Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Identification of pyridine derivative of diselenides as potent inhibitors of main protease of SARS-CoV-2 through *in silico* screening and biochemical evaluation

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Supplementary methods

Synthesis of 2, 2′-Diselenobis-(pyridine) [2-C5H4N-Se]$_2$ or 2-Py$_2$Se$_2$:

The above compound was synthesized using method described previously[1]. In a typical experiment, 2-bromopyridine (0.5 g, 31.5 mmol) was added to an aqueous brown solution of Na2Se2 (prepared from selenium powder (2.5 g, 31.67 mmol) in deoxygenated water and sodium borohydride (1.2 g, 31.5 mmol) at 0 °C under nitrogen. The reaction mixture was refluxed for 3 h till the solution became yellow containing a small amount of suspended selenium. The hot reaction mixture was filtered and allowed to cool to room temperature, whereupon dipyridyl diselenide crystallized out as yellow crystals and was filtered using a Buchner funnel to obtain crystalline Py$_2$Se$_2$ (2.03 g, 42%). M.p. 50-51 °C[1a]; (lit 47.5-50[1b,1c]) °C. Anal. Calcd. for C10H8N2Se2: Calcd. C, 38.23; H, 2.56; N, 8.92%. Found: C, 37.71; H, 2.45; N, 8.45%. IR (KBr, υ cm-1): 3047, 1584, 452, 778. 1H NMR(CDCl3) δ: 7.07 (1H, t, J = 5.6 Hz, C5H4), d 7.53 (1H, t, J = 7.4 Hz, C5H4), d 7.78 (1H, d, J = 7.8 Hz, C5H4), d 8.44 (1H, d, J = 3.6 Hz, C5H4);13C{1H} NMR (CDCl3) δ: 121.2 (C-5),123.5 (C-3), 137.5 (C-4), 149.6 (C-6),154.4 (Se-C); 77Se{1H} NMR (CDCl3) δ: 447.5 ppm.

Synthesis of 2, 2′-Diseleno bis(3-nicotinamide) [2-NC$_5$H$_3$(3-CONH$_2$)Se]$_2$ or Nic$_2$Se$_2$:

The above compound was synthesized using method described previously[2]. To an aqueous suspension of elemental Se (0.378 g, 4.788 mmol) in a three-necked round bottom flask, sodium borohydride (0.18 g, 4.788 mmol) was added slowly with stirring resulting into a dark red solution which was refluxed for 30 min. After cooling to room temperature, 2-choro-3-nicotinamide (0.75 g, 4.788 mmol) (synthesized by reaction 2-chloro-3-nicotinic acid with thionyl chloride ($SOCl_2$) followed by addition of liquid NH_3 and filtered on silica column by $CHCl_3$ eluent) was added slowly with stirring and the solution was refluxed for 5 h till the

yellow-colored clear solution was obtained. The solution was filtered in a beaker and allowed to cool whereupon a yellow solid was separated which was filtered out, washed thoroughly with cold distilled water and dried in vacuo (0.415 g, 43 %). The brownish filtrate remained after this was processed separately. The yellow solid was recrystallized from hot methanol at room temperature to afford yellow crystals of nicotinamide diselenide (0.17 g, 18 %), m. p. 230 ºC (decomp). Analysis for nicotinamide diselenide $C_{12}H_{10}N_4O_2Se_2$: Calcd: C, 36.02; H, 2.52; N, 14.00%. Found C, 35.47; H, 2.59; N, 14.01%. NMR: ^{1}H NMR (dmso-d_6) δ: 7.28 (dd, 4.6, 7.6 Hz, 2H), 7.83, 8.33 (each br s, NH_2), 8.15 (d,d, 1.5, 7.8 Hz, 2H), 8.48 (d,d, 1.5, 4.6 Hz, 2H); ^{13}C{^{1}H} NMR (dmso-d_6) δ: 120.0, 128.3 (C-Se), 135.7, 151.8, 160.3 (C-3, py), 168.6 (CO); ^{77}Se{^{1}H} NMR (dmso-d_6) δ: 524 ppm. The brownish filtrate was extracted with $CHCl_3$ and after evaporation of solvent at room temperature the brownish crystalline compound was obtained. It was authenticated as a selone **[2-NHC$_5$H$_3$(3-CONH$_2$)Se]** analogue of a dinicotinamide diselenide . It was characterized by NMR spectroscopy. It was converted finally to diselenide by aerial oxidation within 2 h. Characterization by NMR: ^{1}H NMR (dmso-d_6) δ: 7.20 (d, d, 4.5, 5.7 Hz, 1H), 7.95 (br s, 2 H, NH_2), 7.99 (d, d, 1.5, 4.5 Hz, 1 H), 8.40 (d, d, 1.2, 5.7 Hz, 1H), 9.79 (br s, NH) (resonances for a small concentration of diselenide were also noted since selone oxidizes slowly to dislenide); ^{13}C{^{1}H} NMR (dmso-d_6) δ: 116.3, 138.1, 141.6, 141.7, 142.5, 168.4; ^{77}Se{^{1}H} NMR (dmso-d_6) δ: 364 ppm.

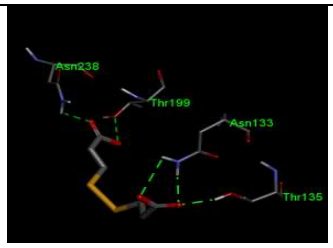
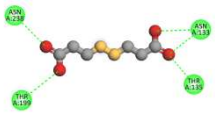
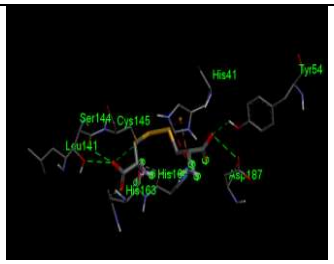
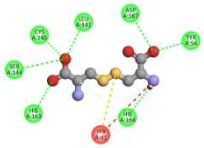
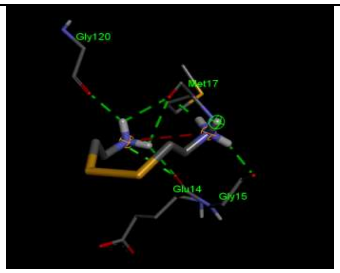
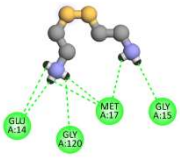
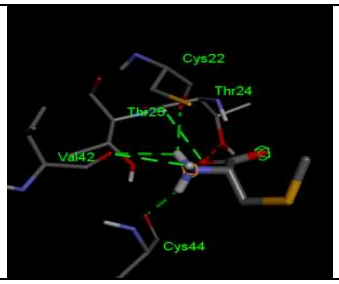
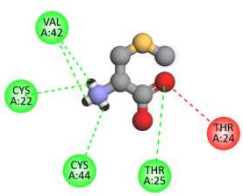
References

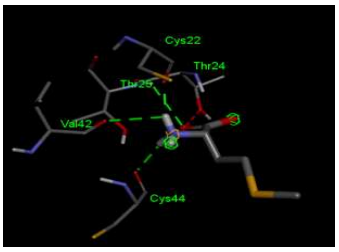
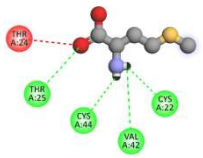
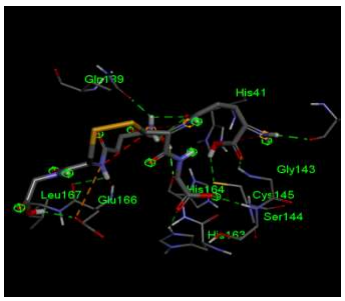
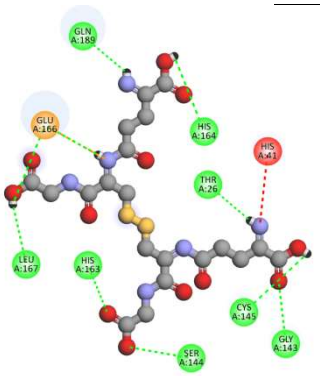
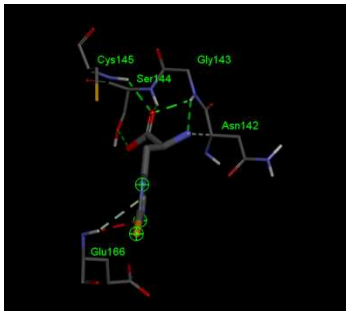
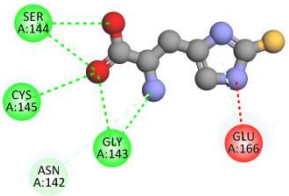
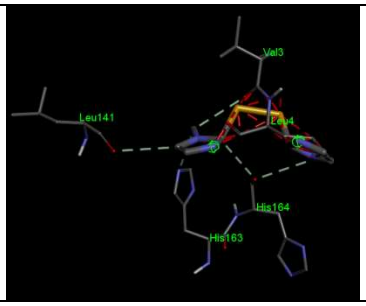
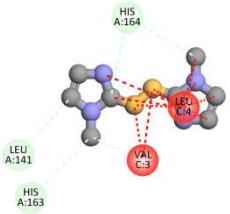
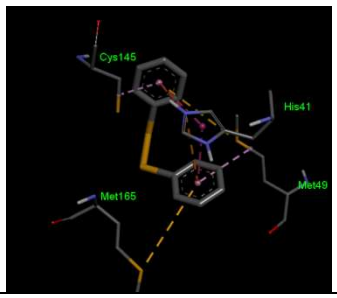
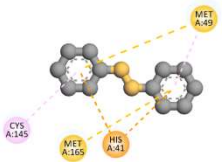
1. (a) A. S. Hodage, C. P.Prabhu, P. P. Phadnis, A.Wadawale, K.I. Priyadarsini and V. K. Jain, Synthesis, characterization, structures and GPx mimicking activity of pyridyl and pyrimidyl based organoselenium compounds, J. Organomet. Chem., 720 (2012) 19-25; (b) F.P. Colonna, G. Distefano, V. Galasso, K. J. Irgolic, G. C. Pappalardo, L. Pope, Dipole moment and helium(I) photoelectron spectroscopic studies of the conformation of di-2-pyridyl and diphenyl dichalcogenides R2X2 (X = S, Se, or Te), J. Chem. Soc. Perkin Trans-II (1981) 281-285; (c) A. Toshimitsu, H. Owada, K. Terao, S. Uemura, M. Okano, Pyridylseleno group in organic synthesis. Preparation and oxidation of α-(2-pyridylseleno) carbonyl compounds leading to α, β-unsaturated ketones and aldehydes, J. Org. Chem., 49(1984) 3796-3800.

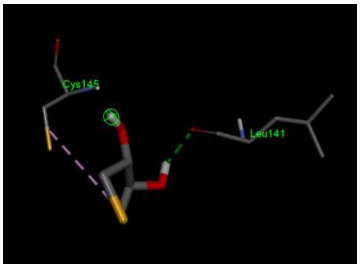
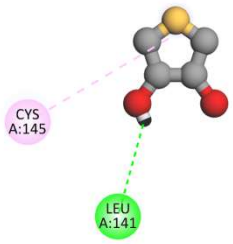
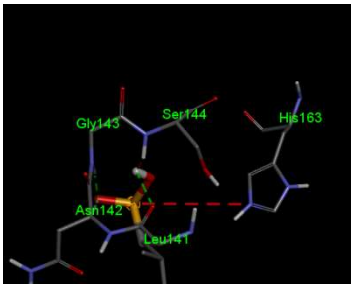
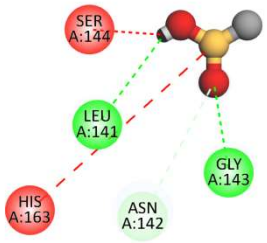
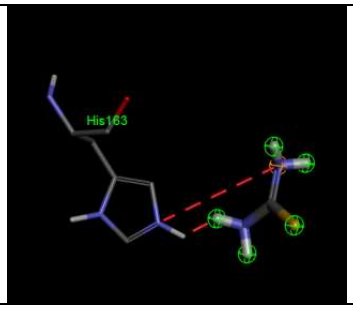
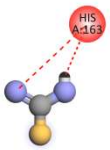
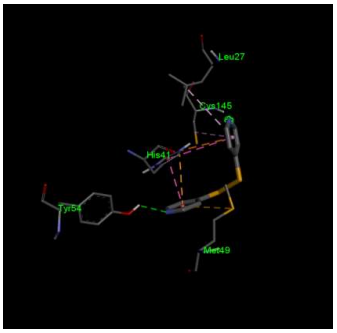
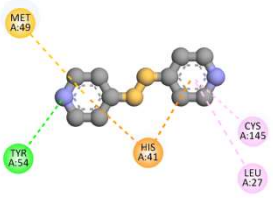
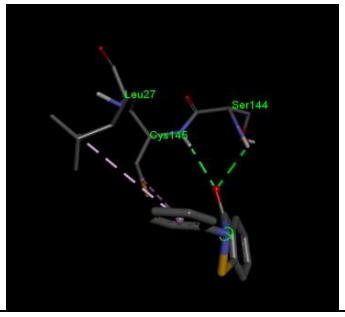
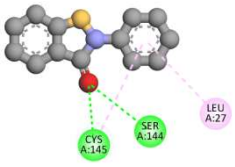
2. M. Raghuraman, P. Verma, A. Kunwar, P.P. Phadnis, V.K. Jain and K.I. Priyadarsini, Cellular evaluation of diselenonicotinamide (DSNA) as a radioprotector against cell death and DNA damage, Metallomics, 9 (2017) 715-725.

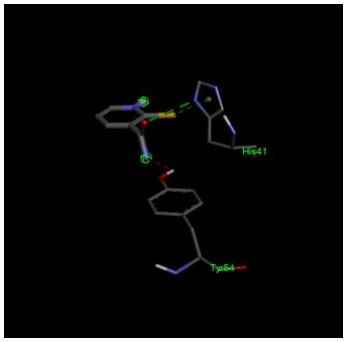
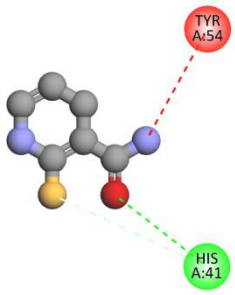
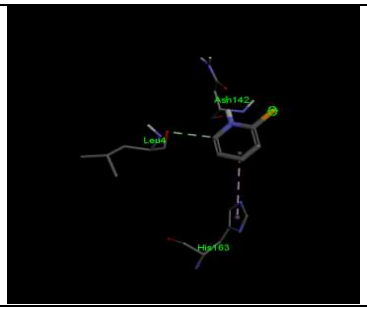
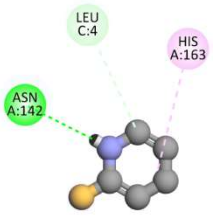
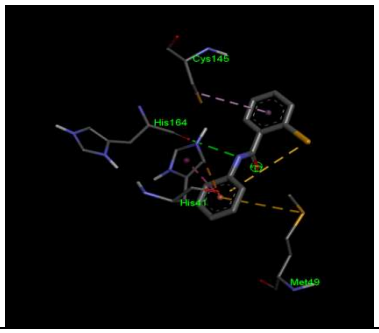
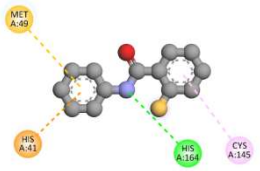
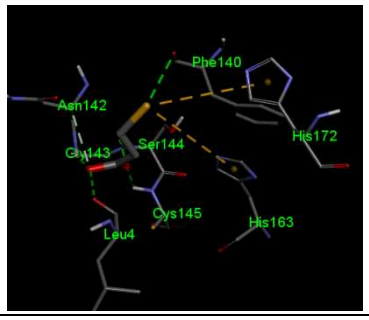
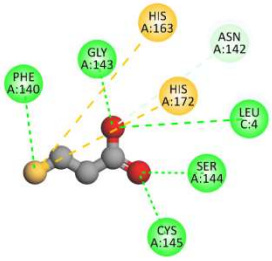
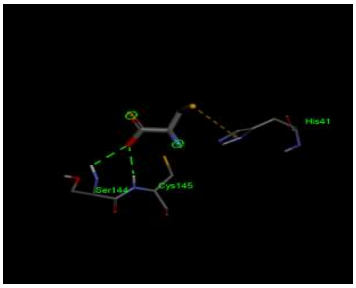
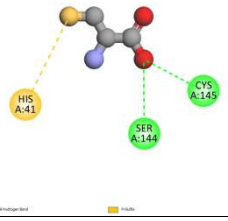
Supplementary tables

Table S1: The amino acid residues involved in binding of the representative orgnoselenium compounds with the SARS-CoV-2 M^{pro} protein (PDB Code: 6LU7).  represents hydrogen bonding, Vander Waals binding, pi-alkyl interaction, pi-cation attraction interaction, repulsive interaction.

Sr. Nos	Compounds	Amino acid in the receptor site involved in interaction with the compounds	Nature of interaction between Amino acids and selenium compounds
1	Diselenodipropanoic acid (DSePA)		
2	Selenocystine (CysSeSeCys)		
3	Selenocystamine (DSePAmine)		
4	Methyl selenocysteine (MeSeCys)		

5	Selenomethionine (SeM)		
6	Diselenodiglutathione (GSeSeG)		
7	Selenoneine (SeHis)		
8	Diseleno bis (2-Methyl imidazole) (Se-IMZ)		
9	Diphenyl diselenide (PhSeSePh)		

10	Dihydroxyl selenolane (DHS)		
11	Methaneseleninic acid (MSeA)		
12	Selenourea (SeU)		
13	4-pyridine diselenide (4-Py2Se2)		
14	Ebselen		

15	2-nicotinyl selone (2Nict-selone)		
16	2-pyridyl selone (2-Pyr-selone)		
17	Ebselenol (EbSeH)		
18	3-Hydroselenopropanoic acid (SePA-SeH)		
19	Selenocysteine (CysSeH)		

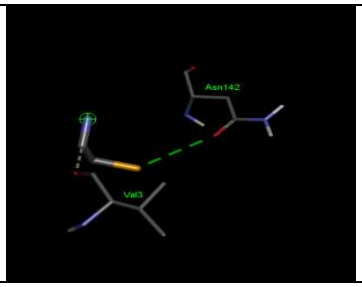
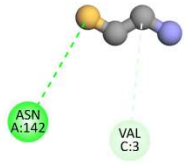
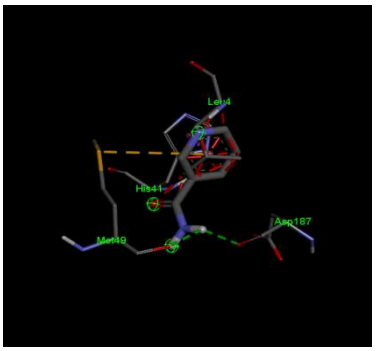
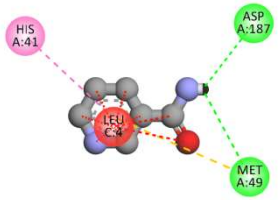
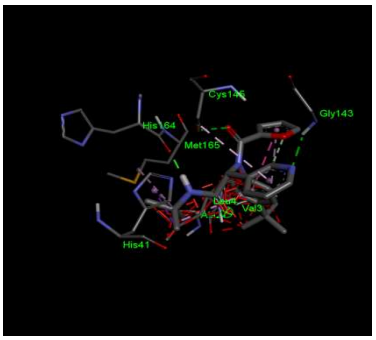
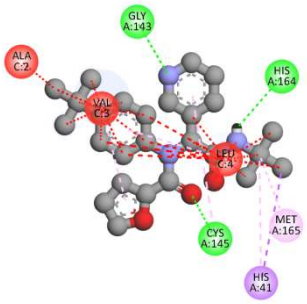
20	3-Hydroselenopropanamine (SePAmine-she)		
21	Nicotinamide		
22	ML188		

Table S2: Concentration dependent changes in secondary structure of M^{pro} protein by $Nict_2Se_2$ (0.1 μM-2.2 μM) in terms of % distribution of peptide chains in various secondary structures (helix, parallel/anti parallel sheets, beta-turns and random coils) at various wavelengths ranges (200-260 nm, 205-260 nm and 210-260 nm).

S.N.	Sample		200-260 nm	205-260 nm	210-260 nm
1.	Native M^{pro} protein	Helix	89.7 %	89.4 %	94.1 %
		Antiparallel	0.7 %	1.2 %	0.6 %
		Parallel	1.5 %	1.1 %	1.0 %
		Beta-Turn	7.1 %	8.3 %	6.8 %
		Rndm. Coil	10.3 %	6.6 %	4.5 %
		Total Sum	**109.2 %**	**106.5 %**	**106.9 %**
2.	M^{pro} protein + 0.1 μM $Nict_2Se_2$	Helix	84.0 %	84.1 %	91.2 %
		Antiparallel	1.0 %	1.6 %	0.8 %
		Parallel	2.1 %	1.5 %	1.3 %
		Beta-Turn	8.1 %	9.3 %	7.6 %
		Rndm. Coil	13.8 %	8.9 %	6.0 %
		Total Sum	**109.1 %**	**105.5 %**	**106.8 %**
3.	M^{pro} protein + 0.3 μM $Nict_2Se_2$	Helix	78.4 %	73.3 %	83.5 %
		Antiparallel	1.4 %	2.5 %	1.4 %
		Parallel	2.9 %	2.6 %	2.0 %
		Beta-Turn	9.0 %	10.9 %	9.2 %
		Rndm. Coil	18.0 %	13.4 %	9.3 %
		Total Sum	**109.7 %**	**102.7 %**	**105.5 %**
4.	M^{pro} protein + 0.7 μM $Nict_2Se_2$	Helix	58.9 %	53.2 %	65.2 %
		Antiparallel	3.1 %	4.5 %	3.0 %
		Parallel	5.3 %	5.0 %	3.8 %
		Beta-Turn	11.7 %	13.5 %	12.0 %
		Rndm. Coil	27.8 %	22.5 %	16.8 %
		Total Sum	**106.8 %**	**98.6 %**	**100.8 %**
5.	M^{pro} protein + 1 μM $Nict_2Se_2$	Helix	78.4 %	73.3 %	83.5 %
		Antiparallel	1.4 %	2.5 %	1.4 %
		Parallel	2.9 %	2.6 %	2.0 %
		Beta-Turn	9.0 %	10.9 %	9.2 %
		Rndm. Coil	18.0 %	13.4 %	9.3 %
		Total Sum	**109.7 %**	**102.7 %**	**105.5 %**
6.	M^{pro} protein + 1.3 μM $Nict_2Se_2$	Helix	30.9 %	24.8 %	30.4 %
		Antiparallel	8.1 %	9.9 %	9.0 %
		Parallel	11.1 %	12.1 %	9.3 %
		Beta-Turn	16.2 %	17.9 %	17.3 %
		Rndm. Coil	43.5 %	41.0 %	35.1 %
		Total Sum	**109.8 %**	**105.7 %**	**101.0 %**
7.	M^{pro} protein + 1.6 μM $Nict_2Se_2$	Helix	31.3 %	27.3 %	31.5 %
		Antiparallel	8.3 %	9.2 %	8.7 %
		Parallel	10.5 %	11.0 %	9.1 %
		Beta-Turn	16.4 %	17.5 %	17.1 %
		Rndm. Coil	40.4 %	38.5 %	34.3 %
		Total Sum	**106.9 %**	**103.6 %**	**100.7 %**
8.	M^{pro} protein + 2.2 μM $Nict_2Se_2$	Helix	27.1 %	24.2 %	27.9 %
		Antiparallel	9.6 %	10.2 %	9.8 %
		Parallel	11.9 %	12.4 %	10.0 %
		Beta-Turn	17.1 %	18.1 %	17.8 %
		Rndm. Coil	43.8 %	41.5 %	37.0 %
		Total Sum	**109.5 %**	**106.2 %**	**102.5 %**

Supplementary Figures

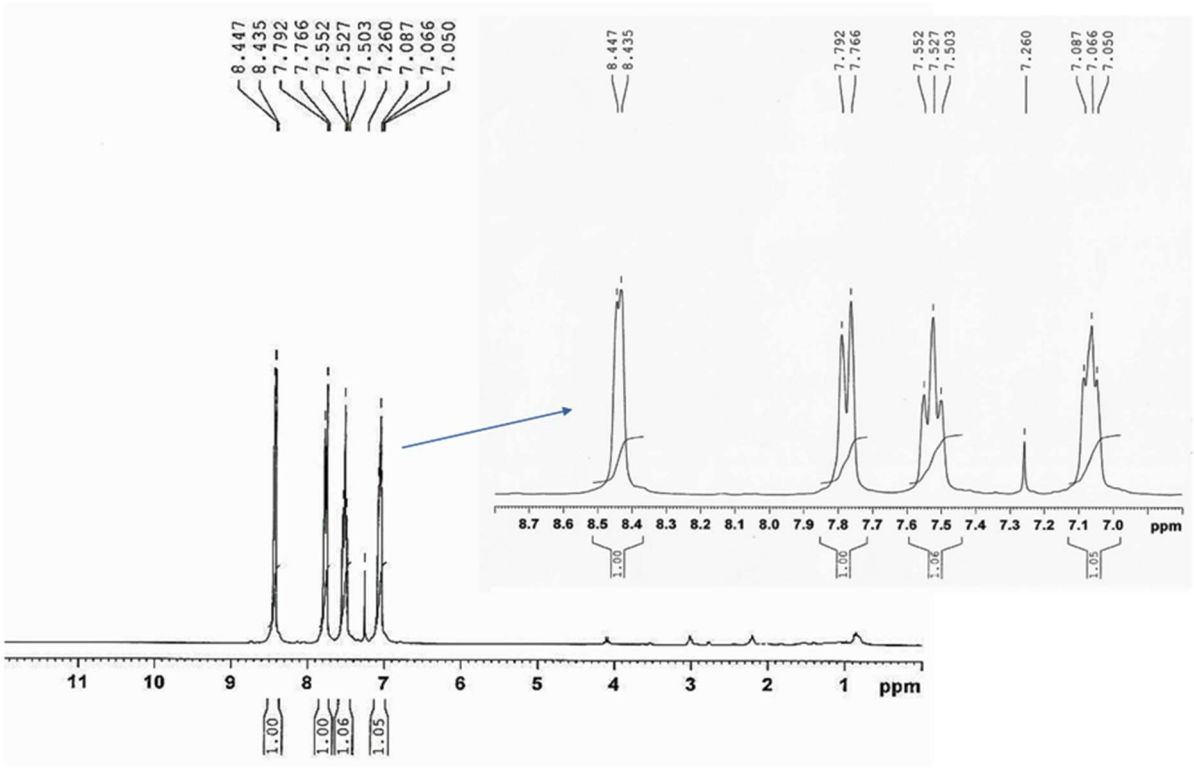


Fig. S1. NMR spectra (^{1}H) of 2, 2′-diselenobis (pyridine) ([2-C_5H_4N-Se]$_2$)

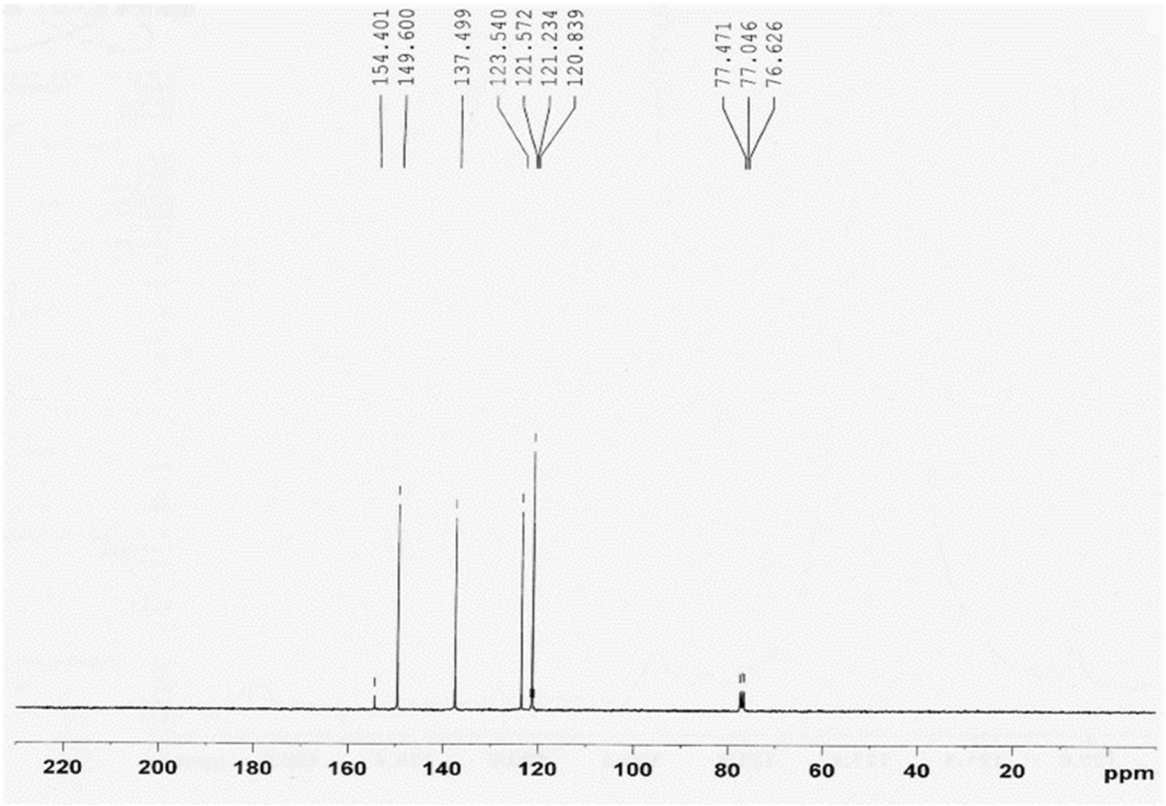


Fig. S2. NMR spectra ($^{13}C\{^{1}H\}$) of 2, 2′-diselenobis (pyridine) ([2-C_5H_4N-Se]$_2$)

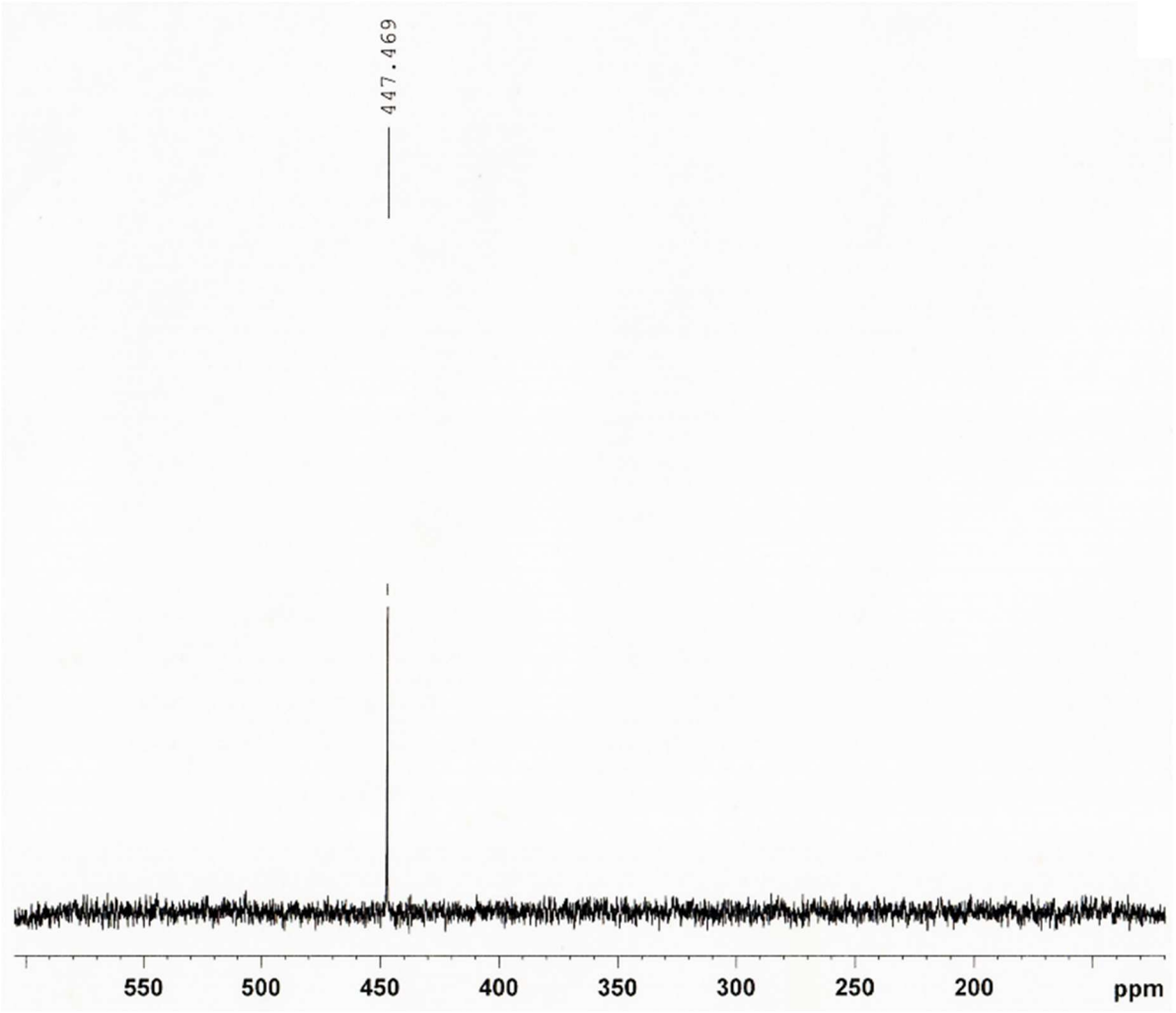


Fig. S3. NMR spectra ($^{77}Se\{^{1}H\}$) of 2, 2′-diselenobis (pyridine) ([2-C_5H_4N-Se]$_2$)

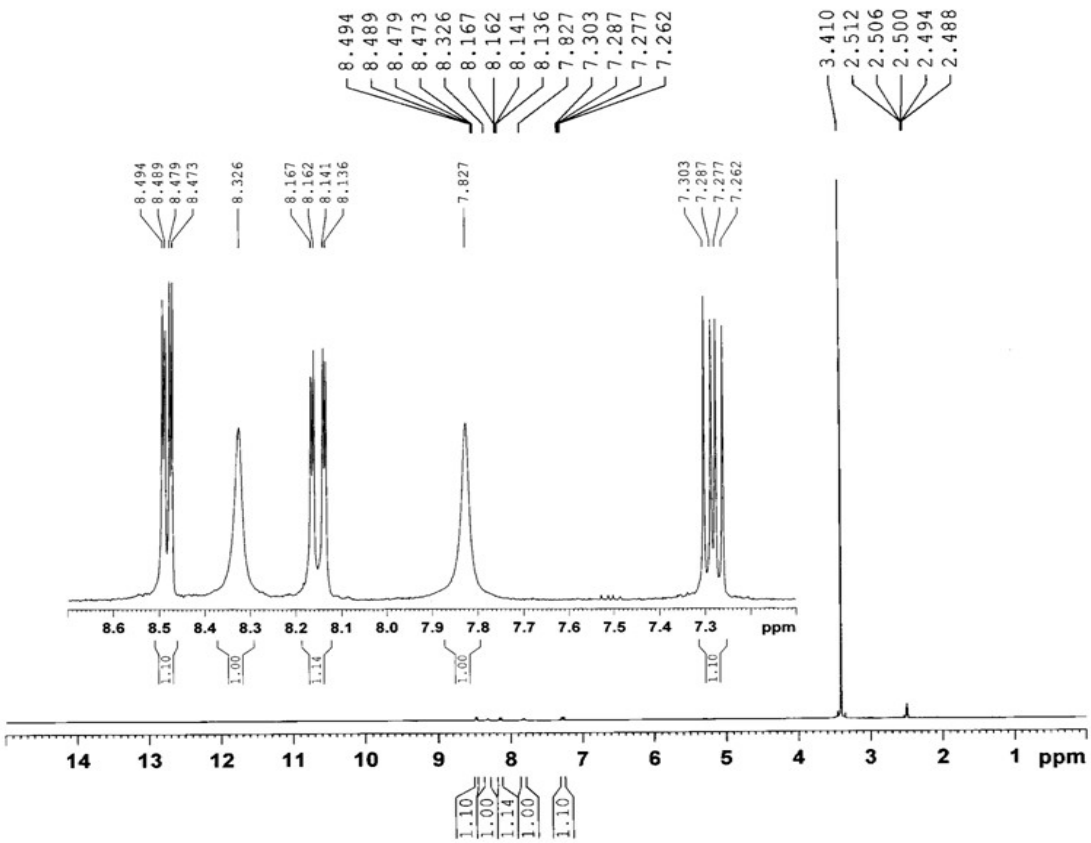


Fig. S4. ^{1}H NMR spectrum of $[2\text{-}C_5H_3N(3\text{-}CONH_2)Se]_2$ in dmso-d_6

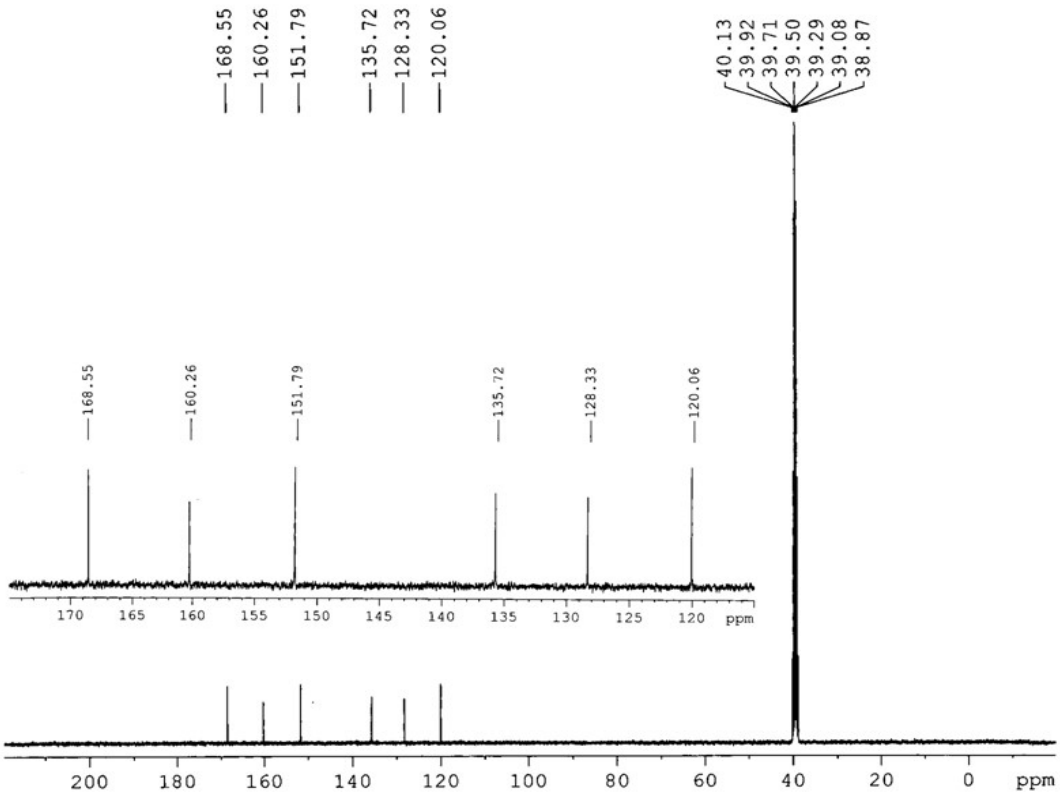


Fig. S5. $^{13}C\{^{1}H\}$ NMR spectrum of $[2\text{-}C_5H_3N(3\text{-}CONH_2)Se]_2$ in dmso-d_6

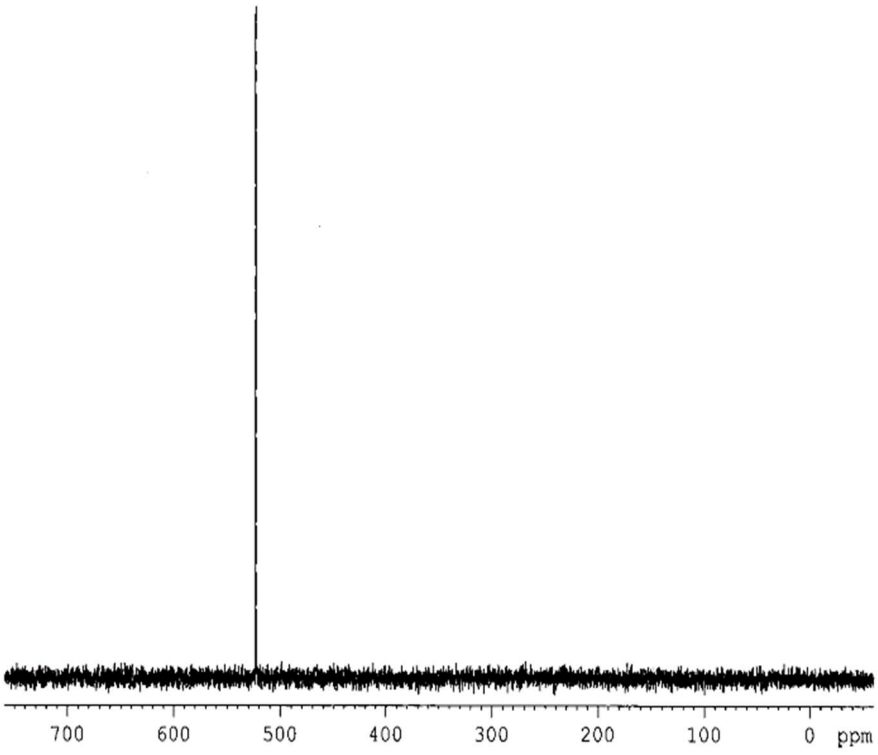


Fig. S6. $^{77}Se\{^{1}H\}$ NMR spectrum of $[2\text{-}C_5H_3N(3\text{-}CONH_2)Se]_2$ in dmso-d_6 (δ: 524 ppm)

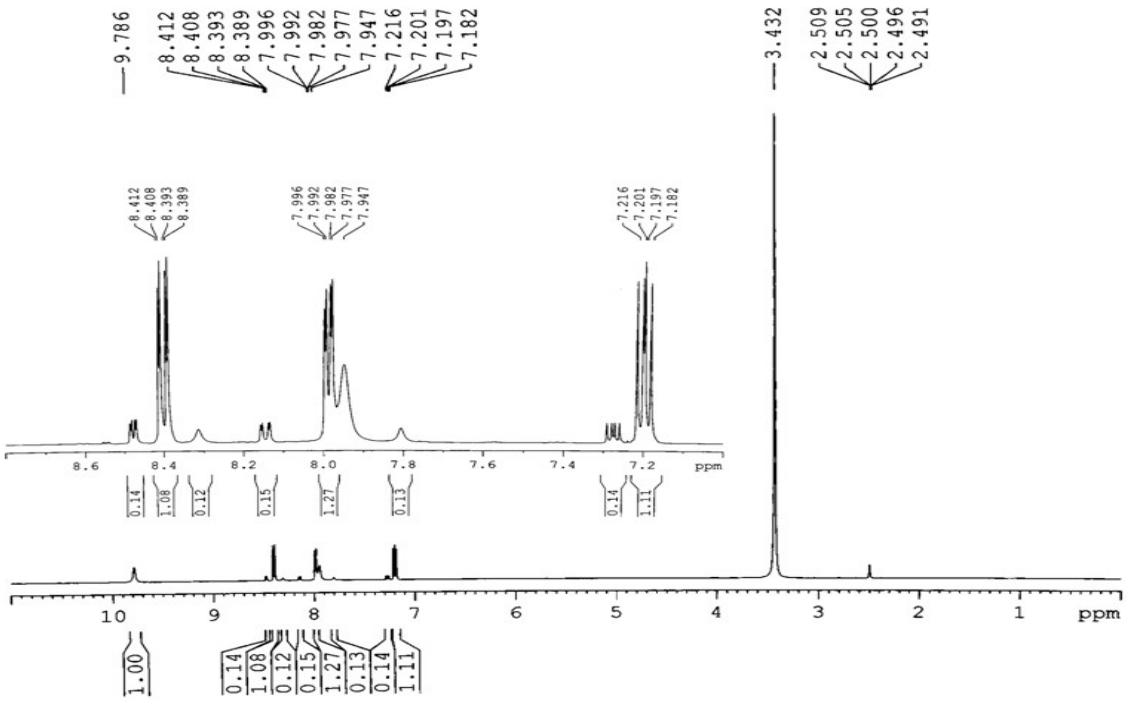


Fig. S7. ^{1}H NMR spectrum of [2-C_5H_3NH(3-$CONH_2$)Se] in dmso-d_6

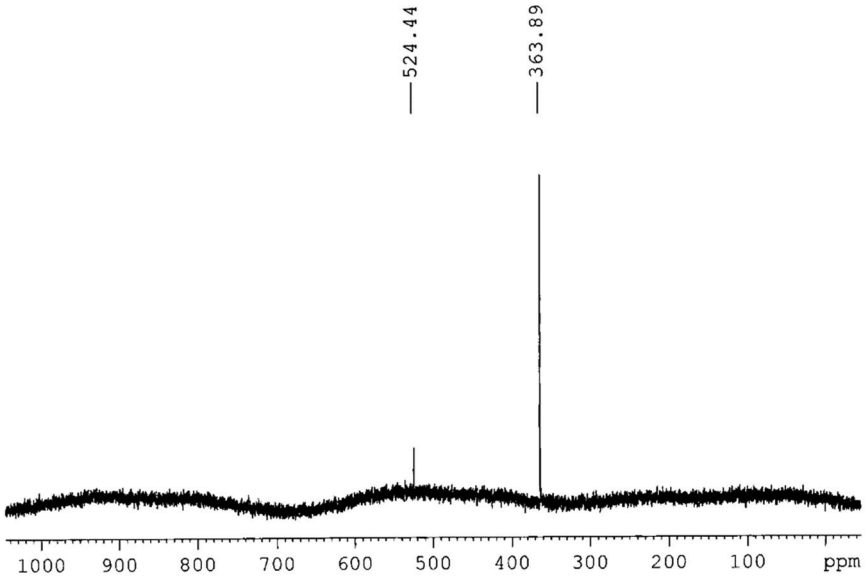


Fig. S8. $^{77}Se\{^{1}H\}$ NMR spectrum of [2-C_5H_3NH(3-$CONH_2$)Se] in dmso-d_6 (δ: 364 ppm)

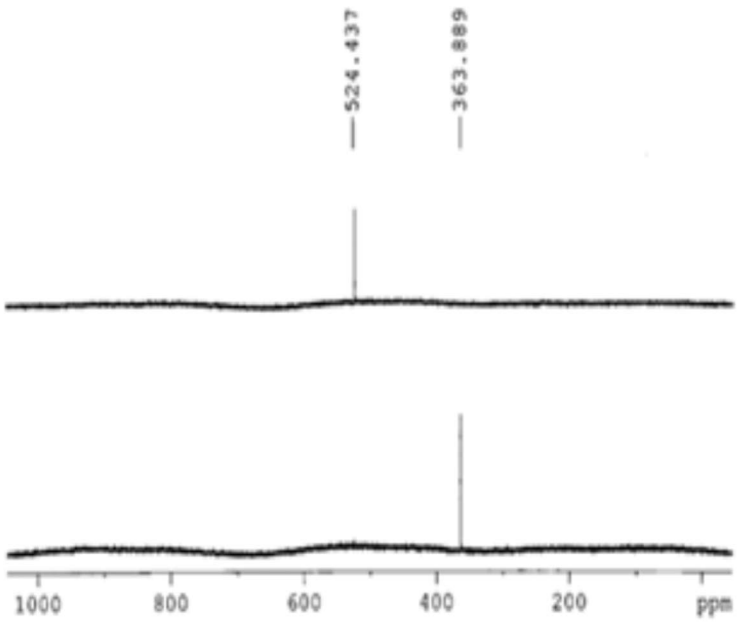


Fig. S9. $^{77}Se\{^{1}H\}$ NMR spectra in dmso-d_6 depicting conversion of [2-C_5H_3NH(3-$CONH_2$)Se] to [2-C_5H_3N(3-$CONH_2$)Se]$_2$ by aerial oxidation

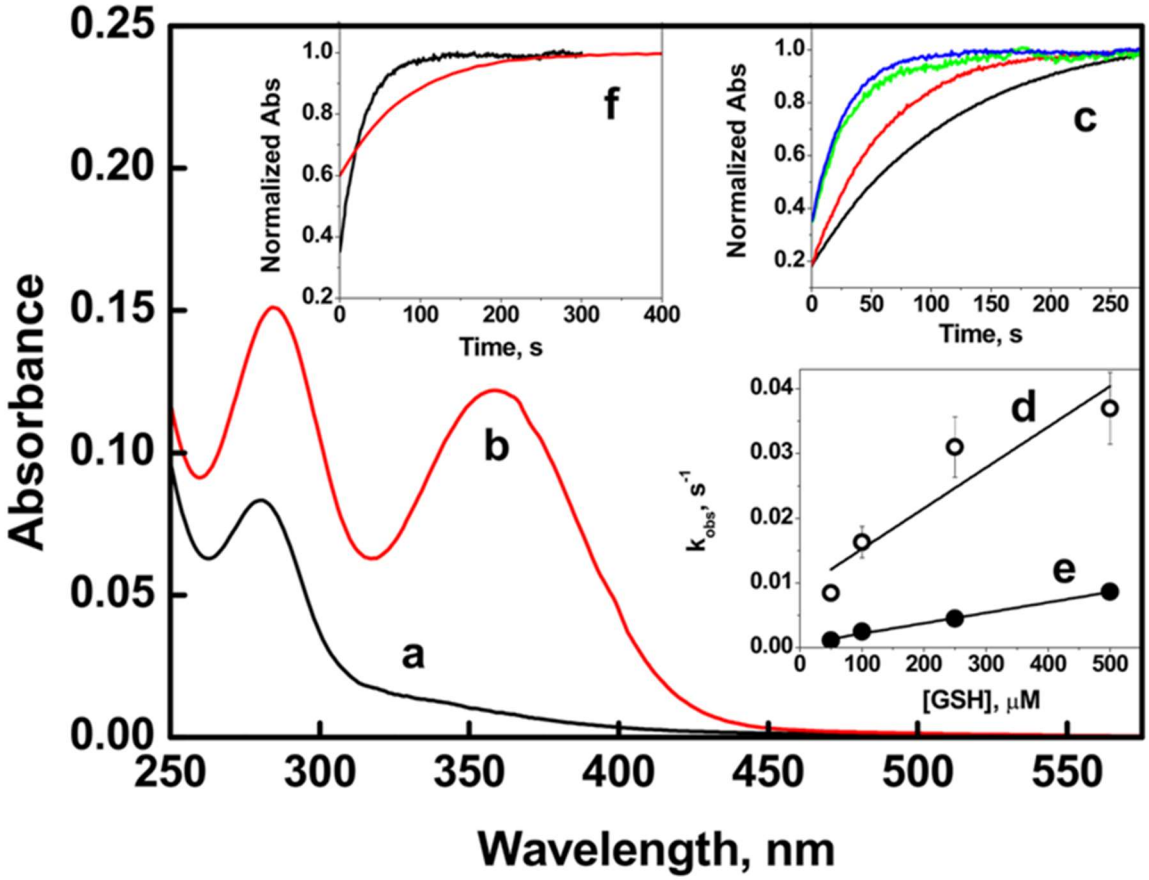


Fig. S10. Absorption spectrum of 10 μM 2-Py_2Se_2 in absence (a) and presence of 1 mM GSH (b). Inset (c) shows the time dependent absorbance at 340 nm obtained on treating 10 μM 2-Py_2Se_2 with varying concentration of GSH (0.1 – 1 mM). These plots were fitted to first order exponential growth equation in origin software (version 8.0) to obtain the observed rate (kobs). Inset (d) and (e) shows the plot of kobs for 2-Py_2Se_2 and $Nict_2Se_2$ at different GSH concentration. Inset (f) shows the relative reactivity of the dislenides with 500 μM GSH (black line = 2-Py_2Se_2 and red line = $Nict_2Se_2$).

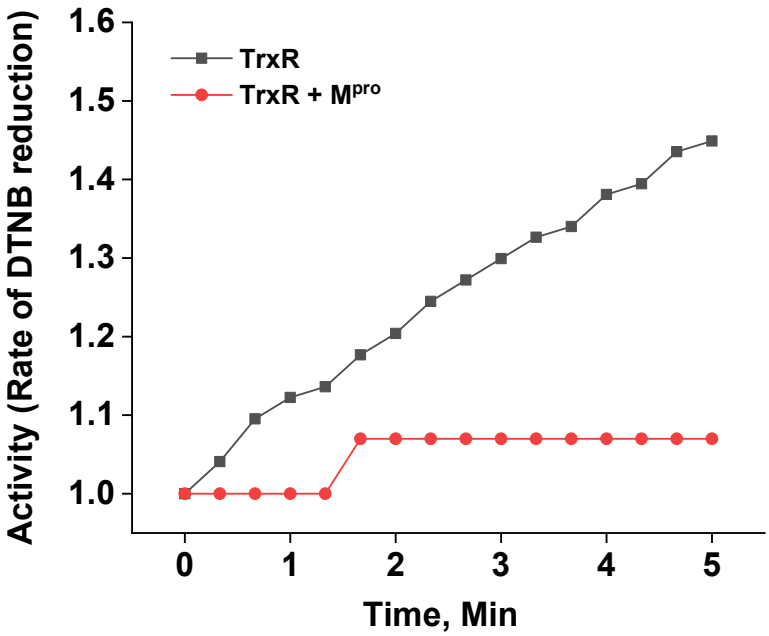


Fig. S11. 50 mM Phosphate-EDTA buffer pH 7.4, containing 100 µM NADPH and 6 mM DTNB was mixed with 50 nM rat liver TrxR in presence and absence of 15 ng M^{pro} (BPC Biosciences, Cat. no. 100823). The formation of TNB was followed by monitoring A412.