>Conductor-like Screening Model</td>
</tr>
<tr>
<td>CPCM</td>
<td>Conductor-like Polarizable Continuum Model</td>
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<tr>
<td>CSD</td>
<td>Cambridge Structural Database</td>
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<tr>
<td>Cys-GPx</td>
<td>Sulfur Mutant of GPx</td>
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<td>D3-BJ</td>
<td>Dispersion Correction by Grimme with Becke-Johnson Damping Function</td>
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<td>DCC</td>
<td>N,N'-Dicyclohexylcarbodiimide</td>
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<tr>
<td>DFT</td>
<td>Density Functional Theory</td>
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<tr>
<td>DIC</td>
<td>N,N'-Diisopropylcarbodiimide</td>
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<tr>
<td>DMAP</td>
<td>4-(Dimethylamino)pyridine</td>
</tr>
<tr>
<td>DMBS</td>
<td>N,N-dimethylbenzylamine diselenide</td>
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<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
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<tr>
<td>EDC</td>
<td>N-(3-Dimethylaminopropyl)-N'-ethylcarbonate</td>
</tr>
<tr>
<td>GAFF</td>
<td>General AMBER Force Field</td>
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<tr>
<td>GGA</td>
<td>General Gradient Approximation</td>
</tr>
<tr>
<td>GPx / Sec-GPx</td>
<td>Glutathione Peroxidase</td>
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<tr>
<td>GSH</td>
<td>Glutathione</td>
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<td>GTO</td>
<td>Gaussian-Type Orbital</td>
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<td>HOBT</td>
<td>1-Hydroxybenzotriazole</td>
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<td>HPLC</td>
<td>High-performance Liquid Chromatography</td>
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<td>MCF-7</td>
<td>Michigan Cancer Foundation-7 (breast cancer cell line)</td>
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<td>MD</td>
<td>Molecular Dynamics</td>
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<td>MP2</td>
<td>Second Order Møller-Plesset (MP) Perturbation Theory</td>
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<td>MP4SDTQ</td>
<td>Fourth-order MP Theory Corrected with Single, Double, Triple and Quadruple Substitutions</td>
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<td>mPW1PW91</td>
<td>Perdew-Wang Hybrid Functional Developed by Adamo-Barone (functional)</td>
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<td>NADPH</td>
<td>Nicotinamide Adenine Dinucleotide Phosphate</td>
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<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
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<td>X: Handy-Cohen, C: Lee-Yang-Parr (functional)</td>
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<td>OPBE</td>
<td>X: Handy-Cohen, C: Perdew-Burke-Emzerhof (functional)</td>
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<td>PES</td>
<td>Potential Energy Surface</td>
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<td>Ph</td>
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<td>PNT1A</td>
<td>A Human Immortalized Prostate Epithelium Cell Line</td>
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<td>QM</td>
<td>Quantum Mechanics</td>
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<td>QZ4P</td>
<td>Quadruple Zeta with 4 Polarization Functions (basis set)</td>
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<td>SAPE</td>
<td>Solvent-Assisted Proton Exchange</td>
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<td>SAR</td>
<td>Structure-Activity Relationship</td>
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<td>Sec</td>
<td>Selenocysteine</td>
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<td>STO</td>
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<tr>
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<td>Thiobarbituric Acid Reactive Substances</td>
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<td>XC</td>
<td>Exchange-Correlation</td>
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<td>ZORA</td>
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CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.
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