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The potential antioxidant activity of 2,3-dihydroselenophene, a prototype drug of 4-aryl-2,3-dihydroselenophenes

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ABSTRACT

Here we present our results in palladium cross-coupling reaction of aryl boronic acids with 4-iodo-2,3-dihydroselenophene derivatives. The cross-coupled products were obtained in satisfactory yields. A dehydrogenation of4,5-diphenyl-2,3-dihydroselenophene was activated by DDQ and the 2,3-diarylselenophene was obtained in good yield. Regarding the antioxidant activity, the selenophene derivative **3a** was effective in counteracting lipid and protein oxidation as well as scavenging ABTS radical. The findings of the present study indicate that **3a** is a prototype for future drug development programs to treat disorders mediated by reactive oxygen species.

1. Biochemistry

Oxidative stress is characterized by an increased concentration of intracellular oxidizing species, such as reactive oxygen species (ROS) and is often accompanied by the loss of antioxidant defense capacity.[1] It is well known that excess ROS attack many organs, and induce oxidative damage directly to critical biological molecules, such as lipoproteins, proteins and nucleotides, causing lipid peroxidation, and protein oxidation. Metabolic oxidative stress has been implicated, directly or indirectly, in the development of diseases and degenerative processes, including inflammation, cancer, dementia and physiological aging.[2] Moreover, oxidative stress also plays a central role in liver pathologies.[3] Antioxidant pharmacotherapy in various forms has emerged as a mean to minimize the biomolecular damage caused by attack of ROS on vital constituents of living organisms.[4] Therefore, there is a great interest in looking for new organoselenides, such as functionalized selenophene rings,[5] which could represent a good pharmacological alternative to counteract oxidative stress.[6]

2. Chemistry

The palladium-catalyzed cross-coupling reactions of aryl halides or triflates with boronic acids, commonly referred to Suzuki–Miyaura reactions, are a powerful, versatile, and popular tool for selective construction of carbon–carbon bonds.[7] The palladium-catalyzed Suzuki–Miyaura cross-coupling reaction of aryl halides with boronic acids and esters has become a common and convenient synthetic method in organic chemistry for biaryl compounds.[8] Many examples of Suzuki coupling reactions, between heterocyclic halides and phenyl boronic acids, have appeared in the literature over the past two decades,[9] being the key stage in the synthesis of many currently interesting heterocycle-incorporated compounds.[10] More recently, significant advances have been made in the use of organoboron reagents as coupling partners in a number of palladium-mediated carbon–carbon bond formation. Among them, the use of potassium organotrifluoroborates, as the organoboron coupling partner, has some advantages in comparison to boronic acids and boronic esters, such as be more nucleophilic, stable in the air, crystalline as solids, and easily prepared.[11]

Chalcogenophene heterocycles (S, Se, and Te) and their derivatives have numerous uses in the fields of biochemistry, physical organic chemistry, materials chemistry, and organic synthesis. For example, selenophene oligomers are compounds of current interest because many of them show photoenhanced biological activities[12] and crystalline polymerizations.[13]

Thus, a wide variety of oligomers and related chalcogen compounds, including mixed thiophene-pyrrole oligomers, have been synthesized mainly with the expectation of obtaining excellent precursor compounds for molecular devices and electroconductive polymers.[14] In addition, chalcogenophenes are widely studied agents with a diverse array of biological effects, these include antioxidant,[15] antinociceptive,[16] and antiinflammatory properties[17] as

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well as efficacy as maturation inducing agents.[18] A great number of these heterocycles have been synthesized and their chemistry has attracted a good deal of interest and activity from a variety of standpoints, such as structures, stereochemistry, reactivities, and applications to organic synthesis.[19] The electrophilic cyclization of alkynes bearing, an organochalcogen moiety, is a powerful approach to the preparation of several functionalized chalcogenophenes with high regioselectivity,[20] particularly, when one considers that there are many ways to transform selectively the resulting halogen, sulfur, selenium, and tellurium functionalities into a great number of interesting substituted heterocycles.

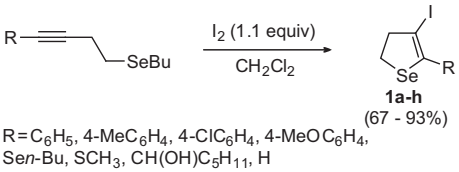
During the course of our research program aiming at structure–activity relationship studies in order to evaluate the pharmacology activity of heterocycles,[5,15–17] we required the synthesis of 2, 3-dihydroselenophenes having a substituted aromatic ring at the 4-position. Since the Suzuki reaction has proven to be successful for the selective arylation of heterocyles,[21] it was of interest to explore this reaction to obtain 4-aryl-2,3-dihydroselenophene derivatives. To our knowledge this methodology has not been explored and we now wish to report the application of 4-iodo-2,3-dihydroselenophenes **1a–h** as substrates on the cross-coupling reaction with boronic acids **2a–j** in the presence of a palladium salt to obtain 4-aryl-2,3-dihydroselenophenes **3a–r** (Scheme 1).

3. Results and discussion

The starting 4-iodo-2,3-dihydroselenophenes **1a–h** were readily available by using the electrophilic cyclization protocol of homo propargyl selenides. The treatment of these selenides with iodine (1.1 equiv) in CH_2Cl_2 at room temperature lead to the formation of the 4-iodo-2,3-dihydroselenophenes **1a–h**. By this methodology a wide range of substituents at 5-position could be used and the starting materials were obtained in isolated yields of 67–93% (Scheme 2).[22]

Our initial studies have focused on the development of an optimum set of reaction conditions. In this way, 4-iodo-5-phenyl-2,3-dihydroselenophene **1a** and phenyl boronic acid **2a** were used as standard substrates. Thus, a mixture of 4-iodo-2,3-dihydroselenophene **1a** (0.25 mmol), phenyl boronic acid **2a** (0.375 mmol) were reacted with different palladium catalysts, bases and solvents, the results are shown in Table 1.

As shown in Table 1, both Pd(0) and Pd(II) catalysts with different ligands were tested, the best result was obtained using $Pd(PPh_3)_4$ 2 mol % which gave the desired product **3a** in 78% yield (Table 1, entry 4). It is important to note that when the amount of catalyst was changed from 2 to 5 mol % and using less than 2 mol % of $Pd(PPh_3)_4$, a decrease in the yields of the coupling products were observed (Table 1, entries 13 and 14). The presence of base was crucial for a clean, high-yielding reaction. For this reason, in our experiments we investigated the influence of other bases. As listed in Table 1, when the reaction was carried out using Na_2CO_3, Cs_2CO_3, KOH, and K_3PO_4 the target product was obtained in poor to moderated yields (Table 1, entries 5–8). The best result was obtained using K_2CO_3, which gave the desired product **3a** in 78% yield (Table 1, entry 4). Regarding the influence of the solvents, all solvents tested (dioxane, toluene, CH_3CN, and THF) gave the product in good yields (Table 1, entries 9–12), however, the highest yield was achieved using a mixture of DMF/H_2O, which furnished the desired product **3a** in 78% yield (Table 1, entry 4). It is also important to mention that when the temperature of the Suzuki reaction was changed from 100 to 70 °C or 110 °C the yields of the coupling products were not improved (Table 1, entries 15 and 16).



Scheme 2.

Table 1
Optimization conditions[a]

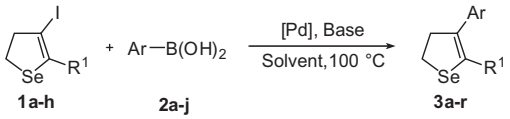
Entry	[Pd]	Base	Solvent	Yield (%)
1	$PdCl_2$	K_2CO_3	DMF	25
2	$Pd(PPh_3)_2Cl_2$	K_2CO_3	DMF	30
3	$Pd(OAc)_2$	K_2CO_3	DMF	10
4	$Pd(PPh_3)_4$	K_2CO_3	DMF	78
5	$Pd(PPh_3)_4$	Na_2CO_3	DMF	46
6	$Pd(PPh_3)_4$	Cs_2CO_3	DMF	57
7	$Pd(PPh_3)_4$	KOH	DMF	10
8	$Pd(PPh_3)_4$	K_3PO_4	DMF	60
9	$Pd(PPh_3)_4$	K_2CO_3	Dioxane	72
10	$Pd(PPh_3)_4$	K_2CO_3	Toluene	77
11	$Pd(PPh_3)_4$	K_2CO_3	CH_3CN	74
12	$Pd(PPh_3)_4$	K_2CO_3	THF	50
13	$Pd(PPh_3)_4$	K_2CO_3	DMF	25[b]
14	$Pd(PPh_3)_4$	K_2CO_3	DMF	63[c]
15	$Pd(PPh_3)_4$	K_2CO_3	DMF	40[d]
16	$Pd(PPh_3)_4$	K_2CO_3	DMF	70[e]
17	$Pd(PPh_3)_4$	K_2CO_3	DMF	55[f]

[a] The reaction was performed using **1a** (0.25 mmol), **2a** (1.5 equiv), [Pd] (2 mol %), base (2 equiv), H_2O (0.4 mL) and solvent (2 mL).
[b] Using 1 mol % of $Pd(PPh_3)_4$.
[c] Using 5 mol % of $Pd(PPh_3)_4$.
[d] Reaction at 70 °C.
[e] Reaction at 110 °C.
[f] Reaction performed using 2 equiv of **2a**.

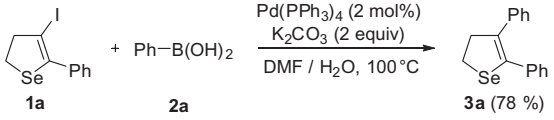
Thus, careful analysis of the optimized reactions revealed that the optimum conditions for this coupling reaction were found to be the use of 4-iodo-5-phenyl-2,3-dihydroselenophene **1a** (0.25 mmol) and phenyl boronic acid **2a** (1.5 equiv), $Pd(PPh_3)_4$ (2 mol %), in DMF/H_2O, at 100 °C. In this condition we are able to prepare 4,5-diphenyl-2,3-dihydroselenophene **3a** in 78% (Scheme 3).

In order to demonstrate the efficiency of this protocol, we explored the generality of our method extending the conditions to other 4-iodo-2,3-dihydroselenophene compounds **1a–h** with different arylboronic acids **2a–j** and the results are summarized in Table 2. First, to determine the real influence of the substituent at aromatic ring of boronic acids, we kept the substrate **1a** invariable. The results revealed that the reaction is not sensitive to the electronic effect of the aromatic ring attached in the boron atom.

For example, arylboronic acid bearing an electron-donating group methoxyl at the *para* position gave a very similar yield than the arylboronic acid bearing an electron-withdrawing group CF_3 (Table 2, entry 3 vs 8). Differentiation in the reactivity between



Scheme 1.



Scheme 3.

Table 2
Products of cross-coupling reaction[a]

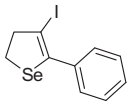
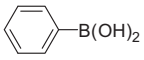
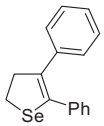
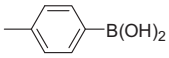
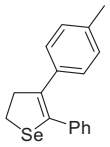
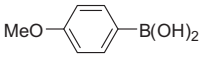
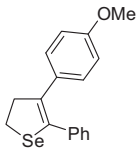
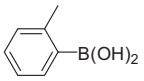
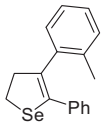
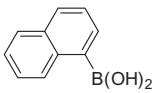
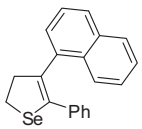
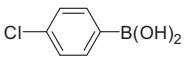
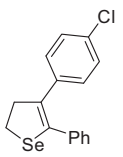
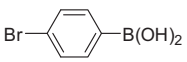
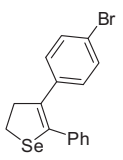
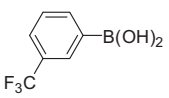
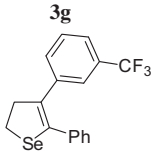
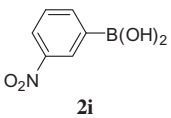
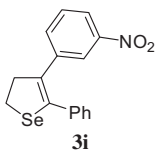
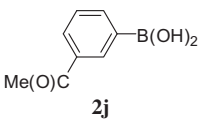
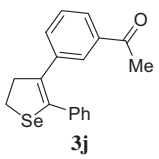
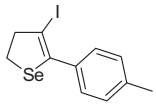
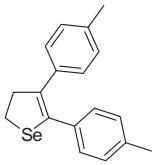
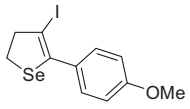
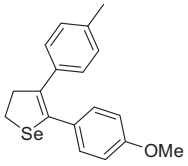
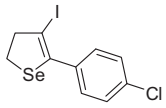
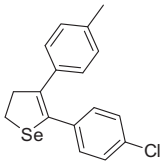
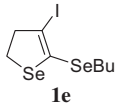
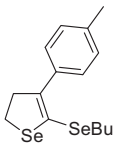
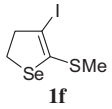
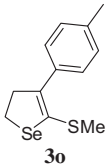
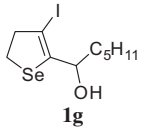
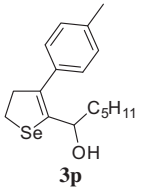
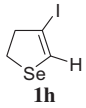
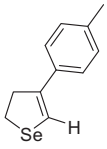
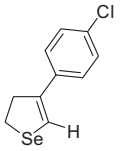
Entry	3-Iodo-dihydroselenophene **1**	Boronic acid **2**	Product **3**	Yield[b] (%)
1	 **1a**	 **2a**	 **3a**	77
2	**1a**	 **2b**	 **3b**	74
3	**1a**	 **2c**	 **3c**	50
4	**1a**	 **2d**	 **3d**	75
5	**1a**	 **2e**	 **3e**	70
6	**1a**	 **2f**	 **3f**	67
7	**1a**	 **2g**	 **3g**	60
8	**1a**	 **2h**	 **3h**	50
9	**1a**	 **2i**	 **3i**	77
10	**1a**	 **2j**	 **3j**	66

Table 2 (*continued*)

Entry	3-Iodo-dihydroselenophene **1**	Boronic acid **2**	Product **3**	Yield[b] (%)
11	 **1b**	**2b**	 **3k**	80
12	 **1c**	**2b**	 **3l**	67
13	 **1d**	**2b**	 **3m**	64
14	 **1e**	**2b**	 **3n**	71
15	 **1f**	**2b**	 **3o**	62
16	 **1g**	**2b**	 **3p**	70
17	 **1h**	**2b**	 **3q**	42
18	**1h**	**2e**	 **3r**	60

[a] The reaction was performed using **1** (0.25 mmol), **2** (1.5 equiv), $Pd(PPh_3)_4$ (2 mol %), K_2CO_3 (2 equiv) in DMF/H_20 (5:1) at 100 °C.
[b] Isolated yields.

halogen and boron atoms can be seen by coupling showed in the experiments described in Table 2, entries 6 and 7, which provide only the Suzuki product, without any homo-coupling product. To the best of our knowledge, aryl halogen could react with boronic acids in the presence of palladium catalysts to afford biaryl products.[23] In our case, the halogen substituent was not affected. We also found that steric effects had a little influence on the coupling reaction. Aryl boronic acid containing a methyl group at the *ortho* position or naphthyl boronic acid, gave slightly difference in the yield of product **3** compared to arylboronic acids no substituted (Table 2, entry 1 vs 4 and 5). In an attempt to broaden the scope of our methodology, the possibility of performing the reaction with other 4-iodo-2,3-dihydroselenophene was also investigated. Then the substrates **1b**–**d**, which have aryl group in the 5-position of

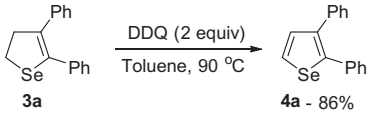
the selenophene ring containing both electron-donating and electron-withdrawing groups, were also cross-coupled efficiently, under the same reaction conditions (Table 2, entries 11–13). Additionally, the substrates **1e** and **1f**, which have an additional chalcogen atom in the 5-position, gave the Suzuki products in good yields (Table 2, entries 14 and 15). These results are highly useful in synthesis, particularly when one considers that there are many ways to transform the resulting chalcogen functionalities into other substituents.[24] Finally, it is worth mentioning that, our reaction system was also suitable for the cross-coupling of 4-iodo-2,3-dihydroselenophene having either propargyl alcohol or with none substituents in the 5 position, giving the desired products in good yields (Table 2, entries 16–18).

Since selenophene derivatives exhibit a broad range of biological activities and applications as intermediates in organic synthesis, we wondered if it would be possible to prepare selenophenes directly from 2,3-dihydroselenophenes. Recently, it was reported that 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) is a useful promoter for the oxidation of 2,3-dihydro-thiophene[25] or -furan[26] to aromatic thiophene or furan. Gratifyingly, we found that the reaction of 2,3-dihydroselenophene **3a** (1 equiv) with DDQ (2 equiv) in toluene at 90 °C gave the selenophene **4a** in 86% yield (Scheme 4).

4. Pharmacology

Since the results from our research group and others have demonstrated that the introduction of a substituent group into unsaturated system of organochalcogen does not alter the pharmacological activity,[6] compound **3a**, a non substituted selenophene, was chosen as a prototype drug to evaluate the antioxidant potential of 4-aryl-2,3-dihydroselenophenes.

Excessive production of ROS is known to affect lipid membrane through lipid peroxidation and also oxidized proteins. Oxidative stress can be accessed through the direct quantification of ROS, antioxidants, and by the measurement of oxidative stress end products. In this study, we investigated the antioxidant effects of compound **3a** on protein and lipid oxidation induced by sodium nitroprusside (SNP). SNP is a good chemical inducer of lipid peroxidation,[27] since it releases NO· in tissue preparations.[28] This radical easily produces peroxynitrite ($ONOO^-$), together with superoxide anion radical ($O_2^{\cdot -}$), thus leading to lipid peroxidation and production of additional free radicals.[29] One product of lipid peroxidation is malondialdehyde, which can be determined by measuring the amount of thiobarbituric acid reactive species.[30] As shown in Fig. 1, compound **3a** at concentrations equal or greater than 30 μM was effective in protecting against lipid peroxidation induced by SNP in rat liver homogenate. The calculated IC_{50} value



Scheme 4.

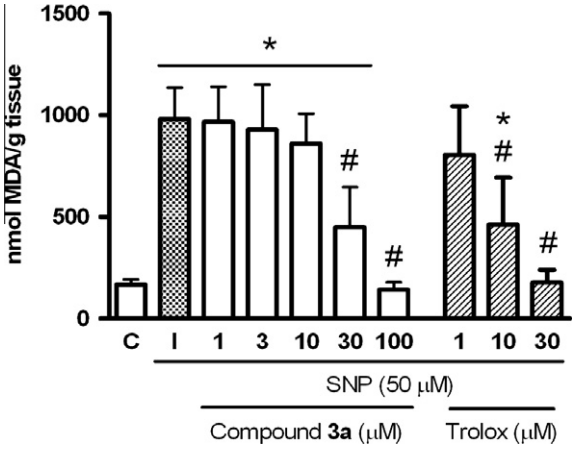


Figure 1. Effect of **3a** on TBARS levels induced by SNP in rat liver homogenate. Data are reported as mean ± SD of four independent experiments. Results were reported as nmol MDA (malondialdehyde)/mg protein. (*) Denotes p <0.05 as compared to the control tube (one-way ANOVA/Duncan). (#) Denotes p <0.05 as compared to the induced tube (one-way ANOVA/Duncan). 'I' means the tube sample containing SNP to induce lipid peroxidation.

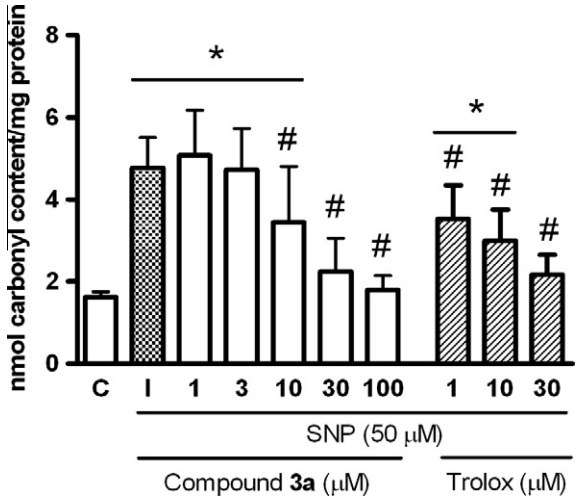


Figure 2. Effect of **3a** on protein carbonyl content induced by SNP in rat liver homogenate. Data are reported as mean ± SD of four independent experiments. Results were reported as nmol carbonyl content/mg protein. (*) Denotes p <0.05 as compared to the control tube (one-way ANOVA/Duncan). (#) Denotes p <0.05 as compared to the induced tube (one-way ANOVA/Duncan). 'I' means the tube sample containing SNP to induce protein carbonylation

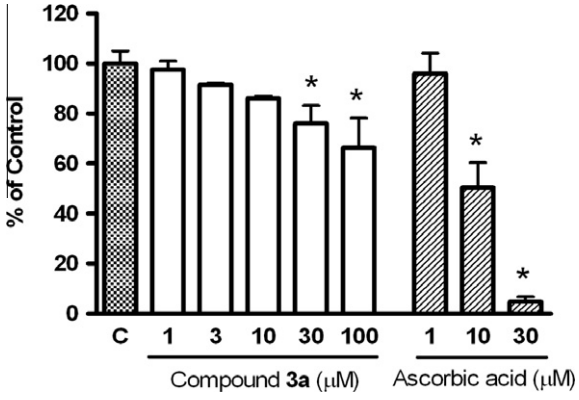
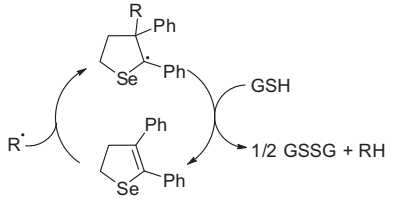


Figure 3. Scavenging activity of compound **3a** on ABTS radicals. Data are reported as mean ± SD of four independent experiments. Results were reported as percentage of the control. (*) Denotes p <0.05 as compared to the control tube (one-way ANOVA/Duncan).



Scheme 5.

was 80 μM. Thus, compound **3a** reduced lipid peroxidation showing a potential antioxidant activity.

A high level of ROS may result in protein oxidation, leading to the production of carbonyl groups. Consequently, the determination of carbonyl content in proteins can be used as a biomarker of oxidative protein damage.[31] Oxidatively modified proteins induced by SNP were significantly reduced by compound **3a** at concentrations equal or greater than 10 μM (Fig. 2). The calculated IC_{50} value was 61 μM. Taken together, these results indicate that compound **3a** was effective in counteracting lipid and protein oxidation.

In addition, **3a** was investigated as a compound able to quench the ABTS radical. Stable radical ABTS has been widely used for the determination of primary antioxidant activities of pure antioxidant compounds, plant and fruit extracts, and foods materials.[32] Compound **3a** showed a significant ABTS radical-scavenging activity at concentrations equal or higher than 30 μM. Although the scavenging activity of **3a** did not outperform ascorbic acid (Fig. 3), the activity of **3a** is better than diphenyl diselenide, a well known antioxidant containing selenium.[33]

Since the selenium atom has a great ability to stabilize radical in the alpha position[34] and higher stability of a tertiary radical intermediate if compared to the possibility of a radical formation in the 2 position, we proposed the mechanism depicted in Scheme 5 to explain the antioxidant activity of compound **3a**. The catalytic cycle shows dangerous radical species adding to the double bond from selenenophene ring and regeneration by thiol reduction of the corresponding 2,3-dihydroselenophene radical.

5. Conclusion

In summary, we have explored the Suzuki–Miyaura cross-coupling reaction of 4-iodo-2,3-dihydroselenophene derivatives with several aryl boronic acids in the presence of catalytic amount of $Pd(PPh_3)_4$ and established a new route to obtain highly substituted 2,3-dihydroselenophene compounds in good to excellent yields. The reaction of compound 2,3-dihydroselenophene **3a** with DDQ afforded the selenophene derivatives **4a** in 86% yield. The findings of the present study indicate that **3a** is a prototype for future drug development programs to treat disorders mediated by reactive oxygen species.

6. Experimental

Proton nuclear magnetic resonance spectra (^{1}H NMR) were obtained at 200 MHz on a DPX-200 NMR spectrometer or at 400 MHz on a DPX-400 NMR spectrometer. Spectra were recorded in $CDCl_3$ solutions. Carbon-13 nuclear magnetic resonance spectra (^{13}C NMR) were obtained either at 50 MHz on a DPX-200 NMR spectrometer or at 100 MHz on a DPX-400 NMR spectrometer. Spectra were recorded in $CDCl_3$ solutions. Column chromatography was performed using Merck Silica Gel (230–400 mesh). Thin layer chromatography (TLC) was performed using Merck Silica Gel GF_{254}, 0.25 mm thickness. For visualization, TLC plates were either placed under ultraviolet light, or stained with iodine vapor, or acidic vanillin. Most reactions were monitored by TLC for disappearance of starting material. MS spectra were determined using a Shimadzu GC–MS-2010P. Elemental analyses were performed on a Vario EL III elemental analysis instrument.

6.1. General procedure for the cross-coupling reaction

To a solution of appropriate 4-iodo-2,3-dihydroselenophene (0.25 mol) in DMF (2 mL) was added $Pd(PPh_3)_4$ (0.0057 g, 2 mol %) and boronic acid (0.375 mmol) under argon. After a solution of K_2CO_3 (0.5 mmol, 0.069 g) in H_2O (0.4 mL) was added. The mixture was then heated at 100 °C. After the required time, the reaction was cooled to rt, diluted with ethyl acetate (20 mL), and washed with brine (2 × 20 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel using hexane and ethyl acetate as eluents.

6.1.1. Selected spectral and analytical data for 4,5-diphenyl-2,3-dihydroselenophene (3a)

Yield 0.055 g (78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.23–7.05 (m, 10H), 3.49–3.44 (m, 2H), 3.38–3.34 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.9, 136.8, 134.0, 133.4, 129.3, 127.1, 128.07, 128.0, 127.4, 126.3, 45.3, 23.3. MS (relative intensity) *m*/*z*: 283 (100), 202 (41), 176 (30), 167 (20), 126 (23), 100 (20). Anal. Calcd for $C_{16}H_{14}Se$ C, 67.37; H, 4.95. Found: C, 67.51; H, 5.01.

6.1.2. 5-Phenyl-4-*o*-tolyl-2,3-dihydroselenophene (3d)

Yield 0.056 g (75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14–7.07 (m, 9H), 3.42–3.38 (m, 2H), 3.32–3.38 (m, 2H), 2.07 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.5, 136.5, 135.6, 135.1, 132.7, 130.2, 128.8, 128.3, 127.8, 127.1, 127.0, 125.8, 46.4, 23.8, 19.7. MS (relative intensity) *m*/*z*: 299 (100), 219 (20), 205 (65), 191 (47), 164 (24), 128 (21), 91 (38). HRMS calcd for $C_{17}H_{16}Se$ $[M+Na]^+$: 323.0315. Found: 323.0321.

6.1.3. 4-(4-Bromophenyl)-5-phenyl-2,3-dihydroselenophene (3g)

Yield 0.054 g (60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27–7.18 (m, 7H), 6.94–6.90 (m, 2H), 3.46–3.33 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.8, 136.5, 134.8, 132.6, 131.1, 129.6, 129.2, 128.3, 127.7, 120.0, 45.0, 23.3. MS (relative intensity) *m*/*z*: 363 (15), 359 (100), 279 (13), 201 (47), 167 (27), 149 (21), 127 (22), 100 (32). HRMS calcd for $C_{16}H_{13}BrSe$ $[M+Na]^+$: 386.9264. Found: 386.9272.

6.1.4. 4,5-Di(*p*-tolyl)-2,3-dihydroselenophene (3k)

Yield 0.062 g (80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12–7.10 (m, 2H), 6.98–6.93 (m, 6H), 3.45–3.40 (m, 2H), 3.34–3.30 (m, 2H), 2.26 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.1, 135.8, 135.2, 134.0, 133.3, 132.5, 129.1, 128.8, 128.6, 127.9, 45.4, 23.0, 21.1, 21.0. MS (relative intensity) *m*/*z*: 314 (100), 233 (20), 218 (36), 202 (21), 182 (29), 141 (12), 115 (19). HRMS calcd for $C_{18}H_{18}Se$ $[M+Na]^+$: 337.0471. Found: 337.0483.

6.1.5. 5-(4-Chlorophenyl)-4-*p*-tolyl-2,3-dihydroselenophene (3m)

Yield 0.053 g (64%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.24–7.12 (m, 4H), 7.01–6.92 (m, 4H), 3.50–3.31 (m, 4H), 2.27 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.4, 135.5, 134.9, 134.7, 133.0, 131.0, 130.6, 128.8, 128.4, 127.9, 45.4, 23.4, 21.1. MS (relative intensity) *m*/*z*: 333 (100), 280 (14), 218 (37), 202 (35), 189 (23), 131 (14). HRMS calcd for $C_{17}H_{15}ClSe$ $[M+Na]^+$: 356.9925. Found: 356.9930.

6.1.6. 5-(Butylselenyl)-4-*p*-tolyl-2,3-dihydroselenophene (3n)

Yield 0.063 g (71%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.25–7.11 (m, 4H), 3.37–3.25 (m, 4H), 2.81 (t, *J* = 7.2 Hz, 2H), 2.33 (s, 3H), 1.66 (qui, *J* = 7.3 Hz, 2H), 1.35 (sext, *J* = 7.3 Hz, 2H), 0.88 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.5, 136.6, 135.3, 128.7, 127.7, 117.1, 44.5, 32.6, 28.9, 24.8, 22.7, 21.2, 13.5. MS (relative intensity) *m*/*z*: 359 (100), 300 (31), 221 (98), 142 (90), 128 (71), 114 (83), 91 (18). HRMS calcd for $C_{15}H_{20}Se_2$ $[M+Na]^+$: 382.9793. Found: 382.9803.

6.1.7. 4-*p*-Tolyl-2,3-dihydroselenophene (3q)

Yield 0.023 g (42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26–7.24 (m, 2H), 7.11–7.09 (m, 2H), 6.93 (t, *J* = 1.7 Hz, 1H), 3.40 (t, *J* = 8.1 Hz,

2H), 3.18 (t, J = 8.1 Hz, 2H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.9, 136.4, 129.1, 125.2, 116.5, 63.9, 38.7, 24.8, 21.1. MS (relative intensity) m/z: 223 (100), 208 (19), 143 (64), 128 (93), 115 (61), 91 (17). HRMS calcd for $C_{11}H_{12}Se$ $[M+Na]^+$: 247.0002. Found: 247.0014.

6.2. General procedure for the aromatization with DDQ

To a solution of dihydroselenophene **3a** (0.25 mmol) in toluene (3 mL), DDQ (2 equiv) was added. The resulting solution was stirred at 90 °C for the desired time. The reaction was diluted with ethyl acetate (30 mL) and washed with aqueous NH_4Cl (3 × 10 mL). After drying the organic phase over anhydrous $MgSO_4$, the solvent was removed under reduced pressure and the residue purified by flash chromatography on silica gel using hexane as eluent.

6.2.1. Selected spectral and analytical data for 2,3-diphenylselenophene (4a)

Yield 0.06 g (86%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.95 (d, J = 5.7 Hz, 1H), 7.43 (d, J = 5.7 Hz, 1H), 7.27–7.18 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.8, 139.8, 137.5, 136.3, 133.7, 129.3, 129.2, 128.9, 128.3, 128.2, 127.1, 126.7. MS (relative intensity) m/z: 283 (91), 268 (22), 202 (100), 189 (17), 176 (16), 101 (27). HRMS calcd for $C_{16}H_{12}Se$ $[M+Na]^+$: 307.0002. Found: 307.0010.

6.3. Biochemistry assay

Animals: Male adult albino Wistar rats (200–300 g) from our own breeding colony were used. The animals were kept on a separate animal room, in a 12 h light/dark cycle, at a room temperature of 22 ± 2 °C, with free access to food (Guabi, RS, Brazil) and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, Federal University of Santa Maria, Brazil.

(a) Thiobarbituric acid reactive species (TBARS) were used as a measure of lipid peroxidation as described by.[30] Rats were euthanized and liver tissue was rapidly removed. Tissue was homogenized in 50 mM Tris–HCl, pH 7.4 (1:10, w/v) and centrifuged at 3000g. The low-speed supernatant (S_1) was separated and used for lipid peroxidation assay. An aliquot of 100 μl of S_1 was incubated at 37 °C for 1 h in the presence of compound **3a** at different concentrations (1–100 μM). TBARS production was stimulated by the incubation of tissues with 50 μM SNP. The absorbance was measured at 532 nm. Trolox was used as a positive control. Results were reported as nmol MDA (malondialdehyde)/mg protein.

(b) Carbonyl content was assayed as described by Levine.[35] Briefly, the liver homogenate was diluted 1:8 (v/v) and incubated 2 h with compound **3a** at different concentrations (1–100 μM) and SNP (50 μM). Then, 1 mL of aliquot was mixed with 200 μl 10 mM 2,4-dinitrophenylhydrazine (DNPH) or 200 μl 2 M HCl. After incubation at room temperature for 1 h in a dark ambient, 500 μl of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing 3% SDS), 1.5 mL of heptane (99.5%) and 1.5 mL of ethanol (99.8%) were sequentially added. The tubes were mixed with vortex agitation for 40 s and centrifuged for 15 min. Next, the protein isolated from the interface was washed two times with 1 mL of ethyl acetate/ethanol 1:1 (v/v) and suspended in 1 mL of denaturing buffer. The absorbance was measured at 370 nm. Trolox was used as a positive control. Results were reported as carbonyl content (nmol/mg protein).

(c) The determination of the ABTS radical scavenging effect of the compound **3a** was performed according to the method of Re,[36] with some modifications. Initially, the ABTS radical was generated by reacting 7 mM ABTS solution in water with 140 mM potassium persulfate in the dark for 12–16 h. In the day of the assay, the pre-formed ABTS radical solution was diluted 1:88 in potassium phosphate buffer, pH 7.0. Briefly, ABTS radical was added to a medium containing the compound **3a** at different concentrations (1–100 μM). The media were incubated for 30 min at 25 °C. The decrease in absorbance was measured at 734 nm. Ascorbic acid was used as a positive control. Results are expressed as percentage of the blank (without compound).

Acknowledgments

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References and notes

1. (a) Arteel, G. E.; Sies, H. *Environ. Toxicol. Pharmacol.* **2001**, *10*, 153; (b) Santos, O. S.; Saraiva, D. F.; Costa, D. C. F.; Scofano, H. M.; Alves, P. C. C. *Exp. Brain Res.* **2007**, *177*, 347.
2. Ren, J. X.; Yang, L. Q.; Liu, J. Q.; Su, D.; You, D. L.; Liu, C. P.; Zhang, K.; Luo, G. M.; Mu, Y.; Yan, G. L.; Shen, J. C. *Arch. Biochem. Biophys.* **2001**, *25*, 250.
3. Vitaglione, P.; Morisco, F.; Caporaso, N.; Fogliano, V. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 575.
4. (a) Day, B. J. *Drug Discovery Today* **2004**, *9*, 557; (b) Mecklenburg, S.; Collins, C. A.; Döring, M.; Burkholz, T.; Abbas, M.; Fry, F. H.; Pourzand, C.; Jacob, C. *Phosphorus, Sulfur Silicon* **2008**, *183*, 863.
5. (a) Wilhelm, E. A.; Jesse, C. R.; Roman, S. S.; Nogueira, C. W.; Savegnago, L. *Exp. Mol. Pathol.* **2009**, *87*, 20; (b) Wilhelm, E. A.; Jesse, C. R.; Bortolatto, C. F.; Nogueira, C. W.; Savegnago, L. *Brain Res. Bull.* **2009**, *79*, 281.
6. (a) Nogueira, C. W.; Rocha, J. B. T. *J. Braz. Chem. Soc.* **2010**, *21*, 2055; (b) Nogueira, C. W.; Zeni, G.; Rocha, J. B. T. *Chem. Rev.* **2004**, *104*, 6255; (c) Prigol, M.; Wilhelm, E. A.; Schneider, C. C.; Nogueira, C. W. *Chem. Biol. Interact.* **2008**, *176*, 129; (d) Rosa, R. M.; Guecheva, T. N.; de Oliveira, I. M.; Braga, A. L.; Henriques, J. A. P. *J. Braz. Chem. Soc.* **2010**, *21*, 2119; (e) Pinton, S.; Luchese, C.; Nogueira, C. W. *Biol. Trace Elem. Res.* doi:10.1007/s12011-010-8645-x.
7. (a) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457; (b) Suzuki, A. *Pure Appl. Chem.* **1985**, *57*, 1749; (c) Suzuki, A. *Pure Appl. Chem.* **1991**, *63*, 419; (d) Suzuki, A. *Pure Appl. Chem.* **1994**, *66*, 213; (e) Suzuki, A. *J. Organomet. Chem.* **1999**, *576*, 147; (f) Oh-e, T.; Miyaura, N.; Suzuki, A. *J. Org. Chem.* **1993**, *58*, 2201; (g) Gronowitz, S.; Hornfeldt, A. B.; Kristjansson, V.; Musil, T. *Chem. Scripta* **1986**, *26*, 305; (h) Goering, B. K. Ph.D. Dissertation, Cornell University, 1995.
8. (a) Suzuki, A. In *Metal-catalyzed Cross-coupling Reactions*; Diederich, F., Stang, P. J., Eds.; Wiley-VCH: Weinheim, 1998; (b) Lloyd-Williams, P.; Giralt, E. *Chem. Soc. Rev.* **2001**, *30*, 145; (c) Buchanan, J. G.; Sable, H. Z. In *Selective Organic Transformations*; Thyagarajan, B. S., Ed.; Wiley-Interscience: New York, 1972; Vol. 2, pp 1–95.
9. (a) Stanforth, S. P. *Tetrahedron* **1998**, *54*, 263; (b) Cailly, T.; Fabis, F.; Legay, R.; Oulyadi, H.; Rault, S. *Tetrahedron* **2007**, *63*, 71; (c) Sicre, C.; Alonso-Gomez, J. L.; Cid, M. M. *Tetrahedron* **2006**, *62*, 11063; (d) Schnurch, M.; Flasik, R.; Khan, A. F.; Spina, M.; Mihovilovic, M. D.; Stanetty, P. *Eur. J. Org. Chem.* **2006**, *15*, 3283.
10. Billingsley, K. L.; Anderson, K. W.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 3484.
11. (a) Molander, G. A.; Ellis, N. *Acc. Chem. Res.* **2007**, *40*, 275; (b) Molander, G. A.; Petrillo, D. E.; Landzberg, N. R.; Rohanna, J. C.; Biolatto, B. *Synlett* **2005**, 1763; (c) Molander, G. A.; Figueroa, R. *Aldrichim. Acta* **2005**, *38*, 49; (d) Molander, G. A.; Felix, L. A. *J. Org. Chem.* **2005**, *70*, 3950; (e) Molander, G. A.; Yun, C. S.; Ribagorda, M.; Biolatto, B. *J. Org. Chem.* **2003**, *68*, 5534; (f) Molander, G. A.; Biolatto, B. *J. Org. Chem.* **2003**, *68*, 4302; (g) Molander, G. A.; Katona, B. W.; Machrouhi, F. *J. Org. Chem.* **2002**, *67*, 8416; (h) Molander, G. A.; Reviro, M. R. *Org. Lett.* **2002**, *4*, 107.
12. (a) Ismail, M. A.; Boykin, D. W.; Stephens, C. E. *Tetrahedron Lett.* **2006**, *47*, 795; (b)*Chemistry and Biology of Naturally-occurring Acetylenes and Related Compounds*; Lam, J., Breteler, H., Arnason, T., Hansen, L., Eds.; Elsevier: Amsterdam, 1988.
13. (a) Nakayama, J.; Konishi, T.; Hoshino, M. *Heterocycles* **1988**, *27*, 1731; (b) Kuroda, M.; Nakayama, J.; Hoshino, M.; Furusho, N.; Kawata, T.; Ohba, S. *Tetrahedron* **1993**, *49*, 3735.
14. Okutan, M. M.; Yerli, Y.; San, S. E.; Yılmaz, F.; Günaydın, O.; Durak, M. *Synth. Met.* **2007**, *157*, 368.
15. Meotti, F. C.; Silva, D. O.; Santos, A. R. S.; Zeni, G.; Rocha, J. B. T.; Nogueira, C. W. *Environ. Toxicol. Pharmacol.* **2003**, *15*, 37.

16. Gonçales, C. E. P.; Araldi, D.; Panatieri, R. B.; Rocha, J. B. T.; Zeni, G.; Nogueira, C. W. *Life Sci.* **2005**, *76*, 2221.
17. (a) Zeni, G.; Lüdtke, D. S.; Nogueira, C. W.; Panatieri, R. B.; Braga, A. L.; Silveira, C. C.; Stefani, H. A.; Rocha, J. B. T. *Tetrahedron Lett.* **2001**, *42*, 8927; (b) Zeni, G.; Nogueira, C. W.; Panatieri, R. B.; Silva, D. O.; Menezes, P. H.; Braga, A. L.; Silveira, C. C.; Stefani, H. A.; Rocha, J. B. T. *Tetrahedron Lett.* **2001**, *42*, 7921.
18. (a) Srivastava, P. C.; Robins, R. K. *J. Med. Chem.* **1983**, *26*, 445; (b) Streeter, D. G.; Robins, R. K. *Biochem. Biophys. Res. Commun.* **1983**, *115*, 544; (c) Kirsi, J. J.; North, J.; McKernan, P. A.; Murray, B. K.; Canonico, P. G.; Huggins, J. W.; Srivastava, P. C.; Robins, R. K. *Antimicrob. Agents Chemother.* **1983**, *24*, 353.
19. (a) Rosário, A. R.; Schumacher, R. F.; Gay, B. M.; Menezes, P. H.; Zeni, G. *Eur. J. Org. Chem.* **2010**, 5601; (b) Stein, A. L.; Alves, D.; da Rocha, J. T.; Nogueira, C. W.; Zeni, G. *Org. Lett.* **2008**, *10*, 4983; (c) Schumacher, R. F.; Alves, D.; Brandão, R.; Nogueira, C. W.; Zeni, G. *Tetrahedron Lett.* **2008**, *49*, 538; (d) Alves, D.; dos Reis, J. S.; Luchese, C.; Nogueira, C. W.; Zeni, G. *Eur. J. Org. Chem.* **2008**, 377; (e) Bui, C. T.; Flynn, B. L. *J. Comb. Chem.* **2006**, *8*, 163.
20. (a) Alves, D.; Luchese, C.; Nogueira, C. W.; Zeni, G. *J. Org. Chem.* **2007**, *72*, 6726; (b) Stein, A. L.; da Rocha, J.; Menezes, P. H.; Zeni, G. *Eur. J. Org. Chem.* **2010**, 705; (c) Alves, D.; Prigol, M.; Nogueira, C. W.; Zeni, G. *Synlett* **2008**, *6*, 914; (d) Kesharwani, T.; Shilpa, A.; Worlikar, S. A.; Larock, R. C. *J. Org. Chem.* **2006**, *71*, 2307; (e) Larock, R. C.; Dawei, Y. *Tetrahedron Lett.* **2001**, *42*, 6011.
21. Barancelli, D. A.; Alves, D.; Prediger, P.; Stangherlin, E. C.; Nogueira, C. W.; Zeni, G. *Synlett* **2008**, 119.
22. Schumacher, R. F.; Rosário, A. R.; Souza, A. C. G.; Menezes, P. H.; Zeni, G. *Org. Lett.* **2010**, *12*, 1952.
23. (a) Arvela, R. K.; Leadbeater, N. E. *Org. Lett.* **2005**, *7*, 2101; (b) LeBlond, C. R.; Andrews, A. T.; Sowa, J. R.; Sun, Y. *Org. Lett.* **2001**, *3*, 1555.
24. (a) Perin, G.; Lenardão, E. J.; Jacob, R. G.; Panatieri, R. B. *Chem. Rev.* **2009**, *109*, 1277; (b) Silveira, C. C.; Braga, A. L.; Vieira, A. S.; Zeni, G. *J. Org. Chem.* **2003**, *68*, 662.
25. (a) Lee, H. S.; Kim, S. H.; Kim, J. N. *Tetrahedron Lett.* **2009**, *50*, 6480; (b) Flynn, B. L.; Flynn, G. P.; Hamel, E.; Jung, M. K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2341.
26. (a) Pohmakotr, M.; Issaree, A.; Sampaongoen, L.; Tuchinda, P.; Reutrakul, V. *Tetrahedron Lett.* **2003**, *44*, 7937; (b) Bellur, E.; Ilia Freifeldb, I.; Langer, P. *Tetrahedron Lett.* **2005**, *46*, 2185.
27. Rauhala, P.; Khaldil, A.; Mohanakumar, K. P.; Chiueh, C. C. *Free Radical Biol. Med.* **1998**, *24*, 1065.
28. Bates, J. N.; Baker, M. T.; Guerra, R.; Harrison, D. G. *Biochem. Pharmacol.* **1991**, *42*, 157.
29. Beckman, J. S.; Beckman, T. W.; Chen, J.; Marshall, P. A.; Freeman, B. A. *Proc. Natl. Acad. Sci.* **1990**, *87*, 1620.
30. Ohkawa, H.; Ohishi, H.; Yagi, K. *Anal. Biochem.* **1979**, *95*, 351.
31. Reznick, A. Z.; Packer, L. *Methods Enzymol.* **1994**, *233*, 357.
32. Chen, C. W.; Ho, C. T. *J. Food Lipids* **1995**, *2*, 35.
33. Luchese, C.; Nogueira, C. W. *J. Pharm. Pharmacol.* **2010**, *9*, 1146.
34. Reich, H. J.; Willis, W. W. *J. Org. Chem.* **1980**, *45*, 5227.
35. Levine, L. R.; Garland, D.; Oliver, C. N.; Amici, A.; Climent, I.; Lenz, A. G.; Ahn, B. W.; Shaltiel, S.; Stadtman, E. *Methods Enzymol.* **1990**, *186*, 464.
36. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M. *Free Radical Biol. Med.* **1999**, *26*, 1231.