Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Supporting Information

for

Mechanistic Investigations on the Efficient Catalytic Decomposition of Peroxynitrite by Ebselen Analogues

Krishna P. Bhabak, Amit A. Vernekar, Surendar R. Jakka, Gouriprasanna Roy and Govindasamy Mugesh*

Contribution from Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore 560012, India, Email: mugesh@ipc.iisc.ernet.in

Peroxynitrite mediated nitration of free L-tyrosine

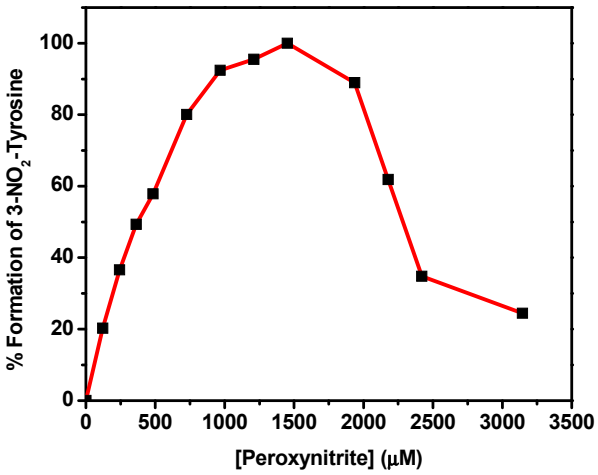


Figure S1. Effect of PN concentration on the formation of 3-nitro-L-tyrosine. The concentration of PN was gradually increased at a fixed concentration of L-tyrosine.

Table S1. Effect of the concentration of compound **1** on the PN-mediated nitration of free L-tyrosine.

[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity	[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity
0	1598	100.00	100	565	35.21
	1599	100.00		563	35.93
20	1293	80.91	130	369	23.08
	1295	80.98		369	22.52
60	806	50.44	160	231	14.46
	811	50.72		238	14.88
80	656	41.05			
	646	40.40			

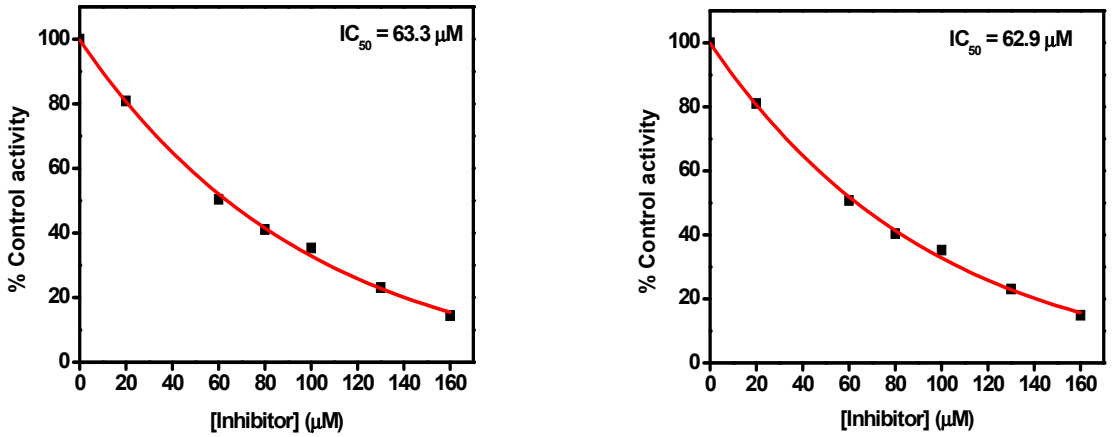


Figure S2. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with the increasing concentration of compound **1**.

Table S2. Effect of the concentration of compound **2** on the PN-mediated nitration of free L-tyrosine.

[Inhibitor] (µM)	Peak area of l-tyrosine	% Control Activity	[Inhibitor] (µM)	Peak area of l-tyrosine	% Control Activity
0	1574	100.00	60	677	43.01
	1581	100.00		673	42.57
5	1199	76.18	90	519	32.97
	1210	76.53		519	32.83
10	1055	67.03	120	350	22.24
	1057	66.86		351	22.20
20	882	56.04	150	218	13.79
	884	55.91		218	13.70
40	779	49.49			
	777	49.15			

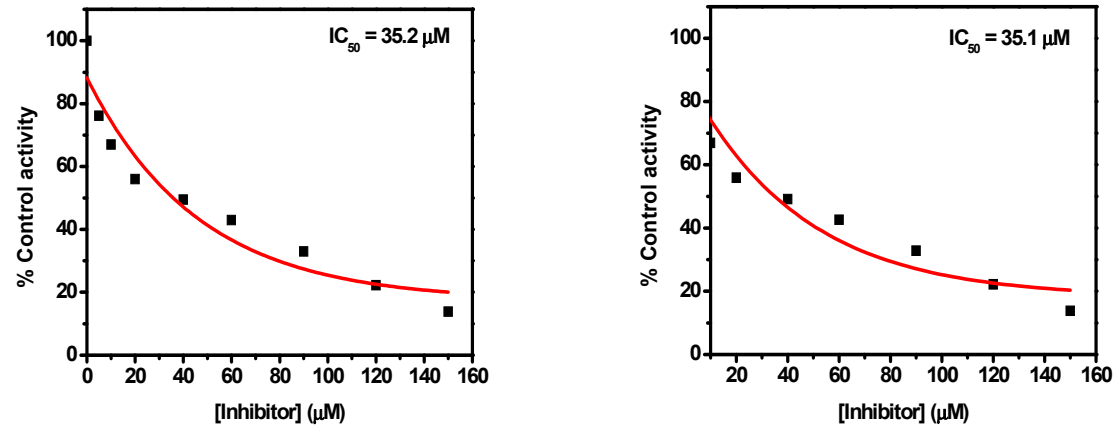


Figure S3. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with the increasing concentration of compound **2**.

Table S3. Effect of the concentration of compound **8** on the PN-mediated Nitration of L-tyrosine.

[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity	[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity
0	1600	100.00	60	680	42.50
	1587	100.00		676	42.60
10	1203	75.19	90	522	32.63
	1175	74.04		525	33.08
20	935	58.44	120	401	25.06
	935	58.89		400	25.20
40	838	52.38	150	276	17.25
	834	52.55		276	17.39

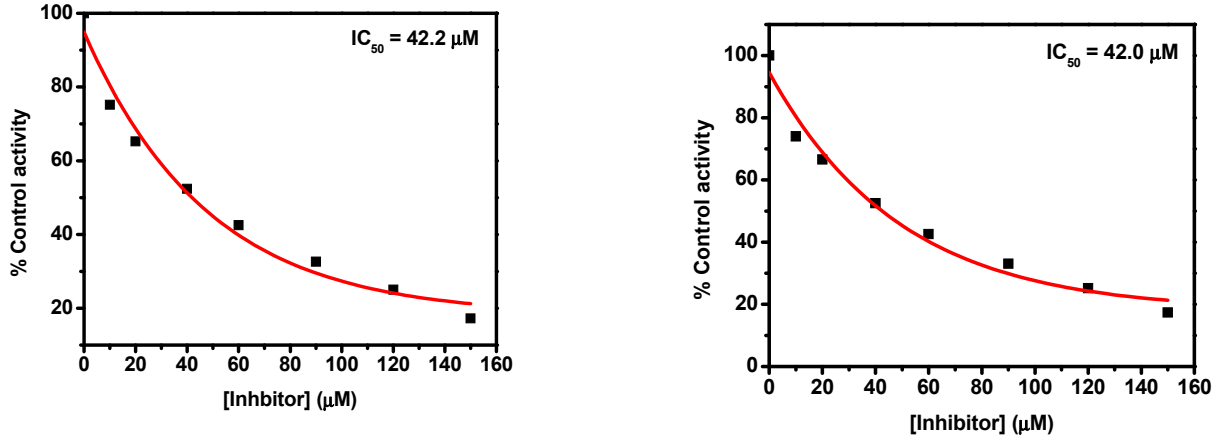


Figure S4. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with the increasing concentration of compound **8**.

Table S4. Effect of the concentration of compound **9** on the PN-mediated nitration of free L-tyrosine.

[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity	[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity
0	1599	100.00	60	666	41.65
	1597	100.00		664	41.58
10	1143	71.48	90	518	32.40
	1146	71.76		518	32.44
20	956	59.79	120	378	23.64
	956	59.86		378	23.67
40	755	47.22	150	225	14.07
	753	47.15		226	14.15

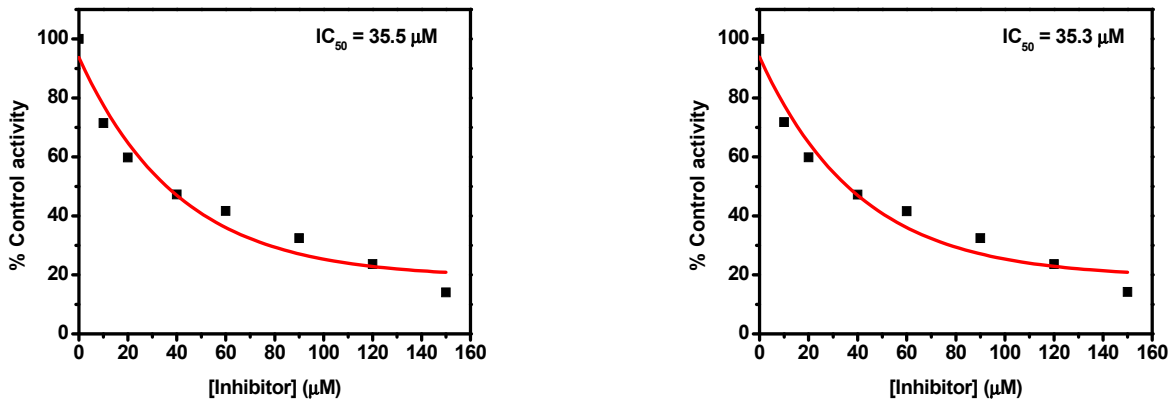


Figure S5. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with the increasing concentration of compound **9**.

Table S5. Effect of the concentration of compound **10** on the PN-mediated nitration of free L-tyrosine.

[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity	[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity
0	1596	100.00	60	655	41.04
	1596	100.00		652	40.85
10	1128	70.68	90	464	29.07
	1129	70.74		464	29.07
20	876	54.89	120	335	20.99
	876	54.89		335	20.99
40	744	46.62			
	745	46.68			

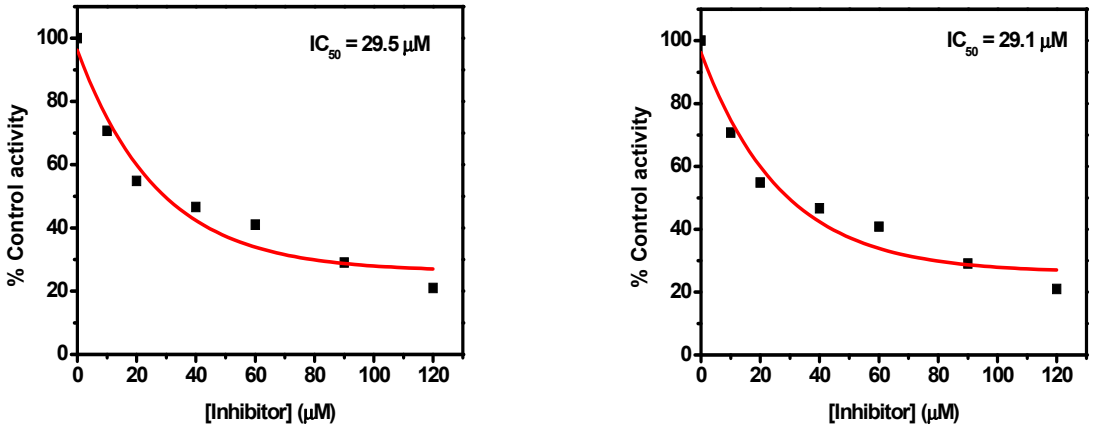


Figure S6. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with the increasing concentration of compound **10**.

Table S6. Effect of the concentration of compound **11** on the PN-mediated nitration of free L-tyrosine.

[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity	[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity
0	1597	100.00	60	721	45.47
	1597	100.00		721	45.47
5	1221	76.46	90	554	34.69
	1221	76.46		554	34.69
10	1019	63.81	120	403	25.24
	1020	63.81		403	25.24
20	919	57.55	150	299	18.72
	917	57.42		300	18.79
40	815	51.03			
	816	51.10			

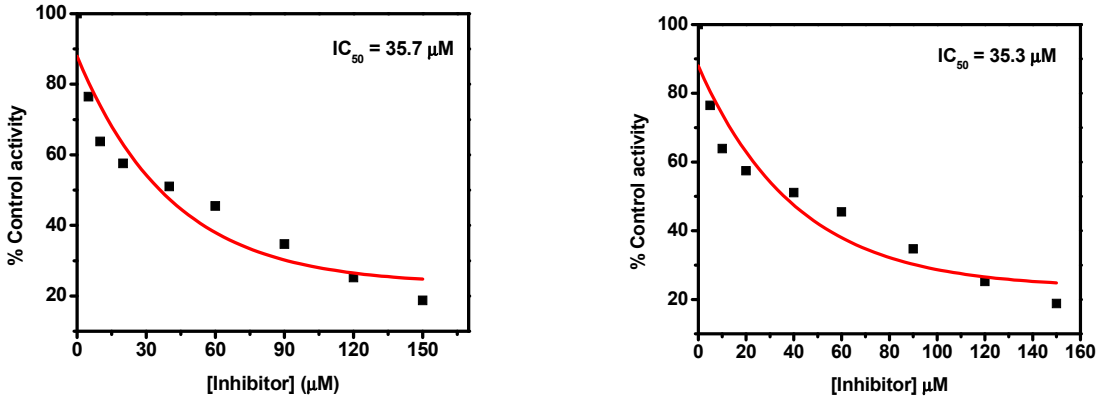


Figure S7. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with the increasing concentration of compound **11**.

Table S7. Effect of the concentration of compound **12** on the PN-mediated nitration of free L-tyrosine.

[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity	[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity
0	1600	100.00	40	727	45.44
	1601	100.00		728	45.47
5	1190	74.38	60	590	36.88
	1191	74.39		586	36.60
10	1009	62.96	90	440	27.50
	1008	62.95		440	27.48
20	861	53.81	120	317	19.81
	861	53.78		317	19.80

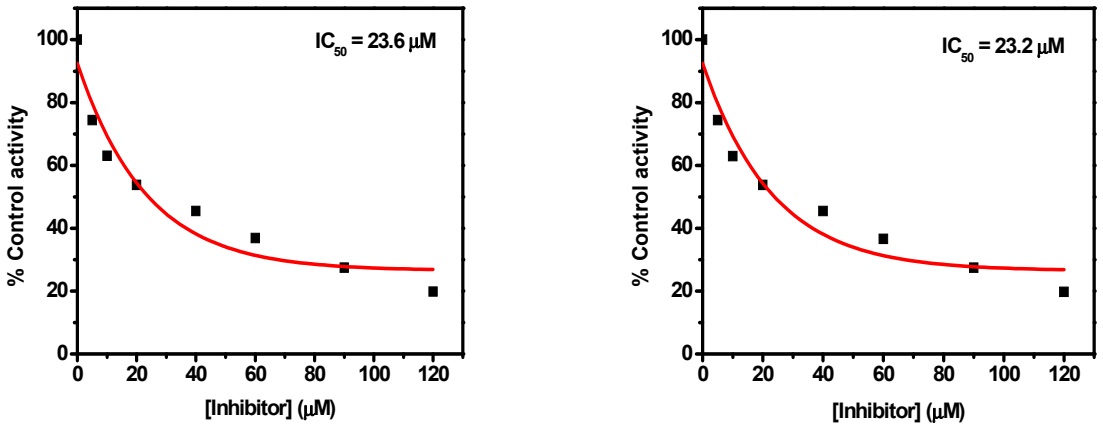


Figure S8. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with the increasing concentration of compound **12**.

Table S8. Effect of the concentration of compound **13** on the PN-mediated nitration of free L-tyrosine.

[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity	[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity
0	1593	100.00	40	675	42.19
	1600	100.00		675	43.18
5	1212	76.08	60	508	31.89
	1213	75.81		509	31.89
10	994	62.40	90	411	25.80
	995	62.59		411	25.69
20	835	52.42	120	290	18.20
	838	52.38		290	18.13

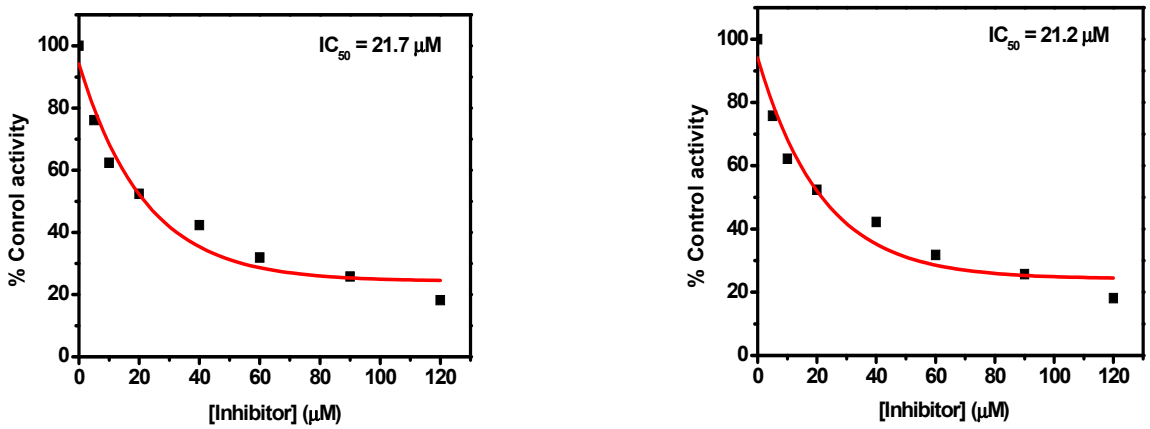


Figure S9. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with the increasing concentration of compound **13**.

Table S9. Effect of the concentration of compound **14** on the PN-mediated nitration of free L-tyrosine.

[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity	[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity
0	1599	100.00	10	599	37.46
	1599	100.00		601	37.59
2.5	1036	64.79	15	501	31.33
	1038	64.49		500	31.27
5	909	56.85	20	388	24.27
	909	56.85		387	24.20
7.5	701	43.84	30	311	19.45
	699	43.72		316	19.76

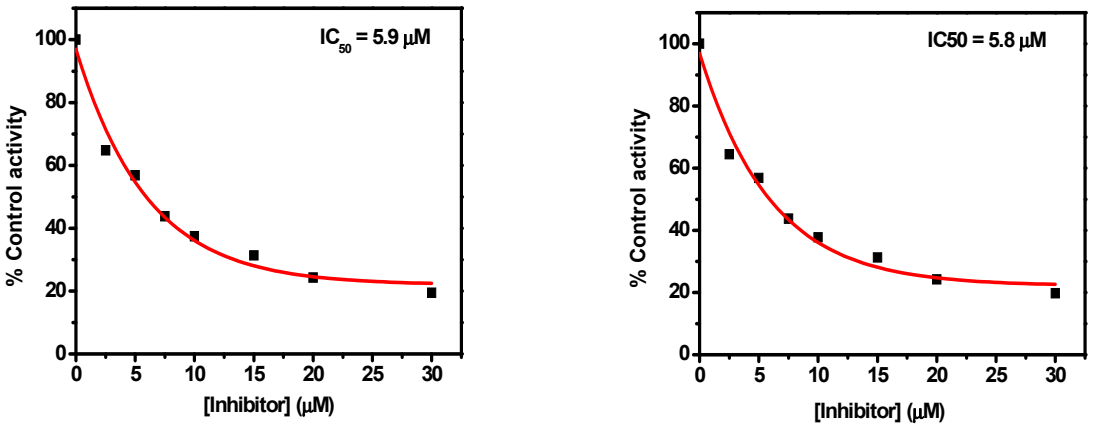


Figure S10. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with increasing concentration of compound **14**.

Table S10. Effect of the concentration of compound **15** on the PN-mediated nitration of free L-tyrosine.

[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity	[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity
0	1601	100.00	10	546	34.10
	1591	100.00		547	34.38
2.5	950	59.34	15	337	21.05
	952	59.84		342	21.50
5	704	43.97			
	711	44.69			

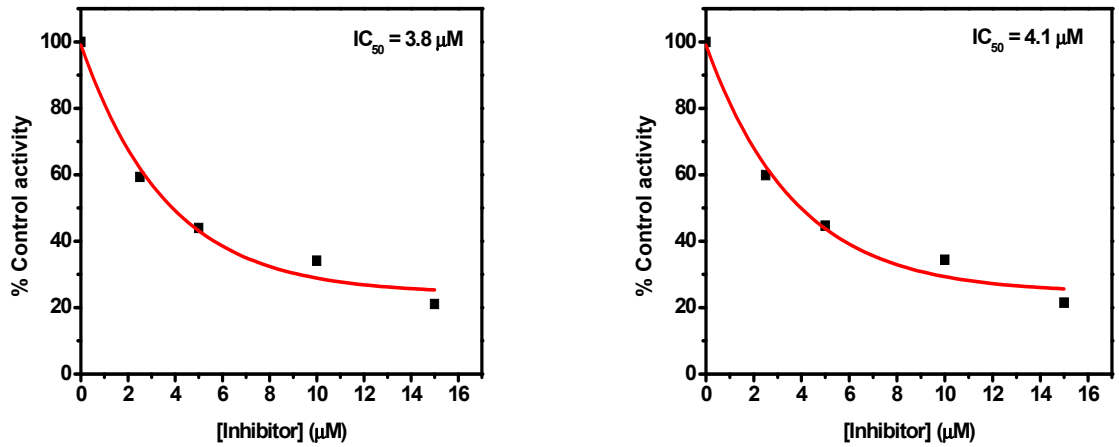


Figure S11. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with increasing concentration of compound **15**.

Table S11. Effect of the concentration of compound **16** on the PN-mediated nitration of free L-tyrosine.

[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity	[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity
0	1599	100.00	30	602	37.65
	1600	100.00		600	37.50
5	1021	63.85	40	502	31.39
	1019	63.69		502	31.38
10	915	57.22	50	403	25.20
	918	57.38		403	25.19
20	701	44.84	60	303	18.95
	702	43.87		302	18.88

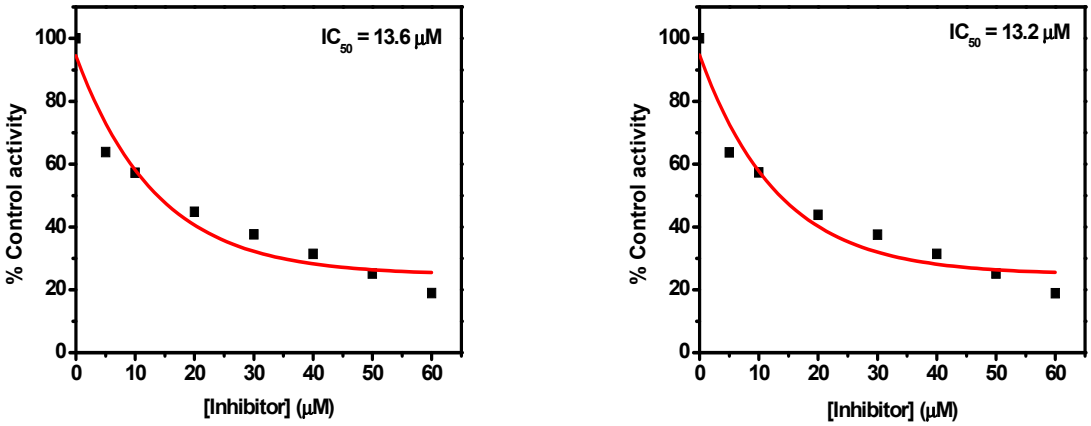


Figure S12. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with increasing concentration of compound **16**.

Table S12. Effect of the concentration of compound **17** on the PN-mediated nitration of free L-tyrosine.

[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity	[Inhibitor] (μM)	Peak area of L-tyrosine	% Control Activity
0	1604	100.00	30	713	44.45
	1598	100.00		713	44.62
5	1182	73.69	40	603	37.59
	1181	73.91		600	37.55
10	1063	66.27	60	454	28.30
	1062	66.46		454	28.41
20	893	55.67	90	306	19.08
	894	55.95		306	19.15

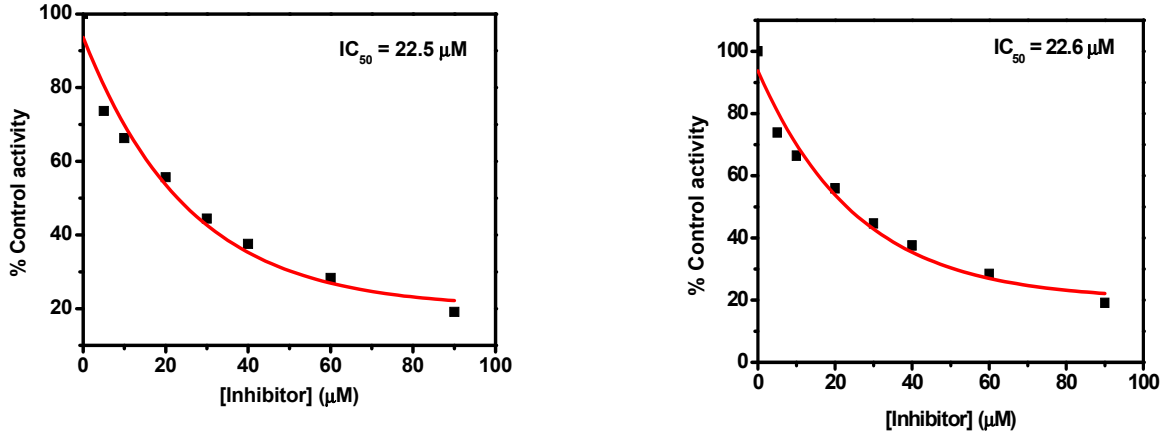


Figure S13. Plot of the inhibition of PN-mediated nitration of free L-tyrosine with increasing concentration of compound **17**.

Inhibition of the PN-mediated oxidation of dihydrorhodamine 123 (DHR):

Table S13. Effect of the concentration of compound **1** on the PN-mediated oxidation of dihydrorhodamine 123.

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	5326800	100.00	1.25	1966910	36.92
	5309190	100.00		1950800	36.74
0.3125	4124600	77.43	1.875	1343700	25.52
	4110330	77.41		1339000	25.22
0.625	3078620	57.79	2.5	992790	18.63
	3074290	57.90		983190	18.51
0.9375	2378200	44.64			
	2350640	44.27			

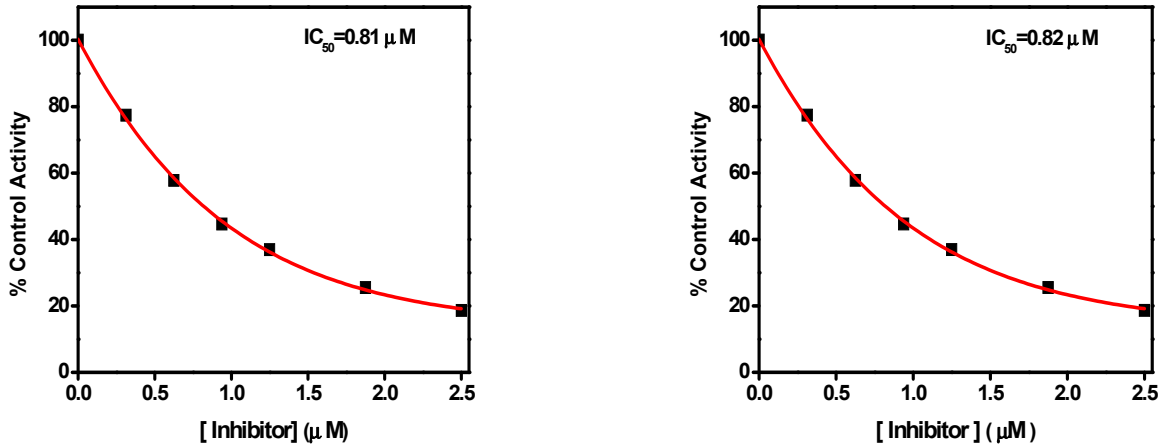


Figure S14. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **1**.

Table S14. Effect of the concentration of compound **2** on the PN-mediated oxidation of dihydrorhodamine 123.

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	5207850	100.00	50.0	2597000	49.86
	5202070	100.00		2570730	49.41
12.5	4608150	88.48	75.0	1542610	29.62
	4590040	88.23		1464040	28.14
25.0	3853230	73.98	100.0	760670	14.60
	3781040	72.68		754790	14.50

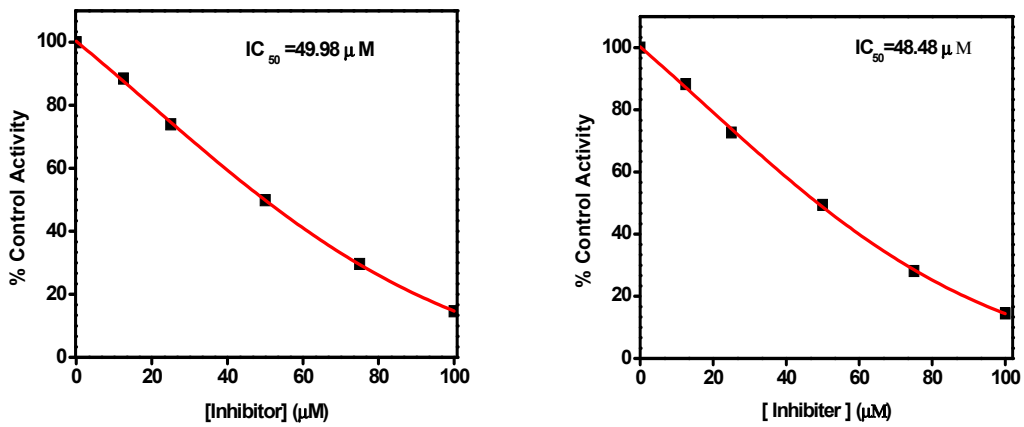


Figure S15. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **2**.

Table S15. Effect of the concentration of compound **8** on the PN-mediated oxidation of dihydrorhodamine 123.

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	5596570	100.00	2.5	2009400	35.90
	5576350	100.00		1952600	35.01
0.625	3672780	65.62	3.125	1756870	31.39
	3660190	65.63		1721820	30.87
1.25	2836140	50.67	5.0	1305570	23.32
	2810170	50.39		1277540	22.90
1.875	2371430	42.37			
	2330080	41.78			

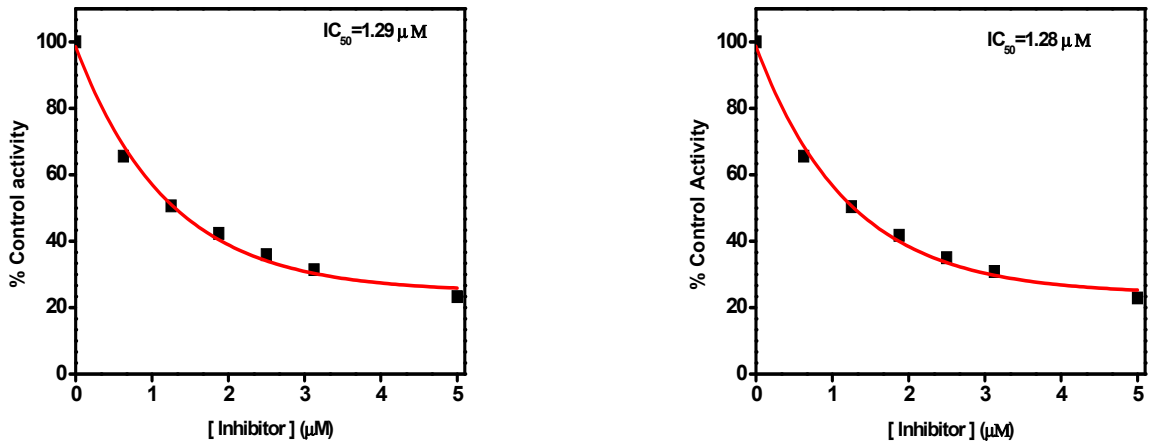


Figure S16. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **8**.

Table S16. Effect of the concentration of compound **9** on the PN-mediated oxidation of dihydrorhodamine 123.

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	5572920	100.00	1.875	2364320	42.42
	5504635	100.00		2362390	42.91
0.625	3381560	60.67	2.50	2120065	38.04
	3359500	61.03		2077460	37.74
1.25	2932485	52.62	3.125	1626220	29.18
	2891360	52.52		1604000	29.13

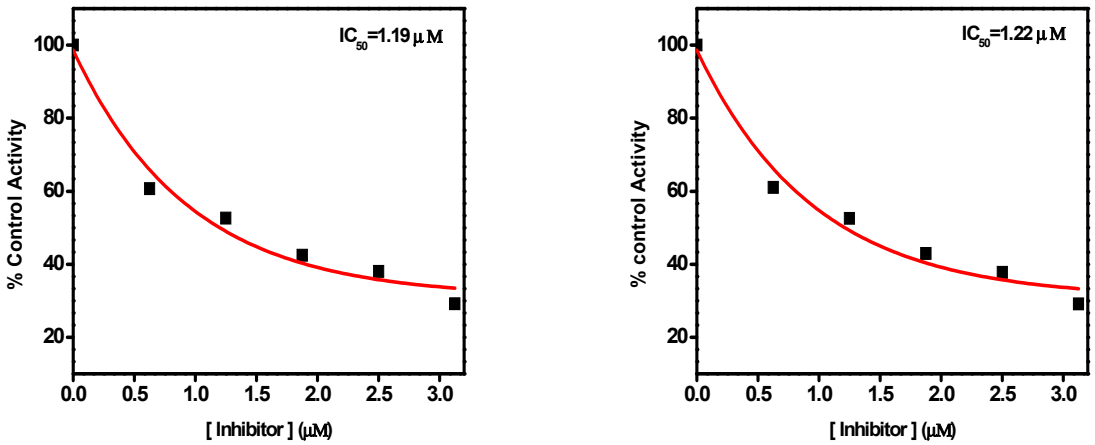


Figure S17. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **9**.

Table S17. Effect of the concentration of compound **10** on the PN-mediated oxidation of dihydrorhodamine 123..

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	5587640	100.00	2.50	2209650	39.92
	5573370	100.00		2206050	39.58
0.625	3689400	66.02	3.75	1784000	31.92
	3678770	66.00		1764050	31.62
1.25	3077880	55.08	5.0	1501790	26.87
	3026200	54.29		1495060	26.82
1.875	2593910	46.42			
	2580120	46.29			

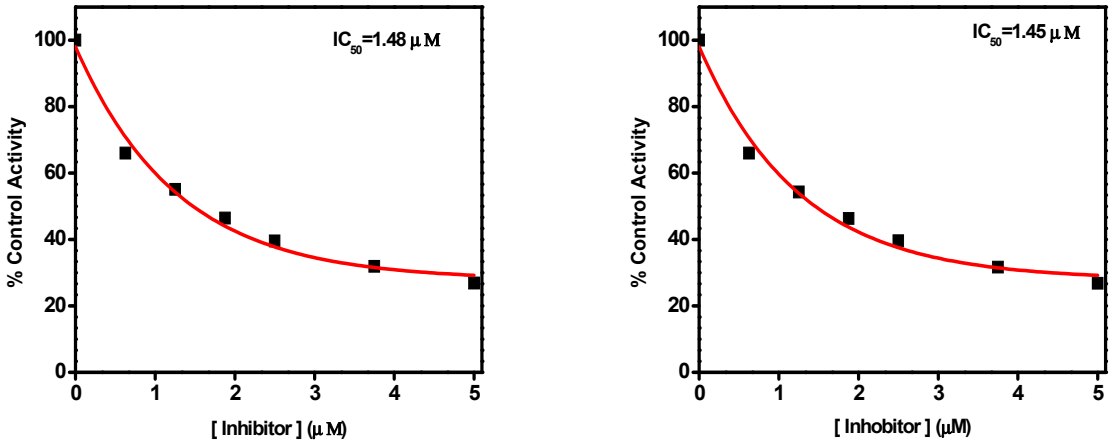


Figure S18. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **10**.

Table S18. Effect of the concentration of compound **11** on the PN-mediated oxidation of dihydrorhodamine 123.

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	5268290	100.00	2.50	1450280	27.52
	5253260	100.00		1434080	27.29
0.625	4509160	85.59	3.75	1206940	22.90
	4498970	85.64		1166510	22.20
1.25	2934470	55.70	5.0	816900	15.50
	2905940	55.31		810890	15.43
1.875	2097610	39.81			
	2093310	39.84			

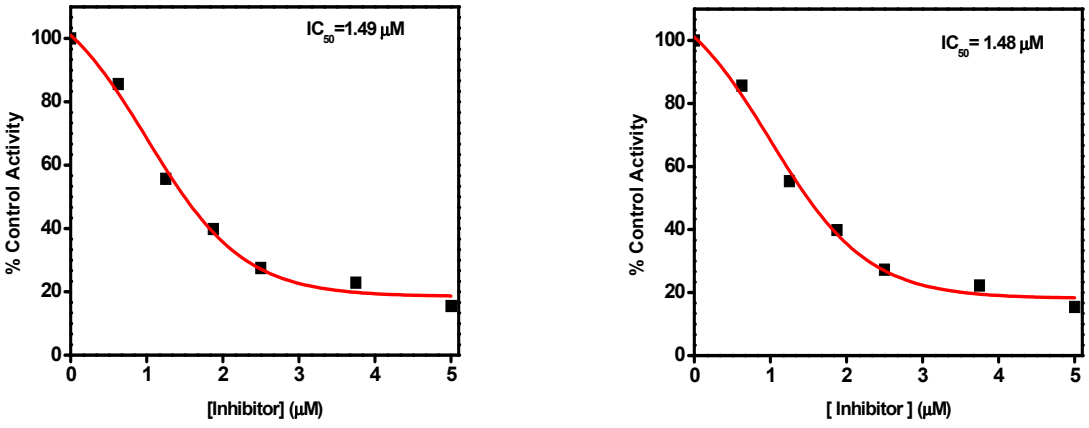


Figure S19. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **11**.

Table S19. Effect of the concentration of compound **12** on the PN-mediated oxidation of dihydrorhodamine 123.

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	4800940	100.00	1.25	1486280	30.95
	4775580	100.00		1480430	31.00
0.3125	3664750	76.33	1.875	1057660	22.03
	3661390	76.66		1048200	21.94
0.625	2785860	58.02	2.50	802340	16.71
	2749350	57.57		798100	16.71
0.9375	1889830	39.36			
	1850270	38.74			

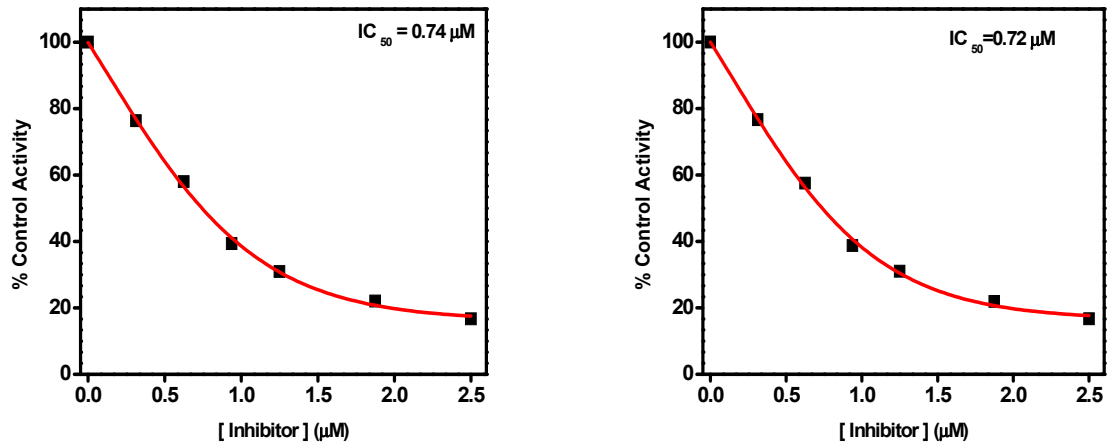


Figure S20. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **12**.

Table S20. Effect of the concentration of compound **13** on the PN-mediated oxidation of dihydrorhodamine 123.

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	5533620	100.00	2.50	2258930	40.82
	5519790	100.00		2239540	40.57
0.625	4180830	75.55	3.75	1881430	33.99
	4178280	75.69		1836600	33.73
1.25	3308440	59.78	5.0	1524560	27.55
	3236760	58.63		1500590	27.02
1.875	2635660	47.62	7.5	1256220	22.70
	2602310	47.14		1160500	21.02

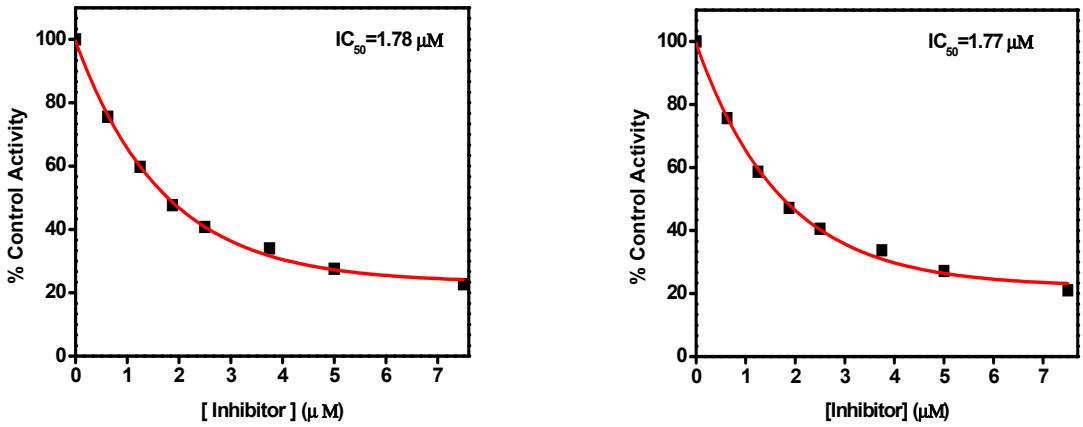


Figure S21. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **13**.

Table S21. Effect of the concentration of compound **14** on the PN-mediated oxidation of dihydrorhodamine 123.

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	5215250	100.00	12.5	2886270	55.34
	5209280	100.00		2863060	54.96
1.25	4546940	87.18	25.0	2226760	42.69
	4502100	86.42		2221190	42.63
2.50	4237310	81.24	50.0	1590180	30.49
	4216580	80.94		1550930	29.77
6.25	3672910	70.42			
	3661470	70.28			

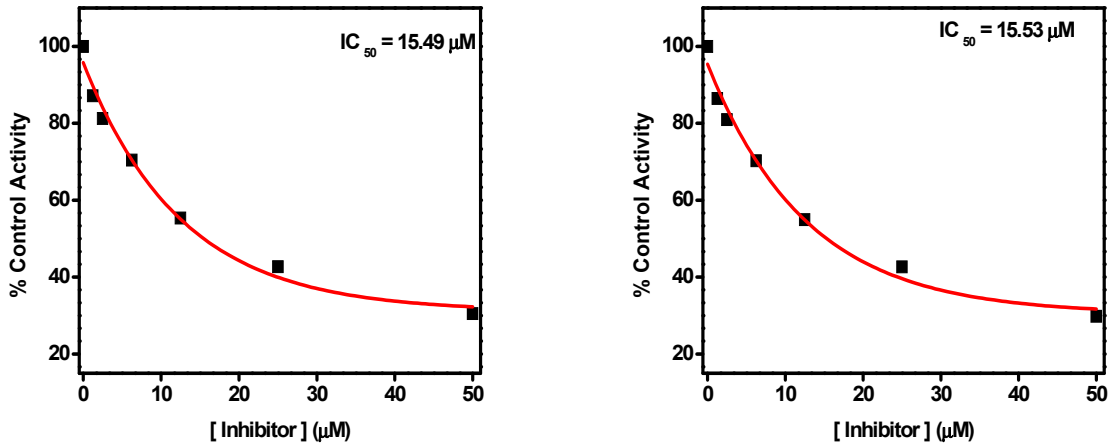


Figure S22. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **14**.

Table S22. Effect of the concentration of compound **15** on the PN-mediated oxidation of dihydrorhodamine 123.

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	5215250	100.00	2.50	2802460	53.73
	5209280	100.00		2783110	53.42
0.625	4235460	81.21	5.0	1592000	30.52
	4214620	80.90		1579260	30.31
1.25	3704300	71.02	10.0	613380	11.76
	3698550	70.99		603150	11.69
1.875	3293460	63.15			
	3260850	62.59			

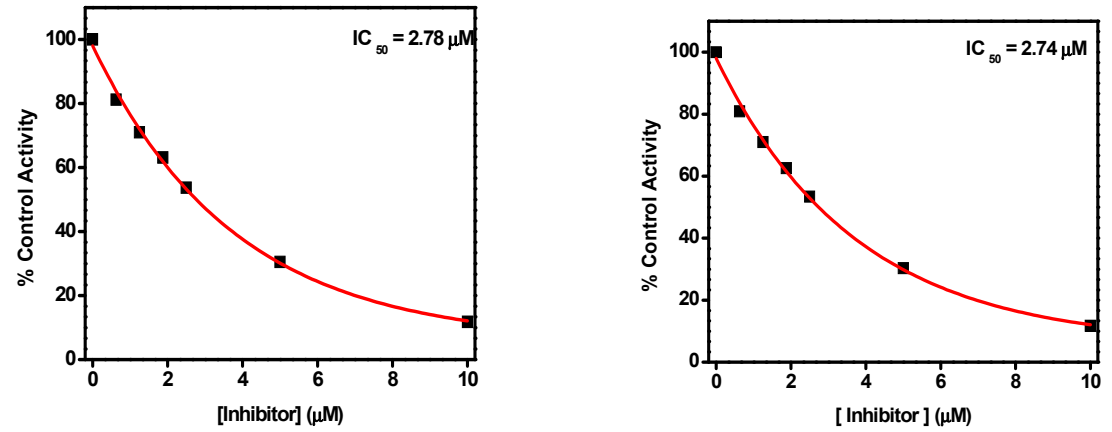


Figure S23. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **15**.

Table S23. Effect of the concentration of compound **16** on the PN-mediated oxidation of dihydrorhodamine 123.

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	5225850	100.00	12.5	3002670	57.45
	5219140	100.00		2984260	57.17
2.50	4709800	90.12	25.0	1968220	37.66
	4686290	89.79		1947320	37.31
5.0	4105040	78.55	50.0	1118010	21.39
	4072560	78.03		1096850	21.01

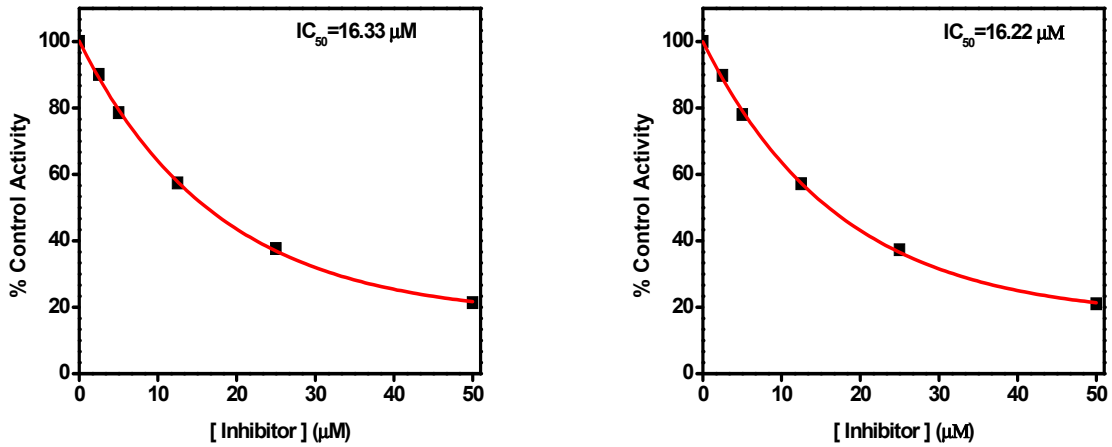


Figure S24. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **16**.

Table S24. Effect of the concentration of compound **17** on the PN-mediated oxidation of dihydrorhodamine 123.

[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity	[Inhibitor] (μM)	Emission intensity of rhodamine 123	% Control Activity
0.00	5235290	100.00	1.875	2141170	40.89
	5230490	100.00		2115570	40.44
0.3125	3747570	71.58	2.50	1808610	34.54
	3706620	70.86		1777710	33.98
0.625	3465910	66.20	5.0	917580	17.52
	3462570	66.19		899080	17.18
1.25	2570350	49.09			
	2542410	48.60			

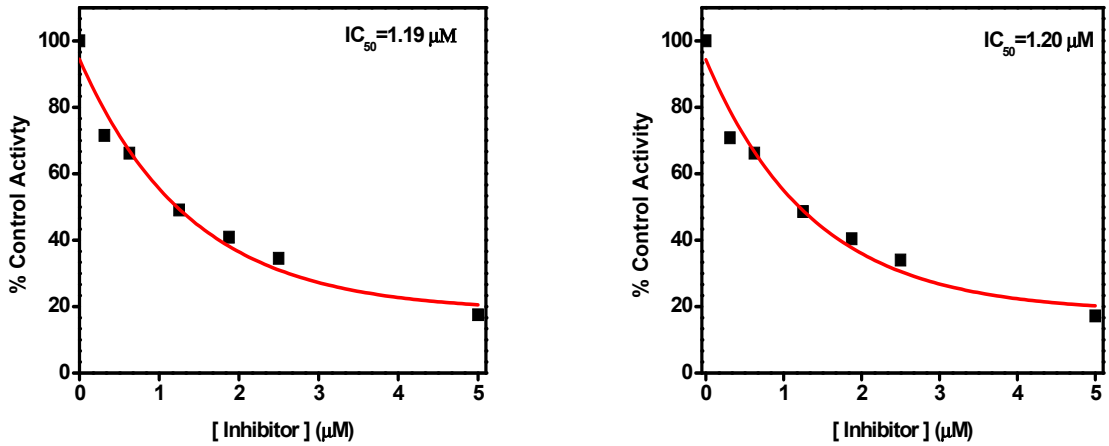


Figure S25. Plot for the inhibition of PN-mediated oxidation of DHR with the increasing concentration of compound **17**.

Table S25. Determination of NO_3^- concentration for the isomerization of peroxynitrite (PN) to NO_3^- in the presence of different selenium compounds

Compound	[NO_2-Resorcinol][a] (μM)
Control	8.64 ± 0.04
1, ebselen	16.18 ± 0.27
13	15.54 ± 0.46
14	14.92 ± 0.01

[a]The isomerization of PN to NO_3^- by the selenium compounds was carried out in phosphate buffered solutions. The reaction mixture was incubated for 30 min to ensure the isomerization. The detailed experimental condition is described in the Experimental Section.

Table S26. Determination of NO_3^- concentration for the conversion of $NaNO_2$ to $NaNO_3$ with the increasing concentration of isolated selenoxide **20** in the assay buffer.

[Selenoxide **20**] (μM)	[NO_2-Resorcinol][a] (μM)
0.0	0.27 ± 0.04
13.0	4.74 ± 0.05
133.0	11.11 ± 0.19
333.0	16.56 ± 0.55
666.0	43.38 ± 0.50

[a]The conversion of $NaNO_2$ to $NaNO_3$ by the selenoxide **20** was carried out in phosphate buffered solutions. The reaction mixture was incubated for 30 min to ensure the conversion. The detailed experimental condition is described in the Experimental Section.

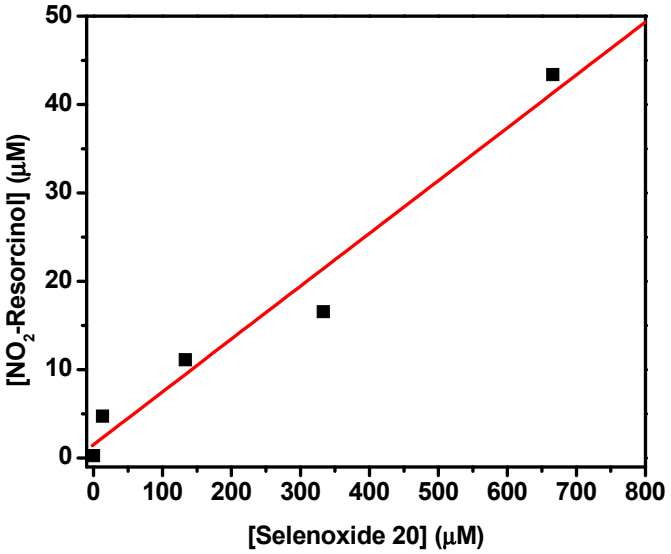


Figure S26. Plot for the formation of NO_2-resorcinol with the increasing concentration of selenoxide **20**.

NMR spectroscopic and ESI-MS spectroscopic characterization of final compounds and some important intermediates

Figure S27. ^{1}H NMR spectrum of pure seleninic acid **7** in MeOH-d_4.

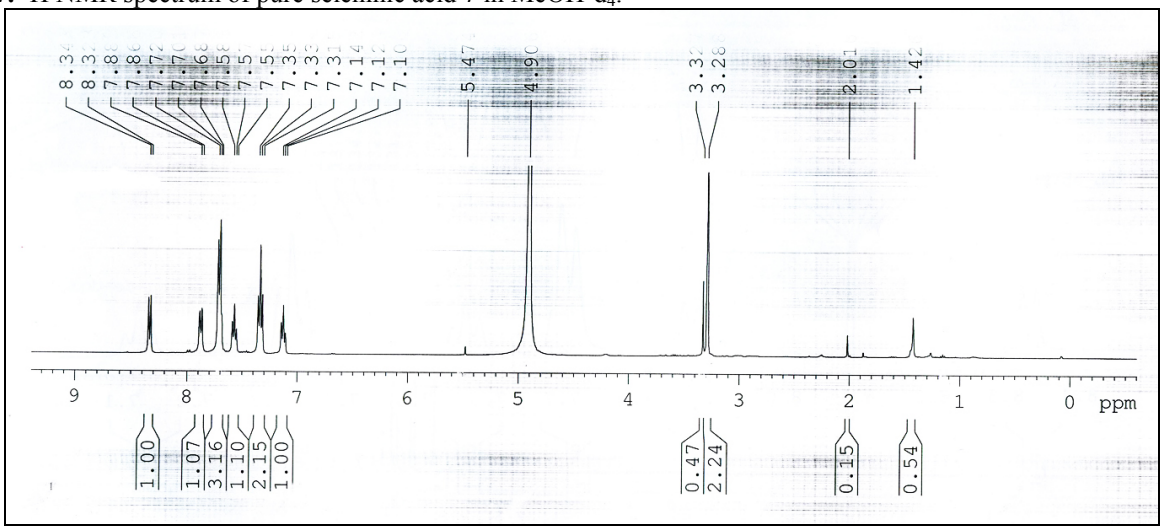


Figure S28. ^{13}C NMR spectrum of pure seleninic acid **7** in MeOH-d_4.

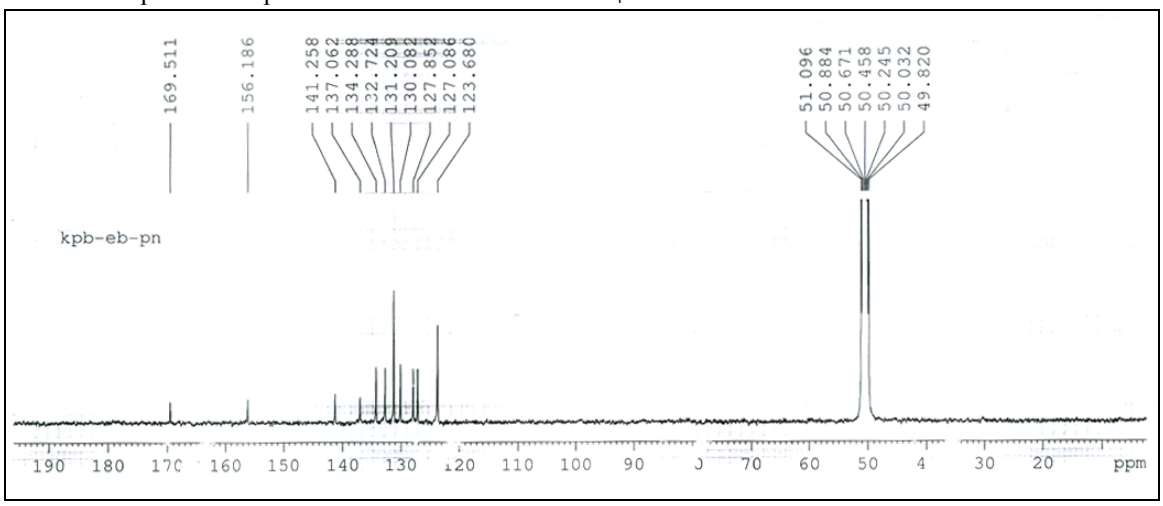


Figure S29. ^{77}Se NMR spectrum of pure seleninic acid **7** in MeOH-d_4.

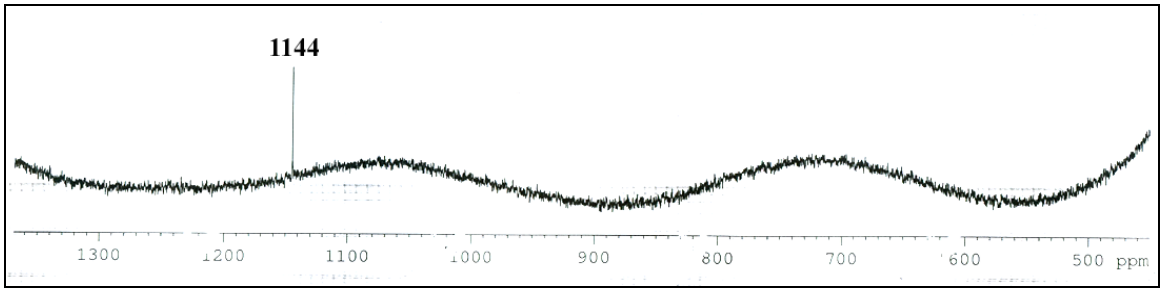


Figure S30. ESI-MS spectrum of pure seleninic acid **7**. Calculated mass $(M-H)^-$: 307.990; Observed mass: 307.699.

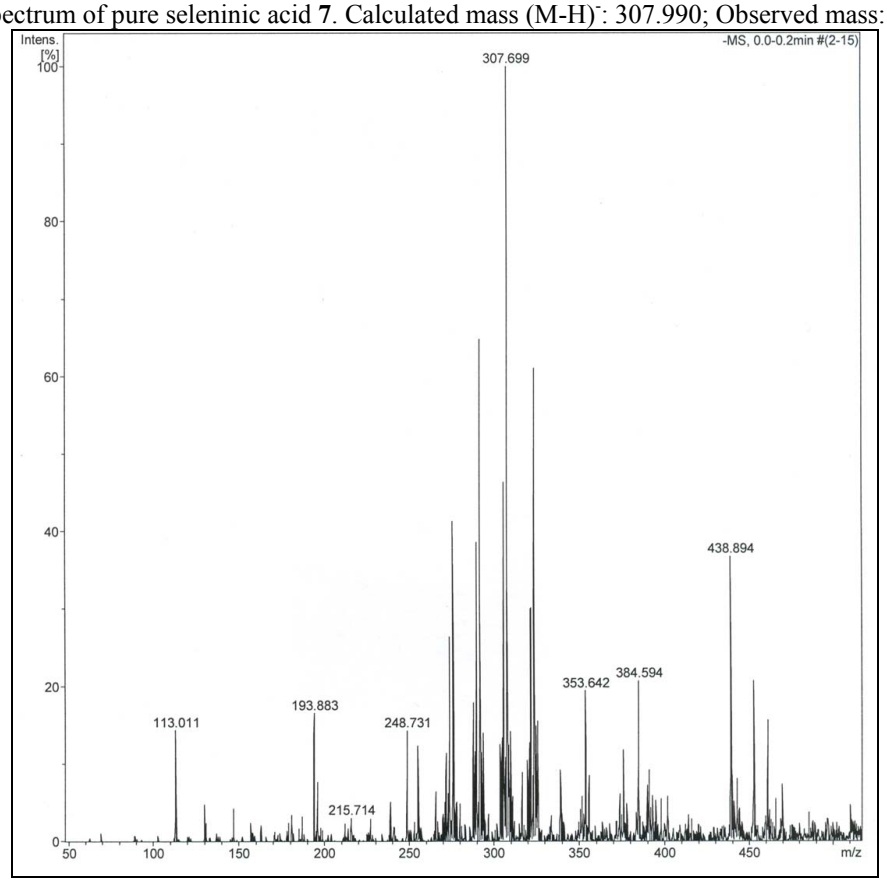


Figure S31. ^{1}H NMR spectrum of compound **16** in DMSO-d_6.

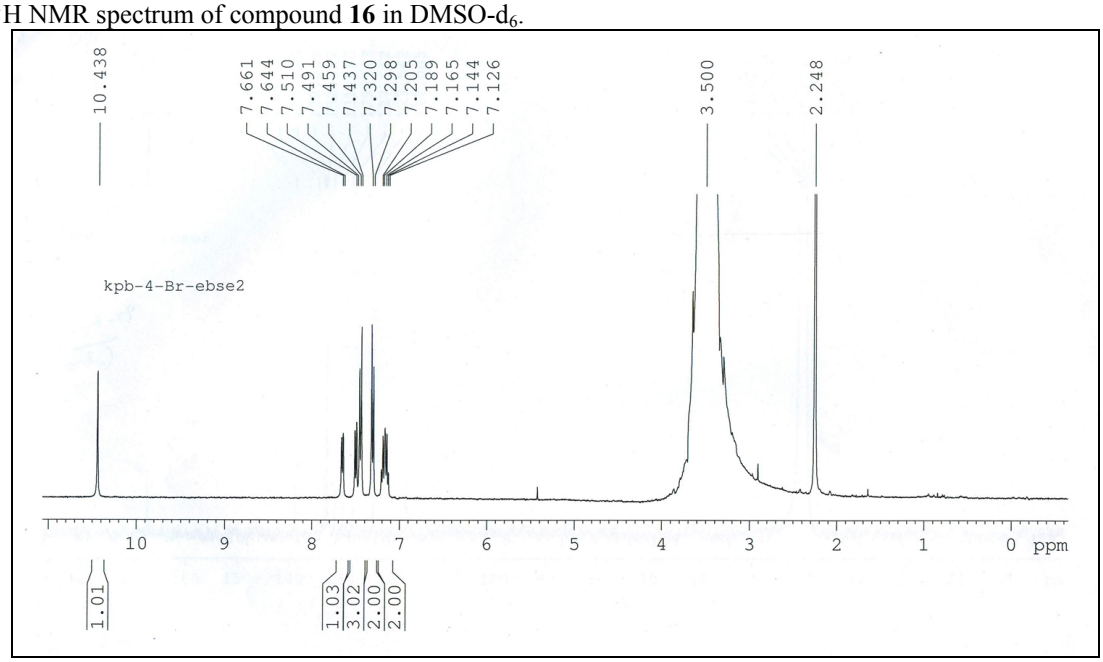


Figure S32. ^{13}C NMR spectrum of compound **16** in DMSO-d_6.

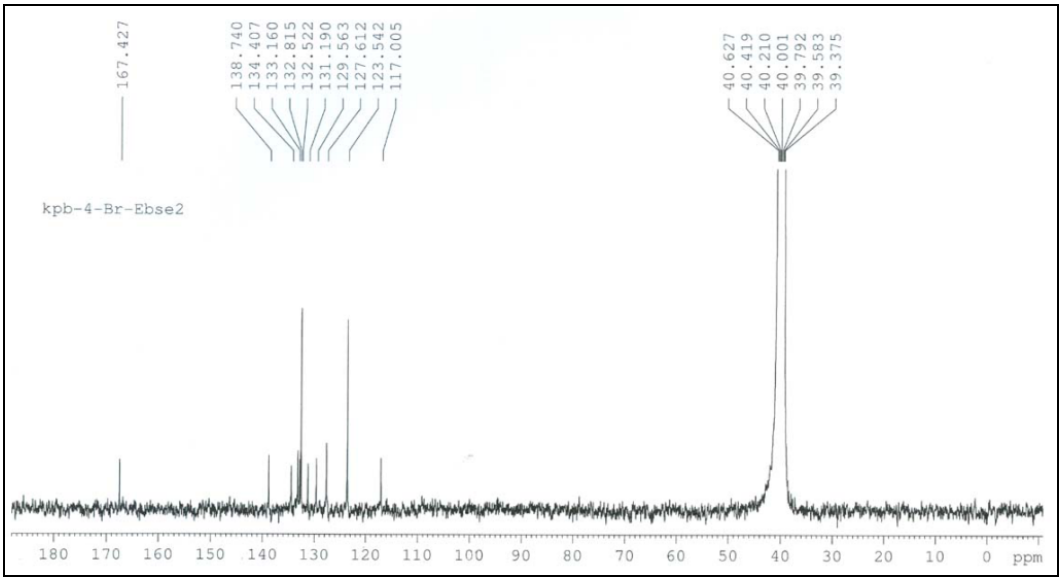


Figure S33. ^{77}Se NMR spectrum of compound **16** in DMSO-d_6.

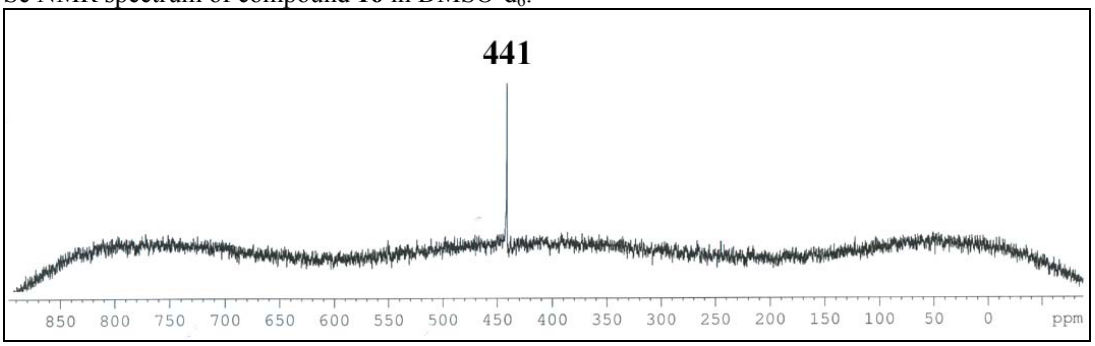


Figure S34. ESI-MS spectrum of compound **16**. Calculated mass $(M+H)^+$: 708.806; Observed mass: 708.783.

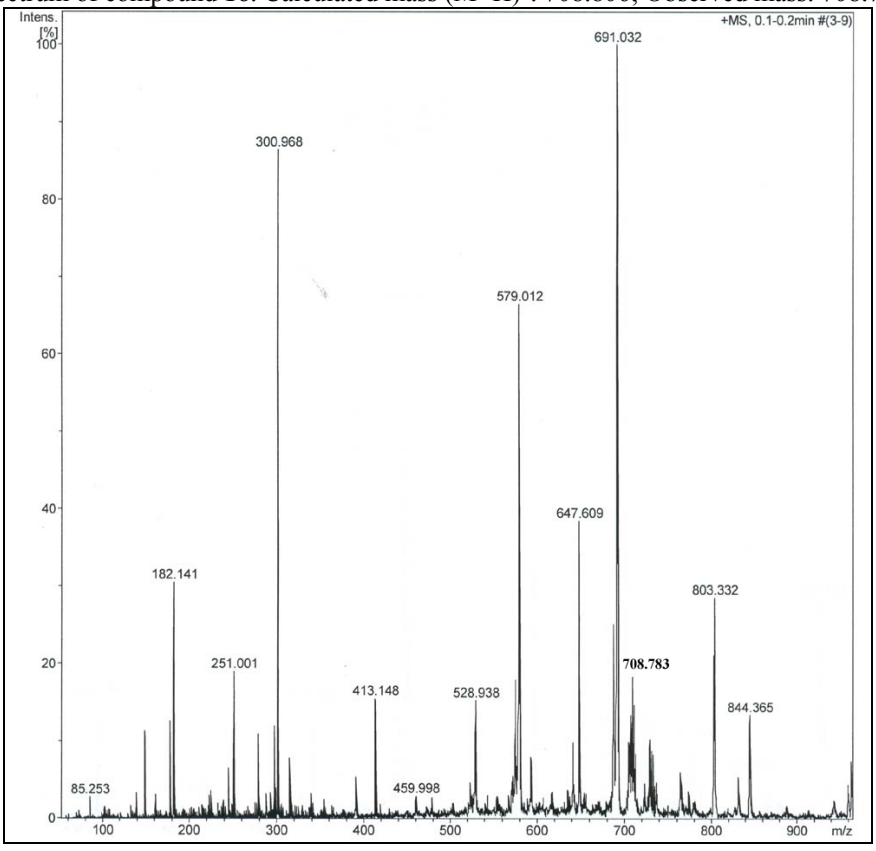


Figure S35. ^{1}H NMR spectrum of compound **17** in DMSO-d_6.

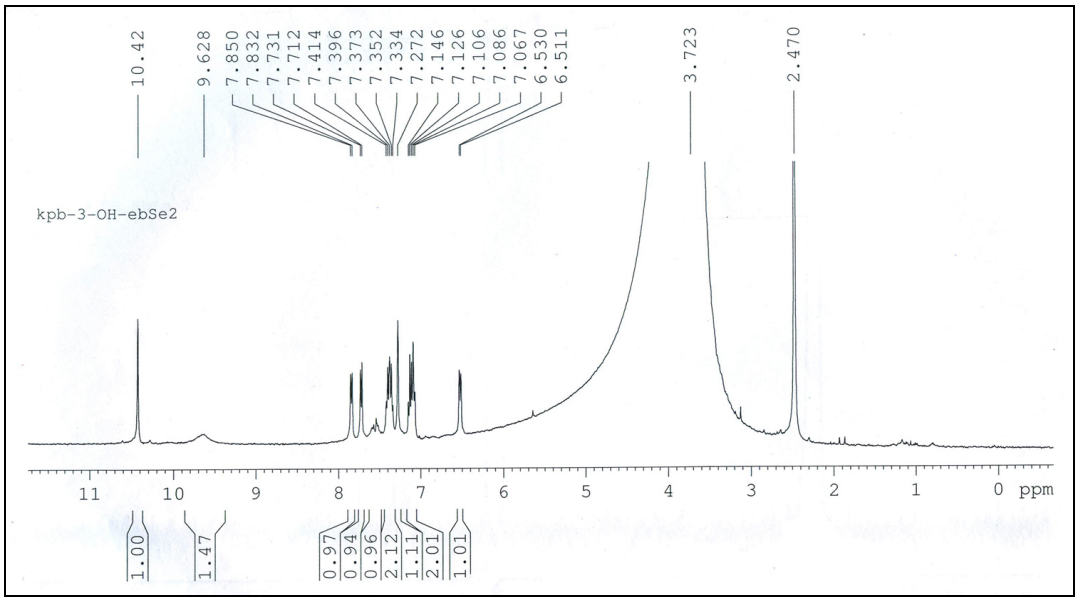


Figure S36. ^{13}C NMR spectrum of compound **17** in DMSO-d_6.

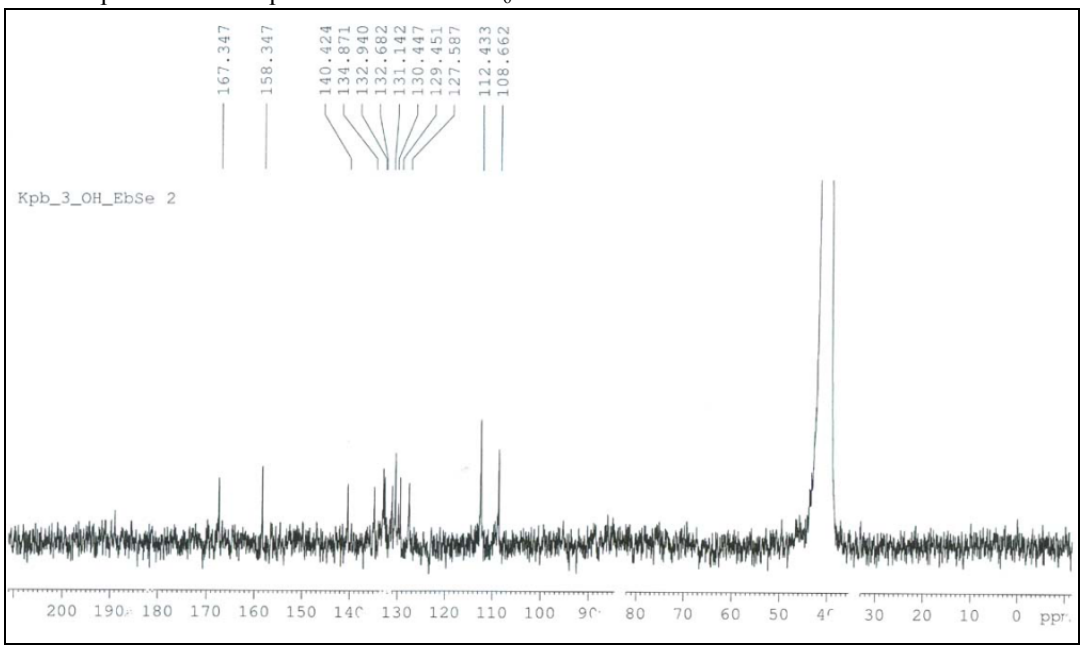


Figure S37. ^{77}Se NMR spectrum of compound **17** in DMSO-d_6.

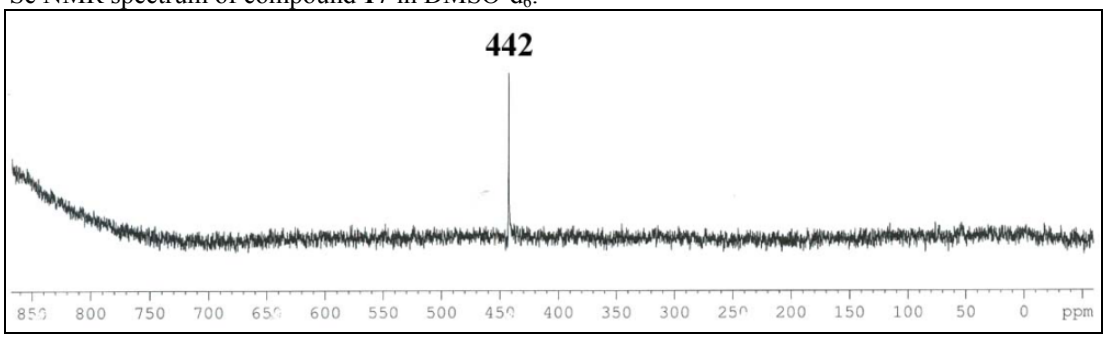


Figure S38. ESI-MS spectrum of compound **17**. Calculated mass $(M+H)^+$: 584.975; Observed mass: 584.810.

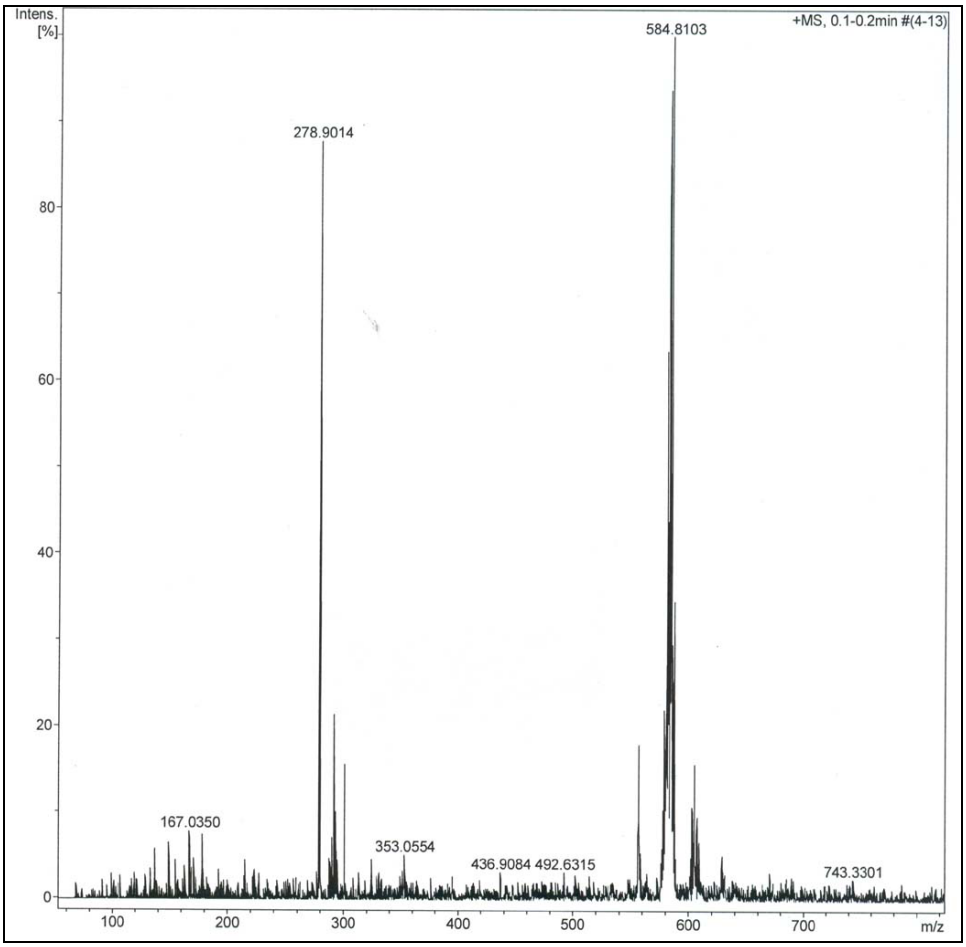


Figure S39. ^{1}H NMR spectrum of compound **19** in MeOH-d_4.

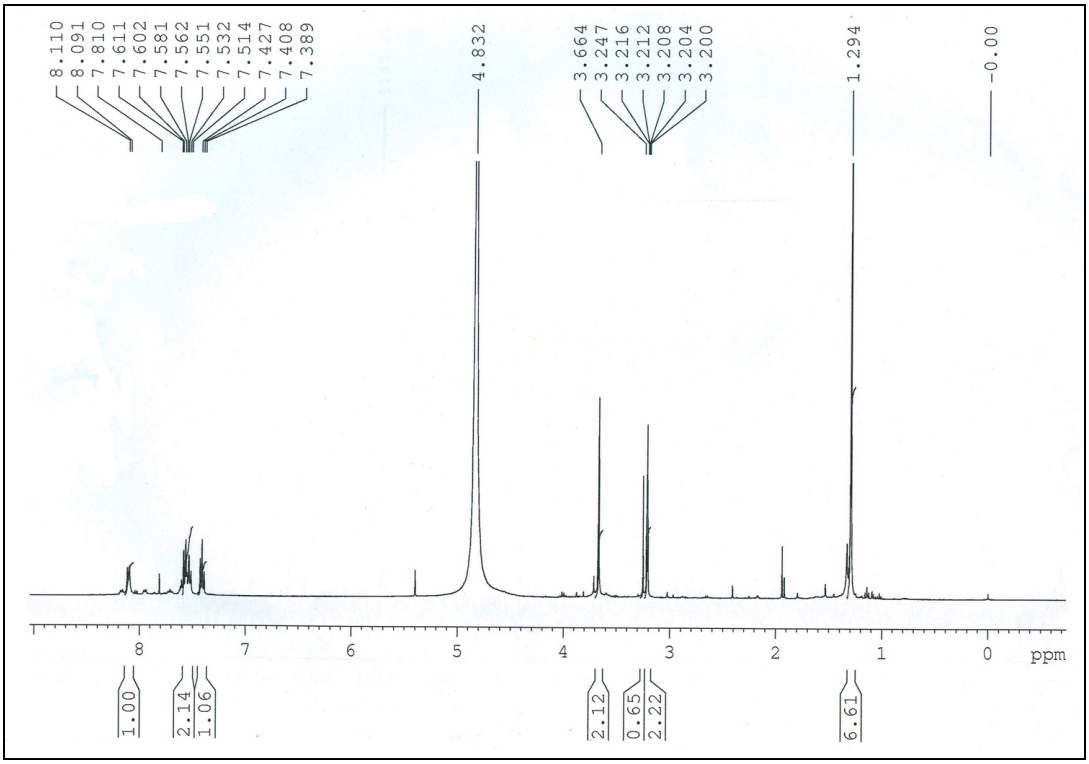


Figure S40. ^{13}C NMR spectrum of compound **19** in MeOH-d_4.

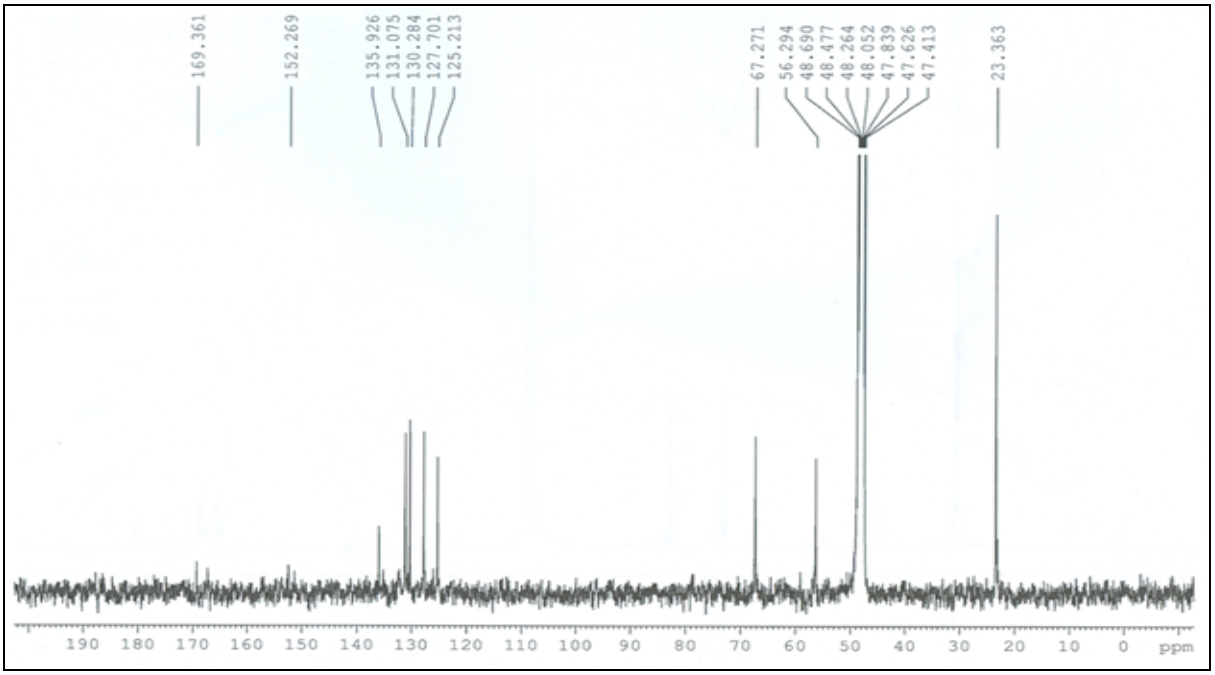


Figure S41. ^{77}Se NMR spectrum of compound **19** in MeOH-d_4.

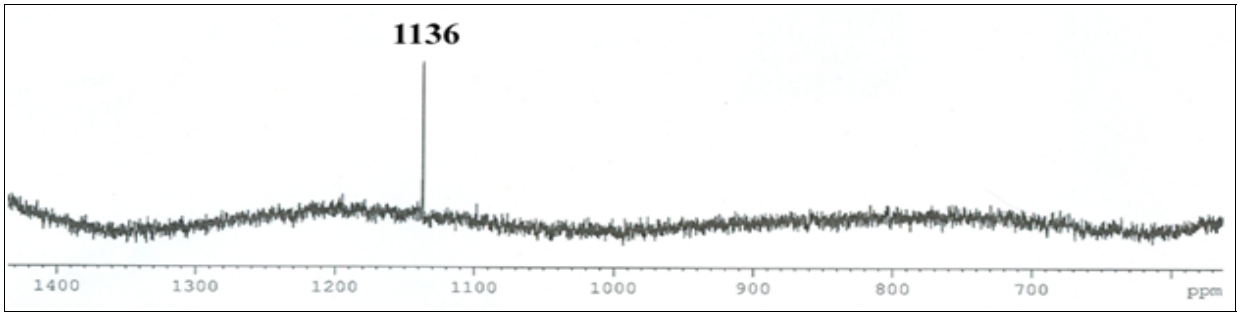


Figure S42. ESI-MS spectrum of compound **19**. Calculated mass $(M+2H)^{2+}$: 307.01; Observed mass: 307.60.

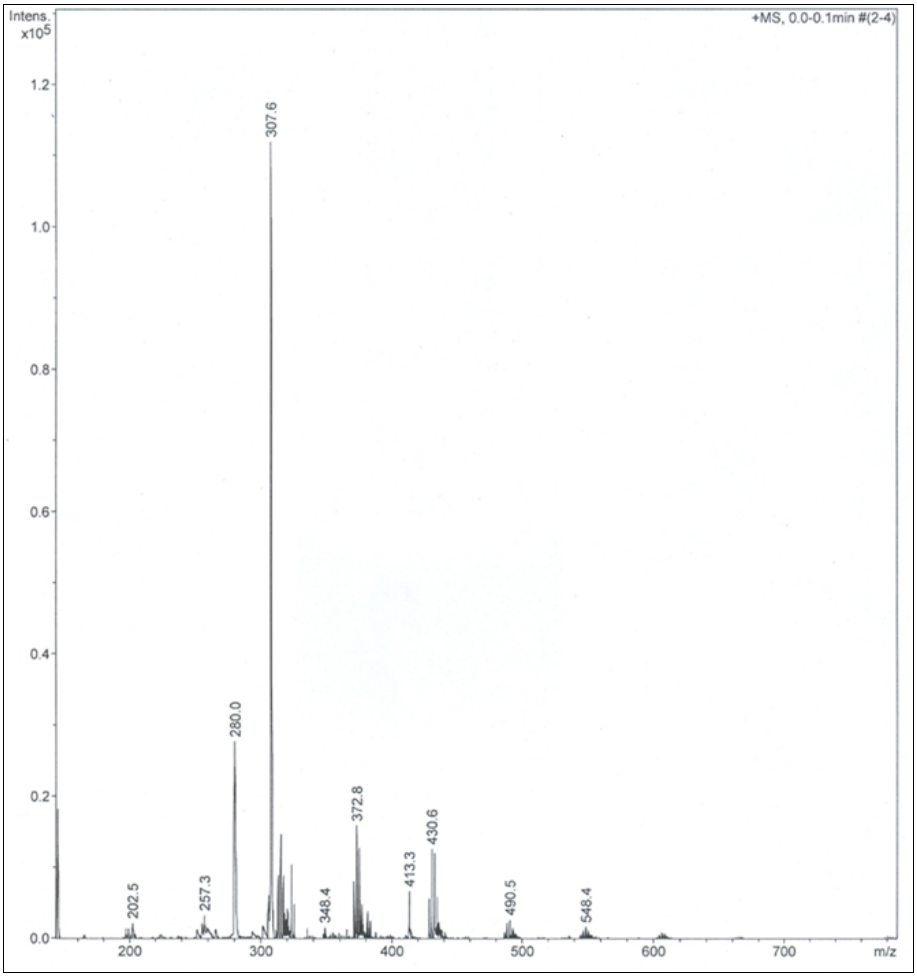


Figure S43. ^{1}H NMR spectrum of compound **20** in $CDCl_3$.

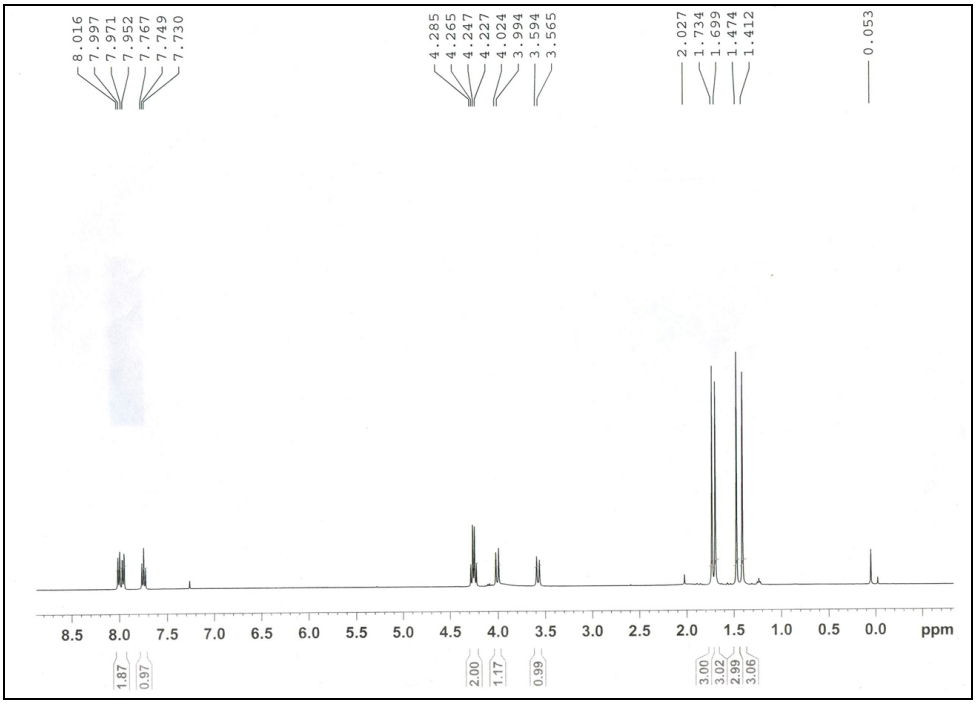


Figure S44. ^{13}C NMR spectrum of compound **20** in $CDCl_3$.

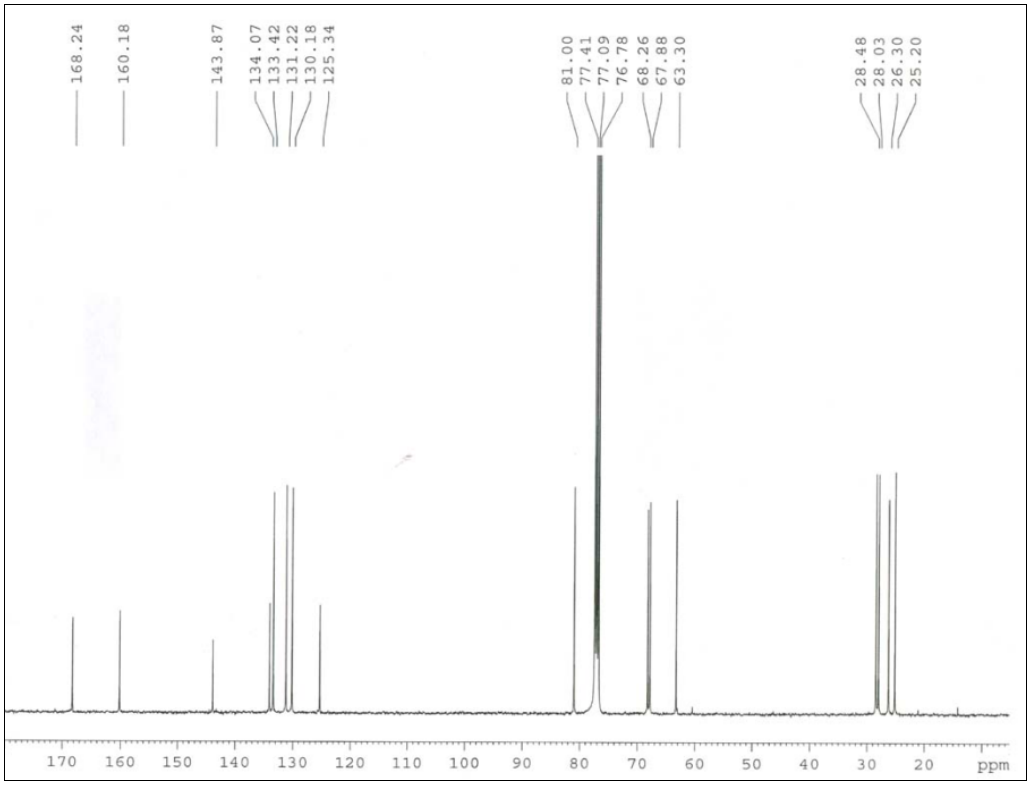


Figure S45. ^{77}Se NMR spectrum of compound **20** in $CDCl_3$.

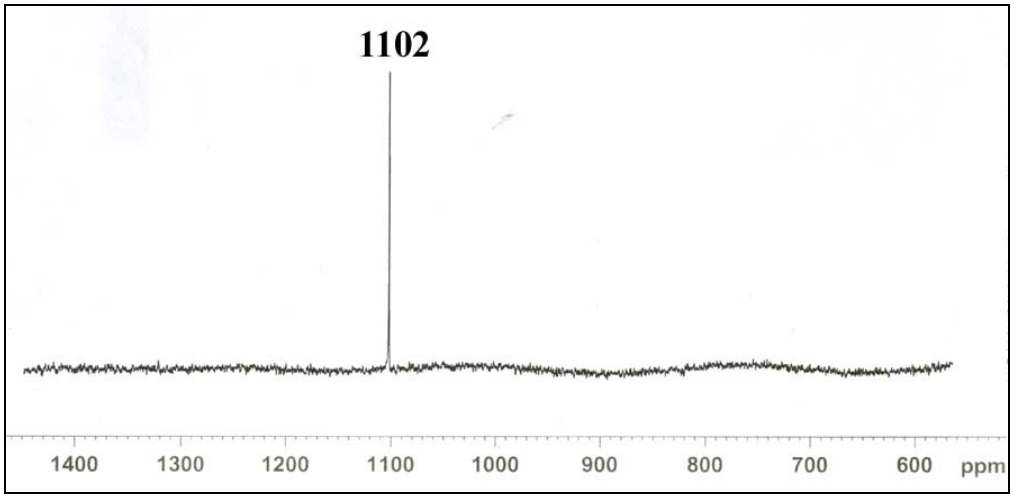


Figure S46. ESI-MS spectrum of compound **20**. Calculated mass $(M+Na)^+$: 407.048; Observed mass: 407.050.

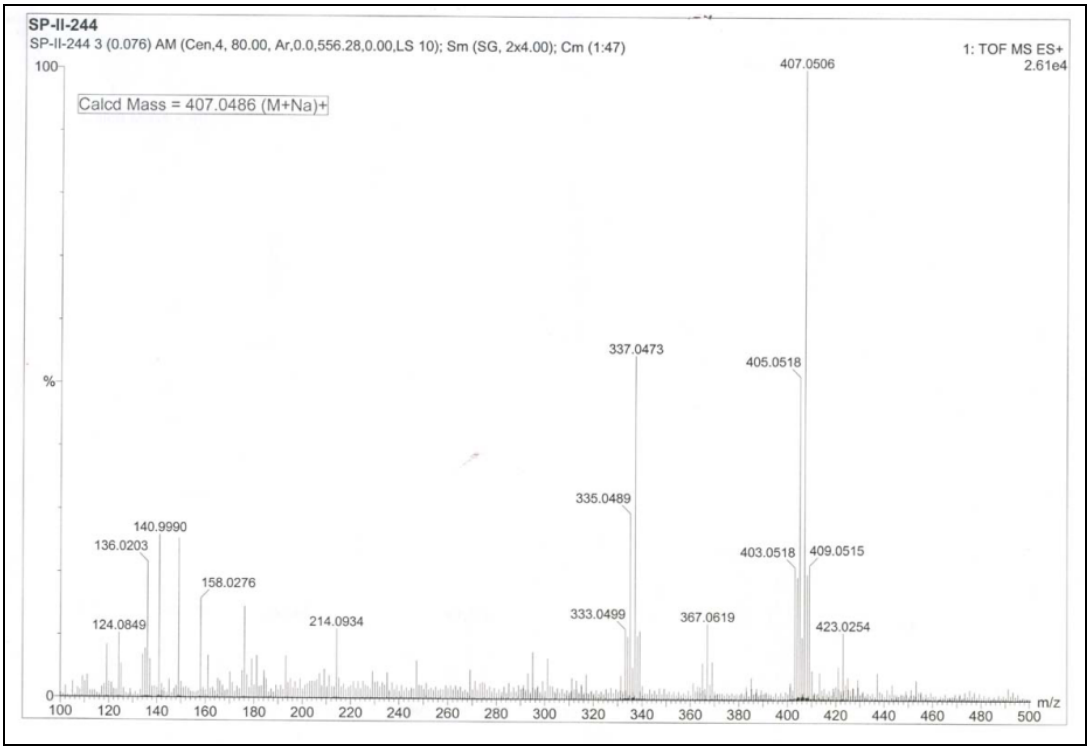


Figure S47. ^{77}Se NMR spectrum of the reaction mixture of ebselen (**1**) with an excess amount of PN in a mixture of MeOH and phosphate buffer (1:1) solution. The peak corresponding to the seleninic acid **7** was observed.

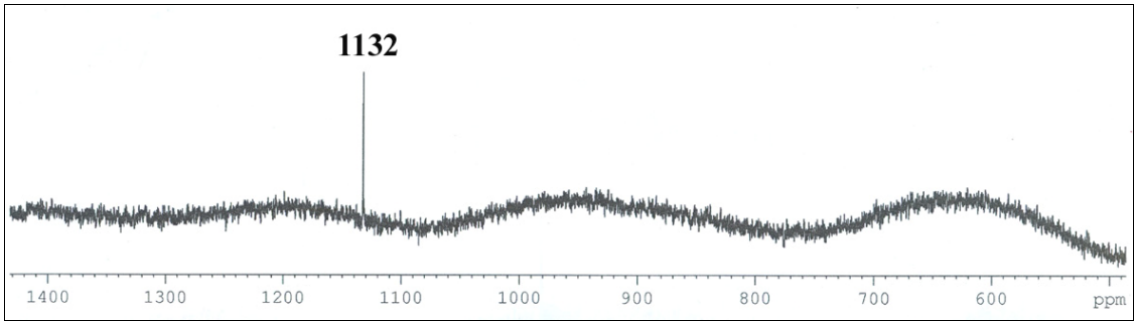


Figure S48. ^{77}Se NMR spectrum of the reaction mixture of ebselen (**1**) with an excess amount of PN in a mixture of MeOH and phosphate buffer (1:1) solution after ~24 h. A partial regeneration of ebselen was observed due to the isomerization of PN to NO_3^-.

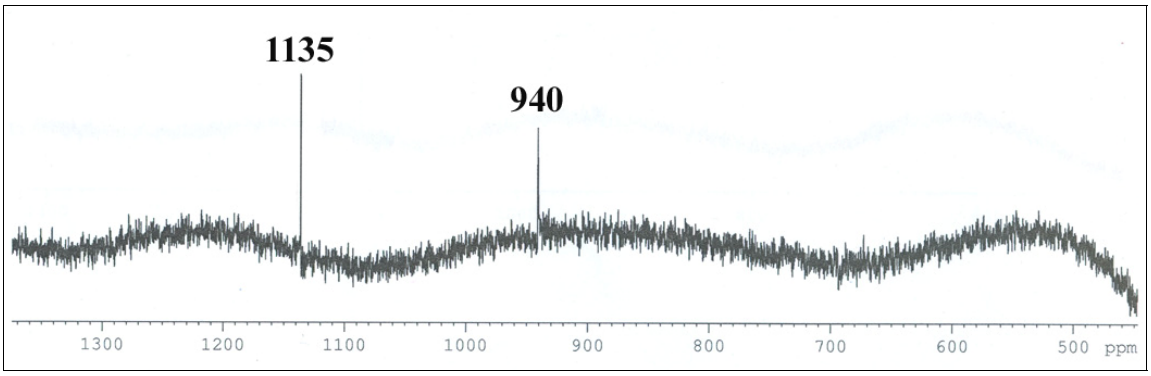


Figure S49. ^{77}Se NMR spectrum of the reaction mixture of compound **13** with an excess amount of PN in a mixture of MeOH and phosphate buffer (1:1) solution. The peak corresponding to the seleninic acid **19** was observed.

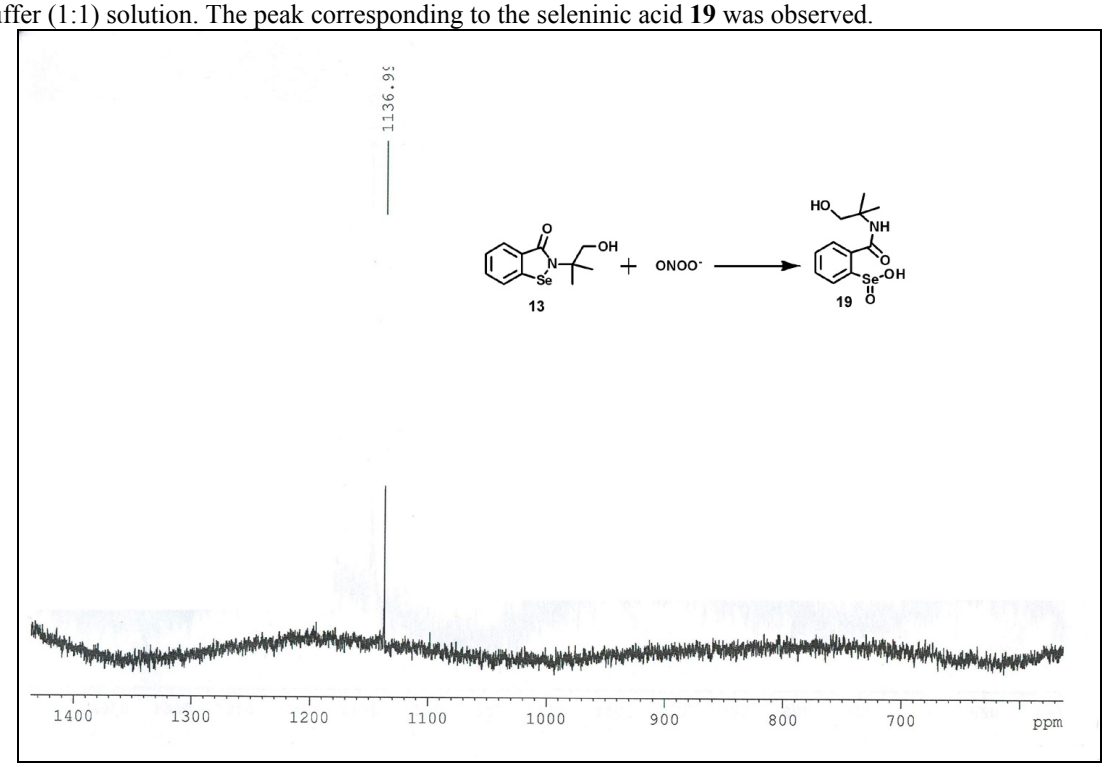


Figure S50. ^{77}Se NMR spectrum of the reaction mixture of compound **14** with an excess amount of PN in a mixture of MeOH and phosphate buffer (1:1) solution. The peak corresponding to the selenoxide **20** was observed.

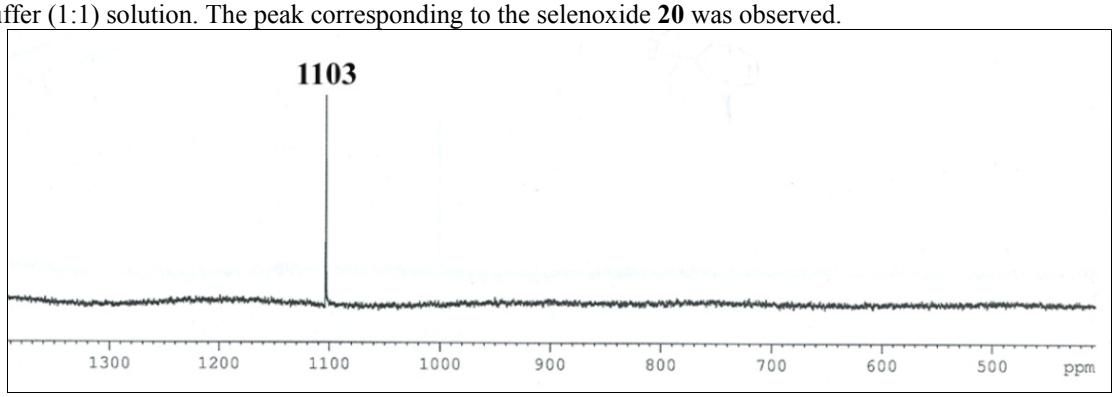


Figure S51. ^{77}Se NMR spectrum of the reaction mixture of compound **14** with an excess amount of PN in a mixture of MeOH and phosphate buffer (1:1) solution after ~24 h. A complete regeneration of selenenyl amide (**14**) was observed due to the isomerization of PN to NO_3^-.

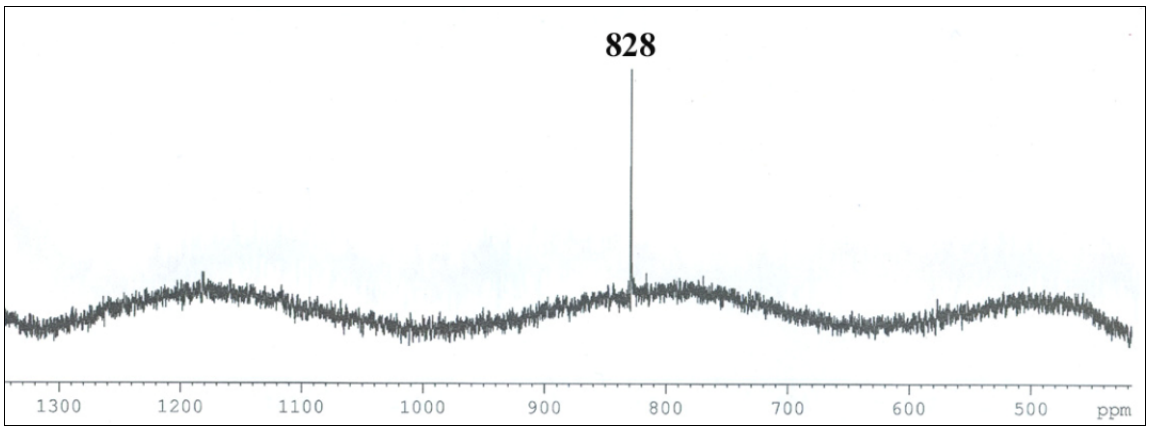


Figure S52. ^{77}Se NMR spectrum of the reaction mixture of compound **15** in the presence of ~5 equiv of PN in a mixture of MeOH and phosphate buffer (1:1) solution. Oxidation of diselenide **15** takes place to generate a mixture of selenenic acid **22** and seleninic acid **7** (1141 ppm). A rapid cyclization of **22** leads to the formation of ebselen (**1**, 964 ppm).

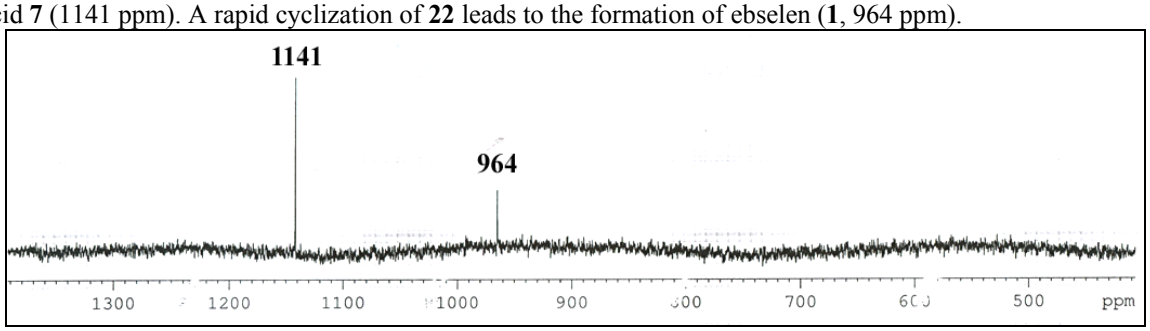


Figure S53. ^{77}Se NMR spectrum of the reaction mixture of compound **15** in the presence of an excess amount of PN (10-12 equiv) in MeOH. A further oxidation of selenenyl amide **1** to the corresponding seleninic acid **7** by PN was observed.

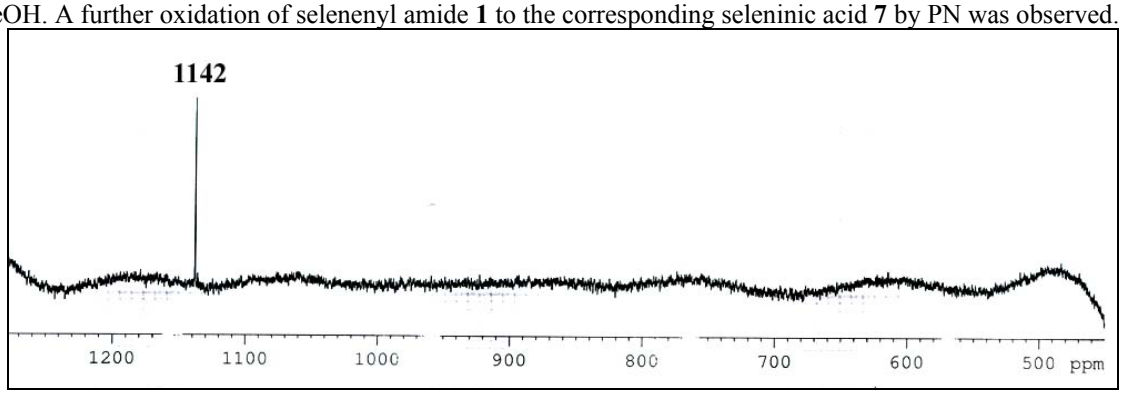


Figure S54. ^{77}Se NMR spectrum of the pure selenoxide **20** in a mixture of MeOH and phosphate buffer (1:1) solution.

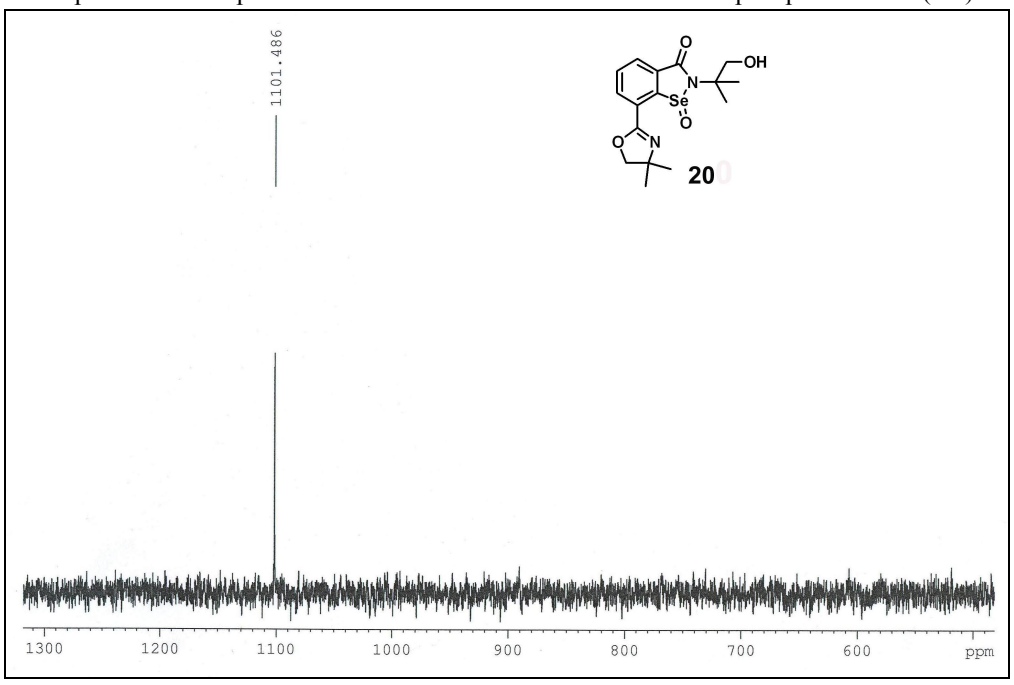


Figure S55. ^{77}Se NMR spectrum of the reaction mixture upon treatment of $NaNO_2$ with pure selenoxide **20** in a mixture of MeOH and phosphate buffer (1:1) solution. The selenoxide **20** reacts with NO_2^- to generate the selenoxide-nitrite complex **24**.

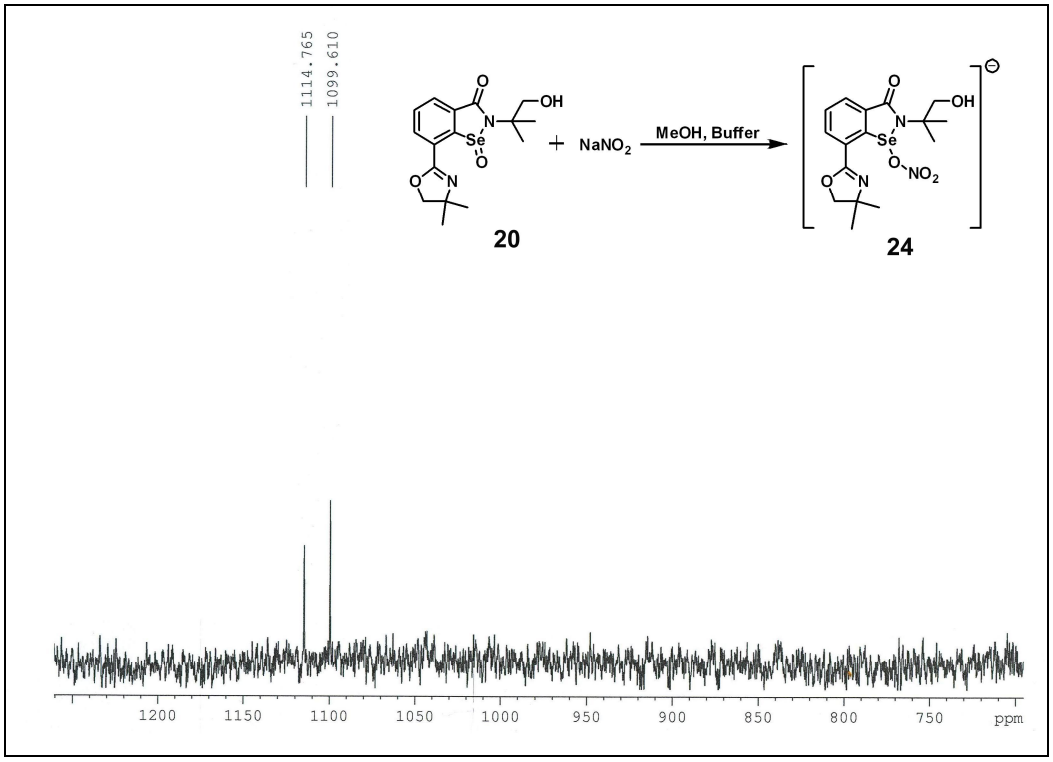
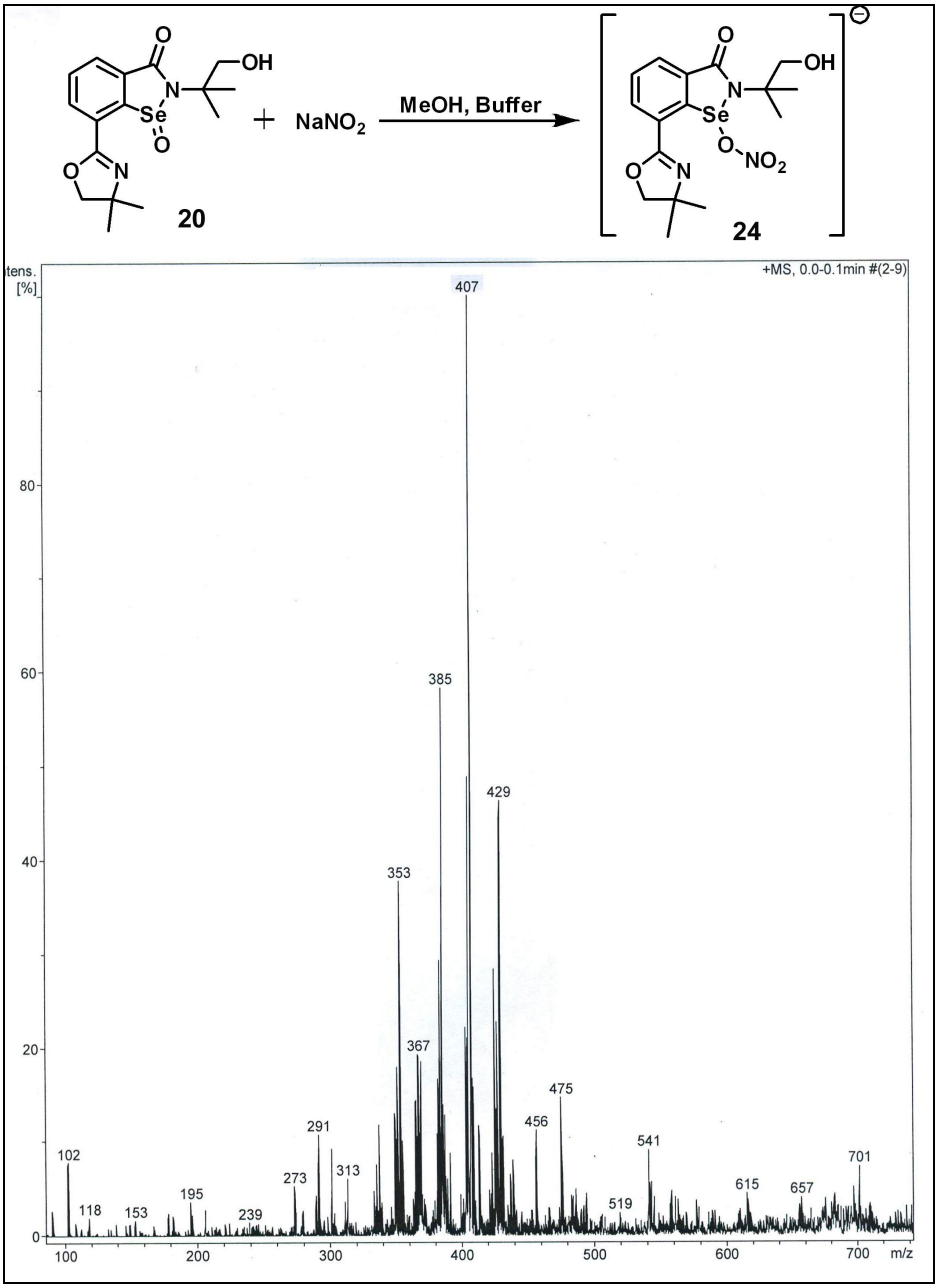


Figure S56. ESI-MS spectrum of the reaction mixture upon treatment of $NaNO_2$ with pure selenoxide **20** in a mixture of MeOH and phosphate buffer (1:1) solution. The selenoxide **20** reacts with NO_2^- to generate the intermediate **24**.
Calculated mass for **20** $(M+H)^+$: 385.0667 $(M+Na)^+$: 407.0486; Observed mass: 385.0236 and 407.0206.
Calculated mass for **24** $(M+H)^+$: 429.3065; Observed mass: 429.0616.



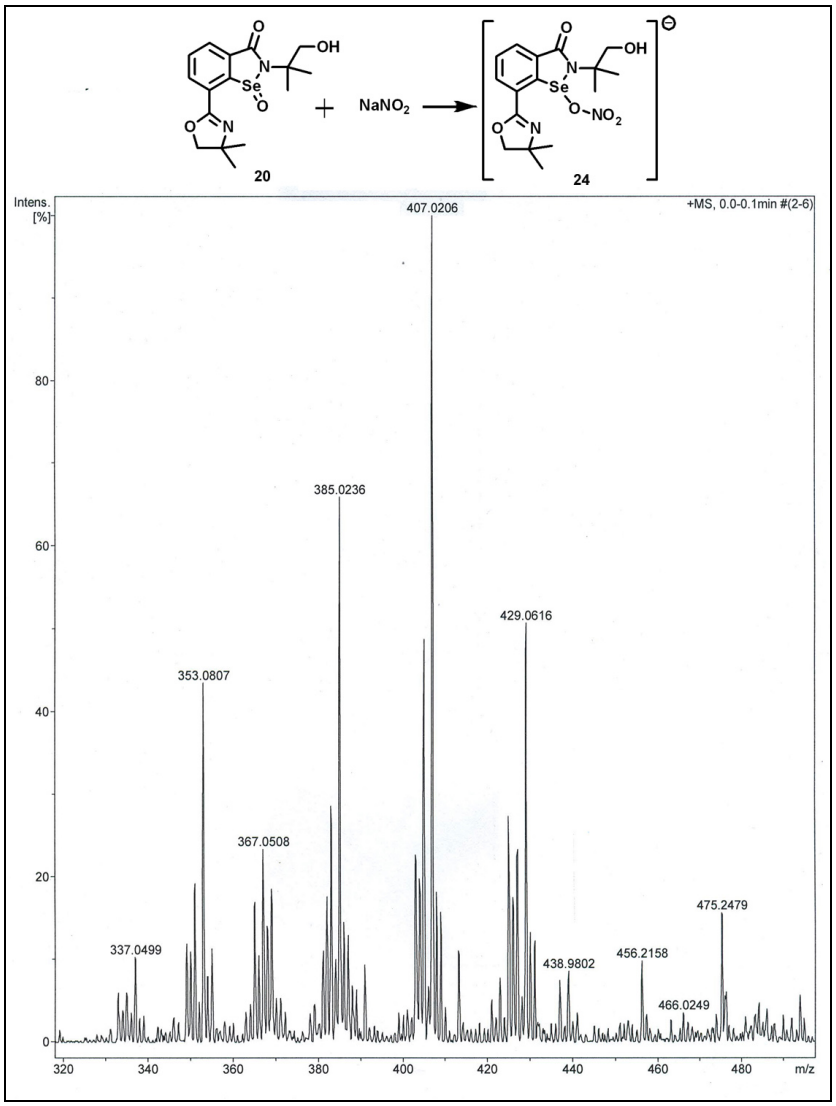


Figure S57. ^{77}Se NMR spectrum obtained after the treatment of the reaction mixture containing **20** and **24** with PN. (Solvent: MeOH and phosphate buffer (1:1)). The regeneration of selenoxide **20** was observed.

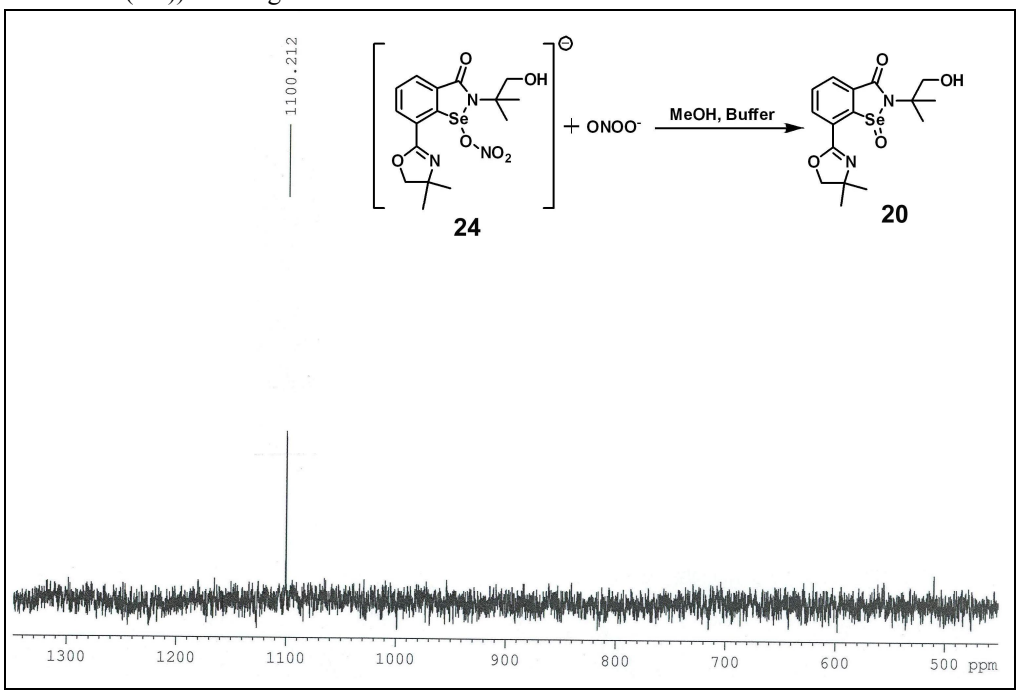


Figure S58. ^{77}Se NMR spectrum of the pure selenenyl amide **14**. (Solvent: MeOH and phosphate buffer (1:1)).

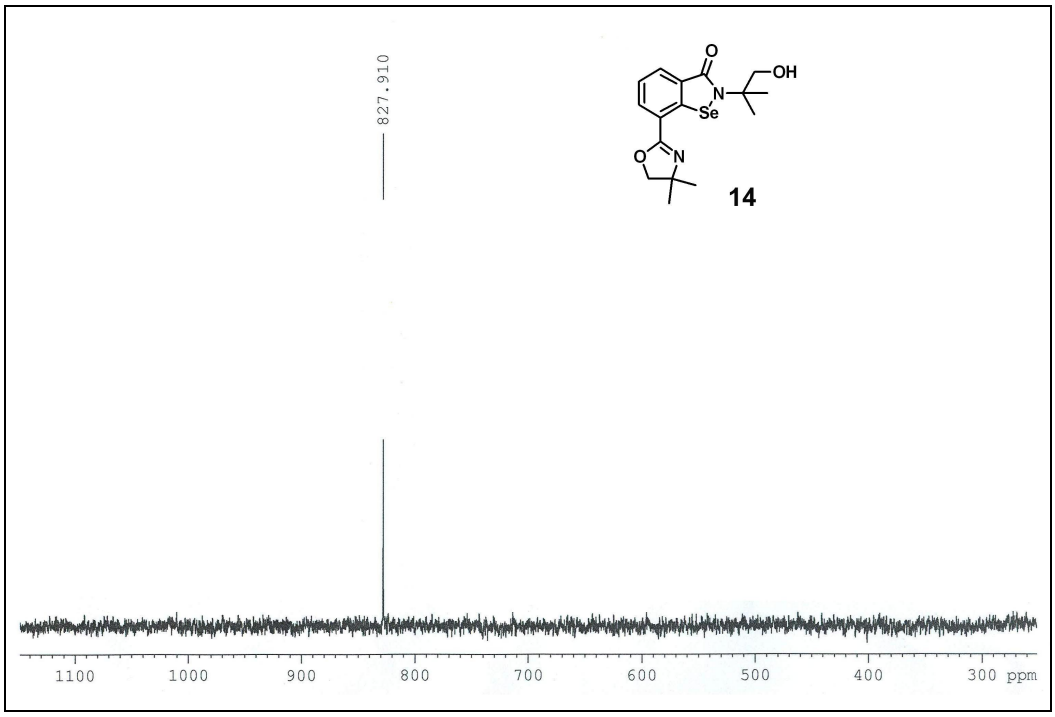


Figure S59. ^{77}Se NMR spectrum obtained for the reaction of selenenyl amide **14** with PN. After 24h, a complete disappearance of the peak due to selenoxide **20** was observed. After this period, the regeneration of **14** was observed. (Solvent: MeOH and phosphate buffer (1:1)).