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ARTICLE

Catalytic and Highly Regenerable Aminic Organoselenium Antioxidants with Cytoprotective Effects

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Novel *N*-methylated ebselenamine antioxidants were prepared from their corresponding diselenides with iodomethane. All ebselenamines showed excellent chain-breaking and glutathione peroxidase (GPx)-like activities. These could also inhibit the lipid peroxidation much more efficiently than α -tocopherol. They could also mimic the functions of the GPx-enzymes nearly two times better than ebselen in the coupled reductase assay. Also, these were found to scavenge the ROS produced at low concentration (10 μ M) with low toxicity effects and would have the therapeutic potential against autoxidation. It is anticipated that these compounds would have the potential to be used in several diseases caused by autoxidation, and thus, providing protection for mammals from cell death.

Introduction

Under pathological conditions, autoxidation is directly linked to oxidative degradation of lipids (i.e. lipid peroxidation) and some biologically important molecules.¹ Both phenols and diarylamines have been considered good radical-trapping antioxidants to inhibit the rate of autoxidation in biological processes, food stuffs, man-made materials and antioxidant pharmacotherapy.²⁻⁴

The 21st century is much devoted for better improvement of radical-trapping activity of amines and phenols.⁵⁻⁶ Nature has provided us α -tocopherol (**1**) as good chain-breaking antioxidant, the most biologically active component of vitamin E family *in vivo* and inhibits the lipid peroxidation by donating

that substituted diphenylamines with electron donating groups (EDGs) and *N*-heterocycles weakened the bond dissociation enthalpy (BDE_{N-H}) of the N-H bond, thus improving the radical-trapping capacity.^{5c,8} The weak BDE_{N-H} of **2** (~82 kcal/mol) is believed for the faster radical-trapping kinetics as compared to phenols (87.2 kcal/mol).⁹ Glutathione peroxidase (GPx) enzymes are the mammalian selenoproteins *in vivo* have preventive antioxidant functions i.e. decomposing hydrogen peroxide and phospholipid hydroperoxides into water and alcohols; respectively using reduced glutathione (GSH) as the stoichiometric agent.¹⁰ Since 1984, ebselen (**3**) has been known for its GPx-like antioxidant activity *in vivo* and *in vitro*.¹¹ It has been under clinical trials for the prevention of diseases as therapeutic potential drug, found to reduce mitochondrial oxidative stress after spinal cord injury and therapeutic potential drugs.¹²⁻¹³ It showed promising antiviral activity of main corona virus protease (M^{Pro}) in SARS-CoV-2 and the corresponding pandemic disease COVID-19.¹⁴ Structurally modified ebselen analogues have been subjected to enhance the antioxidant properties.¹⁵⁻¹⁶ Recently, we described cyclic organoselenium compounds substituted with an OH group that were found to show good chain-breaking and GPx-like antioxidant activity.¹⁷ Herein, we report the syntheses of *N*-methylated ebselenamines organoselenium compounds are as yet, unknown. We also describe the chain-breaking and GPx-like activities as well as the remarkable cytoprotective effects on mammalian cells.

Fig. 1 Some representative antioxidants 1-3.

H-atom from the nearby phenolic OH group to the peroxy radical to give non-radical products.⁷ Since the 1950s, 4,4'-dialkyldiphenylamines (**2**) as the industry standards has attracted great attention as the most effective radical-trapping considered after phenols, used additives as favorite stabilizer to petroleum-derived products.^{5c} In 2002, Pratt et al. found

Results and discussion

Readily prepared diselenides **5a-5d** from **4a-4d** has been used for the construction of novel *N*-methylated ebselenamines **6a-6d**. Reduction of **4a-4d** with *in situ* prepared PhTeNa produced diselenides **5a-5d** (Scheme 1).¹⁸

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Scheme 1 Synthesis of N-methyl ebselenamines **6a-6d**.

Compounds **5a** and **5c** were isolated in 47% and 85% yields, respectively. Identity of **5b** and **5d** was confirmed as previously reported.¹⁸ Furthermore, reactions of diselenides **5a-5d** with four equivalents of iodomethane (MeI) in deuterated dimethyl sulfoxide (DMSO- d_6) at room temperature (rt) afforded **6a** (38%), **6b** (27%), **6c** (29%) and **6d** (33%) in moderate yields. Addition of stoichiometric quantity of MeI was not enough and led to incomplete cyclization. Therefore, four equivalents of MeI were found very crucial to complete the reaction. All reactions were carried out at room temperature in NMR tube containing DMSO- d_6 and the formations of **6a-6d** were monitored by the ^{77}Se NMR spectroscopy. Interestingly, no other traces were observed in the ^{77}Se NMR spectrum except **6a-6d**. The ^{77}Se NMR chemical shifts of **6a-6d** were found very close with the calculated values 933-975 ppm (Table S5 in the Supporting information). The modest isolated yield of **6a-6d** encouraged us to perform the reaction with conventional magnetic stirring with the intention to improve the yield. Surprisingly, compound **6d** was obtained in 42% yield. Our efforts could not cause much in other solvents and the obtained yield was on expected lines as in previous method. In these reactions, we observed that the primary amine group ($-\text{NH}_2$) in diselenides acted as nucleophile towards MeI, leading to the formation of the N-methylated intermediate via the $\text{S}_\text{N}2$ reaction (Scheme S1 in the Supporting Information). Therefore, a strain in the aromatic ring of the molecule bearing two *ortho*-coordinating groups led to unexpected heterocycles.¹⁶⁻²⁰ Further, N-methylation at the $-\text{NHMe}$ group in **6a-6d** could not take place due to steric hindrance at *ortho* to the Se atom as indicated in the crystal structure of **6c** (Fig. 2). An intramolecular H-bonding ($\text{Se}\cdots\text{H}$ 2.842 Å) in molecule was observed significantly shorter than the sum of the van der Waals radii (3.10 Å).²¹

Fig. 2 ORTEP diagram of **6c** with 50% thermal ellipsoids probability.

Peroxidation of linoleic acid was azo-initiated at 42 °C by thermal decomposition of 2,2'-azobis 2,4-dimethylvaleronitrile in lipid phase (chlorobenzene layer) containing lipid-soluble antioxidants **6a-6d** in a two-phase lipid/aqueous system in the presence of atmospheric oxygen.²² L-Ascorbic acid (AscOH), a water soluble co-antioxidant present in the upper aqueous phase could serve regeneration of these antioxidants. The progress of lipid peroxidation was monitored for 700 min by automatic HPLC for the conjugated diene formation of linoleic acid hydroperoxide (LOOH). A graph was plotted against conjugated diene formation versus time. The rate of inhibition (R_{inh}) i.e. slope of the line during the inhibited phase of peroxidation and the inhibition time (T_{inh}) i.e. regenerability of the antioxidant obtained from the graph (Fig. 3, vide infra). Efficient chain-breaking antioxidants could quench peroxy radicals by keeping the low value of R_{inh} .

α -Tocopherol (**1**) and ebselen (**3**) were used as references in this study. Without AscOH (co-antioxidant), compound **1** could inhibit the lipid peroxidation ($R_{\text{inh}} = 28 \pm 2 \mu\text{M/h}$ and $T_{\text{inh}} = 109 \pm 5 \text{ min}$) in Table 1. The stoichiometric number (n) for **1** is 2. It means that each molecule of **1** can quench two peroxy radicals before converted into non radical products. Both R_{inh} and T_{inh} recorded in the presence of co-antioxidant (0.5 and 1.0 mM) were also found to be almost as without AscOH. In the absence of AscOH, **6a-6d** could match **1** when it comes to reactivity towards peroxy radicals and inhibition time. This indicated that the peroxy radical quenching rate was only due to H-atom transfer (HAT). We speculated that in **6a-6d**, the Se atom was readily oxidized from its divalent state (Se^{II}) to tetravalent state (Se^{IV}) (selenoxide **11**, vide infra) due to a small amount of LOOH present in commercially available linoleic acid. The resulting selenoxides formed would be considered as poor retarders of peroxidation than inhibitors. AscOH and other co-antioxidants (thiols) are responsible for the reduction of selenoxide formed and always keep antioxidants in active divalent state.^{22b} While **6a-6d** with ascorbic acid (0.5 and 1.0 mM) outperformed **1** remarkable aminic radical quenching activity and regenerability (Table 1).

Fig. 3 Peroxidation traces (conjugated diene formation vs time) recorded using compounds **1**, and **6b** as antioxidants in the chlorobenzene layer in the presence of AscOH (1 mM) in the aqueous phase.

Compounds **6b** and **6c** were found to be the most active, whether AscOH was 0.5 or 1.0 mM present in the aqueous phase. T_{inh} for **6b** was significantly increased two-fold longer

(252 min) and five-fold longer (588 min) than **1** in the presence of ascorbic acid as 0.5 and 1.0 mM, respectively. Similarly, T_{inh} for **6c** was significantly increased upto two fold- longer (335 min) and five-fold longer (416 min). The R_{inh} values suggested that **6b** and **6c** quenched the peroxy radicals with a stoichiometric numbers as high as $n = 4$ than **1** ($n = 2$). Peroxyl radical quenching efficiency and the regenerability of **6a-6d** was better than **1**. A trend of increasing of T_{inh} (**6a** < **6d** < **6c** < **6b**) was observed. Recorded linoleic acid peroxidation traces of **1** and **6b** were shown in Fig. 3.

As expected, **3** did not cause inhibition but slightly retarded peroxidation. AscOH could regenerate antioxidants **6a-6d** and, therefore, inhibit the peroxidation by keeping very low R_{inh} values. These outcomes indicated that the EDGs would therefore extend the regenerability by increasing the lipophilicity of **6b** and **6c** in the lipid layer. EDGs could cause the lower BDE_{N-H} and, therefore, the rate of HAT to the peroxy radical from the nearby aminic group increased.^{5c,23}

The strength of the N-H bond in **6a-6d** was calculated in gas phase as the energy difference between optimized molecules and the aminyl radical like intermediate **8** (Scheme 3) plus hydrogen radical (see Table S1-S4 in the Supporting Information). The (BDE_{N-H} of the N-H bond values for **6a-6d** were ~ 79 kcal/mol indicating almost ca. 3 kcal/mol lower than **2** (Table S5 in the Supporting Information).^{5c} Since, the N-H bond **6a-6d** is 2 kcal/mol stronger than the O-H bond ($BDE_{O-H} = 77.3$ kcal/mol) in α -tocopherol.⁷ Even though, ebselenamines **6a-6d** quenches the peroxy radicals more efficiently than **1**, which seems likely to be operative by different pathway mechanism.

Table 1. Inhibited Rates (R_{inh}) and Inhibition Times (T_{inh}) of Conjugated Diene Formation with and without AscOH for **1**, **3** and **6a-6d**. The GPx-like activities of **3**, **5a**, **5c** and **6a-6d** determined by the initial rate of NADPH consumption (v_0).

	With AscOH				Without AscOH		GPx activity
	(0.5 mM)		(1.0 mM)				
	$R_{inh}^{(a)}$ ($\mu\text{M}/\text{h}$)	$T_{inh}^{(b)}$ (min)	$R_{inh}^{(a)}$ ($\mu\text{M}/\text{h}$)	$T_{inh}^{(b)}$ (min)	$R_{inh}^{(a)}$ ($\mu\text{M}/\text{h}$)	$T_{inh}^{(b)}$ (min)	
1	21 $\pm$ 3	115 $\pm$ 8	23 $\pm$ 2	125 $\pm$ 3	28 $\pm$ 2	109 $\pm$ 2	----
3	510 $\pm$ 7	0	512 $\pm$ 5	0	537 $\pm$ 4	0	47 $\pm$ 1
5a	----	----	----	----	----	----	240 $\pm$ 1
5c	----	----	----	----	----	----	248 $\pm$ 5
6a	13 $\pm$ 3	146 $\pm$ 8	18 $\pm$ 2	224 $\pm$ 3	38 $\pm$ 4	66 $\pm$ 3	83 $\pm$ 4
6b	9 $\pm$ 1	252 $\pm$ 9	12 $\pm$ 3	588 $\pm$ 6	35 $\pm$ 3	92 $\pm$ 4	120 $\pm$ 3
6c	8 $\pm$ 2	335 $\pm$ 6	11 $\pm$ 3	416 $\pm$ 7	30 $\pm$ 4	85 $\pm$ 3	106 $\pm$ 6
6d	18 $\pm$ 1	206 $\pm$ 5	17 $\pm$ 2	352 $\pm$ 6	31 $\pm$ 4	91 $\pm$ 5	87 $\pm$ 3

(a) Rate of peroxidation during the inhibited phase (uninhibited rate ca. $R_{uninh} = 569$ $\mu\text{M}/\text{h}$). (b) Duration of the inhibited phase of peroxidation. (c) Assay conditions: Phosphate buffer (100 mM), pH 7.5 with EDTA (1 mM), GSH (1 mM), NADPH (0.2 mM), GR (1.3 unit/mL), H_2O_2 (0.80 mM) and catalysts (20 μM). Stock solutions for catalysts **6a-6c** were prepared in DMSO and for **3** and **6d** were prepared in MeOH. Initial rates (v_0) were corrected for the spontaneous oxidation of GSH (27 ± 1 $\mu\text{M}/\text{min}$). Errors correspond to $\pm$ SD for triplicates.

An unconventional mechanism of **6a** proposed involving proton-coupled electron transfer based on experimental and theoretical calculation studies (Scheme 3, Cycle A).^{17, 22b} The mechanism involves the initial O-atom transfer from peroxy radical ($\text{LOO}^\bullet$) to the Se atom resulting alkoxy radical ($\text{LO}^\bullet$) and would then exchange an H-atom from the nearby $-\text{NHMe}$ group *in situ* leading to aminyl radical selenoxide (**7**). The regeneration of **6a** occurs by AscOH at the liquid-aqueous interphase and is together with the dihydroascorbate (DHA) formation to allow for a catalytic mode of action.

Newly prepared diselenides **5a**, **5c** and ebselenamines **6a-6d** have been evaluated for their antioxidant activity in the coupled reductase assay.²⁴ Compounds **6a-6d** (83-120 $\mu\text{M}/\text{min}$) exhibited greater activity than **3** (47 $\mu\text{M}/\text{min}$) as shown in Table 1. The greater activity of **6a-6d** may be due to the presence of the very close proximity effect of the $-\text{NHMe}$ group to the Se atom.

Scheme 3 Proposed mechanism for radical quenching (Cycle A) and the decomposition of H_2O_2 (Cycle B) by **6a**.

The effect of EDGs could also dramatically enhance the activities of **6b** and **6c**, having –Me and –OMe groups at the *N*-phenyl ring, respectively.

Fig. S31 NADPH-consumption with time in the absence (control) and presence of catalysts ebselen (**3**), **6b** and **6c**. Assay conditions: reactions were carried out with phosphate buffer (100 mM), pH 7.5, with EDTA (1 mM), GSH (0.10 mM), NADPH (0.20 mM), GR (1.3 unit/mL), H_2O_2 (20 μM) and catalyst (2 μM).

The performance of the GPx-mimics **6b** and **6c** including **3**, was also followed for much longer time (300 min) during the initial 10 s of the reaction (Fig. 4).

We carried out the ^{77}Se NMR study for the decomposition of H_2O_2 by **6a** in DMSO-d_6 (Scheme 3, Cycle B). Addition of (2 + 2) equiv of H_2O_2 to the NMR tube containing a solution of **6a** (926 ppm) in DMSO-d_6 showed no change in the ^{77}Se NMR spectrum. When 3 equiv of PhSH was added to the NMR tube, signal at 926 ppm of **6a** disappeared completely. Two new signals were observed at 407 and 50 ppm corresponding to selenosulfide **9** and selenol **10**, respectively. This is in contrast with the ebselen where thiol exchange reactions take place and prevent the formation of the recetive selenol specie.²⁵ Further, addition of 3 equiv of PhSH to the above mixture, major signals for **9** and **10** were present. Addition of 6 equiv H_2O_2 to the NMR mixture, signals for **9** and **10** were disappeared. Only the signals for selenoxide **11** and **6a** were observed at 1150 and 938 ppm, respectively. The selenol **10** was over oxidized in excess of H_2O_2 to **11**. The regeneration of **6a** was observed due to the reduction of **11** from PhSH present in the mixture. The mechanistic study suggested that selenosulphide **9**, selenol **10** and selenoxide **11** would be involved in the catalytic cycle, responsible for the decomposition of H_2O_2 .

To understand the antioxidant behavior of **3**, **5a-5d** and **6a-6d**, C6 astroglial cell lines were treated with H_2O_2 to generate ROS. The observed cellular ROS levels were quantified by a DCF-DA (2',7'-dichlorodihydrofluoresce diacetate) fluorescence probe. As shown in Fig. 5, the fluorescence

intensity of **3**, **5a-5d** and **6a-6d** was significantly decreased, and thus suggesting the ROS quenching capacity of antioxidants.

Fig. 5 Fluorescence (normalized to positive control) monitored at ($\lambda = \text{ex } 488$ and $\text{em } 525$ nm) in DCF-DA-stimulated C6 astroglial cells exposed to 10 and 20 μM concentrations of antioxidants ebselen (**3**), **5a-5d**, and **6a-6d**.

Cell viability of **5a-5d** and **6a-6d**, C6 astroglial cells were exposed to different concentrations in MTT assay that showed normal cell growth after 24 h at low concentration (10 μM), (Fig. 6, MTT = (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)).

Fig. 6 Relative cell viability of C6 cells in the presence of 10 and 20 μM of **5a-5d**, and **6a-6d** as determined by MTT measurements after 24 h at 570 nm.

Some cell death or decreased proliferation was observed at higher concentrations (see also Figure S35 in the Supporting Information).

Conclusions

In conclusion, we have described the straight forward synthesis of novel *N*-methyl ebselenamine compounds. These are found to be better quenchers of peroxy radicals than α -tocopherol and are highly regenerable in the presence of aqueous AscOH. The regenerability was increased up to 5-fold for the best compound **6b** higher than α -tocopherol. We therefore believe that it would contribute better understanding of ebselenamine antioxidants as potential drugs for oxidative damages cell prevention, in pharmacotherapy,

stabilizer in petroleum-derived products, and as food preservative.

Author Contributions

Manish Kumar performed the methodology, data curation, investigation and validation. Babli Chhillar and Manisha Yadav involved in methodology. Poonam Sagar performed biological studies. Nitin K. Singhal supervised the biological study part. Paul J. Gates collected the HRMS data. Ray J. Butcher performed X-ray crystallographic analysis. Vijay P. Singh performed supervision, writing-original draft and conceptualization.

Conflicts of interest

There are no conflicts to declare.

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Experimental

^1H and ^{13}C NMR spectra for all compounds prepared were recorded on 400 MHz (^1H : 399.97 MHz; ^{13}C : 100.6 MHz) and 500 MHz (^1H : 499.93 MHz) spectrometers, using the residual solvent peaks of CDCl_3 (^1H : δ 7.26; ^{13}C : δ 77.2), as an indirect reference to TMS (δ = 0 ppm). The ^{77}Se NMR spectra were recorded on 400 MHz (^{77}Se : 76 MHz) spectrometers with Ph_2Se_2 (δ = 460 ppm) as an indirect reference to Me_2Se (δ = 0 ppm). Flash column chromatography was performed using silica gel (0.04-0.06 mm). Melting points are uncorrected for solid compounds. The high resolution mass spectra (HRMS) were obtained using MALDI-TOF instrument equipped with electron ionization (EI) operating in the positive ion mode. Tetrahydrofuran (THF) was dried in a solvent purification system by passing it through an activated alumina column before use. Compounds **4a-4d**, **5b**, and **5d** were prepared according to literature procedure.¹

Typical Procedure for the Synthesis of Diselenides **5a** and **5c**:

Bis[3-amino-*N*-phenyl-benzamide-2-yl] Diselenide (5a**):** The azo-bis-ebsele **4a** (440 mg, 0.77 mmol) was added to an *in situ*-prepared colorless solution of PhTeNa prepared from Ph_2Te_2 (627 mg, 1.53 mmol) and NaBH_4 (116 mg, 3.06 mmol) in EtOH (20 mL) at room temperature under an inert atmosphere. The mixture was then heated at 80 °C for 1 h and allowed to cool at room temperature. After addition of water, the mixture was extracted with CHCl_3 and dried over anhydrous MgSO_4 . Evaporation of excess of the solvent and purification by column chromatography, using 60% ethyl acetate/*n*-pentane mixture as an eluent afforded the title product as an orange crystalline solid. Yield: 210 mg (47%); Mp 222-224 °C; ^1H NMR (400 MHz, $[\text{D}_6]\text{DMSO}$): δ = 5.47 (s, 4H),

6.61 (dd, J = 1.2, 5.6 Hz, 2H), 6.79 (dd, J = 1.2, 7.2 Hz, 2H), 7.04 (t, J = 7.6 Hz, 2H), 7.11 (t, J = 7.6 Hz, 2H), 7.25 (t, J = 8.0 Hz, 4H), 7.47 (d, J = 7.6 Hz, 4H), 10.16 ppm (s, 2H). ^{13}C NMR (100 MHz, $[\text{D}_6]\text{DMSO}$): δ = 114.6, 115.1, 120.0, 123.5, 128.5, 128.6, 130.4, 138.9, 144.6, 150.8, 168.0 ppm. ^{77}Se NMR (76 MHz, $[\text{D}_6]\text{DMSO}$): δ = 370 ppm. HRMS (MALDI TOF) m/z calcd for $\text{C}_{26}\text{H}_{22}\text{N}_4\text{O}_2\text{Se}_2$ [$M + \text{Na}$] $^+$: 604.9977; found: 604.9970.

Bis[3-amino-*N*-(4-methoxyphenyl)benzamide-2-yl] Diselenide (5c**):** Diselenide was synthesized from **4c** (1.0 g, 1.57 mmol) and *in situ*-prepared PhTeNa prepared from Ph_2Te_2 (1.28 g, 3.14 mmol) and NaBH_4 (237 mg, 6.28 mmol) in EtOH (20 mL) according to the procedure described for diselenide **6a**. Purification by column chromatography, using 80% ethyl acetate/*n*-pentane mixture as an eluent afforded the title product as an orange crystalline solid. Yield: 860 mg (85%); Mp 218-220 °C; ^1H NMR (400 MHz, $[\text{D}_6]\text{DMSO}$): δ = 3.72 (s, 6H), 5.47 (s, 4H), 6.58 (d, J = 7.6 Hz, 2H), 6.77-6.83 (several peaks, 6H), 7.10 (t, J = 7.2 Hz, 2H), 7.34 (t, J = 9.2 Hz, 4H), 10.03 ppm (s, 2H). ^{13}C NMR (100 MHz, $[\text{D}_6]\text{DMSO}$): δ = 55.2, 110.2, 113.5, 114.5, 114.9, 121.5, 130.4, 132.0, 144.7, 150.8, 155.4, 167.6 ppm. ^{77}Se NMR (76 MHz, $[\text{D}_6]\text{DMSO}$): δ = 369 ppm. HRMS (MALDI TOF) m/z calcd for $\text{C}_{28}\text{H}_{26}\text{N}_4\text{O}_4\text{Se}_2$ [$M + \text{Na}$] $^+$: 665.0182; found: 665.0189.

Similarly, compounds **5b** and **5d** were also prepared in 74% (lit. 87%) and 80% yield (lit. 52%) according to the literature procedure.¹⁸

Typical Procedure for the Synthesis of *N*-Methyl Ebselenamines **6a-6d**:

Methyl iodide (4 equivalents) was added to an NMR tube containing one equivalents of diselenides **6a-6d** (50 mg, 0.086 mmol) in 0.5 mL of $\text{DMSO}-d_6$ solvent at room temperature. The NMR tube was kept for 2h at the same temperature. The progress of the reaction was monitored by the ^{77}Se NMR spectroscopy. The reaction mixture was poured into water and then extracted with ethyl acetate. The combined extracted organic layers were dried over anhydrous MgSO_4 , filtered and evaporated under vacuum to give black semi-solid as crude. Further, the crude was chromatographed on silica-gel using 50% ethyl acetate/*n*-pentane as eluent to afford the pure products **6a-6d**.

7-(Methylamino)-2-phenyl-benzisoselenazol-3(2H)-one (6a**):** Light green solid. Yield: 20 mg (38%); mp 206-210 °C; ^1H NMR (400 MHz, $[\text{D}_6]\text{DMSO}$): δ = 2.83 (s, 3H), 6.78 (d, J = 7.6 Hz, 1H), 7.18 (d, J = 7.2 Hz, 1H), 7.29-7.37 (several peaks, 2H), 7.47 (t, J = 7.2 Hz, 2H), 7.61 ppm (d, J = 7.6 Hz, 2H). ^{13}C NMR (100 MHz, $[\text{D}_6]\text{DMSO}$): δ = 29.7, 111.2, 115.4, 122.5, 125.0, 126.4, 127.8, 128.2, 129.4, 139.4, 145.2, 165.8 ppm. ^{77}Se NMR (76 MHz, $[\text{D}_6]\text{DMSO}$): δ = 926 ppm. HRMS (MALDI TOF MS EI $^+$) m/z calcd for $\text{C}_{14}\text{H}_{12}\text{N}_2\text{OSe}$ [$M + \text{Na}$] $^+$: 327.0008; found: 327.0016.

7-(Methylamino)-2-(*p*-tolyl)benzisoselenazol-3(2H)-one (6b**):** Yellowish green solid. Yield: 14 mg (27%); mp 180-183 °C; ^1H NMR (400 MHz, CDCl_3): δ = 2.35 (s, 3H), 2.96 (s, 3H), 6.88 (d, J = 7.2 Hz, 1H), 7.20 (d, J = 8.0 Hz, 2H), 7.39 (t, J = 7.2 Hz, 1H),

7.46 (d, $J = 7.6$ Hz, 2H), 7.55 ppm (d, $J = 7.2$ Hz, 1H). ^{13}C NMR (100 MHz, CDCl_3): $\delta = 21.3, 31.2, 113.6, 119.3, 125.7, 125.8, 128.1, 128.4, 130.0, 136.8, 137.0, 144.2, 166.9$ ppm. ^{77}Se NMR (76 MHz, CDCl_3): $\delta = 928$ ppm. HRMS (TOF MS EI^+) m/z calcd for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{OSe}$ [$M + \text{Na}$] $^+$: 341.0164; found: 341.0154.

7-(Methylamino)-2-(*p*-methoxyphenyl)benziselenazol-

3(2H)-one (6c): Black solid. Yield: 15 mg (29%); mp 174–176 °C; ^1H NMR (400 MHz, CDCl_3): $\delta = 2.95$ (s, 3H), 3.81 (s, 3H), 6.87–6.92 (m, 3H), 7.39 (t, $J = 7.2$ Hz, 1H), 7.45 (d, $J = 8.4$ Hz, 2H), 7.55 ppm (d, $J = 7.2$ Hz, 1H) ppm. ^{13}C NMR (100 MHz, CDCl_3): $\delta = 31.2, 55.7, 113.5, 114.6, 119.1, 125.8, 127.7, 127.8, 128.3, 131.9, 144.2, 158.6, 167.1$ ppm. ^{77}Se NMR (76 MHz, CDCl_3): $\delta = 934$ ppm. HRMS (TOF MS EI^+) m/z calcd for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_2\text{Se}$ [M] $^+$: 334.0220; found: 334.0223.

7-(Methylamino)-2-(1-naphthyl)benziselenazol-3(2H)-one

(6d): Brown solid. Yield: 17 mg (33%); mp 195–198 °C; ^1H NMR (400 MHz, CDCl_3): $\delta = 2.94$ (s, 3H), 6.91 (d, $J = 7.6$ Hz, 1H), 7.40–7.48 (several peaks, 4H), 7.56 (d, $J = 7.2$ Hz, 1H), 7.61 (d, $J = 7.6$ Hz, 1H), 7.71–7.74 (m, 1H), 7.85 ppm (dd, $J = 3.2, 8.0$ Hz, 2H). ^{13}C NMR (100 MHz, CDCl_3): $\delta = 31.3, 113.8, 119.5, 123.4, 125.6, 126.8, 126.9, 127.1, 127.2, 127.7, 128.4, 128.5, 129.6, 131.0, 134.7, 135.0, 144.2, 168.2$ ppm. ^{77}Se NMR (76 MHz, CDCl_3): $\delta = 962$ ppm. HRMS (MALDI TOF MS EI^+) m/z calcd for $\text{C}_{18}\text{H}_{14}\text{N}_2\text{OSe}$ [$M + \text{Na}$] $^+$: 377.0164; found: 377.0160.

Alternatively, compound **6d** was also obtained by treating diselenide **5c** (50 mg) with MeI (4 equiv.) in deuterated DMSO- d_6 under stirring condition in inert atmosphere and gentle heating for 4 h at 40 °C. Column chromatographic separation as above yielded solid compound **6d** (22 mg, 42%).

X-ray Crystallographic Analysis. X-ray crystallographic studies of **6c** were carried out using graphite-monochromatized Mo K α radiation ($\lambda = 0.7107$ Å). The structure was solved by direct methods (SHELXS-2016) and refined by a full-matrix least-squares procedure on F^2 for all reflections using SHELXL-2016 software.²⁶ Hydrogen atoms were localized by geometrical means. A riding model was chosen for refinement. The isotropic thermal parameters of hydrogen atoms were fixed at 1.5 times and 1.2 times $U(\text{eq})$ of the corresponding carbon atoms for sp^3 C–H and sp^2 C–H bonds, respectively. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC) as supplementary publications. CCDC 2011383 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk/structures.

Crystal data for 6c. $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_2\text{Se}$, $M_r = 333.24$, triclinic, space group $P-1$, $a = 9.0455(5)$ Å, $b = 12.1947(6)$ Å, $c = 13.2917(7)$ Å, $\alpha = 101.367(4)^\circ$, $\beta = 90.571(4)^\circ$, $\gamma = 109.919(5)^\circ$, $V = 1346.68(13)$ Å 3 , $\lambda = 0.71073$ Å, $Z = 4$, $T = 100(2)$ K, $\rho_{\text{calcd}} = 1.644$ Mg/m 3 , $\text{GOF} = 1.062$, $R_1 = 0.0888$, $wR_2 = 0.2418$ [$I > 2\sigma(I)$]; $R_1 = 0.1014$, $wR_2 = 0.2492$ (all data). Of the 7914 reflections that were collected, 7914 were unique ($R_{\text{int}} = 0.1121$).

Computational Details. Computational calculations for compounds **6a–6d** were executed by using the Gaussian 09 suite of quantum chemical programs.²⁷ The hybrid Becke 3-Lee-Yang-Parr (B3LYP) exchange correlation functional was implemented for density functional theory (DFT) calculations.²⁸ The geometry optimizations and frequencies were carried out at the B3LYP/6-311+G(d) basis sets. The quantifications of orbital interaction were done by natural bond analysis at B3LYP/6-311+G(d,p) level.²⁹ The ^{77}Se NMR calculations were performed at B3LYP/6-311+G (d,p) level on B3LYP/6-311+G(d)-level-optimized geometries by using the gauge-including atomic orbital (GIAO) method (referenced with respect to the peak of Me_2Se).³⁰

HPLC Lipid Peroxidation Assay: The experimental setup for recording inhibition times (T_{inh}) and inhibited rates of peroxidation (R_{inh}) during azo-initiated peroxidation of linoleic acid in a two-phase system were recently described. Fresh linoleic acid was used as purchased. Values reported in Tables 1 for reactions performed in the presence and absence of AscOH are means $\pm$ SD based on triplicates. As R_{inh} and T_{inh} values showed little variations depending on the amount of linoleic acid hydroperoxide present in the commercial sample as an impurity, and increases upon storage for longer.

Coupled Reductase Assay. The GPx-like activities of prepared compounds **6a**, **5c** and **6a–6d** including ebselen as reference were determined by UV-spectroscopy by following the protocol in literature with slight modifications. The test mixture contained GSH (1 mM), ethylene diamine tetra acetate (EDTA, 1 mM), glutathione reductase (GR) (1.3 unit/mL), and β -nicotinamide adenine dinucleotide phosphate (NADPH, 0.2 mM) in potassium phosphate buffer (100 mM), pH 7.5. Catalysts (20 μM) were added to the test mixture at 21 °C and the reaction was initiated by addition of H_2O_2 (0.8 mM). Initial reaction rates (v_0) were based on the consumption of NADPH as assessed by UV-spectroscopy at 340 nm. Rates (v_0) were triplicated and calculated from the initial 10 seconds of reaction by using 6.22 $\text{mM}^{-1}\text{cm}^{-1}$ as the molar extinction coefficient for NADPH. GPx-data reported as means $\pm$ SD.

Mechanistic Study of Compound 6a. A solution of two equiv of 30% H_2O_2 (15 μL , 0.132 mmol) was added to the NMR tube containing **6a** (20 mg, 0.066 mmol) in 0.5 mL of DMSO- d_6 . Further, two more equiv of H_2O_2 were added to the above mixture. The ^{77}Se NMR spectra were recorded after each addition to the NMR tube. A solution of three equiv of PhSH (20 μL , 0.198 mmol) was added to the NMR tube. The NMR spectra were recorded followed by ^{77}Se NMR spectroscopy. Then another three more equiv of PhSH was added and the ^{77}Se NMR spectrum recorded again. For the spectra, please see the Supporting Information.

Biological Studies.

ROS assay: C6 astroglial cells were seeded in a 96 well black plate at passage number 24. After cells were 80% confluent,

they were treated with 500 μM hydrogen peroxide for five minutes followed by treatment with 10 and 20 μM compounds and incubated for 15 minutes. Dichlorodihydrofluorescein diacetate (25 μM) was used to quantify the amount of ROS generated. DCHFDA oxidises to dichlorofluorescein (DCF) in presence of ROS. Fluorescence of DCF was recorded at 488nm and 525nm excitation and emission respectively using fluorescence plate reader (SpectraMax i3x, molecular devices).

MTT Assay: Materials: C6 astroglial cells (NCCS Pune, India), MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was obtained from Sigma, Cell culture media DMEM, Fetal bovine serum (FBS) and antibiotic (penicillin-streptomycin) were supplied by Gibco, Invitrogen, Dimethylsulfoxide (DMSO) was obtained from MP biomedical. C6 cell line was maintained in DMEM with 10% FBS and 1% antibiotic and seeded in 96 well sterile cell culture plate (SPL life science) at passage no 21 and incubated at 37 $^{\circ}\text{C}$ and 5% CO_2 (v/v). After cells were 70% confluent, media was aspirated out, washed with PBS, and 0-120 $\mu\text{M}/\text{ml}$ dosages of the compounds were given to cells in triplicate and incubated for 24 hr at 37 $^{\circ}\text{C}$ and 5% CO_2 . After 24 hours, media was removed and 100 μl of 0.5 mg/mL MTT reagent was added and incubated for another 4 hours. After the incubation period, the media containing MTT reagent was aspirated out, Formazan crystals were solubilized in DMSO, and absorbance was recorded at 570 nm using Nano-quant. The cell viability was calculated using the following equation:

Cell viability (%) = {Absorbance (test)/Absorbance (control)} $\times$ 100

Statistical Analysis

The values were calculated and expressed as average $\pm$ SD. The grouped were compared among each other following ANOVA and analysed by post hoc test (Tukey) in Sigma Stat.

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