Synthesis and Biological Evaluation of Ebselen and Its Acyclic Derivatives

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Five ebselen and three acyclic ebselen derivatives were synthesized. These compounds were screened for their glutathione peroxidase (GSH Px)-like activity and scavenging activity against 1,1-diphenyl-2-pycryl-hydrazyl (DPPH) and peroxynitrite radical. All tested compounds displayed similar significant GSH Px-like activity, which are slightly higher than that of ebselen. The peroxynitrite scavenging activity showed that the acyclic allylseleno 4c was five times more potent than ebselen.

Key words ebselen derivative; selenoorganic compound; antioxidant; peroxynitrite; glutathione peroxidase

Biological systems use enzymatic (superoxide dismutase, glutathione peroxidase, *etc.*) and nonenzymatic (uric acid, creatinine, polyamine, retinal, *etc.*) antioxidant systems to prevent oxidative stress. However, once the systems are disturbed the uncontrolled oxidative stresses initiate a series of harmful biochemical events, or generate them as a consequence of earlier tissue injury, thus aggravating the final damages. Such damages include brain dysfunction, cancer, and cardiovascular disease and inflammation.[1,2)]

Glutathione peroxidase (GSH Px, EC 1.11.1.9), a selenoenzyme, is one of the enzymes in the mammalian antioxidant systems, which perform the reduction of H_2O_2 and other hydroperoxides.[3)] It is well accepted that selenium (as selenocysteine), an essential component of the active sites of glutathione peroxidase, is responsible for the scavenging of reactive oxygen species and protecting biomembrane from oxidative stress. Although possessing potent antioxidative activity, like other protein drugs GSH Px is limited for the clinical use because of its instability, poor availability, and easy metabolism. Therefore many efforts have been directed to design and synthesize small selenium-containing organic molecules with GSH Px activity.[4)] Ebselen (2-phenyl-1,2-benzisoselenazole-3(2*H*)-one), a nontoxic low molecular weight selenium-containing heterocyclic compound, has been reported to mediate the reduction of hydroperoxides by glutathione, thereby mimicking the enzymatic activity of GSH Px.[5)] Beside possessing antioxidative activity, ebselen also demonstrate a number of pharmacologic activities, such as antiinflammatory, antiatherosclerotic, and cytoprotective properties.[6)] Studies in our laboratory have recently focused on the design and synthesis of antioxidants as potential antiinflammatory agents. Based on the unique structure of ebselen, we speculated that simple modification of the chemical structure of ebselen might provide potent antioxidative activity. In this study, the ebselen molecule and its acyclic derivative were modified and their ability to scavenge free radicals and GSH Px-like effects were also investigated.

Chemistry Ebselen has been prepared previously by several methods.[7)] In the earliest approach 2,2′-diselenobis (benzoic acid) was converted to selenyl chloride benzoyl chloride, which was treated with aniline to give ebselen. More recent advance involves ortholithiation of benzanilide, subsequent insertion of selenium into benzanilide-derived dianion and cyclization of selenium-containing dianion to ebselen.[7)] Because of the short synthetic and easy handling steps, the latter method has been used in our synthetic work. Chart 1 summarizes our results and illustrates the structures of the new products obtained from benzoic acid. The *N*-substituted benzoylamides were conveniently synthesized by the treatment of benzoic acid with thionyl chloride, followed by substituted arylamine. Lithiation of *N*-substituted benzoylamides with two equivalents of *n*-butyllithium at room temperature in dry tetrahydrofuran under nitrogen atmosphere gave the dilithiobenzoylamides. Addition of selenium to these intermediates resulted in the expected insertion of selenium into the carbon lithium bond giving dianion intermediates. Cyclization of the dianion intermediates with two equivalents of copper (II) bromide at −78 °C provided compounds **3a**—**f**, respectively. Treatment of **2a**—**c** with two equivalents of *n*-butyllithium, followed by selenium gave the dianion intermediates. Without isolation, these intermediates reacted with either methyl iodide or allyl bromide to furnish compounds **4a**—**c**. It is interesting that **4a** and **4b** were both *N*- and Se-methylated products while **4c** was only a Se-allylic compound. No allylation was observed at the nitrogen atom of **4c**.

Results and Discussion

The free radical-scavenging effects are expressed as the

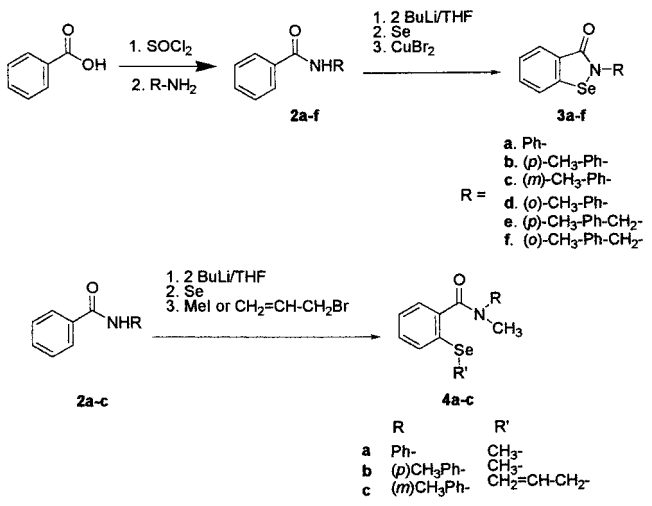

Chart 1

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scavenging concentration (SC_{50}) needed to reduce 50% of the initial amount of 1,1-diphenyl-2-pycryl-hydrazyl radical (DPPH).[8] Ebselen and other tested compounds exhibited little radical-scavenging activity (SC_{50}>5 mM) against DPPH radicals.

The increasing evidence for a role of peroxynitrite in biological processes prompted us to investigate the reaction of compounds with peroxynitrite. Indeed, it has been reported that ebselen rapidly reacts with peroxynitrite and protects against DNA damage caused by peroxynitrite.[9] Peroxynitrite-scavenging activities were determined according to the method reported by Balavoine and Geletii[10] and Bailly *et al.*[11] Briefly, peroxynitrite induced the bleaching of Pyrogallol red (PR) dye, which was measured at 550 nm. Consumption of PR in the presence and absence of the test compounds was measured over a range of peroxynitrite concentrations (0—62.5 μM). Figure 1 shows the D_0/D_A data for different concentrations of antioxidant plotted against $[\text{antioxidant}]_0/[PR]_0$. D_0 and D_A are the stoichiometries for the reaction of peroxynitrite with PR in the absence and presence of the tested antioxidant compounds, respectively. k_A and k_{PR} are the rate constants for the reaction of peroxynitrite with the tested antioxidants and PR, respectively. The ratio k_A/k_{PR}, which represents the relative antioxidant activities, was calculated from the slope of the straight line plotting D_0/D_A against $[A]_0/[PR]_0$. The greater the ratio of k_A/k_{PR}, the more potent the peroxynitrite-scavenging activity of the tested compounds. All tested compounds except for **3c**, **4a**, and **4b** exhibited higher k_A/k_{PR} values than ebselen (Table 1). Compound **4c**, containing the allylseleno moiety, was the most potent one with five-fold greater activity than ebselen. The scavenging potency was in the order of **4c**>**3b**>**3d**>**3f**>**3e**=**3a** (ebselen).

To evaluate the efficacy of the tested compounds as peroxide scavengers, the GSH Px-like activity was investigated. The GSH Px-like activity of ebselen results from two consecutive reactions: the oxidation of the thiols by peroxide and the reduction of the disulphides by glutathione. The consumption of NADPH[11,12] upon the addition of H_2O_2 in the absence of test compounds was 0.8 μM NADPH/min. There was no detectable activity with the solvent DMSO alone. Upon the addition of test compounds, catalytic NADPH consumption occurred, as summarized in Table 1. A typical reaction kinetic plot for compounds **3b**—**f** is also shown in Fig. 2, with the compound **3a** (ebselen) as standard control. The catalytic rates of other compounds are listed in Table 1. Our results indicate that all of the tested compounds have higher GSH Px-like activity than ebselen.

In conclusion, ebselen has been shown to be involved in a variety of effects such as antioxidation, free radical-scavenging, and antiinflammatory activities. Much of these activities can be attributed to its seleno-containing moiety with GSH Px-like activity. In the present study, we synthesized derivatives of ebselen and investigated their potential roles as antioxidants. All of the derivatives showed higher GSH Px-like activity than ebselen. Several of the derivatives possessed potent peroxynitrite-scavenging activity. In particular, **4c**, con-

Table 1. GSH Peroxidase-Like Activity and Inhibition of Test Compounds against Peroxynitrite-Mediated Oxidation

Compound	GSH Px-like activity (relative to ebselen)[a]	Inhibition of peroxynitrite-dependent oxidation	
		k_A/k_{PR}	Activity relative to ebselen
3a (Ebselen)	1.00	0.31	1.00
3b	1.36	0.77	2.48
3c	1.47	0.29	0.94
3d	1.17	0.68	2.19
3e	1.60	0.43	1.39
3f	1.60	0.56	1.81
4a	1.60	0.23	0.74
4b	1.33	0.27	0.87
4c	1.47	1.66	5.35

a) The consumption of NADPH upon addition of H_2O_2 in the absence of test compounds was 0.8 μM/min and the consumption of NADPH for ebselen was 10.9 μM/min.

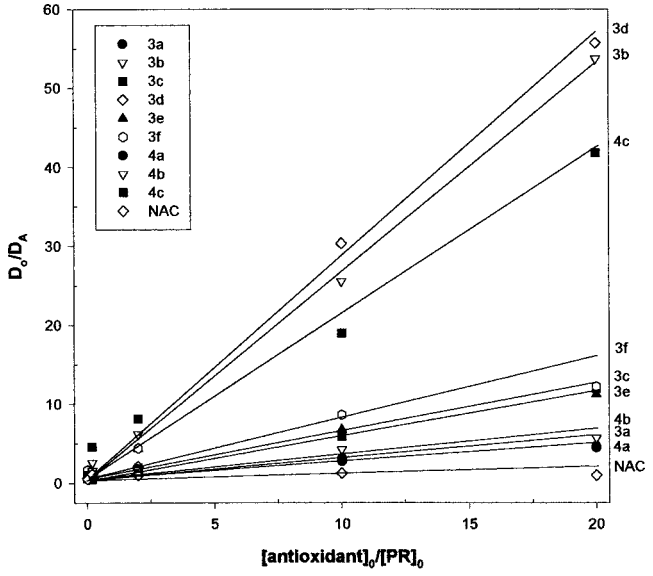

Fig. 1. Plot of D_0/D_A against $[\text{Antioxidant}]_0/[PR]_0$

Reactions were carried out at room temperature by adding peroxynitrite (12.5 μM) into tubes containing phosphate buffer (50 mM, pH 7.0) and 50 μM of $[PR]_0$. Results shown are the mean of two independent experiments.

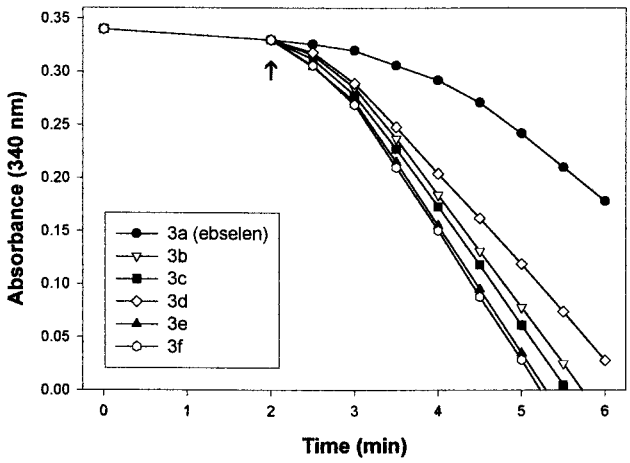

Fig. 2. GSH Px-Like Activity of Tested Compounds and Ebselen (**3a**)

GSH Px-like activity was measured by the disappearance of NADPH. Reactions were initiated with the addition of 1 mM H_2O_2 to the assay mixture containing 1.0 mM GSH, 0.28 mM NADPH, and 1 U/ml glutathione reductase. Ebselen 100 μM and its synthetic derivatives were added 2 min later (arrow). The reaction plot for compound **4a**, **4b**, and **4c** are not shown here, but their activity is listed in Table 1. Results shown here represent the mean of at least two independent experiments.

taining the allylseleno moiety, displayed the highest activity and could serve as a lead compound to be further developed as antiinflammation agent.

Experimental

General Melting points (mp) were taken on a BUCHI 530 apparatus and are uncorrected. Merck Art No 105554 plates precoated with Silica gel 60 containing a fluorescent indicator were used for thin-layer chromatography, and Silica gel 60 (Merck Art No 109385, 230—400 mesh) was employed for column chromatography. Evaporations were carried out at <50 °C using a rotary evaporator at reduced pressure (water aspirator). ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian 300 NMR spectrometer at 300 and 75 MHz, respectively. Where necessary, deuterium exchange experiments were used to obtained proton shift assignments. Mass spectra were recorded on a JEOL J.M.S-300 spectrophotometer. Analytical samples were dried under reduced pressure at 78 °C in the presence of P_2O_5 for at least 12 h unless otherwise specified. Elemental analyses were obtained using a Perkin-Elmer 2400 Elemental Analyzer.

***N*-Substituted-benzoylamide (2a—f), General Procedure** Freshly distilled thionyl chloride (20 ml) was added to a solution of benzoic acid (5.0 g, 41.0 mmol), pyridine (2.0 ml), and dichloromethane (20 ml) and the reaction mixture was heated under reflux for 2 h. The mixture was concentrated under reduced pressure and the residue was coevaporated with toluene (5 ml) three times. Toluene (50 ml) and aniline (3.8 ml, 50 mmol) were added to the residue and the mixture was heated under reflux for 3 h. The solvent was removed under reduced pressure, 10% sodium bicarbonate (20 ml) was added, and the mixture was extracted with dichloromethane (30 ml×3). The organic layer was concentrated and the residue was crystallized with enthanol to give pure **2a** (6.4 g, 80%).

2b—**f** were obtained under the same conditions.

N-Phenyl-benzoylamide (**2a**): *Rf*: 0.40 (*n*-hexane/ethyl acetate=3/1). mp 161—162 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 7.84 (d, 2H, J=5.2 Hz, H-2, H-6), 7.62 (d, 2H, J=7.7 Hz, H-2′, H-6′), 7.52 (t, 1H, J=6.8 Hz, H-4), 7.46 (t, 2H, J=6.9 Hz, H-3, H-5), 7.35 (t, 2H, J=7.0 Hz, H-3′, H-5′), 7.14 (t, 1H, J=7.4 Hz, H-4′). ^{13}C-NMR (DMSO-d_6) δ: 132.9, 130.0, 129.7, 129.0, 125.0, 121.8, 107.4. *Anal.* Cacld for $C_{13}H_{11}NO$: C, 79.16; H, 5.62; N, 7.10. Found: C, 79.06; H, 5.32; N, 7.06.

N-*p*-Toluidyl-benzoylamide (**2b**): *Rf*: 0.36 (*n*-hexane/ethyl acetate=3/1). mp 158—159 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 7.88 (d, 2H, J=6.7 Hz, H-2, H-6), 7.75 (s, 1H, H-4), 7.56—7.50 (m, 4H, H-3, H-5, H-2′, H-6′), 7.19 (d, 2H, J=8.3 Hz, H-3′, H-5′), 2.35 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ: 137.0, 136.7, 135.8, 133.2, 131.1, 130.3, 128.5, 121.9, 22.4. *Anal.* Cacld for $C_{14}H_{13}NO$: C, 79.59; H, 6.20; N, 6.63. Found: C, 79.26; H, 6.32; N, 6.96.

N-*m*-Toluidyl-benzoylamide (**2c**): *Rf*: 0.34 (*n*-hexane/ethyl acetate=3/1). mp 119—120 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 7.95 (d, 2H, J=6.7 Hz, H-2, H-6), 7.63—7.51 (m, 5H, H-3, H-4, H-5, H-2′, H-6′), 7.24 (t, 1H, J=8.3 Hz, H-5), 6.93 (d, 1H, J=7.7 Hz, H-4′), 2.32 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 137.0, 136.7, 135.8, 133.2, 132.1, 130.3, 129.5, 128.6, 121.7, 22.5. *Anal.* Cacld for $C_{14}H_{13}NO$: C, 79.59; H, 6.20; N, 6.63. Found: C, 79.33; H, 6.04; N, 6.86.

N-*o*-Toluidyl-benzoylamide (**2d**): *Rf*: 0.36 (*n*-hexane/ethyl acetate=3/1). mp 144—145 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 7.98—7.89 (m, 3H, H-2, H-6, H-6′), 7.69 (s, 1H, H-4), 7.58—7.49 (m, 3H, H-3, H-5, H-5′), 7.30—7.24 (m, 1H, H-4′), 7.15 (d, 1H, J=7.7 Hz, H-3′), 2.35 (s, 3H, CH_3). ^{13}C-NMR δ (75 MHz, $CDCl_3$): 137.0, 136.7, 135.8, 133.2, 132.1, 130.3, 129.5, 121.7, 23.4. *Anal.* Cacld for $C_{14}H_{13}NO$: C, 79.59; H, 6.20; N, 6.63. Found: C, 79.41; H, 6.45; N, 6.47.

N-(4-Methoxybenzyl)-benzoylamide (**2e**): *Rf*: 0.32 (*n*-hexane/ethyl acetate=4/1). mp 91—92 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 8.99 (s, 1H, NH), 7.89 (d, 2H, J=7.7 Hz, H-2, H-6), 7.53—7.44 (m, 3H, H-3, H-4, H-5), 7.25 (d, 2H, J=8.2 Hz, H-2′, H-6′), 6.90 (d, 2H, J=8.6 Hz, H-3′, H-5′), 4.41 (s, 2H, CH_2), 3.35 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 169.0, 137.0, 136.5, 133.7,132.8, 131.9, 120.0, 128.5, 121.7, 57.6, 39.7. *Anal.* Cacld for $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.41; H, 6.42; N, 6.02.

N-(2-Methoxybenzyl)-benzoylamide (**2f**): *Rf*: 0.36 (*n*-hexane/ethyl acetate=4/1). mp 79—80 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 8.87 (s, 1H, NH), 7.92 (d, 2H, J=7.3 Hz, H-2, H-6), 7.55—7.46 (m, 3H, H-3, H-4, H-5), 7.24—7.16 (m, 2H, H-3′, H-5′), 7.01—6.90 (m, 2H, H-4′, H-6′), 4.45 (s, 2H, CH_2), 3.83 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 169.0, 136.7, 133.5, 133.2, 132.1, 131.9, 129.5, 128.6, 121.7, 58.4, 40.1. *Anal.* Cacld for $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.98; H, 6.22; N, 5.92.

***N*-Substituted-1,2-benzisoselenazol-3(2*H*)-one (3a—f), General Procedure** To a stirred solution of **2a** (1.0 g, 5.1 mmol) in dry tetrahydrofuran (35 ml) under nitrogen at 0 °C was added *n*-butyllithium (6.4 ml, 1.6 M; 10.2 mmol). After 30 min, black selenium powder (0.4 g, 5.1 mmol) was added to the reaction mixture and the reaction was continued for another 30 min. Upon cooling to −78 °C, $CuBr_2$ (2.3 g, 10.2 mmol) was added and the mixture was stirred for 30 min. The cooling bath was removed after 30 min, and the temperature was allowed to reach ambient temperature. After 2 h, the reaction mixture was poured into water (100 ml) containing acetic acid (1 ml) and, after filtration, extracted with dichloromethane. The organic layer was concentrated under reduced pressure. Column chromatography on silica gel with 3 : 1 *n*-hexane/ethyl acetate as eluent gave **3a** (0.2 g, 14%) as a solid white product.

3b—**f** were obtained under the same conditions.

2-Phenyl-1,2-benzisoselenazol-3(2*H*)-one (**3a**): *Rf*: 0.33 (*n*-hexane/ethyl acetate=3/1). mp 180—181 °C (lit.[13]) mp 182—183 °C). ^{1}H-NMR (DMSO-d_6) δ: 8.08 (d, 1H, J=7.9 Hz, H-4), 7.90 (d, 1H, J=7.7 Hz, H-7), 7.71—7.43 (m, 3H, H-6, H-2′, H-6′), 7.47 (dd, J=7.7, 7.9 Hz, 3H, H-5, H-3′, H-5′), 7.27 (t, J=6.9 Hz, 1H, H-4′). ^{13}C-NMR (DMSO-d_6) δ: 132.6, 129.6, 128.3, 126.6, 126.2, 125.0. IR (KBr) cm^{-1}: 3071, 1654. UV λ_{max} (MeOH) nm (log ε): 327 (3.98). FAB-MS *m*/*z*: 276 (M+H$^+$). *Anal.* Cacld for $C_{13}H_9NOSe$: C, 56.95; H, 3.31; N, 5.11. Found: C, 56.78; H, 3.22; N, 5.22.

2-(4-Methylphenyl)-1,2-benzisoselenazol-3(2*H*)-one (**3b**): *Rf*: 0.36 (*n*-hexane/ethyl acetate=3/1). mp 163—164 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.07 (d, 1H, J=8.4 Hz, H-4), 7.89 (d, 1H, J=7.6 Hz, H-7), 7.67 (t, 1H, J=7.7 Hz, H-6), 7.51—7.44 (m, 3H, H-5, H-2′, H-6′), 7.25 (d, 2H, J=8.6 Hz, H-3′, H-5′), 2.32 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 132.5, 130.0, 128.3, 126.6, 126.1, 125.0, 20.9. IR (KBr) cm^{-1}: 3014, 1652. UV λ_{max} (MeOH) nm (log ε): 327 (3.98). FAB-MS *m*/*z*: 289 (M$^+$). *Anal.* Cacld for $C_{14}H_{11}NOSe$: C, 58.34; H, 3.85; N, 4.86. Found: C, 58.68; H, 3.72; N, 5.02.

2-(3-Methylphenyl)-1,2-benzisoselenazol-3(2*H*)-one (**3c**): *Rf*: 0.35 (*n*-hexane/ethyl acetate=3/1). mp 154—155 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.13 (d, 1H, J=8.1 Hz, H-4), 7.66 (d, 2H, J=5.7 Hz, H-2′, H-6′), 7.50—7.27 (m, 4H, H-5, H-6, H-7, H-5′), 7.11 (d, 1H, J=7.1 Hz, H-4′), 2.41 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 140.0, 133.5, 130.4, 129.3, 128.0, 127.6, 127.2, 126.6, 123.2, 22.4. IR (KBr) cm^{-1}: 3004, 1654. UV λ_{max} (MeOH) nm (log ε): 327 (3.81). FAB-MS *m*/*z*: 289 (M$^+$). *Anal.* Cacld for $C_{14}H_{11}NOSe$: C, 58.34; H, 3.85; N, 4.86. Found: C, 58.01; H, 3.70; N, 4.66.

2-(2-Methylphenyl)-1,2-benzisoselenazol-3(2*H*)-one (**3d**): *Rf*: 0.29 (*n*-hexane/ethyl acetate=3/1). mp 173—174 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.89 (d, 2H, J=6.5 Hz, H-4, H-7), 7.61—7.50 (m, 4H, H-5, H-6, H-5′, H-6′), 7.33 (d, 2H, H-3′, H-4′), 2.33 (s, 1H, CH_3). ^{13}C-NMR ($CDCl_3$) δ: 139.0, 135.6, 131.1, 129.3, 128.4, 127.7, 127.2, 125.6, 123.2, 23.4. IR (KBr) cm^{-1}: 3065, 1647. UV λ_{max} (MeOH) nm (log ε): 327 (3.82). FAB-MS *m*/*z*: 289 (M$^+$). *Anal.* Cacld for $C_{14}H_{11}NOSe$: C, 58.34; H, 3.85; N, 4.86. Found: C, 58.21; H, 3.67; N, 4.51.

2-(4-Methoxybenzyl)-1,2-benzisoselenazol-3(2*H*)-one (**3e**): *Rf*: 0.30 (*n*-hexane/ethyl acetate=4/1). mp 150—151 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (d, 1H, J=8.2 Hz, H-4), 7.56 (d, 2H, J=5.7 Hz, H-5, H-7), 7.41—7.27 (m, 3H, H-6, H-3′, H-5′), 6.94 (dd, 2H, J=8.19, 8.13 Hz, H-2′, H-6′), 5.05 (s, 2H, CH_2), 3.91 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ: 131.7, 130.8, 129.7, 128.7, 125.9, 123.7, 120.8, 110.5, 55.3, 43.4. IR (KBr) cm^{-1}: 3065, 1647. UV λ_{max} (MeOH) nm (log ε): 319 (3.80). FAB-MS *m*/*z*: 318 (M$^+$). *Anal.* Cacld for $C_{15}H_{13}NO_2Se$: C, 56.61; H, 4.12; N, 4.40. Found: C, 56.23; H, 3.97; N, 4.51.

2-(2-Methoxybenzyl)-1,2-benzisoselenazol-3(2*H*)-one (**3f**): *Rf*: 0.35 (*n*-hexane/ethyl acetate=4/1). mp 144—145 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.08 (d, 1H, J=7.7 Hz, H-4), 7.57 (d, 2H, J=5.7 Hz, H-5, H-7), 7.45—7.41 (m, 2H, H-6, H-5′), 7.31 (d, 1H, J=8.6 Hz, H-3′), 6.90 (t, 2H, H-4′, H-6′), 4.95 (s, 2H, CH_2), 3.82 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ: 161.4, 133.4, 131.6, 130.4, 127.7, 125.5, 115.8, 56.8, 49.8. IR (KBr) cm^{-1}: 3045, 1642. UV λ_{max} (MeOH) nm (log ε): 319 (3.79). FAB-MS *m*/*z*: 318 (M$^+$). *Anal.* Cacld for $C_{15}H_{13}NO_2Se$: C, 56.61; H, 4.12; N, 4.40. Found: C, 56.33; H, 4.07; N, 4.21.

***N*-Phenyl-(2-alkylseleno)benzoylamide (4a—c), General Procedure** To a stirred solution of **2a** (1.5 g, 7.6 mmol) in dry tetrahydrofuran (50 ml) under nitrogen at 0 °C was added *n*-butyllithium (11 ml, 1.6 M; 17.4 mmol). After 30 min, black selenium powder (0.6 g, 7.6 mmol) was added to the reaction mixture and the reaction was continued for another 4 h. To the reaction mixture methyl iodide (4.6 ml, 7.6 mmol) was added and the mixturre was poured into water. Precipitated solid was recrystallized from methanol to give pure **4a** (0.9 g, 42%) as a white solid.

4a—**c** were obtained under the same conditions.

N-Phenyl-*N*-methyl-(2-methylseleno)benzoylamide (**4a**): *Rf*: 0.3 (*n*-hexane/ethyl acetate=3/1). mp 113—114 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.34—7.13 (m, 8H, H-3, H-4, H-5, H-6, H-2′, H-3′, H-5′, H-6′), 7.0 (t, 1H, J=7.2 Hz, H-4′), 3.49 (s, 3H, N-CH_3), 2.31 (s, 3H, SeCH_3). ^{13}C-NMR ($CDCl_3$) δ: 132.4, 130.8, 130.4, 129.5, 128.3, 128.2, 127.1, 39.3, 9.3. IR

(KBr) cm^{-1}: 3052, 1638. UV λ_{max} (MeOH) nm (log ε): 318 (3.79). FAB-MS *m/z*: 305 (M^+). *Anal.* Cacld for $C_{15}H_{15}NOSe$: C, 59.22; H, 4.97; N, 4.60. Found: C, 58.97; H, 4.74; N, 4.52.

N-Toluidyl-*N*-methyl-(2-methylseleno)benzoylamide (**4b**): *Rf*: 0.28 (*n*-hexane/ethyl acetate=3/1). mp 77—78 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.32 (d, 1H, J=7.59 Hz, H-6), 7.14—7.00 (m, 7H, H-4, H-5, H-3, H-2′, H-3′, H-5′, H-6′), 3.45 (s, 3H, N-CH_3), 2.31 (s, 3H, $SeCH_3$), 2.24 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ: 138.1, 132.4, 131.1, 130.7, 129.5, 128.0, 127.1, 39.3, 22.5, 9.3. IR (KBr) cm^{-1}: 3023, 1633. UV λ_{max} (MeOH) nm (log ε): 318 (3.79). FAB-MS *m/z*: 319 (M^+). *Anal.* Cacld for $C_{16}H_{17}NOSe$: C, 60.38; H, 5.38; N, 4.40. Found: C, 60.49; H, 5.19; N, 4.34.

N-Phenyl-(2-allylseleno)benzoylamide (**4c**): *Rf*: 0.46 (*n*-hexane/ethyl acetate=3/1). mp 106—107 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.15 (s, 1H, H-6), 7.73—7.58 (m, 3H, H-3, H-4, H-5), 7.41—7.27 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.19 (t, 1H, J=7.40 Hz, H-4′), 6.00—5.86 (m, 1H, $CH=CH_2$), 5.10—4.98 (m, 2H, $CH=CH_2$), 3.55 (d, 2H, J=7.14 Hz, $SeCH_2$). IR (KBr) cm^{-1}: 3282, 3052, 1638. UV λ_{max} (MeOH) nm (log ε): 321 (3.82). FAB-MS *m/z*: 317 (M^+). *Anal.* Cacld for $C_{16}H_{15}NOSe$: C, 60.76; H, 4.78; N, 4.43. Found: C, 60.54; H, 4.57; N, 4.51.

Determination of the Scavenging Effect on 1,1-Diphenyl-2-pycryl-hydrazyl radical (DPPH·) The experimental procedure was adapted from Ohnishi *et al.*[14] The ethanolic solution of DPPH· was added to 2 ml of the test compounds at different concentrations in ethanol (12.5, 25, 37.5, 50 μM). Each mixture was then shaken vigorously and kept for 30 min at room temperature in the dark. The decrease in absorption of DPPH· at 517 nm was measured. Ethanol was used as a blank solution and DPPH· solution in ethanol served as the control. The percentage of remaining DPPH· was then calculated, and the radical-scavenging effects of the tested compounds were compared in terms of IC_{50} (the concentration needed to reduce 50% of the initial amount of DPPH· and expressed as the molar ratio of each compound to the radical). All tests were performed in triplicate.

Determination of the Scavenging Effect on Peroxynitrite Peroxynitrite synthesis was carried out as described by Radi *et al.*[15] Briefly, acidified hydrogen peroxide (1 M in 0.7 M HCl, 20 ml) and sodium nitrite (0.2 M, 20 ml) solution were drawn into two separate syringes. The contents of both syringes were simultaneously injected into an ice-cooled beaker containing 1.5 M potassium hydroxide (40 ml). Manganese dioxide was added to the solution to remove excess hydrogen peroxide. The solution was filtered and the concentration of the resulting stock was determined spectrophotometrically at 302 nm (ε=1670 M^{-1} cm^{-1}). The typical yield of freshly prepared peroxynitrite was 30 mM. The peroxynitrite was diluted in 0.1 M NaOH. Experiments were conducted at 25 °C in 50 mM phosphate-buffered saline containing 0.1 mM diethylenetriaminepentaacetic acid, 90 mM NaCl, and 5 mM KCl, pH 7.4. Blanks using DMSO alone in the absence of test compounds and peroxynitrite allowed to degrade for 5 min in phosphate-buffered saline, pH 7.4, were also examined. There was no interference by DMSO and degraded peroxynitrite on the PR. Peroxynitrite induced the bleaching of PR dye, which was measured at 542 nm (ε=24000 M^{-1} cm^{-1}). Consumption of PR (50 μM) in the presence and absence of test compounds was measured over a range of peroxynitrite concentrations (0—62.5 μM). Antioxidative activities were determined according to the methods reported by Balavoine *et al.*[10] The ratios of rate constants k_A/k_{PR}, which represent the relative antioxidant activities, were determined by plotting D_0/D_A against $[\text{antioxidant}]_0/[PR]_0$. k_A and k_{PR} are the rate constants for reaction of peroxynitrite with the antioxidants and PR, respectively. D_0 and D_A are the stoichiometries for the reaction of peroxynitrite with PR in the absence and presence of the antioxidant compounds, respectively. *N*-Acetyl cysteine was used as a control compound and our results showed that it has a very low level of peroxynitrite-scavenging activity.

Determination of GSH Px-Like Activity GSH Px-like activity was determined by the reduction of GSSG formed *via* the NADPH-glutathione reductase system as a continuous indicator system.[16] Loss of NADPH was monitored continuously at 340 nm using a spectrophotometer (Perkin-Elmer, Shelton, CT, U.S.A.) and a molar absorption coefficient of 6220 M^{-1} cm^{-1}. Experiments were conducted at room temperature in 50 mM phosphate-buffered saline, pH 7.6, containing 1.0 mM EDTA and 1.0 mM NaN_3, 1.0 mM GSH, 0.25 mM NADPH, 1 U of GSH reductase and 10—100 μM of test compounds. The reaction was initiated by addition of 0.5 mM *tert*-butylhyroperoxide, and ebselen or synthetic compounds were added later. GSH Px-like activity was proportional to the decrease in NADPH absorption at 340 nm.

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