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Towards New Catalytic Antioxidants: A Simple and Mild Synthesis of Selenenylsulfides

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Abstract: A new methodology for the synthesis of small molecules containing the S-Se bond is reported. Aryl- and alkyl-selenols react smoothly with *N*-thiophthalimides to afford the corresponding selenenylsulfides through a clean S_N2 path occurring at the sulfur atom. The reaction proceeds under very mild conditions in DMF in absence of catalysts for most of the substrates. The scope of the reaction was found to be broad, allowing a wide series of selenols and *N*-thiophtalimides to be efficiently employed in this procedure. Owing to the instability of the S-Se bond, selenenylsulfides exhibited a remarkable tendency to disproportionate to the corresponding symmetric diselenides and disulfides. Preliminary evaluation of the catalytic antioxidant properties of novel selenenylsulfides showed their behaviour as GPx mimics.

Keywords: selenols; *N*-thiophtalimides; selenenylsulfides; antioxidants; selenium; glutathione peroxidase (GPx) mimics; sulfur

1. Introduction

Antioxidants are compounds able to prevent or inhibit the oxidation of other molecules, and can be natural or synthetic [1,2]. Natural systems include both low molecular weight derivatives, such as polyphenols, ascorbic acid, lipoic acid and vitamin A, and also several enzymes, namely glutathione peroxidase (GPx), thioredoxine reductase (TrxR), superoxide dismutase (SOD), and catalase. Elements like zinc, selenium, iron, and copper are responsible of the activity of certain enzymes. Also, synthetic antioxidants found an extensive use in medicinal and food chemistry.

In this context, selenium and its organic derivatives play an essential role in biochemistry and medicine. In fact, organoselenium compounds, together with some sulfur and tellurium derivatives, find application as antioxidants, anticancer, chemo-protectors, and enzymes modulators [3–9]. Several studies established the role of selenium in biological systems [10,11].

The catalytic activity of enzymes and of synthetic antioxidants has been ascribed to the heteroatom present in the active site. Selenium derivatives are well known to behave as mimics of the selenoenzyme GPx [12–14]. Besides this activity, the positive effect of selenium containing molecules against various diseases has been also attributed to other antioxidant mechanisms, such as ROS (reactive oxygen species) scavenging properties and metal coordination (iron and copper) to prevent DNA damage [15]. Organoselenium compounds usually behave as more efficient systems with respect to sulfur analogues in cancer prevention or as radical scavengers.

On the basis of the catalytic cycle proposed for GPx, the selenol moiety of the enzyme (EnzSeH) is oxidized by the peroxide, forming a selenenic acid (EnzSeOH), which reacts with a thiol (typically glutathione, GSH) leading to a selenenylsulfide (EnzSeSG). The mixed selenosulfide reacts with

a second equivalent of GSH, to reform the selenol, together with oxidized glutathione GSSG. The selenenylsulfide, containing the Se-S bridge, represents the key intermediate for the activity of the enzyme [7,16–19]. Taking into account the importance of this process, several synthetic approaches to obtain selenated small molecules as enzyme mimics have been developed. However, to the best of our knowledge, a limited number of methods are reported for the preparation of selenenylsulfides. These methods typically exploited the reactivity of diselenides in thiol-diselenide exchange or foresee the reaction of sulfenyl derivatives with thiols [16,17,20–22].

It is well established that the Se-S bridge is unstable and undergoes disproportionation reaction to afford diselenides and disulfides. Some examples are reported on selenenylsulfides stabilized by sterically bulky groups or by intramolecular Se $\cdots$ Het (N, O) interactions [23].

Owing to the interest in these selenium containing compounds, the search for novel procedures to prepare small molecules containing the Se-S unit is of great interest.

Our long-dated interest in the chemistry of silyl chalcogenides allowed to disclose novel procedures to access a plethora of sulfur and selenium containing molecules exploiting the nucleophilic behaviour of the chalcogen atom towards various electrophiles [24–29]. Recently we reported the reaction of bis(trimethylsilyl)selenide (HMDSS) with *N*-thiophtalimides as a mild, general metal free procedure for the synthesis of variously substituted disulfides [30].

Indeed, despite the interest in the reactivity of selenols as nucleophilic selenium transfer reagents, their use has been critically limited by their instability. However, HMDSS was efficient in the opening of ring strained heterocycles, namely epoxides, episulfides and aziridines, leading to a wide range of β-functionalized selenols, which were stable enough to react with electrophiles under controlled catalytic conditions [31]. Differently substituted stable aryl selenols were also prepared by reduction of the parent diselenides to explore their activity as enzyme inhibitors [32].

On the basis of our findings on the behaviour of selenols we report here a new approach for the preparation of selenenylsulfides using *N*-thiophtalimides as suitable electrophiles.

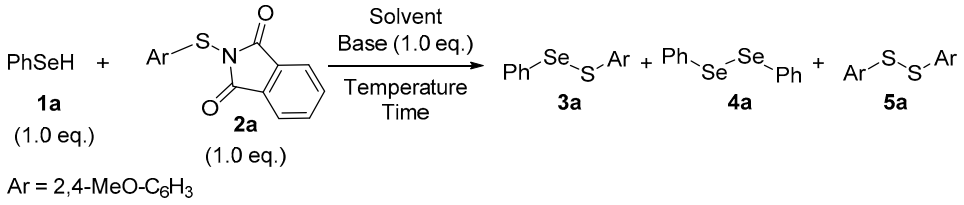
2. Results and Discussion

We began our investigations by studying the reaction of benzeneselenol **1a** with 2,4-dimethoxyphenyl *N*-thiophthalimide **2a** (Table 1). The reaction was initially performed at 0 °C for 2 h in the presence of Et_3N, using $CHCl_3$ as the solvent (Table 1, entry 1). Interestingly, under these conditions the formation of the desired selenenylsulfide **3a** was observed, albeit together with a comparable amount of diselenide **4a** and disulfide **5a**. Furthermore, ca. 30% of the starting *N*-thiophthalimide **2a** remained unreacted. Similar results, both in terms of conversion and **3a**:**4a**:**5a** ratio, were obtained upon performing the reaction at −78 °C for 4 h (entry 2). We evaluated whether the solvent could have an effect on the reaction outcome. We found that the use of acetonitrile in place of chloroform gave the diselenide **4a** and the disulfide **5a** as main reaction products, together with a larger amount of unreacted **2a** (ca. 50%, entry 3). The use of toluene gave slight **3a**:**4a**:**5a** ratio improvements, although poor conversion was achieved (Table 1, entry 4). Remarkably, when DMF was used as solvent in the presence of Et_3N or Cs_2CO_3/TBAI, complete consumption of **2a** was observed within 2 h. Under these conditions a comparable mixture of compounds **3a**, **4a**, and **5a** was observed (entries 5 and 6). Finally, we were delighted to find that the desired selenenylsulfide **3a** was smoothly formed as major product simply treating selenol **1a** with *N*-thiophthalimide **2a** in DMF, in absence of any catalyst (entry 7). This observation seems to suggest that the formation of selenenylsulfide **3a** is the result of a clean S_N2 at sulfur of **2a** by the nucleophile **1a**, favoured in polar aprotic solvents such as DMF.

In our hands compound **3a** proved to be rather unstable, as the selenenylsulfide exhibited a remarkable tendency to form the corresponding symmetrical diselenide **4a** and disulfide **5a**. Indeed, selenenylsulfide **3a** significantly decomposed on silica gel during flash chromatography purification (36% isolated yield). Similar behaviour was observed when using a different stationary phase such as neutral Al_2O_3. We also observed that both bases and acids could promote the conversion of **3a** into **4a**

and **5a**. Intriguingly, the mild acidity of chloroform proved to be sufficient to induce fast conversion of **3a**, which could also be observed while acquiring the ^{1}H NMR spectrum in $CDCl_3$.

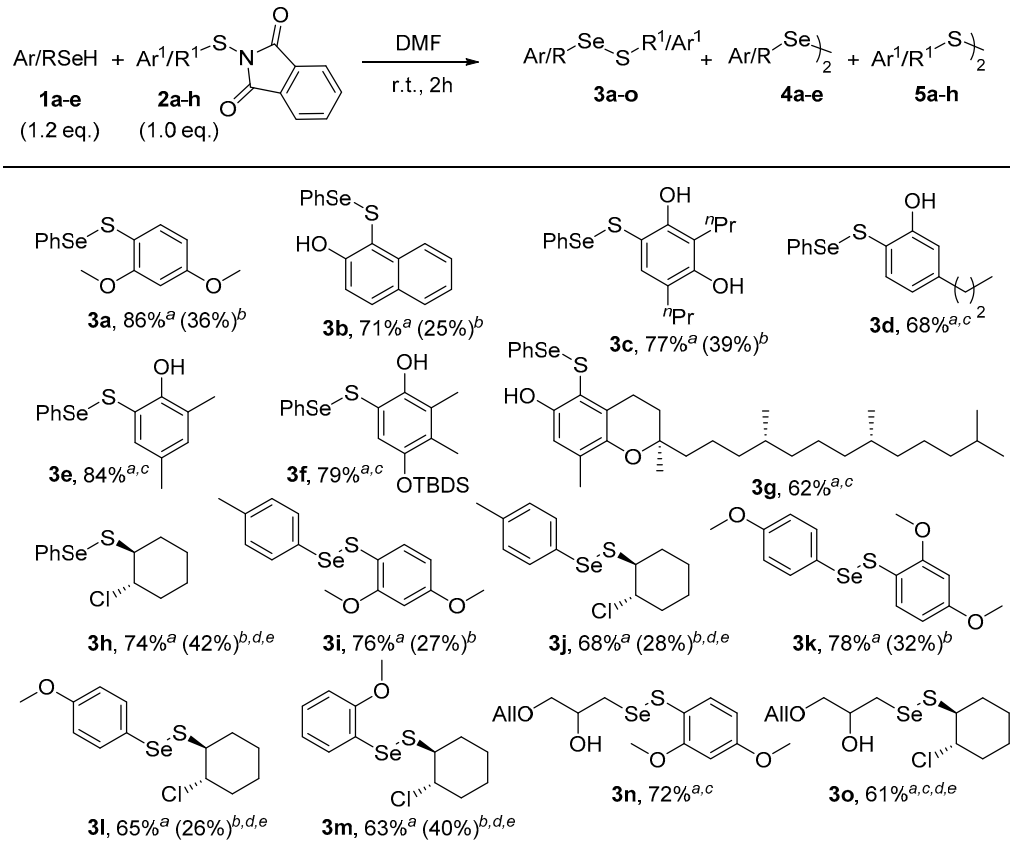
Table 1. Optimization of the reaction conditions for the synthesis of **3a** from **1a** and **2a**.



Entry	Solvent	Base	Temperature	Time	3a:4a:5a [a]	Conversion (%) [a]
1	$CHCl_3$	Et_3N	0 °C to r.t.	2 h [b]	36:32:32	71 [c]
2	$CHCl_3$	Et_3N	−78 °C	4 h	34:33:33	88 [c]
3	CH3CN	Et_3N	0 °C to r.t.	4 h	18:41:41	52 [c]
4	PhMe	Et_3N	0 °C to r.t.	24 h	42:29:29	67 [c]
5	DMF	Et_3N	0 °C to r.t.	2 h	32:34:34	>95
6	DMF	Cs_2CO_3/TBAI	0 °C to r.t.	2 h	44:28:28	>95
7	DMF	-	r.t.	4 h	72:14:14	>95

[a] Determined by ^{1}H NMR of the crude reaction mixture. [b] Longer reaction time led to improved conversion but lower **3a**:**4a**:**5a** ratio. [c] 29%, 12%, 48%, and 33% of starting *N*-thiophthalimide **2a** remained unreacted, respectively for entries 1, 2, 3, and 4.

Having established optimum reaction conditions for the conversion of selenols and *N*-thiophthalimides into the corresponding selenenylsulfides, we explored the scope and the limitation of the reaction. We initially focused our attention on variations of the *N*-thiophthalimide structure. We found that, under the optimised reaction conditions, variously substituted *N*-arylthiophthalimides **2a**–**f**, bearing different substituents, such as alkyl, unprotected hydroxyl, methoxy, and silyloxy moieties at different positions of the aromatic ring, reacted smoothly with benzeneselenol **1a**, enabling the formation of aryl(phenylselanyl)sulfanes **3a**–**f** (Scheme 1). Furthermore, the reaction was also applied to the *N*-arylthio derivative of δ-tocopherol **2g**, leading to the formation of compound **3g** bearing a vitamin E-like skeleton and the phenylseleno moiety. Intriguingly, the 2-chloro-1-*N*-cyclohexylthiophthalimide **2h** proved to be scarcely reactive under the standard conditions. Nonetheless, **1a** and **2h** readily reacted in the presence of Cs_2CO_3 and TBAI to afford alkyl(phenylselanyl)sulfane **3h**. However, as previously observed for compound **3a**, although selenenylsulfides **3b**–**h** were formed in good yields, a significant decomposition occurred during purification. Whereas pure **3b**,**c**,**h** could be isolated in moderate yields, aryl(phenylselanyl)sulfane **3d**–**g** gave only the corresponding diselenide **4a** and disulfides **5d**–**g** upon flash column chromatography (Scheme 1). Having demonstrated that a variety of aryl- and alkyl- *N*-thiophthalimides could be successfully employed in this reaction, we next explored the scope of the reaction with respect to differently substituted selenols. Pleasingly, arylselenols **1b**–**d**, bearing methyl and methoxy groups onto different position of the aromatic ring, reacted efficiently with *N*-arylthiophthalimide **2a** and *N*-alkylthiophthalimide **2h** to yield the corresponding substituted selenenylsulfides **3i**–**m**. Despite the instability exhibited by S-Se bonds, compounds **3i**–**m** were obtained in good to moderate isolated yields (Scheme 1).



[a]Conversion of selenol **1** and *N*-thiophthalimide **2** into the corresponding selenenylsulfide **3**; determined by ^{1}H NMR of the crude mixture. [b]Yields in parentheses refer to isolated yields. [c]Complete transformation of selenenylsulfides **3d**-**g**,**n**,**o** into the corresponding diselenides **4a**,**e** and disulfides **5a**,**d**-**h** occurred during any attempt of purification on silica gel. [d]The reaction was performed in the presence of Cs_2CO_3/TBAI, as reported in Table 1, entry 6 (see experimental section for details). [e]Racemic mixture.

Scheme 1. Scope of the synthesis of selenenylsulfides from selenols and *N*-thiophthalimides.

Finally, in order to evaluate whether this reactivity could also be extended to functionalized alkyl selenols, β-hydroxy selenol **1e** was treated with *N*-thiophthalimides **2a** and **2h**. Unfortunately, albeit selenenylsulfides **3n** and **3o** were efficiently formed, complete disproportionation occurred during purification on silica gel, leading exclusively to the isolation of diselenide **4e** and disulfides **5a**,**h** (Scheme 1).

Worthy of mention is the complete chemoselectivity observed in the reactions of selenols with *N*-thiophthalimide **2h**. In fact, despite working in the presence of a remarkably strong nucleophilic selenate ion and a potentially very good leaving group, no trace of the attack on the chloro substituted carbon was observed, being the sulfur atom of **3h** the unique electrophilic partner of these reactions.

The amount of diselenides **4** and disulfides **5** could not be reduced working under strictly controlled conditions (i.e., using dry-box, in absence of light, fast manipulation). The observed behaviour is in accordance with previous reports on the low stability of this kind of structures which, amongst other factors, depends on the nature of substituents on the sulfur or selenium atoms [19,23].

As stated above, a selenenylsulfide is postulated to be the key intermediate of the GPx catalytic cycle. The selenium atom of a selenocysteine residue (Sec) of the GPx catalytic triad and the thiol group of a molecule of GSH are indeed involved in the formation of the selenenylsulfide which, upon reaction with a second molecule of GSH regenerates the catalytically active selenol moiety of Sec. This biochemical mechanism allows the reduction, in living cells, of harmful hydroperoxides to safe alcohols and water, therefore accounting for the biological relevance of S-Se bonds formation [1,3,4].

Thus, having developed a novel procedure for the synthesis of small molecules containing the S-Se moiety, we sought to preliminary test their GPx-like activity. The thiol peroxidase-like activity of compounds **3h** and **3l** was studied according to the DTT oxidation test (Figure 1) [33–35]. Indeed, selenenylsulfides **3h**,**l** behaved as medium effective catalysts with T_{50} around 70 min [36] Furthermore, in line with previous findings on the antioxidant properties of different organoselenium compounds [37,38], the presence of a methoxy group onto the arylseleno moiety renders selenenylsulfide **3l** more efficient than **3h** (Figure 1). To get more insight on the GPx activity of these new compounds [39–42], in Figure 1 are reported data obtained for diselenides **4a** and **4c** and disulfide **5h** measured under the same conditions. Thus, while diselenides **4a**,**c** promoted a faster oxidation than selenenylsulfides with T_{50} around 20 min, disulfide **5h** did not exhibit significant catalytic antioxidant properties (Figure 1).

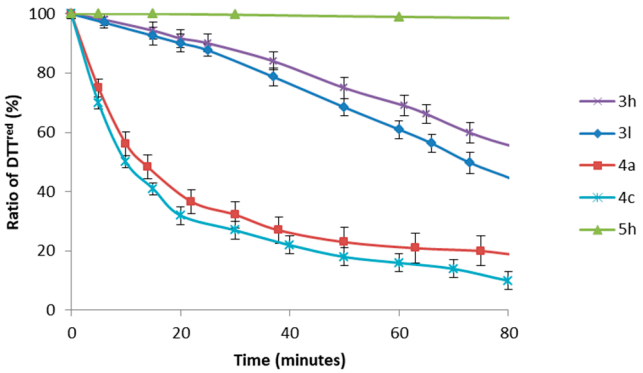


Figure 1. Oxidation of DTT^{red} with H_2O_2 in the presence of Se- or S-containing catalysts (10 mol%). Reaction conditions: $[DTT^{red}]_0$ = 0.14 M, $[H_2O_2]_0$ = 0.14 M, [catalyst] = 0.014 M, CD_3OD (0.6 mL). Reaction progress was monitored by means of ^1H NMR. The mean ± SD values of three separate experiments are reported.

3. Materials and Methods

3.1. Experimental Section

All the reactions were performed under a positive pressure of nitrogen and were monitored by TLC using commercially available precoated plates (silica gel 60 F 254) and compounds were visualised by fluorescence quenching or by staining the plates with acidic *p*-anisaldehyde solution. Silica gel 60, 230–400 mesh, was used for flash column chromatography. Dry solvents were obtained using a Pure Solv™ Micro system. Commercially available reagents were used as obtained from freshly opened containers without further purification. Aryl selenols **1a**–**d** [32], alkyl selenol **1e** [31], and *N*-thiophthalimides **2** [43–48] were prepared according to reported procedures. Spectroscopic data of diselenides **4a**–**e** [26,49] and disulfides **5a**–**h** [30] matched those previously reported in the literature.

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or C_6D_6 with a Varian Mercury Plus instrument or with a Varian INOVA instrument at 400 and 100 MHz, respectively. The corresponding residual non-deuterated solvent was used as a reference ($CDCl_3$: 7.26 ppm for ^1H and 77.0 ppm for ^{13}C; C_6D_6: 7.16 ppm for ^1H and 128.0 ppm for ^{13}C). ^{77}Se NMR spectra were recorded using Bruker 400 Ultrashield spectrometer (Bruker, Milan, Italy), operating at 76 MHz. Diphenyl diselenide $(PhSe)_2$ was used as an external reference for ^{77}Se NMR (δ = 461 ppm). ^1H NMR data are reported as follows: chemical shift, integration, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublet, etc.), coupling constant (*J*) or line separation (ls), and assignment. Mass spectra (MS) were determined by ESI (Thermo Fisher Scientific, Milan, Italy). See Electronic Supplementary Material for details.

3.2. General Procedure for the Synthesis of Selenenylsulfides **3**

A solution of selenol **1** (0.20 mmol, 1.0 eq.) in dry DMF (0.5 mL) was added to a solution of *N*-thiophthalimide **2** (0.24 mmol, 1.2 eq.) in dry DMF (1.5 mL) at room temperature under inert atmosphere (N_2); the reaction mixture was stirred for 4 h (reaction progress monitored by TLC). Afterwards, the mixture was diluted with Et_2O (5 mL) and then H_2O (3 mL) was added. The organic phase was extracted with Et_2O (5 mL), washed with water (3 × 5 mL) and brine (5 mL), and then dried over Na_2SO_4; filtered and concentrated in vacuo. The crude material was purified by flash chromatography to afford selenenylsulfides **3**.

3.2.1. Synthesis of (2,4-Dimethoxyphenyl)(phenylselanyl)sulfane **3a**

Following the general procedure, *N*-thiophthalimide **2a** (57 mg, 0.18 mmol) and benzeneselenol **1a** (24 mg, 0.15 mmol) gave, after flash chromatography (petroleum ether/Et_2O 5:1, **3a**: R_f = 0.47; **4a**: R_f = 0.95; **5a**: R_f = 0.14), selenenylsulfide **3a** (18 mg, 36%) as a yellowish oil. ^{1}H NMR (400 MHz, C_6D_6) δ (ppm): 7.63-7.69 (2H, m), 7.52 (1H, d, J = 8.5 Hz), 6.93-7.04 (3H, m), 6.28 (1H, d, J = 2.5 Hz), 6.11 (1H, dd, J = 2.5, 8.5 Hz), 3.22 (3H, s, CH_3O), 3.17 (3H, s, CH_3O). ^{13}C NMR (100 MHz, C_6D_6) δ (ppm): 162.1, 160.3, 135.3, 130.9, 128.8, 128.0, 127.5, 127.1, 104.9, 99.3, 54.8, 54.6. HRMS m/z calcd for $C_{14}H_{15}O_2SSe$ 326.9958, found 326.9963.

3.2.2. Synthesis of 1-((Phenylselanyl)thio)naphthalen-2-ol **3b**

Following the general procedure, *N*-thiophthalimide **2b** (58 mg, 0.18 mmol) and benzeneselenol **1a** (24 mg, 0.15 mmol) gave, after flash chromatography (petroleum ether/Et_2O 20:1, **3b**: R_f = 0.48; **4a**: R_f = 0.83; **5b**: R_f = 0. 38), selenenylsulfide **3b** (12 mg, 25%) as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.05 (1H, d, J = 8.4 Hz), 7.75–7.80 (2H, m), 7.59 (2H, ap.d., J = 8.1 Hz), 7.32–7.43 (2H, m), 7.25–7.29 (3H, m), 7.16 (1H, d, J = 8.4 Hz), 6.70 (1H, s, OH). ^{13}C NMR (100 MHz, CDCl3) δ (ppm): 156.9, 135.0, 135.0, 132.8, 131.5, 129.5, 129.3, 129.2, 128.5, 128.5, 127.4, 125.1, 123.8, 116.7. ^{77}Se NMR (76 MHz, $CDCl_3$) δ (ppm): 637.9. HRMS m/z calcd for $C_{16}H_{12}NaOSSe$ 354.9672, found 354.9681.

3.2.3. Synthesis of 4-((Phenylselanyl)thio)-2,6-dipropylbenzene-1,3-diol **3c**

Following the general procedure, *N*-thiophthalimide **2c** (89 mg, 0.24 mmol) and benzeneselenol **1a** (31 mg, 0.20 mmol) gave, after flash chromatography (petroleum ether/Et_2O 8:1, **3c**: R_f = 4.2; **4a**: R_f = 0.92; **5c**: R_f = 0.26), selenenylsulfide **3c** (30 mg, 39%) as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.62 (2H, app.d, J = 8Hz), 7.28–7.41 (3H, m), 6.89 (1H, s), 6.21 (1H, s, OH), 4.90 (1H, s, OH), 2.60 (3H, t, J = 8 Hz, CH_3), 2.40 (3H, t, J = 8 Hz, CH_3), 1.46–1.63 (4H, m), 0.81–1.02 (4H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 155.0, 154.7, 134.1, 134.0, 132.2, 129.2, 128.9, 120.5, 114.6, 111.1, 31.4, 26.0, 22.7, 22.0, 14.1, 13.8. ^{77}Se NMR (76 MHz, $CDCl_3$) δ (ppm): 641.8. HRMS m/z calcd for $C_{18}H_{22}NaO_2SSe$ 405.0403, found 405.0397.

3.2.4. Synthesis of (2-Chlorocyclohexyl)(phenylselanyl)sulfane **3h**

Following a slightly modified general procedure, a solution of benzeneselenol **1a** (31 mg, 0.20 mmol) in dry DMF (2.5 mL) was cooled at 0 °C under inert atmosphere and treated with Cs_2CO_3 (65 mg, 0.20 mmol, 1.0 eq.) and TBAI (74 mg, 0.20 mmol, 1.0 eq.). Afterwards, a DMF solution (0.5 mL) of *N*-alkylthiophthalimide **2h** (70 mg, 0.24 mmol, 1.2 eq.) was added and the mixture was allowed to warm to room temperature and stirred for 4 h. Then, the mixture was diluted with Et_2O (5 mL) and then saturated aq. NH_4Cl (3 mL) was added. The organic phase was extracted with Et_2O (5 mL), washed with brine (3 × 8 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified by flash chromatography (petroleum ether, **3h**: R_f = 0.54; **4a**: R_f = 0.65) to afford selenenylsulfide **3h** (26 mg, 42%) as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.58–7.64 (2H, m), 7.22–7.31 (3H, m), 3.99–4.04 (1H, m, C*H*Cl), 2.97–3.02 (1H, m, C*H*S), 2.27–2.29 (1H, m), 2.16–2.27 (1H, m), 1.55–1.73 (4H, m), 1.32–1.42 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 131.5, 129.7, 129.1,

127.3, 62.6, 55.2, 34.5, 31.6, 24.0, 23.7. ^{77}Se NMR (76 MHz, $CDCl_3$) δ (ppm): 463.6. HRMS m/z calcd for $C_{12}H_{16}ClSSe$ 306.9826, found 306.9833.

3.2.5. Synthesis of (2,4-Dimethoxyphenyl)(*p*-tolylselanyl)sulfane **3i**

Following the general procedure, *N*-thiophthalimide **2a** (57 mg, 0.18 mmol) and 4-methylbenzeneselenol **1b** (26 mg, 0.15 mmol) gave, after flash chromatography (petroleum ether/Et_2O 15:1, **3i**: R_f = 0.43; **4b**: R_f = 0.95; **5a**: R_f = 0.10), selenenylsulfide **3i** (14 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.49 (2H, ap.d., J = 8.0 Hz), 7.41 (1H, d, J = 9.2 Hz), 7.07 (2H, ap.d., J = 8.0 Hz), 6.38–6.46 (2H, m), 3.80 (3H, s), 3.79 (3H, s), 2.34 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.9, 155.3, 136.1, 132.9, 132.4, 130.5, 105.7, 99.6, 56.4, 56.0, 21.6. HRMS m/z calcd for $C_{15}H_{17}O_2SSe$ 341.0114, found 341.0102.

3.2.6. Synthesis of (2-Chlorocyclohexyl)(*p*-tolylselanyl)sulfane **3j**

Following a slightly modified general procedure, a solution of 4-methylbenzeneselenol **1b** (26 mg, 0.15 mmol) in dry DMF (2.5 mL) was cooled at 0 °C under inert atmosphere and treated with Cs_2CO_3 (49 mg, 0.15 mmol) and TBAI (56 mg, 0.20 mmol). Afterwards, a DMF solution (0.5 mL) of *N*-alkylthiophthalimide **2h** (53 mg, 0.18 mmol) was added and the mixture was allowed to warm to room temperature and stirred for 4 h. Then, the mixture was diluted with Et_2O (5 mL) and then saturated aq. NH_4Cl (3 mL) was added. The organic phase was extracted with Et_2O (5 mL), washed with brine (3 × 8 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified by flash chromatography (petroleum ether, R_f = 0.45) to afford selenenylsulfide **3j** (13 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.53 (2H, ap.d., J = 8.1Hz), 7.12 (2H, ap.d., J = 8.1 Hz), 4.03-4.08 (1H, m, C*H*Cl), 2.99-3.04 (1H, m, C*H*S), 2.34 (3H, s, CH_3), 2.29–2.33 (1H, m), 2.17–2.28 (1H, m), 1.62–1.75 (4H, m), 1.36–1.43 (2H, m). ^{13}C NMR (100MHz, $CDCl_3$) δ (ppm): 138.2, 132.9, 131.1, 130.6, 63.2, 55.7, 34.8, 32.0, 24.5, 24.2, 21.7. HRMS m/z calcd for $C_{13}H_{17}ClNaSSe$ 342.9802, found 342.9818.

3.2.7. Synthesis of (2,4-Dimethoxyphenyl)((4-methoxyphenyl)selanyl)sulfane **3k**

Following the general procedure, *N*-thiophthalimide **2a** (57 mg, 0.18 mmol) and 4-methoxybenzeneselenol **1c** (28 mg, 0.15 mmol) gave, after flash chromatography (petroleum ether/Et_2O 10:1, **3k**: R_f = 0.25; **4c**: R_f = 0.42; **5a**: R_f = 0.12), selenenylsulfide **3k** (17 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.53 (2H, ap.d., J = 8.8 Hz), 7.43 (1H, d, J = 9.1 Hz), 6.83 (2H, ap.d., J = 8.8 Hz), 6.40–6.46 (2H, m), 3.83 (3H, s), 3.82 (3H, s), 3.81 (3H, s). ^{13}C NMR (100MHz, $CDCl_3$) δ (ppm): 160.2, 159.9, 155.6, 136.6, 136.0, 134.0, 124.3, 115.3 105.6, 99.5, 56.4, 56.0, 55.9. ^{77}Se NMR (76 MHz, $CDCl_3$) δ (ppm): 504.8. HRMS m/z calcd for $C_{15}H_{16}NaO_3SSe$ 378.9883, found 378.9896.

3.2.8. Synthesis of (2-Chlorocyclohexyl)((4-methoxyphenyl)selanyl)sulfane **3l**

Following a slightly modified general procedure, a solution of 4-methoxybenzeneselenol **1c** (37 mg, 0.20 mmol) in dry DMF (2.5 mL) was cooled at 0 °C under inert atmosphere and treated with Cs_2CO_3 (65 mg, 0.20 mmol, 1.0 eq.) and TBAI (74 mg, 0.20 mmol, 1.0 eq.). Afterwards, a DMF solution (0.5 mL) of *N*-alkylthiophthalimide **2h** (70 mg, 0.24 mmol, 1.2 eq.) was added and the mixture was allowed to warm to room temperature and stirred for 4 h. Then, the mixture was diluted with Et_2O (5 mL) and then saturated aq. NH_4Cl (3 mL) was added. The organic phase was extracted with Et_2O (5 mL), washed with brine (3 × 8 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified by flash chromatography (petroleum ether, R_f = 0.26) to afford selenenylsulfide **3l** (17 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.58 (2H, app.d., J = 8.8 Hz), 6.85 (2H, app.d., J = 8.8 Hz), 4.01–4.15 (1H, m), 3.81 (3H, s, CH_3O), 2.90-3.11 (1H, m), 2.07–2.38 (2H, m), 1.52–1.81 (4H, m), 1.28–1.41 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.4, 135.6, 134.0, 115.5, 63.2, 55.9, 55.6, 34.5, 31.8, 24.3, 24.0. ^{77}Se NMR (76 MHz, $CDCl_3$) δ (ppm): 503.6. HRMS m/z calcd for $C_{13}H_{17}ClNaOSSe$ 358.9752, found 358.9741.

3.2.9. Synthesis of (2-Chlorocyclohexyl)((2-methoxyphenyl)selanyl)sulfane **3m**

Following a slightly modified general procedure, a solution of 2-methoxybenzeneselenol **1d** (28 mg, 0.15 mmol) in dry DMF (2.5 mL) was cooled at 0 °C under inert atmosphere and treated with Cs_2CO_3 (49 mg, 0.15 mmol) and TBAI (56 mg, 0.15 mmol). Afterwards, a DMF solution (0.5 mL) of *N*-alkylthiophthalimide **2h** (53 mg, 0.18 mmol) was added and the mixture was allowed to warm to room temperature and stirred for 4 h. Then, the mixture was diluted with Et_2O (5 mL) and then saturated aq. NH_4Cl (3 mL) was added. The organic phase was extracted with Et_2O (5 mL), washed with brine (3 × 8 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude material was purified by flash chromatography (petroleum ether/Et_2O 100:1, R_f = 0.30) to afford selenenylsulfide **3m** (20 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.82 (1H, dd, *J* = 1.6, 7.7 Hz), 7.23-7.28 (1H, m), 7.00–7.04 (1H, m), 6.85 (1H, dd, *J* = 1.1, 8.1 Hz), 4.05–4.09 (1H, m, C*H*Cl), 3.91 (3H, s, CH_3O), 2.96–3.01 (1H, m, C*H*S), 2.34–2.36 (1H, m), 2.23–2.33 (1H, m), 1.62-1.78 (4H, m), 1.36-1.41 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 157.0, 129.1. 128.5, 126.1, 122.4, 110.9, 63.5, 56.5, 55.5, 35.2, 32.3, 24.6, 24.4. HRMS *m*/*z* calcd for $C_{13}H_{17}ClNaOSSe$ 358.9752, found 358.9745.

3.3. GPx-Like Catalytic Activity Measurments

GPx-like activity was determined according to a reported NMR assay [28–30]. DTT^{red} (0.14 mmol) and catalyst (0.014 mmol) were dissolved in CD_3OD (0.6 mL), and the solution was added to 35% H_2O_2 (14 µL, 0.14 mmol) to start the reaction. ^{1}H NMR spectra were measured at a variable reaction time at 25 °C. The relative populations of DTT^{red} and DTT^{ox} were determined by integration of the ^{1}H NMR signals.

4. Conclusions

In summary, we have disclosed a novel procedure to convert selenols and *N*-thiophthalimides into the corresponding selenenylsulfides under very mild procedure. These small molecules, containing the S-Se bond, resemble the key intermediate involved in the GPx catalytic cycle and, therefore, could be employed as useful and simple models to gain insight into the GPx mechanism. Furthermore, preliminary evaluation of the catalytic antioxidant properties of selenenylsulfides showed that they possess GPx-like activity.

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