Deuterium labeling improves the therapeutic index of 3,3′-diselenodipropionic acid as anticancer agent: Insights from redox reactions

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Methods

X-ray Crystallography

A Rigaku-Oxford make XtaLAB Synergy, Dualflex X-ray diffractometer was employed for crystal screening, unit cell determination, and data collection. The goniometer was controlled using the APEX3 software suite [1]. The X-ray radiation employed was generated from a Mo X-ray tubeKα(λ = 0.71073 Å). All data were integrated with [unknown integration program] and a multi-scan absorption correction using SCALE3 ABSPACK was employed to correct the data for absorption effects [2]. Systematic reflection conditions and statistical tests of the data suggested the space group *Cc*. The structure was solved by direct methods using SHELXS and refined by full-matrix least-squares methods against F^2 by SHELXL-2017/1 [2,3].Hydrogen atoms were placed in idealized positions and were set riding on the respective parent atoms. All non-hydrogen atoms were refined with anisotropic thermal parameters. ORTEP and Mercury was employed for the final data presentation and structure plots [4,5].

References:

[1] CrysAlisPRO, Oxford Diffraction /Agilent Technologies UK Ltd, Yarnton, England.

[2] G. M. Sheldrick, *Acta Cryst.2008*, A64, 112–122, doi:10.1107/S0108767307043930.

[3] G. M. Sheldrick, *Acta Cryst.2015*, C71, 3–8, doi:10.1107/S2053229614024218.

[4] L. J. Farrugia, *J. Appl. Crystallogr.*, 1997, 30, 565.

[5] C. F. Macrae, P. R. Edgington, P. McCabe, E. Pidcock, G. P. Shields, R. Taylor, Towler and van der Streek, *J. Appl. Crystallogr.,* 2006, 39, 453-457.

Table S1: Crystallographic and structure refinement data for **D-DSePA**.

Compounds	**D-DSePA**
Chemical Formula	$C_6H_2D_8O_4Se_2$
Formula weight	312.08
Crystal Size (mm^3)	0.10 x 0.05 x 0.05
Diffractometer	Rigaku-Oxford make XtaLAB Synergy, Dualflex X-ray diffractometer
T/K	298(2)
λ/Å	MoKα (λ = 0.71073 Å)
Crystal system	Monoclinic
Space group	C 2/c
a/Å	5.5158(6)
b/Å	9.1291(9)
c/Å	19.1868(19)
α/º	90
β/º	94.815(9)
γ/º	90
V/$Å^3$	962.73(17)
ρ_{calc}/g cm^{-3}	2.153
Z	2
μ/mm^{-1}	7.655
Reflection collected/ unique	3830/ 1202
Data/restraints/parameters	1202/ 5/ 69
Final R_1, wR_2 indices	R1 = 0.0917, wR2 = 0.2470
R_1, wR_2 (all data)	R1 = 0.1183, wR2 = 0.2572
Largest diff. peak & hole [$eÅ^{-3}$]	2.745 and -0.703

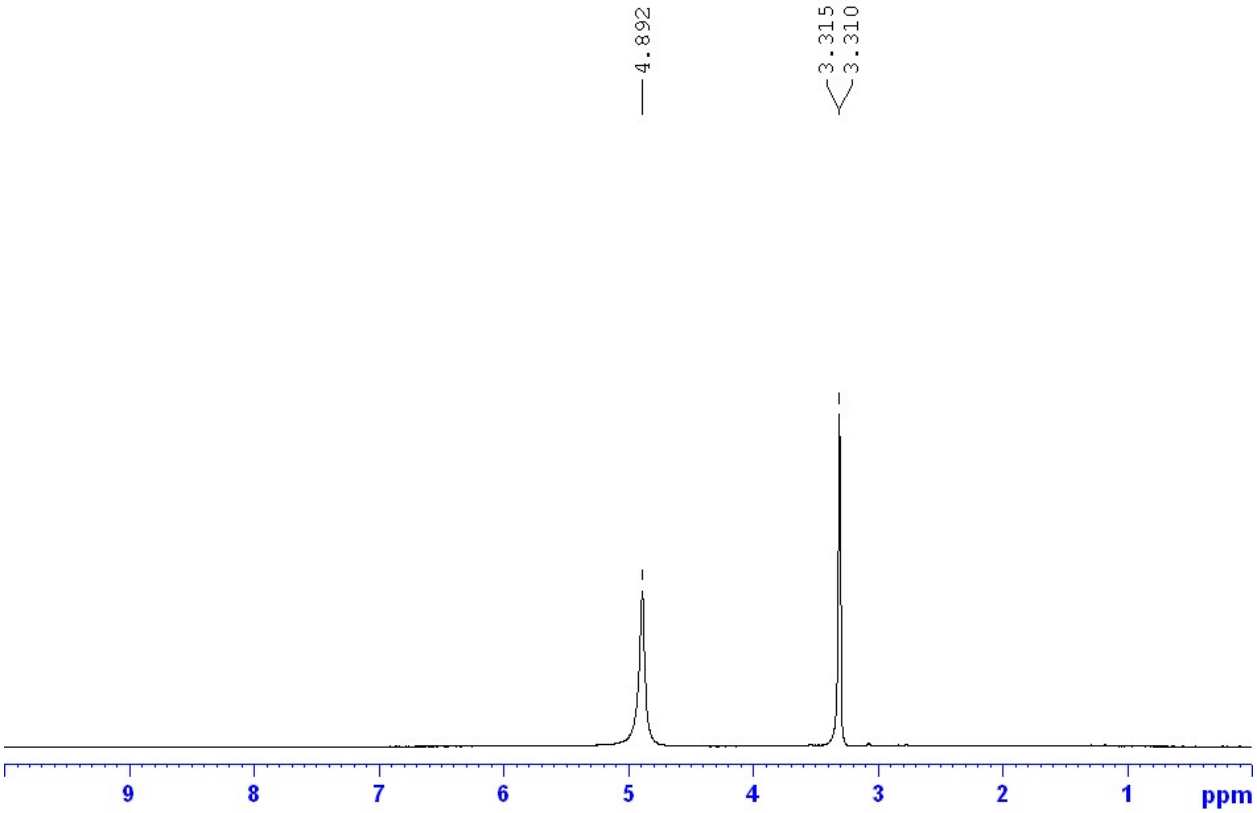

Figure S1. ^{1}H NMR (300 MHz, CH_3OH-d_4) of D-DSePA.

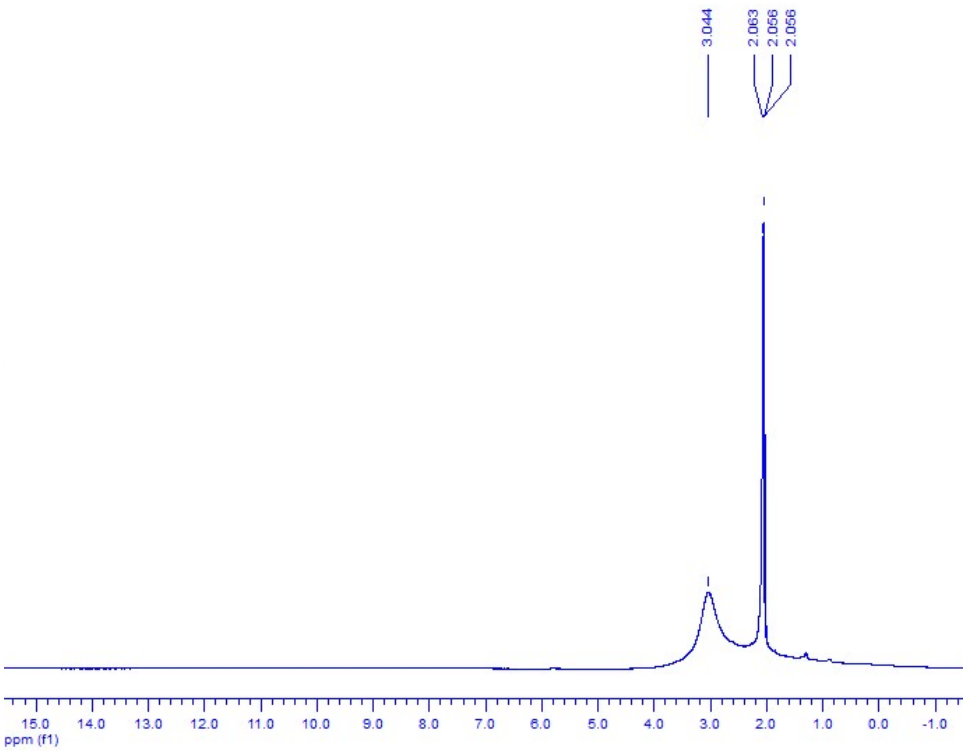

Figure S2. ^{1}H NMR (300 MHz, CH_3COCH_3-d_6) of D-DSePA.

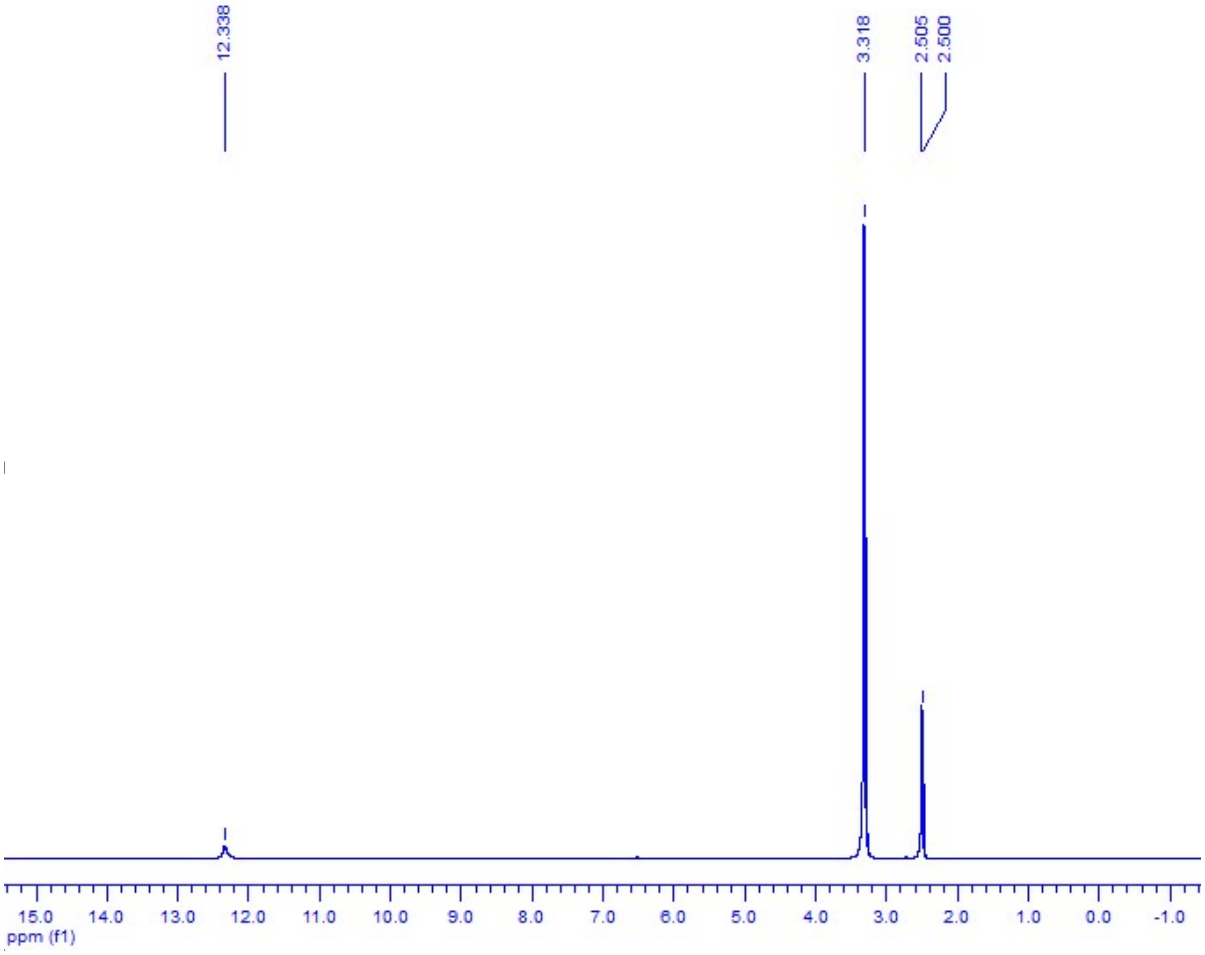

Figure S3. ^{1}H NMR (300 MHz, DMSO-d_6) of D-DSePA.

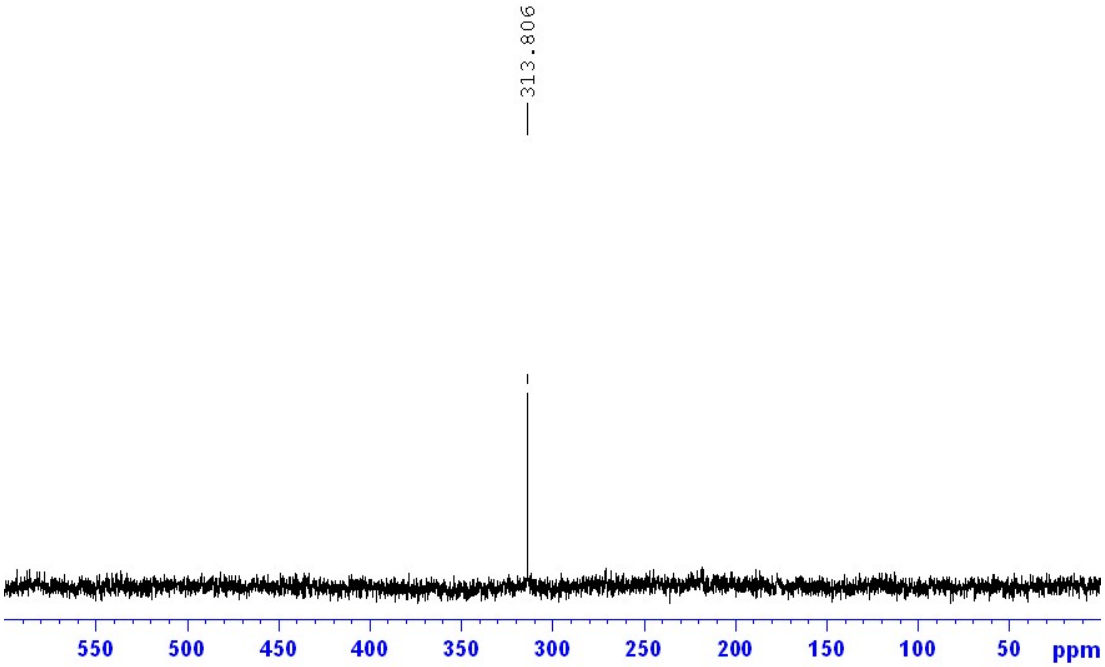

Figure S4. $^{77}Se\{^{1}H\}$ NMR (57.2 MHz, CH_3OH-d_4) of D-DSePA

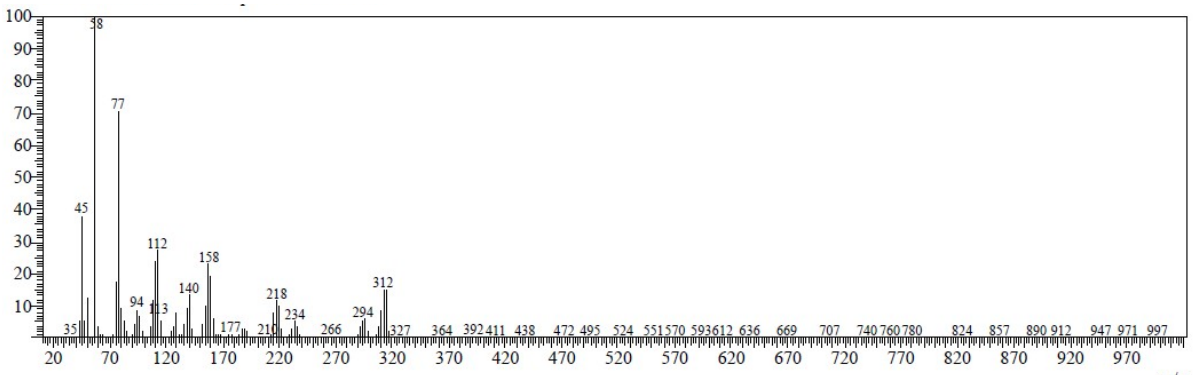

Figure S5. GC-MS spectrum of D-DSePA.

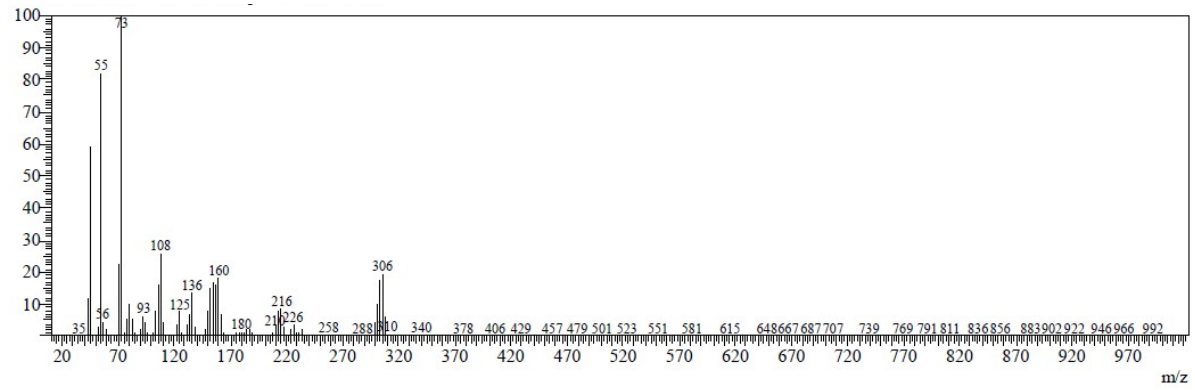

Figure S6. GC-MS spectrum of DSePA.

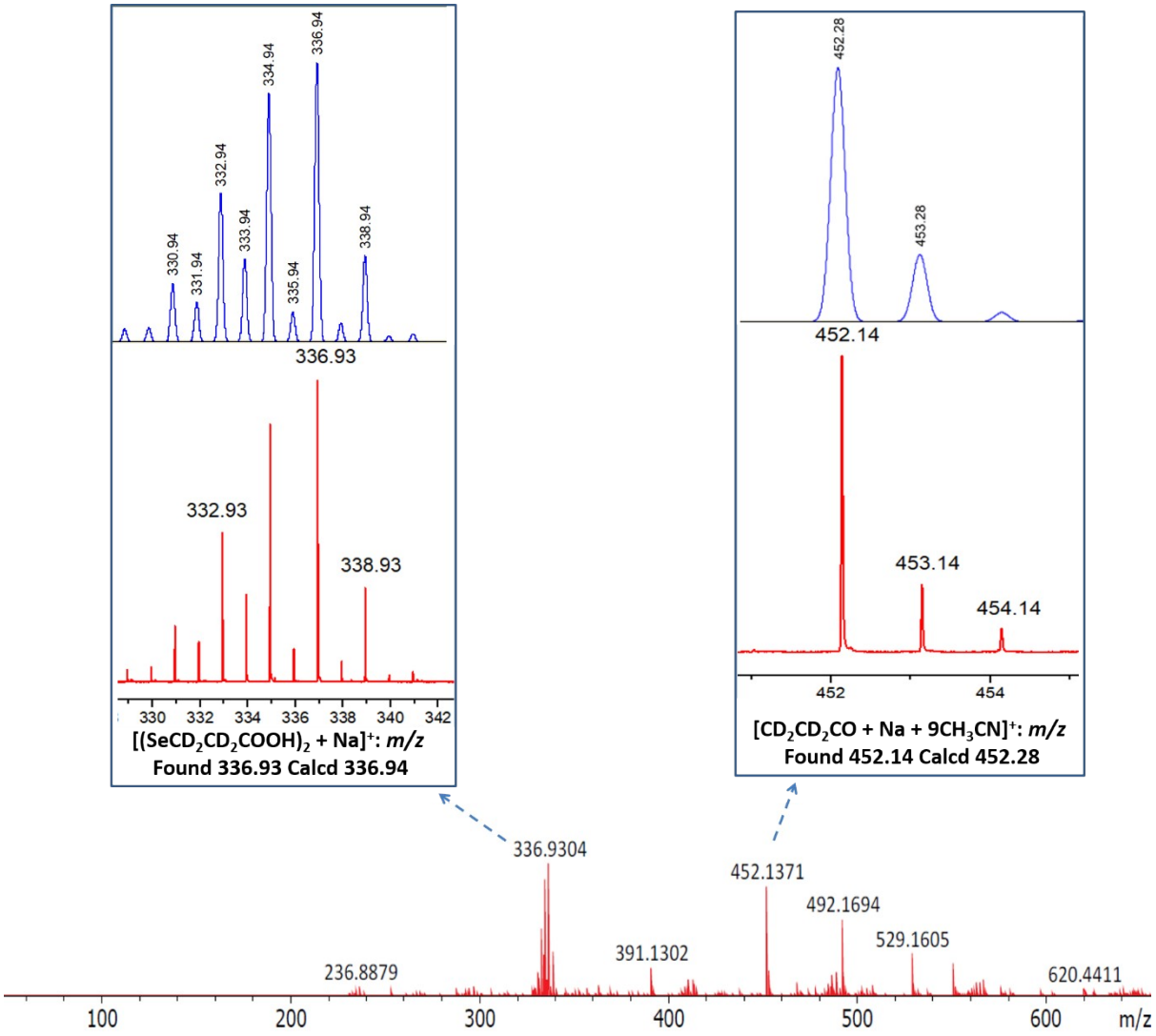

Figure S7. ESI-MS spectrum of D-DSePA.The insets show the experimentally obtained (below) isotope patterns of the fragments with those simulated (above) on the basis of natural isotope abundances. The found and calcd values are for the most abundant peak of ion.

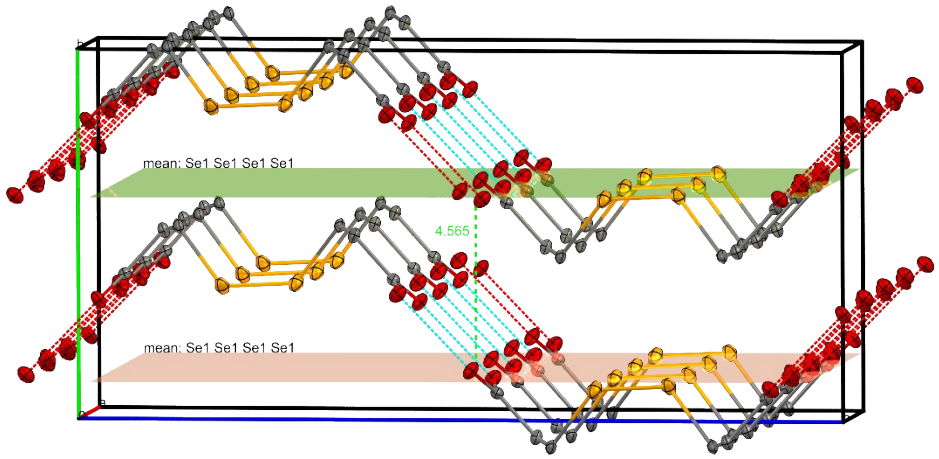

Figure S8. Stacking of molecules of **D-DSePA** in Unit cell observed along the *b* axis(a-axis perpendicular to the plane of paper).

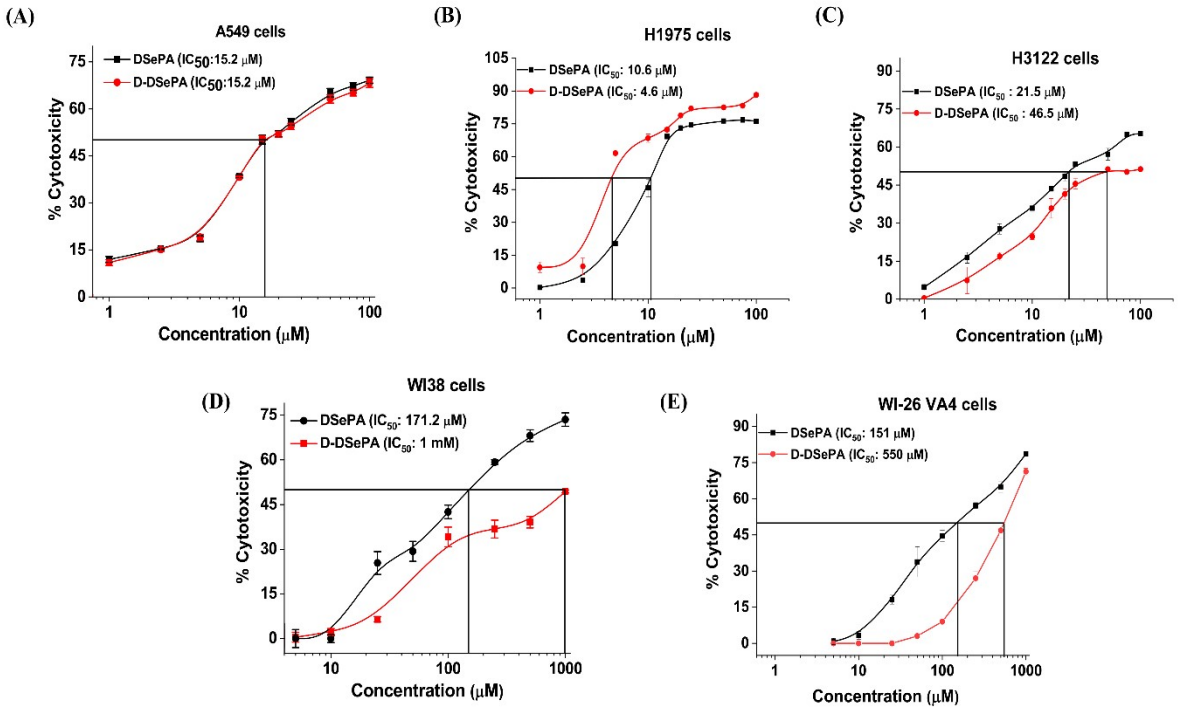

Figure S9. Cytotoxicity of DSePA and D-DSePA in (A) A549(B) H1975 (C) H3122 (D) WI-38 and (E) WI-26 SV4A is presented. Cells like A549, H1975 and H3122 are the representative of human NSCLC. Cells like WI-38 and WI-26 SV4A are the representative of human lung fibroblast and epithelial respectively. The cells were treated with DSePA/D-DSePA for 48 hours prior to MTT assay. The experiments were done in triplicates. The results are presented as mean ± SEM (n = 3).

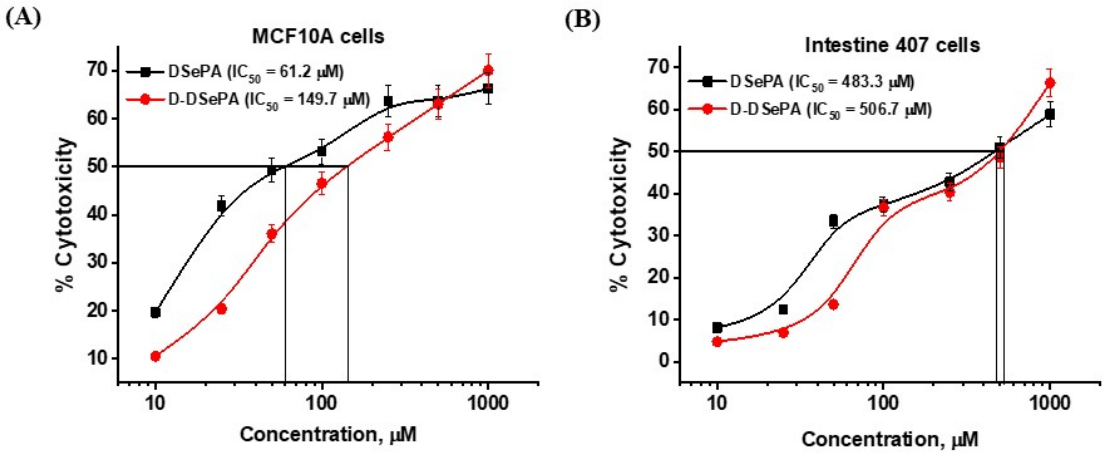

Figure S10. Cytotoxicity of DSePA and D-DSePA in (A) MCF10A (B) intestine 407 is presented. Cells like MCF10A and intestine 407 are the representative of human breast epithelial and intestinal epithelial respectively. The cells were treated with DSePA/D-DSePA for 48 hours prior to MTT assay. The experiments were done in triplicates. The results are presented as mean ± SEM (n = 3).

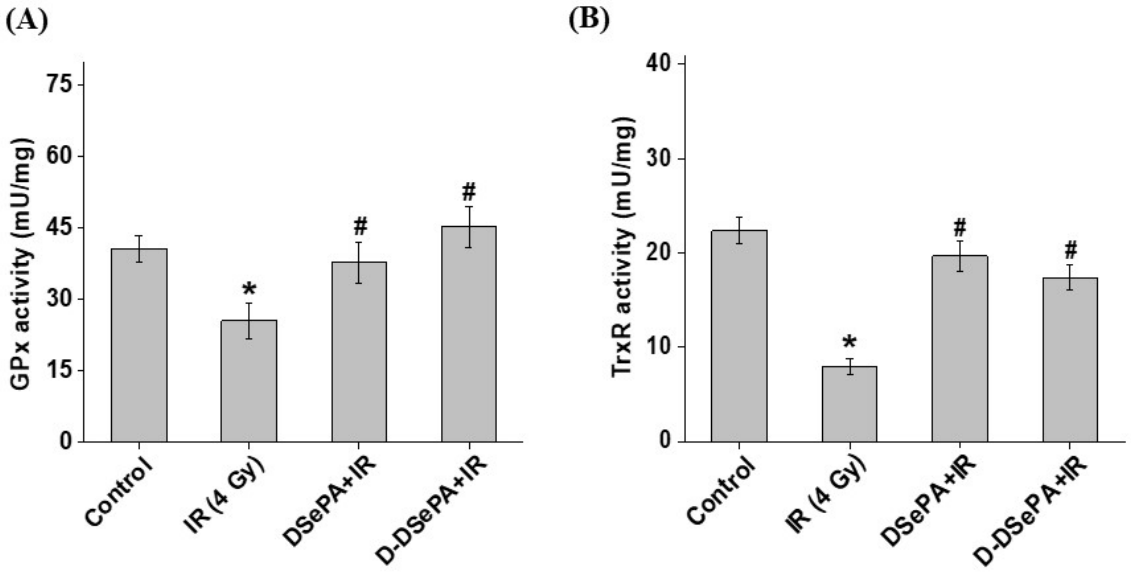

Figure S11. (A & B) Effects of DSePA and D-DSePA on the activity levels of GPx and TrxR enzymes in the γ-irradiated non-cancerous lung fibroblast (WI38) cells are presented. The cells were treated with 10 μM of DSePA/D-DSePA for 24 hours prior to γ-irradiation. The activity was measurement at 24 h post -iiradiation by biochemical assays.

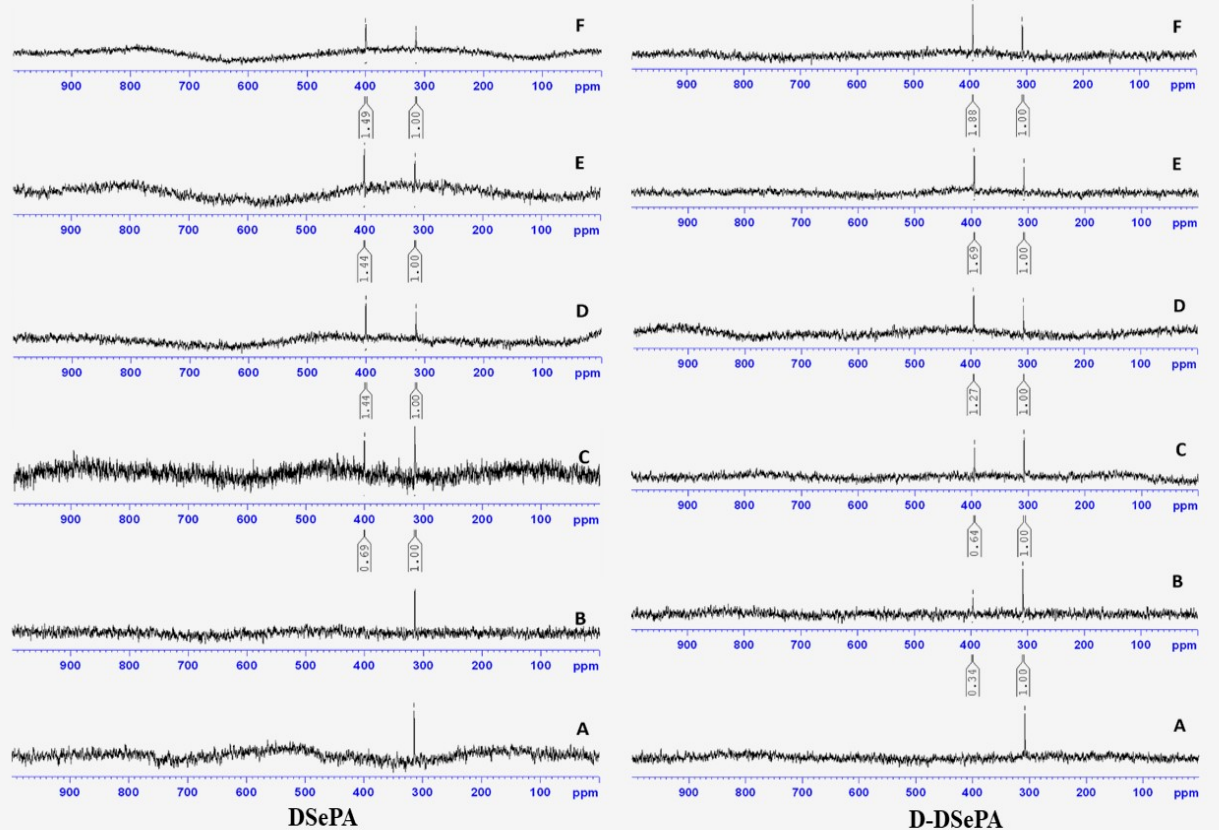

Figure S12: $^{77}Se\{^{1}H\}$ NMR in DMSO-d_6 for DSePA/D-DSePA (A) and reaction mixture of DSePA/D-DSePA + N-Acetyl Cysteine (1:10) after (B) 2h, (C) 6h, (D) 1 day, (E) 2 days and (F) 3 days.

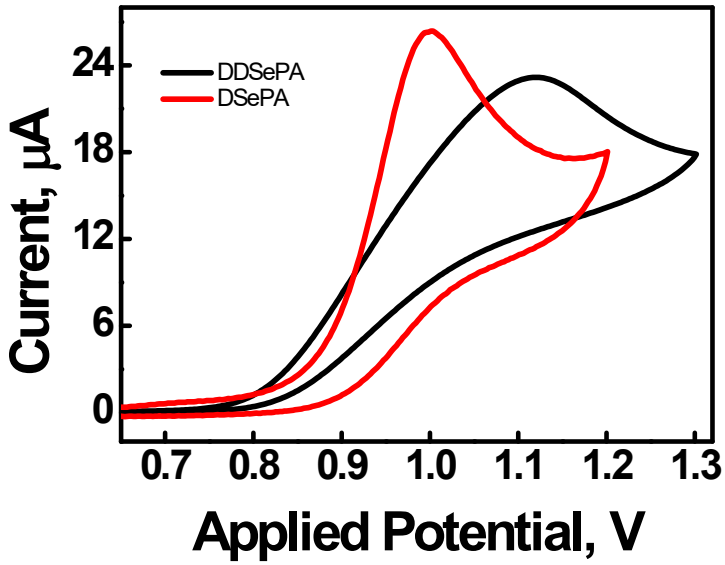

Figure S13: Cyclic voltamogram of 1 mM D-DSePA and DSePA obtained at scan rate of 100 mV/s and 50 mV/s, respectively.

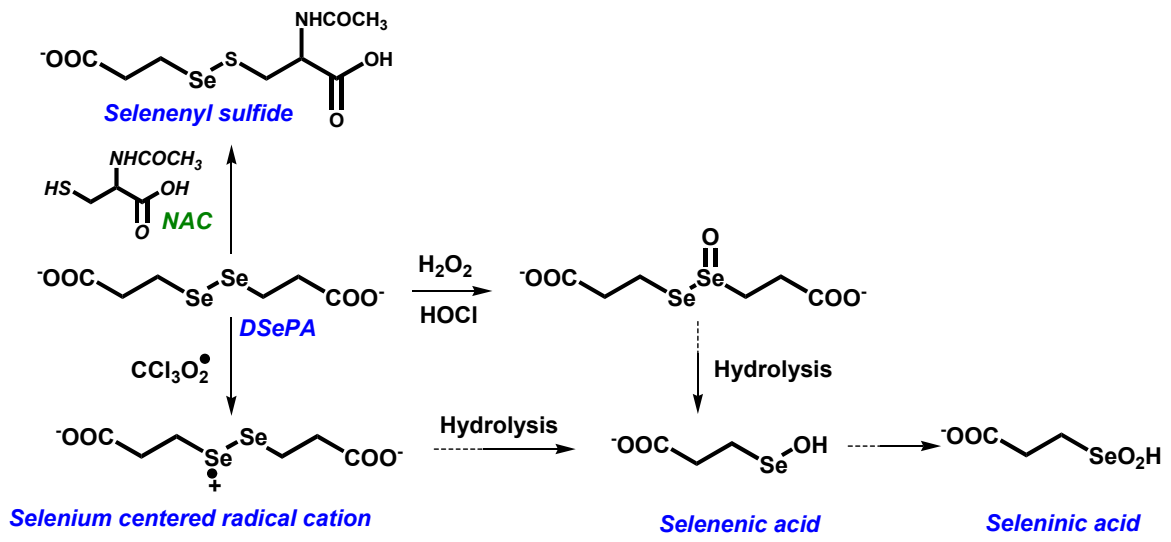

Figure S14: Plausible chemical steps of the reaction between D-DSePA/DSePA and the molecular oxidants/reductant.