Resveratrol-based benzoselenophenes with an enhanced antioxidant and chain breaking capacity†

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The structural modification of the resveratrol scaffold is currently an active issue in the quest for more potent and versatile antioxidant derivatives for biomedical applications. Disclosed herein is an expedient and efficient strategy to a novel class of resveratrol derivatives featuring an unprecedented 2-phenylbenzoselenophene skeleton. The new compounds were obtained in good yields by direct selenenylation of resveratrol with Se(0) and SO2Cl2 in dry THF. Varying the [Se : SO2Cl2 : resveratrol] ratio resulted in the formation of the parent benzoselenophene (1) and/or mono (2) and/or dichloro (3) benzoselenophene derivatives. All the benzoselenophene derivatives proved to be more efficient than resveratrol in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing/antioxidant power (FRAP) assays, with 1 showing an activity nearly comparable to that of Trolox. 1–3 also proved to be more efficient inhibitors than the parent resveratrol in kinetic experiments of styrene autoxidation. DFT calculations of the O–H bond dissociation enthalpy (BDE) revealed that the introduction of the Se-atom causes a significant decrease of the BDE of 3-OH and 5-OH, with just a small increase of the 4-′-OH BDE. Compounds 1–3 showed no cytotoxicity at 5 μM concentrations on human keratinocyte (HaCaT) and intestinal (CaCo-2) cell lines.

Introduction

Natural or natural product-inspired phenolic antioxidants have attracted widespread interest as food supplements and as additives for a broad range of applications. Because of the growing demand for cheap, efficient and non-toxic antioxidants on the part of food, pharmaceutical and polymer industries, considerable efforts are currently devoted to develop practical manipulation strategies for potentiating and tailoring the antioxidant properties of natural phenolic scaffolds based on the structure–property relationships.1

Among the polyphenol-based natural products, stilbene phytoalexin resveratrol is one of the most widely distributed. Isolated from a broad range of plants, resveratrol owes its notoriety to its occurrence in red wine and its implication in the “French paradox”.2

Currently, it is on the market as a food supplement because of its manifold positive activities on human health3 and has been extensively investigated as a versatile platform for the design of natural product-like compounds.4 Some of the biological effects of resveratrol are related to its antioxidant activity, as it prevents the oxidation of LDL in vitro and reduces the markers of oxidative stress in vivo.5 The antioxidant activity depends on the 4′-OH group, which exhibits a superior H-atom transfer and peroxy radical scavenging capacity compared to the resorcinol-type OH groups, due to the olefinic double bond contribution to resonance stabilization of the phenoxy radical.6 However, despite many favorable properties, resveratrol antioxidant activity cannot be compared with that of other natural phenols. Accordingly, the development of new derivatives with a stronger chain breaking and free radical scavenging capacity is an important research goal for both scientific and practical interests.

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1 Electronic supplementary information (ESI) available: NMR spectra of 1–3; optimized stoichiometry ratios for the isolation of 1–3; computational data. See DOI: 10.1039/c5ob00193e
It is well known that the electron-donating groups in the para and ortho positions to the phenolic hydroxyl lower the O–H bond dissociation enthalpy (BDE) and increase the rate of H-atom transfer to peroxyl radicals. In addition, the incorporation of nitrogen atoms into the aromatic ring of phenolic compounds to obtain pyridinols and pyrimidinols has enabled the development of potent radical-trapping antioxidants, characterized by an increased stability under air with respect to the parent phenols.

Several studies support the introduction of 3rd period and higher chalcogen atoms as another effective means of enhancing the antioxidant capacity of phenolic compounds. Selenium-substitution was found to enhance the chain breaking activity via a decrease in the BDE values of the phenolic groups. Similarly, the introduction of an octyltelluro group into the β- or δ-tocopherol system resulted in more efficient quenching of peroxyl radicals via an unusual oxygen transfer process to the chalcogen in the presence of N-acetylcysteine.

In this paper, we have reported selenium-substitution as a means of boosting the resveratrol antioxidant capacity via conversion to 2-phenylbenzoselenophene derivatives showing a Trolox-like antioxidant and chain-breaking capacity.

Results and discussion

Preparation of the benzoselenophene derivatives

The proposed approach for the preparation of the benzoselenophene derivatives hinges on an efficient selenenylation protocol involving in situ generation of SeCl2, a convenient source of electrophilic Se2+ by the reaction of Se(0) with SO2Cl2 (eqn (1)), neat for 10 min, for SO2 evolution, and then in dry THF, followed by the reaction with resveratrol in dry DMF.

\[
\text{Se} + \text{SO}_2\text{Cl}_2 \rightarrow \text{SeCl}_2 + \text{SO}_2 \quad (1)
\]

\[
\text{SO}_2\text{Cl}_2 \rightleftharpoons \text{SO}_2 + \text{Cl}_2 \quad (2)
\]

Using a [Se : SO2Cl2 : resveratrol] ratio of [1 : 0.8 : 0.4], the reaction leads to the benzoselenophene derivative 1 in almost 50% yield (Scheme 1). Compared to other methods examined, the reaction is advantageous in terms of costs and expedient operational conditions, without the need for phenol group protection, a key requirement for facile and scalable procedures. To the best of our knowledge, there is no precedent for one-pot reactions of stilbenes with an electrophilic selenyl species to obtain benzoselenophenes. Although several methods are described for the synthesis of benzoselenophene derivatives, the most efficient ones are based on electrophilic intramolecular cyclization of alkynyl selenocompounds.

The choice of suitable solvents required several attempts. In the first step of the reaction, THF was used since it significantly reduces the well-known disproportionation of monochalcogen dihalides into oligo-chalcogen dihalides. For the second step of the reaction, among the few solvents able to dissolve resveratrol, protic solvents such as water and methanol, or acetone, were avoided because of their reactivity with SO2Cl2 and the formation of electrophilic seleno-species. The best results were eventually obtained with DMF that afforded a good solubility of resveratrol and was compatible with the reaction conditions. On the other hand, when DMSO was used, selenophenes 1–3 (vide infra) were obtained in much lower yields.

Interestingly, analysis of the reaction mixture revealed the presence of small amounts of chlorinated side-products. Accordingly, suitable modification of the stoichiometry ratio between Se(0) and SO2Cl2 allowed one to gain selective access to two additional derivatives identified as the mono- and dichloro derivatives substituted on the resorcinol ring. Thus, the reaction of Se(0) (1 equiv.) with 1 equiv. of SO2Cl2 and resveratrol (0.4 equiv.) led to the isolation of the 4-chlorobenzoselenophene derivative 2 in 71% yield (Table S1, ESI†). On the other hand, using a 1 : 2 ratio between Se(0) and SO2Cl2, the dichlorinated derivative 3 became the major product and was isolated in 82% yield.

These results show that the distribution of the products 1–3 is strictly dependent on the amount of SO2Cl2 used (see Fig. S1 in the ESI† for further details). Notably, the scale of reagents

![Scheme 1](image_url)  
**Scheme 1** One-pot formation of selenophenes 1–3 from the reaction of resveratrol with Se(0)/SO2Cl2.
did not affect the product distribution, which remained substantially the same in reactions run with up to 1.5 g of resveratrol.

Formation of the benzoselenophene derivatives involves probably an electrophilic aromatic substitution as the first step, followed by an electrophilic addition to the double bond, and then HCl elimination leading to 1 (Scheme 1). The efficient ring closure of the selenophene ring implies that 'in situ' generated SeCl₂ reacts almost exclusively with the C-2 nucleophilic carbon of the resorcin moiety of resveratrol and, hence, that the reactivity at C2 is much higher than at C4. Due to the presence of Cl₂ in the reaction mixture (eqn (2))¹⁹ a subsequent chlorination may be envisaged to give 2 and 3. The actual intermediacy of 1 in the process has been demonstrated in separate experiments in which the formation of 2 and 3 was observed by reacting 1 with Se(0)/SO₂Cl₂. Reacting resveratrol with commercially available Se₂Cl₂ did not lead to any detectable selenophene formation. On the other hand, the use of SeCl₄ led to some ability selenophene formation. On the other hand, the use of SeCl₄ led to some (10–15% yield) supporting the superior synthetic value of the adopted procedure.

Whenever chlorination occurred, only one regioisomer of the monochloro derivative was observed under all the reaction conditions examined. The assignment of structure the monochloro derivative was observed under all the reaction conditions examined. The assignment of structure the monochloro derivative was observed under all the reaction conditions examined. The assignment of structure the monochloro derivative was observed under all the reaction conditions examined. The assignment of structure the monochloro derivative was observed under all the reaction conditions examined. The assignment of structure the monochloro derivative was observed under all the reaction conditions examined.

The antioxidant capacity of 1–3 was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assayagainst Trolox in methanol. The time course of DPPH decolorization (Fig. 1) shows that all the selenophene derivatives are more efficient than resveratrol when tested under the same conditions.

Notably, the percentage of DPPH reduced after 10 min in the case of 1 was comparable to that obtained with Trolox (Table 1). The superior activity of selenophenes is also apparent from the data analysis (Table 1), with a ca. four-fold increase of the rate constant for the H-atom transfer in the fast step (k₁ value) in the case of 1 with respect to resveratrol. The stoichiometry (nₜₒₜ in Table 1) of 3.68 and 3.54 found for Trolox and 1 respectively indicates that under the reaction conditions, the oxidation products of these compounds are still capable of reacting with DPPH, as observed in the case of other polyphenols.²¹

The reducing capacity of the selenophene derivatives was measured by the ferric reducing/antioxidant power (FRAP) assay.²² Table 1 reports the results expressed as Trolox equivalents. 1 again showed an activity comparable to that of Trolox, while the chlorinated derivatives 2 and 3, though more active than resveratrol, proved to be less efficient.

### Inhibited autoxidation studies

The chain-breaking antioxidant activity was tested by studying the inhibited autoxidation of styrene in chlorobenzene or acetonitrile (50% v/v) at 30 °C, initiated by AIBN (0.05 M), in the presence of variable amounts of 1–3 and resveratrol (indicated as ArOH in eqn (7) and (8)).¹¹,¹²,¹³ 2,2,5,7,8-Pentamethyl-6-chromanol (α-TOH), an α-tocopherol analogue lacking the phytyl tail, was used as the reference antioxidant.²³
The autoxidation was followed by monitoring the oxygen consumption in an oxygen uptake apparatus based on a differential pressure transducer (Fig. 2). The slope of the oxygen consumption trace during the inhibited period afforded the rate constant for the reaction with peroxyl radicals \( k_{inh} \) (eqn (7)), while its length enabled the determination of the stoichiometric coefficient \( n \) (Table 2).

In the apolar solvent chlorobenzene, the \( k_{inh} \) values of the selenophene derivatives are larger than that of resveratrol, the order being 1 > 2 > 3 > resveratrol (Table 2). This reactivity order is believed to be derived from two overlapping effects: the electron-donating activity of the Se-atom, which causes a lowering of the bond dissociation enthalpy (BDE) of the OH groups, and the H-bond accepting ability of chlorine atoms which increases the BDE of the OH groups, \( \Delta BDE \), of the investigated compounds and phenol, and by adding this value to the known experimental \( BDE(\text{OH}) \) of phenol in benzene (86.7 kcal mol\(^{-1}\)).

Autoxidation experiments in chlorobenzene also provided the stoichiometry of the radical trapping (Table 2), which in all the Se-containing compounds were significantly smaller than 2, that is the value expected for antioxidants acting by eqn (7) and (8) \( \text{i.e. by H-atom transfer followed by radical-radical recombination.} \) It may be suggested that under autoxidation conditions, \text{i.e. in the presence of peroxyl radicals and hydroperoxides, the selenocompounds are partially converted into Se-oxides, which, however, are expected to be poor antioxidants} (Table S2, ESI†). Kinetic data with peroxyl radicals in chlorobenzene are in agreement with the results of the DPPH assay indicating that the selenophenes are more reactive than resveratrol. However, the reactivity order among the selenophenes varies with the solvent, due to the effect of the interplay between the solvent characteristics and the different reaction mechanisms for the reaction with ROO' and DPPH radicals.

**DFT calculations**

To obtain deeper insight into the antioxidant activity of 1–3, the bond dissociation enthalpy (BDE) of the phenolic O–H bonds was investigated by DFT calculations at the B3LYP/LANL2DZdp level. The BDE(\text{OH}) values were obtained by using an isodesmic approach, that consists of calculating \( \Delta \text{BDE} \) between the investigated compounds and phenol, and by adding this value to the known experimental BDE(\text{OH}) of phenol in benzene (86.7 kcal mol\(^{-1}\)). This procedure has been previously tested with phenols having alkyl-selenium substituents in the ortho or para positions, and it reproduced the experimental BDE(\text{OH}) values within ±0.3 kcal mol\(^{-1}\), and therefore, it was expected to provide accurate BDE values for the 3-OH and 5-OH groups. On the other hand, when comparing the calculated 4'-O-H BDE of resveratrol to the experimental value (82.6 kcal mol\(^{-1}\) in benzene), it was found that this BDE value was underestimated by 1.8 kcal mol\(^{-1}\). Considering that an almost identical underestimation is obtained with other DFT-based methods also, the values for 4'-O-H reported in Table 3 and S2 (ESI†) were scaled by adding 1.8 kcal mol\(^{-1}\).

The results reported in Table 3 show that the introduction of the Se-atom causes a significant decrease of the 3-OH and 5-OH BDEs, and an increase of the 4'-OH BDE. The effect on the

### Table 2 Rate constants \( (k_{inh}) \) and number of radicals trapped \((n) \) for the reaction of 1–3 with peroxyl radicals at 30 °C

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Chlorobenzene ( k_{inh}/10^5 ) (M(^{-1}) s(^{-1}))</th>
<th>Acetonitrile ( k_{inh}/10^5 ) (M(^{-1}) s(^{-1}))</th>
<th>( n^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.8 ± 1.8</td>
<td>0.24 ± 0.02</td>
<td>0.66 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>4.9 ± 0.4</td>
<td>0.48 ± 0.05</td>
<td>0.83 ± 0.13</td>
</tr>
<tr>
<td>3</td>
<td>3.5 ± 0.5</td>
<td>0.44 ± 0.04</td>
<td>0.85 ± 0.09</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>1.8 ± 0.2</td>
<td>0.10 ± 0.02</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>α'-TOH(^b)</td>
<td>32(^c)</td>
<td>6.8(^d)</td>
<td>2(^c)</td>
</tr>
</tbody>
</table>

\(^a\) \( n \) Values were measurable only in chlorobenzene. \(^b\) 2,2,5,7,8-Pentamethyl-6-chromanol (reference antioxidant). \(^c\) From ref. 23. \(^d\) From ref. 25.

### Table 3 Calculated BDE(\text{OH}) in the gas-phase at the B3LYP/LANL2DZdp level

<table>
<thead>
<tr>
<th>Compd.</th>
<th>4'-OH</th>
<th>3-OH</th>
<th>5-OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83.8</td>
<td>82.4</td>
<td>83.0</td>
</tr>
<tr>
<td>2</td>
<td>84.5</td>
<td>82.0</td>
<td>84.4</td>
</tr>
<tr>
<td>3</td>
<td>84.8</td>
<td>83.5</td>
<td>83.4</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>82.6</td>
<td>85.0</td>
<td>85.3</td>
</tr>
</tbody>
</table>

\(^a\) For the numbering of the OH groups, see Fig. 3.
3 and 5 positions can be interpreted as being derived from the electron-releasing, radical-stabilizing effect of the chalcogen atom. Calculations showed that a significant spin density is delocalized on selenium (Fig. 3, structures (c) and (d)). However, for the 3 position, a larger BDE would be expected because the 3-OH is H-bonded to the Se-atom, as previously reported for 2-alkylselenophenols. The phenolic OH groups in ortho to hydrogen bond-accepting groups possess larger BDEs than the free ones because the cleavage of the O–H bond also involves the loss of the intramolecular H-bond. Although the Se-atom is not considered a strong H-bond acceptor, nevertheless it has been reported that an ortho octyl-seleno substituent raises the BDE of a phenolic OH by about 3 kcal mol$^{-1}$. Notably, the calculations show that there is no H-bond between Se and the 3-OH, as the most stable structures have the 3-OH group pointing away from the Se-atom (Fig. 3). In the case of 1, for instance, the 3-OH “away” isomer is more stable by 1.5 kcal mol$^{-1}$ than the “toward” one, as already observed in benzo-fused heterocycles containing a sulfur atom ortho to a phenolic OH group.

For what concerns the 4′-OH, the small increase of the BDE(OH) observed on moving from resveratrol to benzoselenophenones may be explained as being due to the perturbation of the stilbene system caused by the Se-atom, which causes a decrease of spin delocalization (Fig. S2, ESI†). The introduction of a Cl-atom in 2 had a small BDE-lowering effect on the 3-OH, in line with the reported effect of chlorine on the BDE of phenols, while it increased the BDE of the 5-OH by 1.4 kcal mol$^{-1}$ because of the formation of a weak intra-molecular H-bond, as Cl-atoms are not good H-bond acceptors. Similarly, the BDE of the 3-OH and 5-OH in 3 are larger than those in 1 because both OH are involved in weak intramolecular H-bonds with the Cl-atoms.

**Cytotoxicity evaluation**

With the aim of evaluating the possible use of the selenoderivatives of resveratrol as topical or systemic antioxidants, the cytotoxicity of 1–3 was preliminarily evaluated in comparison with resveratrol by measuring the proliferation of human keratinocyte cells (HaCaT) and human intestinal (CaCo-2) cells in a time-dependent assay. The results are illustrated in Fig. 4, which reports no significant decrease in cell survival measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction after 24, 48 and 72h of incubation following the addition of benzoselenophene compounds at 5 μM final concentration. No alterations in cell morphology (light microscopy, data not shown) could be observed.

![Optimized geometry of the most stable conformations of 1 (a) and of the phenoxy radicals obtained from the abstraction of the H-atom from the 4′-OH (b), 3-OH (c) and 5-OH (d); spin density in the radicals is also shown.](image-url)

**Fig. 3** Optimized geometry of the most stable conformations of 1 (a) and of the phenoxy radicals obtained from the abstraction of the H-atom from the 4′-OH (b), 3-OH (c) and 5-OH (d); spin density in the radicals is also shown.

![Effect of resveratrol and benzoselenophenones 1–3 on the viability of HaCaT and CaCo-2 cells. The mean values ± SD from three independent experiments run in triplicate are shown.](image-url)

**Fig. 4** Effect of resveratrol and benzoselenophenones 1–3 on the viability of HaCaT and CaCo-2 cells. The mean values ± SD from three independent experiments run in triplicate are shown.
Conclusions

Resveratrol manipulation by selenium chemistry leads to benzoselenophene derivatives as promising lead structures for innovative antioxidants with Trolox-like capacity. Ring closure allowed installment of the selenium center onto the resorcinol moiety enhancing the H-atom transfer ability of the 3-OH and 5-OH groups with respect to the main resveratrol active site, the 4′-OH group, via a consistent decrease of their BDE(O–H).

The marked solvent-dependence of the chain-breaking activity of unsubstituted 1 compared to chlorinated derivatives underscored the role of chlorine atoms as modulators of the deactivating effect of H-bonding with polar solvents on the H-atom donor capacity. The possible advantages of the new benzoselenophene antioxidants relative to the parent compound is the donor capacity. The possible advantages of the new benzoselenophene antioxidants relative to the parent compound is the combination within a single scaffold of the H-atom donor groups (OH) with a hydroperoxide-scavenging center (Se) and the availability of activity-tuning sites, e.g. the chlorine substituents, on the active resorcinol moiety.

Experimental section

Materials and methods

All the commercial materials were used as received without further purification. THF and DMF were dried using a solvent purification system (Pure-Solv™). NMR spectra were recorded in CD2OD at 200, 300 or 400 MHz, 50 or 100 MHz, 38 or 76 MHz for 1H, 13C and 77Se NMR, respectively. The chemical shifts were referenced according to residual solvent signals at 3.31 ppm (1H) and 44.9 ppm (13C). For 77Se spectra, PhSeSePh δSe (76 MHz, CD3OD, PhSeSePh) 462.5. Compound 1 was used as the external reference (461 ppm). Mass spectra were determined by electrospray ionization (ESI) in negative ion mode. Flash column chromatography was performed using silica gel (230–400 mesh). The purity of the isolated products was estimated by 1H NMR analysis. All products were obtained with ≥95% purity.

General procedure for preparation of the benzoselenophene derivatives

All the reactions were carried out using oven-dried glassware under an inert atmosphere (N2). Fresh distilled SO2Cl2 (0.8 mmol, 1 mmol or 2 mmol to obtain 1, 2 or 3 as major products, in that order) was added dropwise to selenium powder (79 mg, 1 mmol) and stirred at rt for 10 min, and then 2.5 mL of distilled THF was added. After 1 h, resveratrol (94 mg, 0.4 mmol) dissolved in 0.8 mL of dry DMF was added. The mixture was stirred for 24 h at rt. The brownish red product was filtered off Celite before extraction with ethyl acetate (3 × 15 mL) and the organic layer was washed with water and brine and dried over anhydrous Na2SO4. The solvent was evaporated under vacuum to afford the crude product, which was purified by flash column chromatography (chloroform/methanol 9 : 1, 1% acetic acid).

2-(4-Hydroxyphenyl)benzo[b]selenophene-5,7-diol (1)

Following the general procedure, from 200 mg of resveratrol, compound 1 was obtained together with 2 (70 : 30 ratio determined by NMR), and purified by flash column chromatography to give a hygroscopic brownish red powder (128 mg, 48% yield). Found C 54.7; H 3.6%; ESI-MS: m/z 304 [M – H]−. C14H10O3Se requires C, 55.1%, H, 3.3%; M, 305.19. NMR: δH (400 MHz, CD2OD, MeSi) 6.25 (1H, d, J = 2.2 Hz, 6-H), 6.72 (1H, d, J = 2.2 Hz, 4-H), 6.81 (2H, d, J = 8.0 Hz, 3′-H, 5′-H), 7.44 (1H, s, 3-H), 7.48 (2H, d, J = 8.0 Hz, 2′-H, 6′-H); δC (50 MHz, CD2OD, MeSi) 99.6 (C-6), 103.1 (C-4), 116.6 (C-3′, C-5′), 122.4 (C-3, C-7a), 128.7 (C-2′, C-6′), 129.4 (C-1′), 146.7 (C-3a), 149.1 (C-2), 155.2 (C-5 or C-7), 157.7 (C-7 or C-5), 158.9 (C-4′); δSe (76 MHz, CD2OD, PhSeSePh) 462.5. Compound 1 was exposed to Se/SO2Cl2 under the standard reaction conditions, and the products formed were analyzed by TLC (eluant chloroform/methanol 9 : 1, 1% acetic acid).

4-Chloro-2-(4-hydroxyphenyl)benzo[b]selenophene-5,7-diol (2)

Following the general procedure, starting from 200 mg of resveratrol, compound 2 was obtained as an 85 : 15 mixture (ratio determined by NMR) with 3. After flash chromatography purification, 2 (211 mg, 71% yield) was isolated as a hygroscopic dark brownish green compound. Found C 49.8; H 2.4%; ESI-MS: m/z 339 ([M – H]−). C14H8Cl2O3Se requires C, 49.5%, H, 2.7%; M, 393.63. NMR: δH (300 MHz, CD2OD, MeSi) 6.41 (1H, s, 6-H), 6.85 (2H, d, J = 9.0 Hz, 3′-H, 5′-H), 7.47 (2H, d, J = 9.0 Hz, 2′-H, 6′-H), 7.66 (1H, s, 3-H); δC (100 MHz, CD2OD, MeSi) 101.2 (C-6), 107.7 (C-4), 117.8 (C-3′, C-5′), 120.8 (C-7a), 121.2 (C-3), 129.8 (C-2′, C-6′), 129.9 (C-1′), 144.6 (C-3a), 151.7 (C-2), 153.6 (C-5 or C-7), 154.7 (C-7 or C-5), 160.2 (C-4′); δSe (76 MHz, CD2OD, PhSeSePh) 498.6.

4,6-Dichloro-2-(4-hydroxyphenyl)benzo[b]selenophene-5,7-diol (3)

Following the general procedure, starting from 200 mg of resveratrol, after flash column chromatography purification, 3 (269 mg, 82% yield) was obtained as a hygroscopic dark brownish compound. Found C 44.9; H 2.4%; ESI-MS: m/z 373 ([M – H]−). C14H8Cl2O3Se requires C, 44.95%, H, 2.1%; M, 374.08. NMR: δH (400 MHz, CD2OD, MeSi) 6.83 (2H, d, J = 8.8 Hz, 3′-H, 5′-H), 7.50 (2H, d, J = 8.8 Hz, 2′-H, 6′-H), 7.65 (1H, s, 3-H); δC (100 MHz, CD2OD, MeSi) 108.2 (C-6), 109.3 (C-4), 117.7 (C-3′, C-5′), 120.9 (C-3), 129.5 (C-1′), 129.9 (C-2′, C-6′), 133.0 (C-7a), 142.2 (C-3a), 149.7 (C-2), 150.4 (C-5 or C-7), 152.1 (C-7 or C-5), 160.4 (C-4′); δSe (76 MHz, CD2OD, PhSeSePh) 510.9.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The assay was performed as described.20 Briefly, to 1.98 mL of 200 μM DPPH in methanol, 20 μL of 5 mM methanolic solution of compounds 1, 2, 3 or resveratrol were added and rapidly mixed. The reaction was followed by spectrophotometric analysis measuring the absorbance at 515 nm every 30 s for 10 min. Trolox was used as the standard.

Ferric reducing/antioxidant power (FRAP) assay

The assay was performed as described.22 To 3.6 mL solution of FRAP reagent, 10–30 μL of 5 mM methanolic solution of compounds 1, 2, 3 or resveratrol (10–70 μM final concentration).
were added. After 10 minutes, the absorbance at 593 nm was measured. Trolox was used as the standard. The FRAP reagent was prepared freshly by mixing 0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-tris(2-pyridyl)-1,3,5-triazine in 40 mM HCl, and 20 mM ferric chloride in water, in the ratio 10:1:1, in that order.

Kinetics with peroxy radicals
The chain-breaking antioxidant activity of the title compounds was evaluated by studying the inhibition of the thermally initiated autoxidation of styrene in chlorobenzene or acetonitrile. Autodissociation experiments were followed by measuring the O2 consumption by using a gas-uptake recording apparatus. In a typical experiment, an air-saturated mixture of styrene in acetonitrile or chlorobenzene (50% v/v) containing AIBN (5 × 10^-5 M) was equilibrated with the reference solution containing also an excess of 2,2,5,7,8-pentamethyl-6-chromanol (α-TOH) in the same solvent at 30 °C. After equilibration, a concentrated solution of the antioxidant was injected into the sample flask (final concentration from 5 × 10^-6 to 5 × 10^-5 M), and the oxygen consumption in the sample was measured. From the slope of the oxygen consumption during the inhibited period, kobs values were obtained as previously reported, while the α coefficient was determined from the length of the inhibited period using α-TOH (n = 2) as a reference.†

Calculations
DFT calculations were carried out using the Gaussian03 program.† Gas phase geometries were optimized at the B3LYP/LANL2DZ level,† with the added diffuse and polarization basis function, i.e. the B3LYP/LANL2DZdp level. Basis sets were from the EMSL basis set library. This level has been previously adopted to calculate the geometries and bond dissociation enthalpies of S, Se and Te containing phenols. The nature of the located stationary points was determined by computation of harmonic vibrational frequencies (zero imaginary frequency). The enthalpies at 298 K were computed at the B3LYP/LANL2DZ level,31 with the added diatomic basis function, Ethanol, methanol, acetaldehyde, pyridine, and acetonitrile. The electronic structure of the title compounds was investigated by density functional theory computations, using the B3LYP/LANL2DZ level31 with the added diatomic basis function.

Cell viability assay
The cytotoxicity of resveratrol and benzoselenophene derivatives 1–3 was evaluated by performing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. Non-tumorigenic human keratinocyte cells (HaCaT) and HEK 293 cells were maintained at 37 °C in a humidified incubator containing 5% CO2. The cells were plated on 96-well plates at a density of 2.5 × 10^3 cells per well in 100 μL of medium containing 10 μL of a 5 mg mL^-1 stock MTT solution in PBS, corresponding to a final concentration of 0.5 mg L^-1 in DMEM (final volume 100 μL) were added to the cells. After 4 h incubation, the MTT solution was removed and the MTT formazan salts were dissolved in 100 μL of 0.1 N HCl in anhydrous isopropanol. Cell survival was expressed as the absorbance of blue formazan measured at 570 nm with an automatic microplate reader.

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References


