Paradoxical behavior of organodiselenides: pro-oxidant to antioxidant

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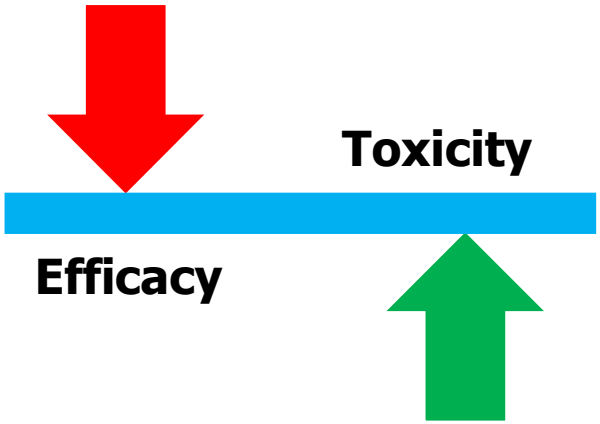
Challenge in selenium research

Selenium compounds have gained a lot of interest in therapeutic research

"Anti-cancer agent , Neuroprotective agent, Antioxidant, Radioprotective agent"

Limitation → Narrow margin between essentiality and toxicity



❖ **Organoselenium compounds exhibit lesser toxicity compared to inorganic selenium**

(*Int. J. Cancer* 1995, *63*, 428–434; *Arch Toxicol.* 2011, 85, 1313-1359; *Molecules* 2018, *23*, 628)

Organodiselenides

R-Se-Se-R **R – Alkyl or Aryl group**

(Organodiselenide)

- ✓**Pharmacologically relevant class of molecules**
- ✓**Antioxidant activity as glutathione peroxidase (GPx) mimic**
- ✓**Antioxidant activity as substrate of thioredoxin reductase (TrxR)**
- ✓**Pro-oxidant activity leading to toxicity in biological systems**

Well known organo-diselenide - Diphenyl diselenide (Ph_2Se_2)
Selenocystine (SeCys)
Diselenodipropionic acid (DsePA)

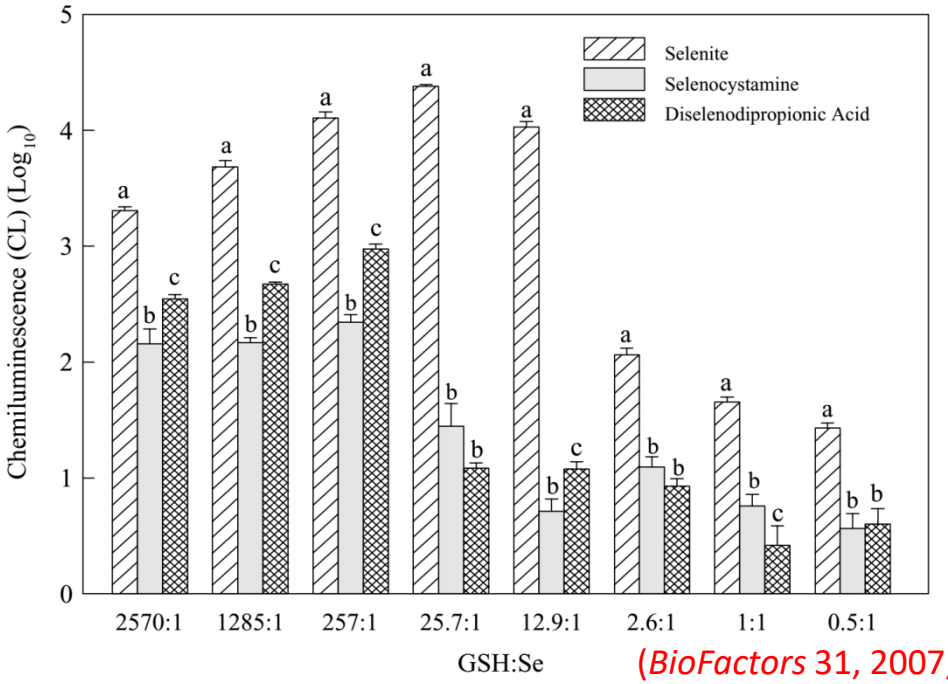
(*Molecules* 2010, 15, 7292–7312; *Molecules* 2018, 23, 628)

Pro-oxidant activity of organodiselenides

Pro-oxidants - Agents that induce ROS generation and in turn oxidise bio-molecules

Thiol (GSH) oxidase activity : Cell free condition

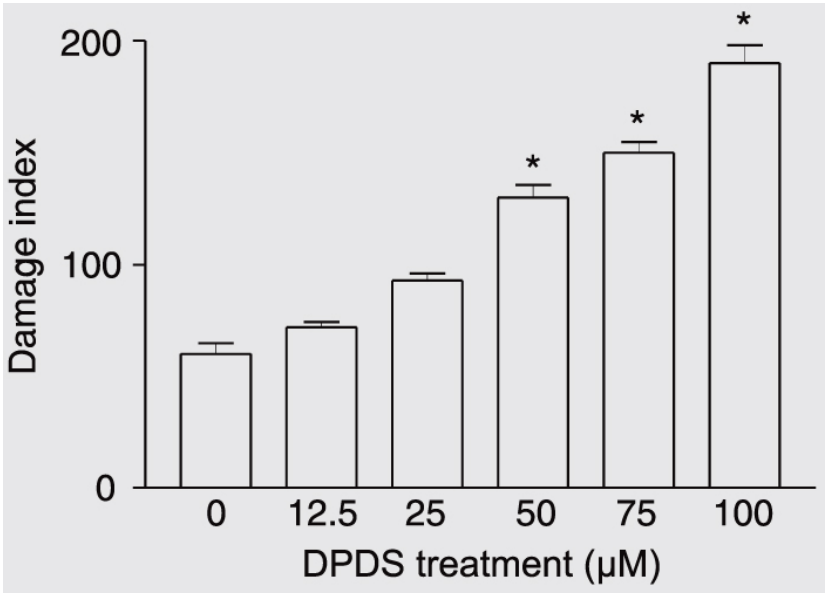
Method : Chemiluminescence (CL)



(*BioFactors* 31, 2007, 55–66)

Pro-oxidant activity of Ph_2Se_2 in MCF7 cells

Method : DNA damage by comet assay



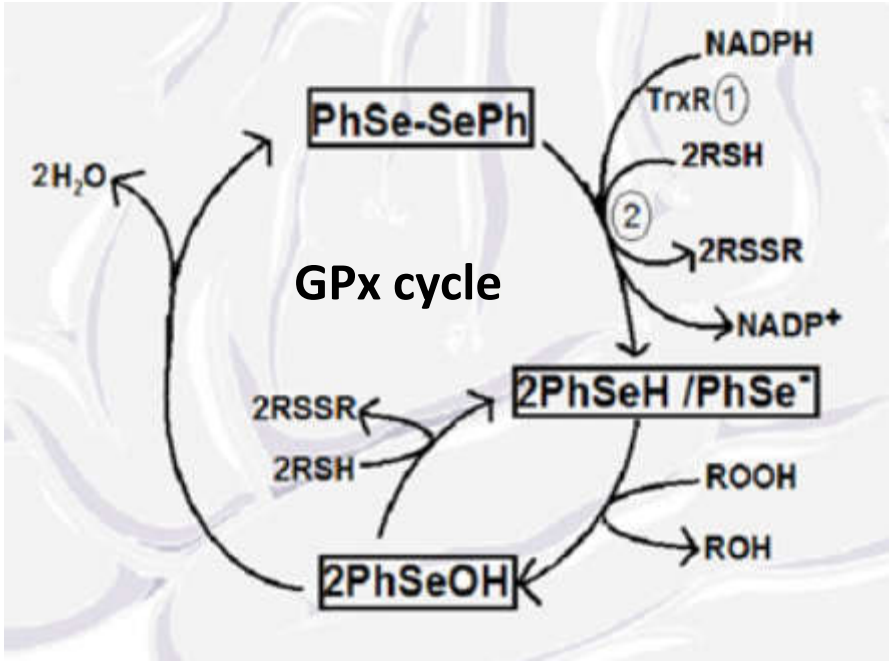
(*Braz J Med Biol Res*, 2007, 40, 1287-1304)

Probable mechanism of pro-oxidant activity :

- ❖GSH oxidation
- ❖GSH depletion via conjugation
- ❖Oxidation of thiol (-SH) containing proteins

Antioxidant activity of organodiselenides

- GPx is an antioxidant enzyme with reduces hydroperoxide to protect the organisms from oxidative damage
- TrxR maintains thiol containing proteins in reduced state by catalyzing reduction of thioredoxin (Trx)



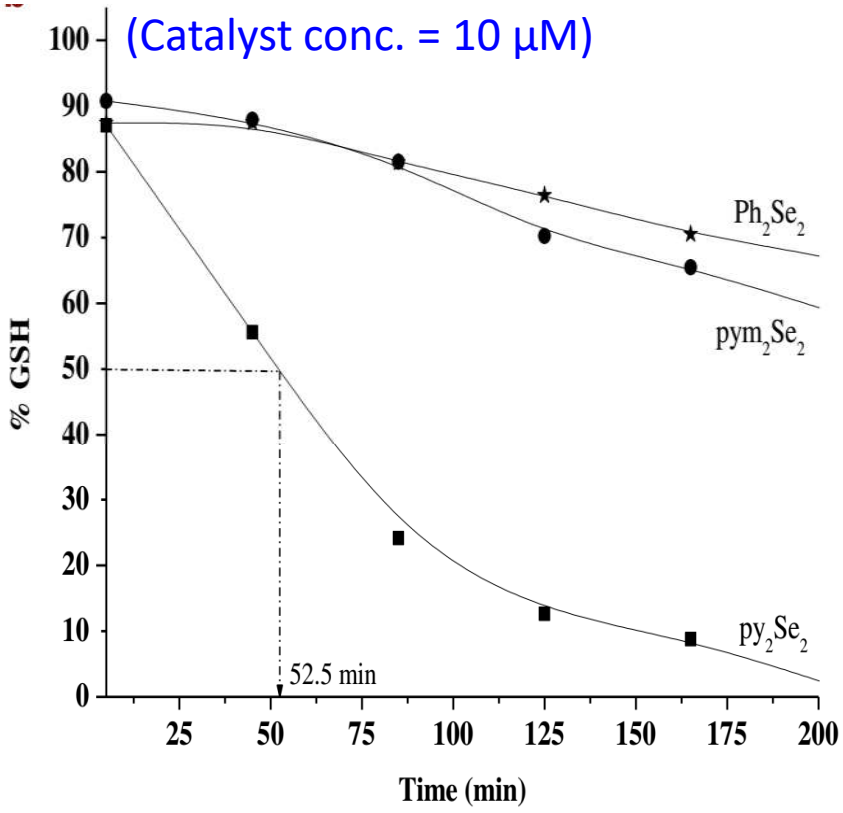
GPx cycle of Ph_2Se_2

(*Neuroscience Letters* 503, 2011, 1–5)

Cont.

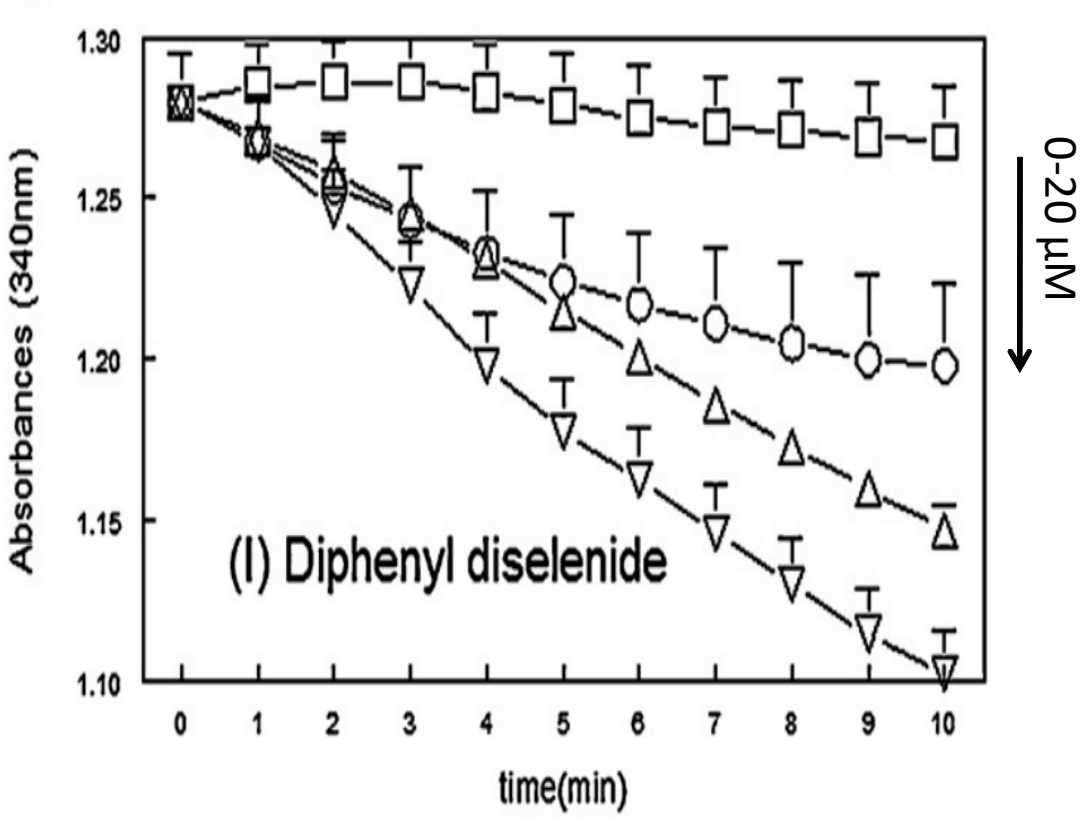
GPx like activity : Cell free condition

Method : t_{50} (min) of GSH consumption by HPLC



TrxR substrate : Cell free condition

Method : NADPH (340 nm) decay



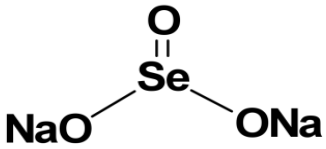
✓**Diselenides catalyses reduction of toxic H_2O_2 in to water by using GSH as redox equivalent**

✓**Diselenides act as a substrate for TrxR forming intermediates taking part in GPx reaction**

(*Journal of Organometallic Chemistry* 720 , 2012, 19-25; *Neuroscience Letters* 503, 2011, 1–5)

Organoselenium compounds studied by our group

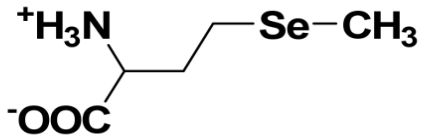
Selenone



Sodium selenite

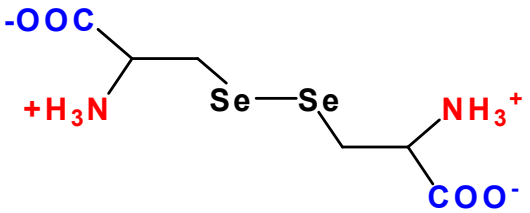
Biol Trace Elem Res. 2017, 179,130-139

Amino acids



Selenomethionine (SeMet)

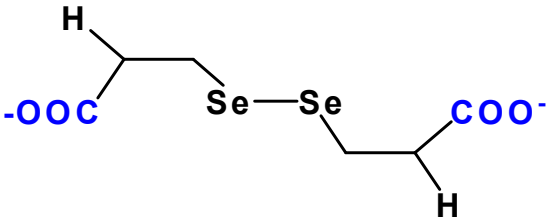
Current Chemical Biology 2013, 7, 37-46
Radiat. Environ. Biophys. 2011, 50, 271–280



Selenocystine (SeCys)

Radiat. Environ. Biophys. 2009, 48, 379-384
Biol Trace Elem Res. 2011, 140: 127-138

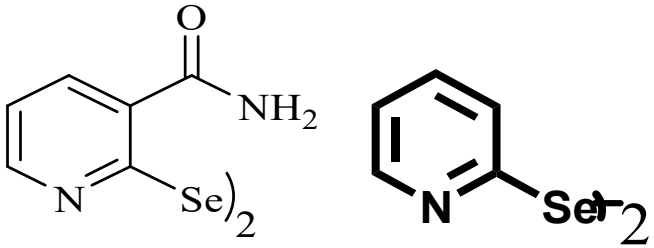
Aliphatic diselenides



Diselenodipropionic acid (DSePA)

Che. Res. Toxicol. 2007, 20,1482-1487
Free Radic. Biol. Med. 2010,*48,399-410*
Arch. Toxicol. 2011, 85,1395-1405
Am J Respir Cell Mol Biol. 2013, 49, 654-661
Eur J Drug Metab Pharmacokinet. 2016, 41, 839-844
Radiotherapy and Oncology 2018, 127: S584-S585
Regulatory Toxicology and Pharmacology 2018, 99: 159-167
Free Radic. Biol. Med. 2019,*145,8-19*

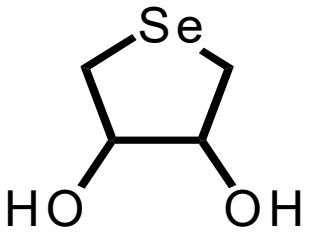
Aromatic diselenides



2,2′-diselenobis[3-amidopyridine]
2,2′-dipyridyl diselenide

Metallomics, 2017, 9, 715-725
Journal of Organometallic Chemistry 2017, 852, 1-7
New J. Chem. 2020, 44, 7329-7337.
Metallomics 2020, 12, 1253-1266.

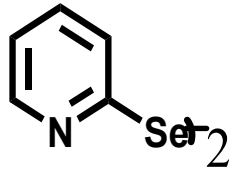
Cyclic monoselenide



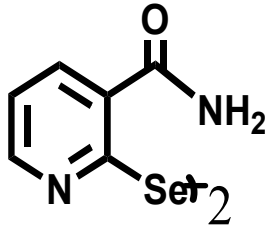
3,4-dihydroxy-1-selenolane (DHS_{red})

Biochimie 2018,144, 122-133
Mutation Research 2016, 807, 33-46
Toxicology Research 2016, 5, 434-445
Molecules 2015, 20,12364-12375;
ChemBiochem 2015,16,1226-1234

Structure & synthesis of pyridine diselenides



(Py_2Se_2)
Dipyridine dislenide

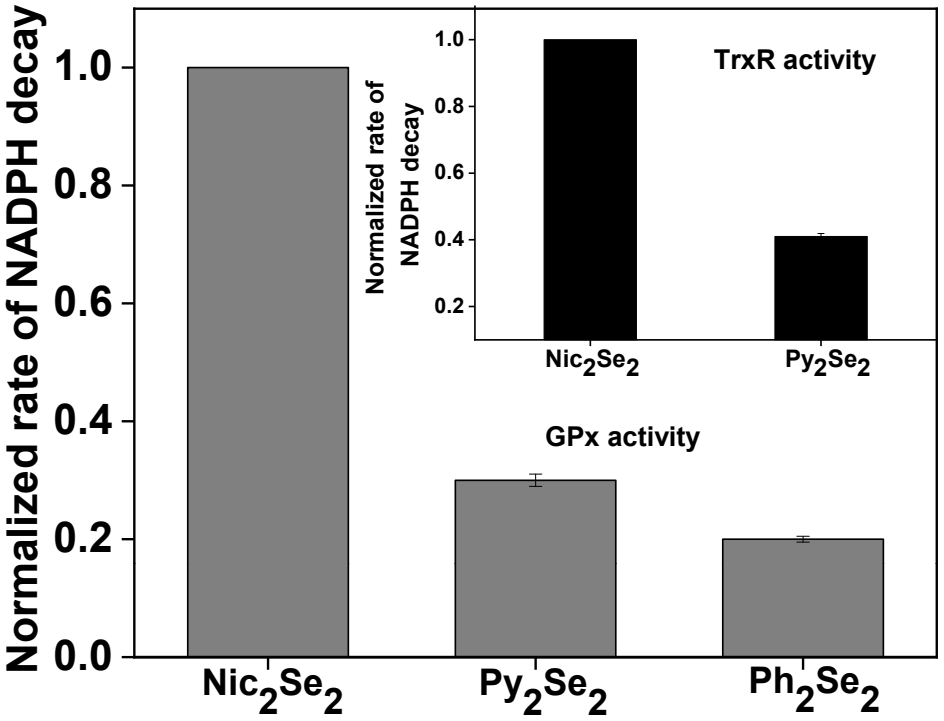


(Nic_2Se_2)
Dinioctinamide diselenide

- Both Py_2Se_2 and Nic_2Se_2 were synthesized in house as per the reported literature
- Compounds were characterized by NMR, IR and Mass spectroscopy.

(*Journal of Organometallic Chemistry* 713, 2012, 42-50; *Journal of Organometallic Chemistry* 720, 2012, 19-25)

GPx and TrxR activity of Py_2Se_2 and Nic_2Se_2

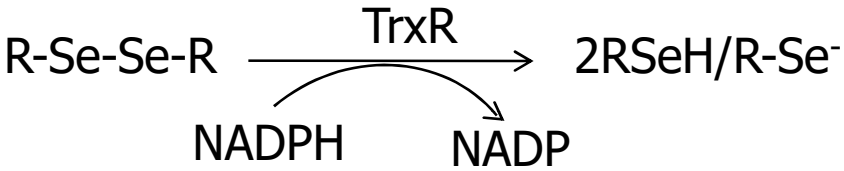


GPx reaction

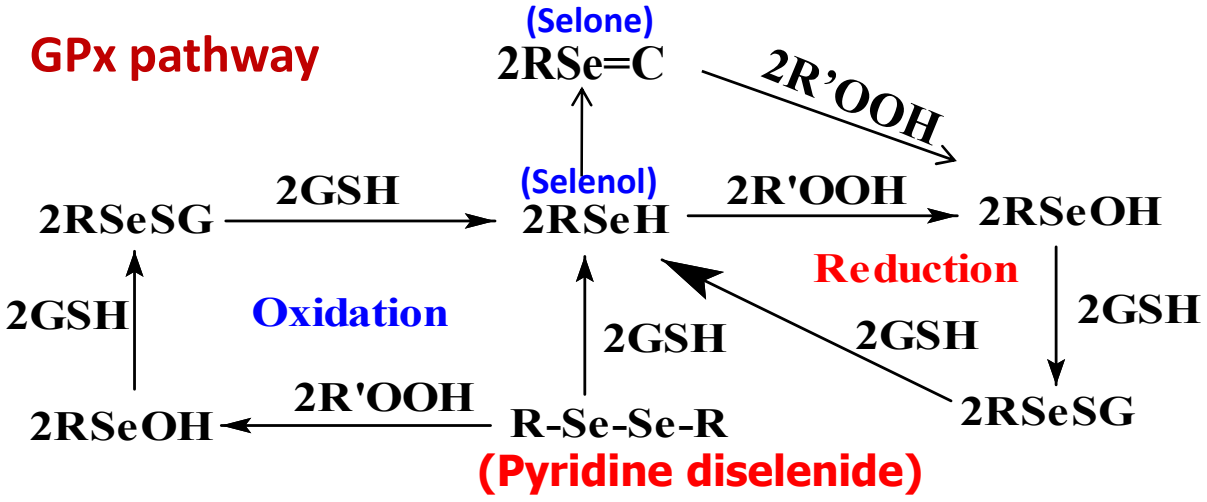
$$CuOOH + 2GSH \xrightarrow{DSePA} CuOH + GSSG + H_2O$$

$$NADPH + GSSG + H^+ \xrightarrow[reductase]{Glutathione-} 2GSH + NADP^+$$

TrxR reaction



GPx pathway



- ✓ GPx-like activity of Py_2Se_2 and Nic_2Se_2 predominantly follow reduction path
- ✓ GPx and TrxR substrate activities follow order of $Nic_2Se_2 > Py_2Se_2$
- ✓ Reduction of Py2Se2 and Nic2Se2 generate selone as a stable intermediate
- ✓ Selone of Nic_2Se_2 is more stable compared to that of Py_2Se_2

(*Org. Biomol. Chem.* 2014, 12, 2404–2412)

Cytotoxicity of Py_2Se_2 and Nic_2Se_2 in different cells

Method – MTT assay **Time point – 48 h Post treatment**

Compounds	CHO (Normal ovary epithelium)	WI38 (Normal lung fibroblast)	A549 (Lung carcinoma)	MCF7 (Breast carcinoma)
Py_2Se_2	~6 µM	~8 µM	~5 µM	~5 µM
Nic_2Se_2	>100 µM			~70 µM

✓ **Cytotoxicity: Py_2Se_2 > Nic $_2Se_2$**

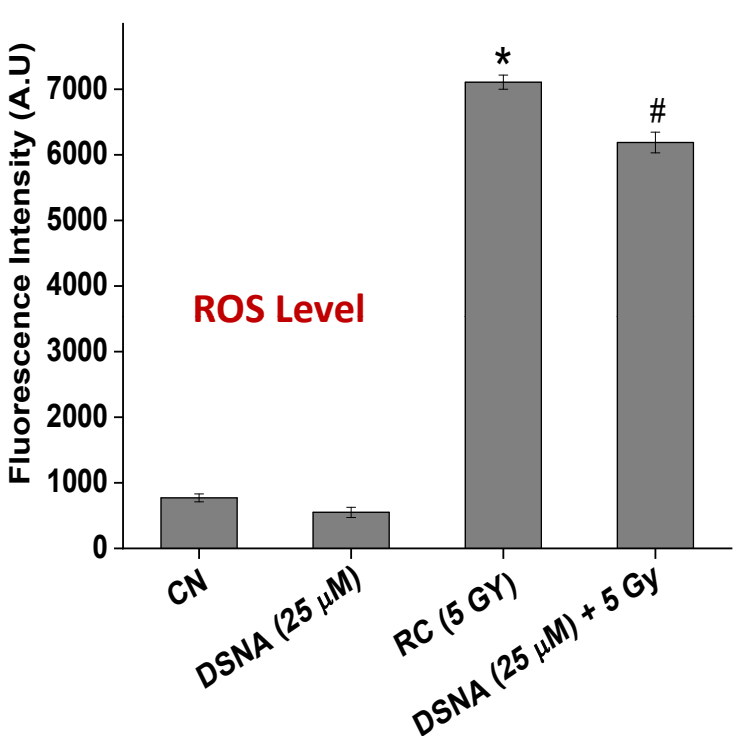
✓ **Nic_2Se_2 exhibits differential toxicity in tumor versus normal cells**

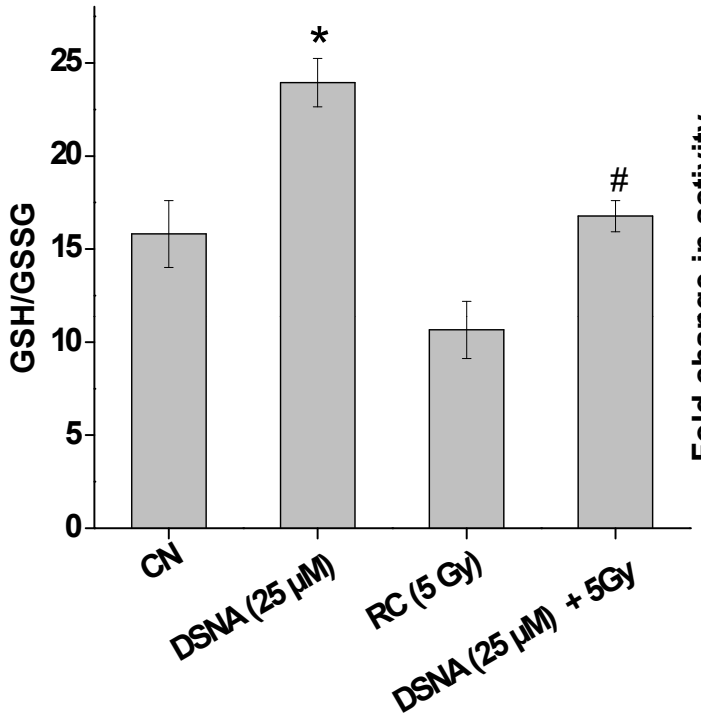
(*Metallomics* 2017, 9, 715-725; *New J. Chem.* 2020, 44, 7329-7337; *Metallomics* 2020, 12, 1253-1266)

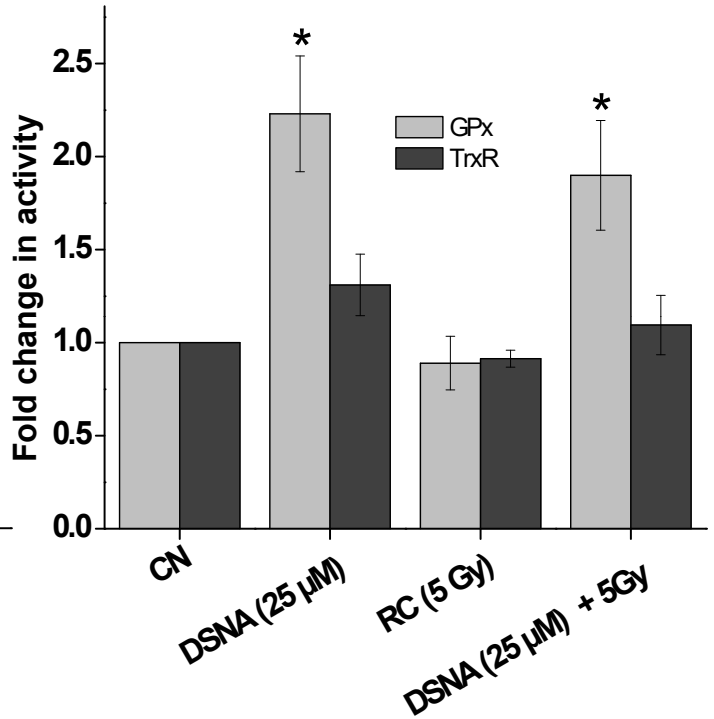
Redox modulatory activity of Nic_2Se_2 in normal CHO cells

NIc_2Se_2 (DSNA) treatment – 16 h prior to irradiation by Co^{60} γ-radiation

Method – Biochemical assays; **RC** – Radiation control







✓ **Nic_2Se_2 per se induced reductive environment in cells marked by increase and decrease respectively in GSH/GSSG and ROS levels**

✓ **Nic_2Se_2 pre-treatment reduced γ-radiation induced oxidative stress**

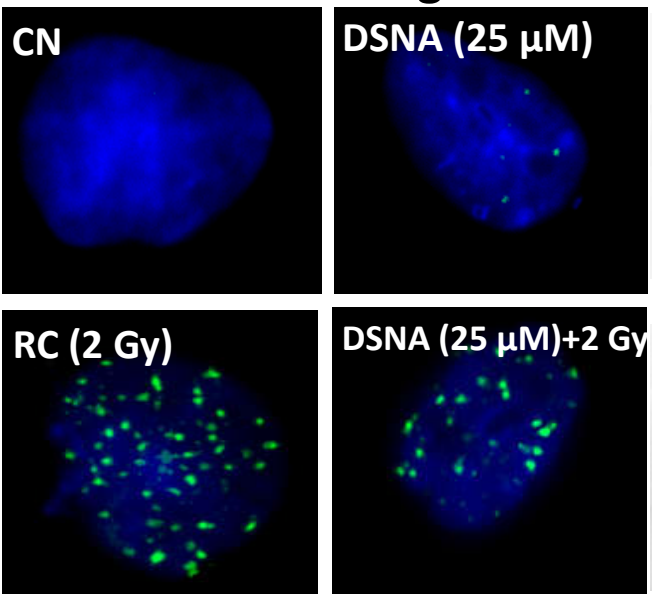
(*Metallomics* 2017, 9, 715-725)

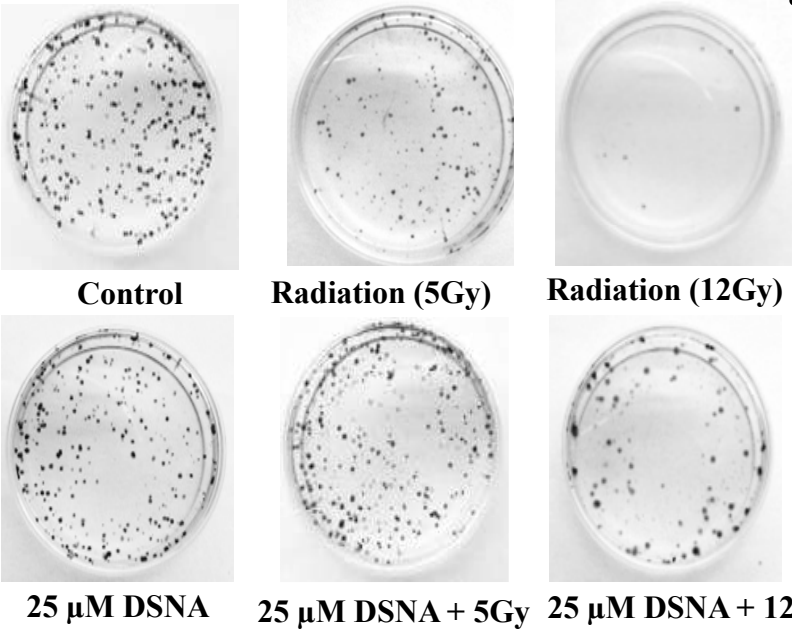
Radio-protective activity of Nic_2Se_2 in CHO cells

DNA damage – γ-H2AX assay; **Cell viability** – Clonogenic agency

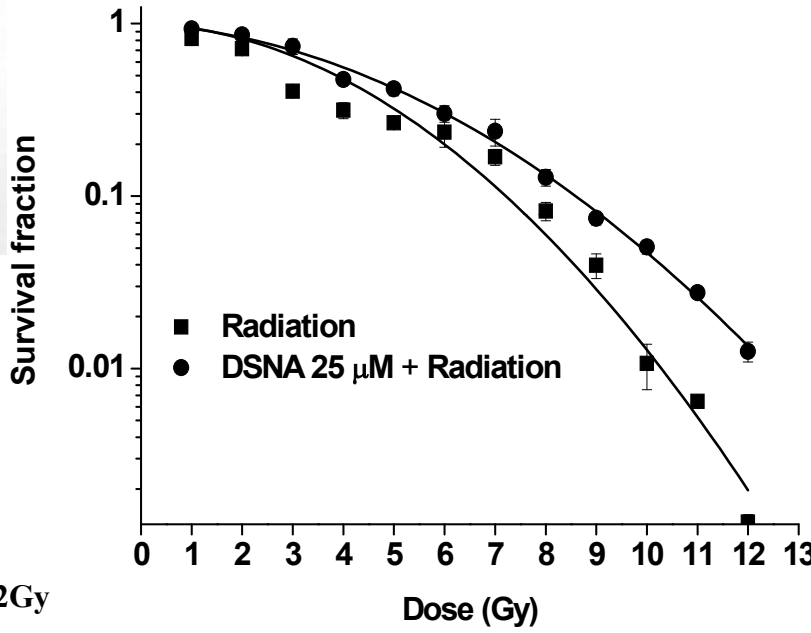
NIc_2Se_2 (DSNA) treatment – 16 h prior to irradiation by Co^{60} γ-radiation

DNA damage





Clonogenic assay



✓ Nic_2Se_2 pre-treatment protects CHO from γ-radiation induced DNA damage

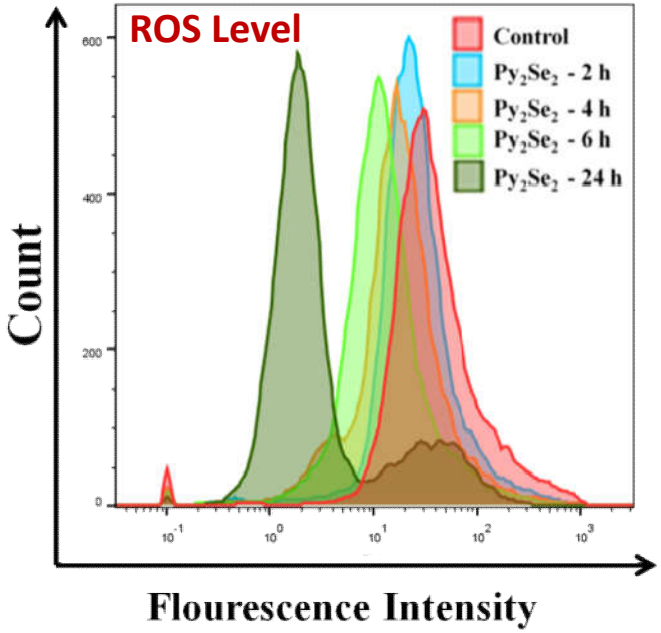
✓ Nic_2Se_2 pre-treatment protects CHO from γ-radiation induced cell death

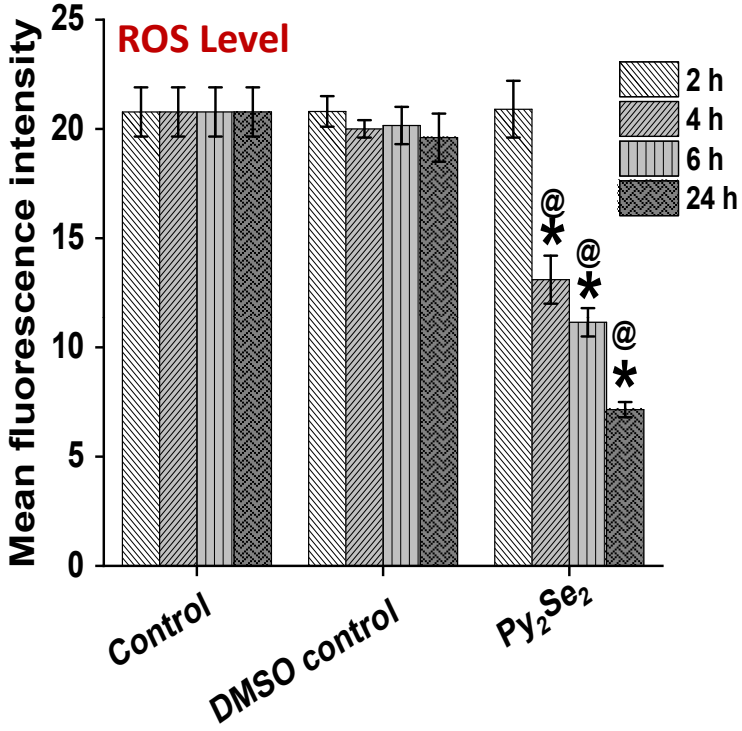
(*Metallomics* 2017, 9, 715-725)

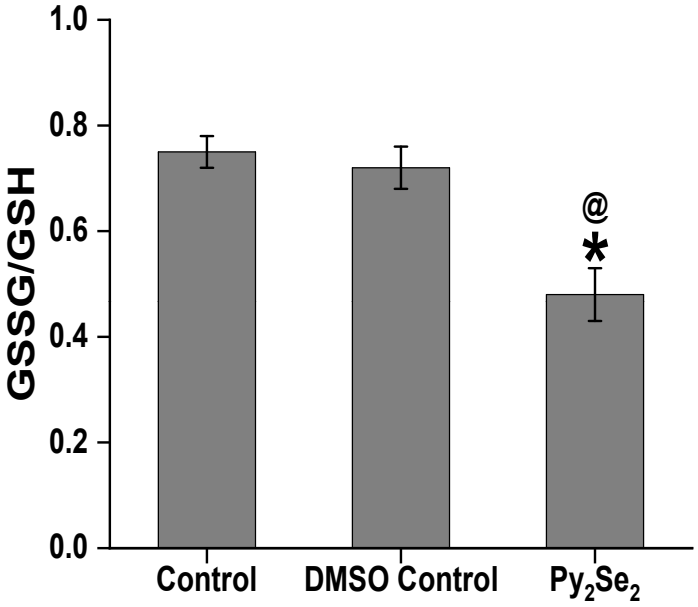
Redox modulatory activity of Py_2Se_2 in lung cancer (A549) cells

Py_2Se_2 treatment – 24 h

Method – DCFDA staining followed by FACS, Biochemical determination of GSH and GSSG



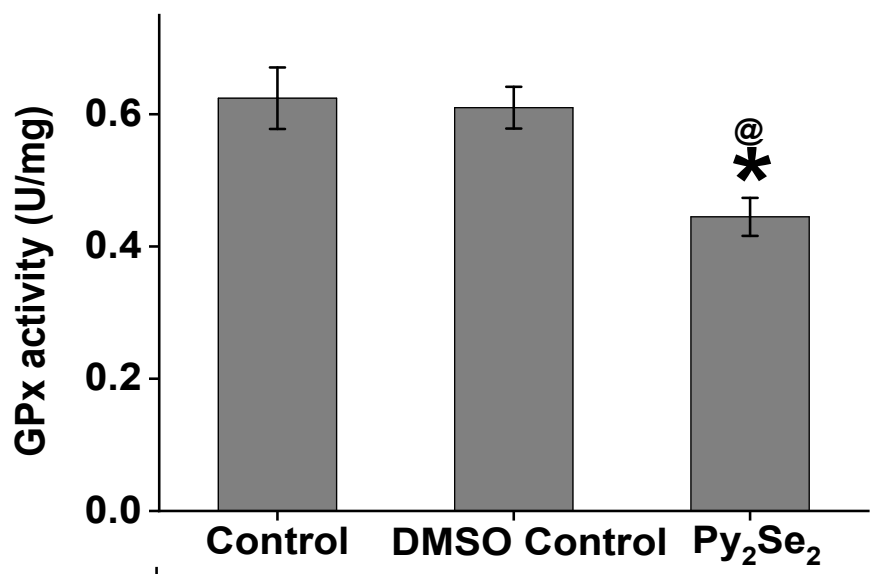


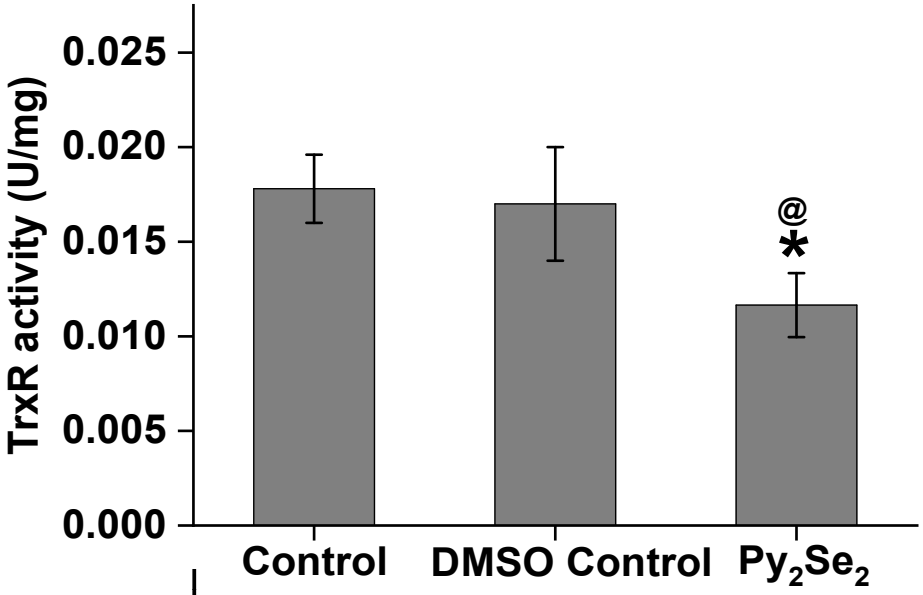


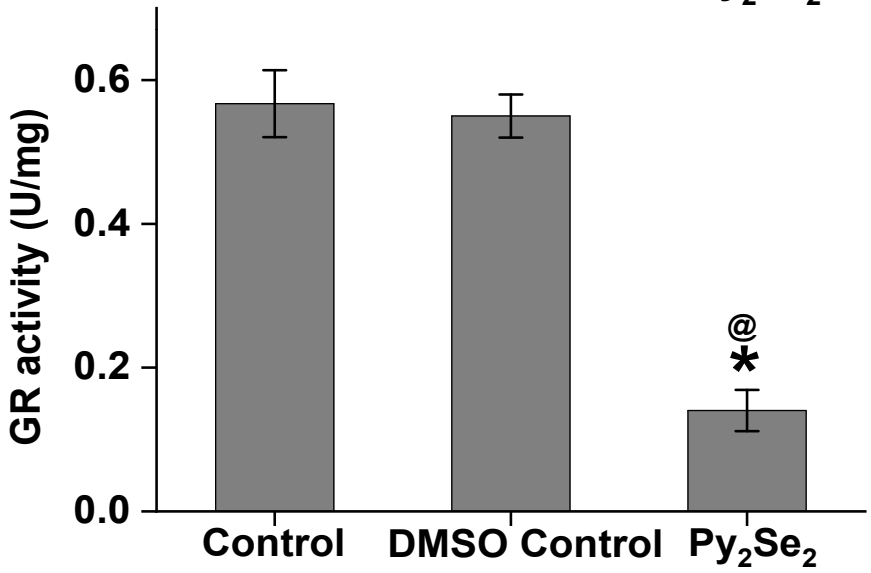
✓ Py_2Se_2 treatment induces reductive stress in A549 cells

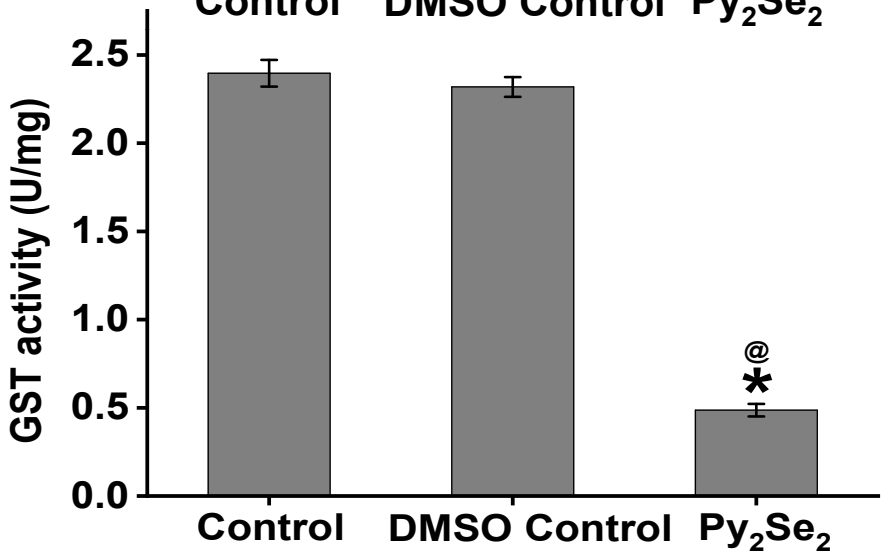
(*Metallomics* 2020, 12, 1253-1266)

Effect of Py_2Se_2 treatment on the activity of thiol and selenoproteins





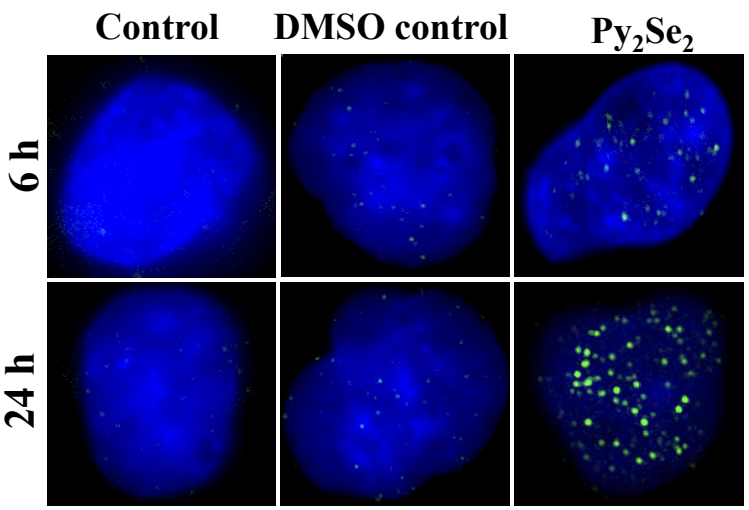


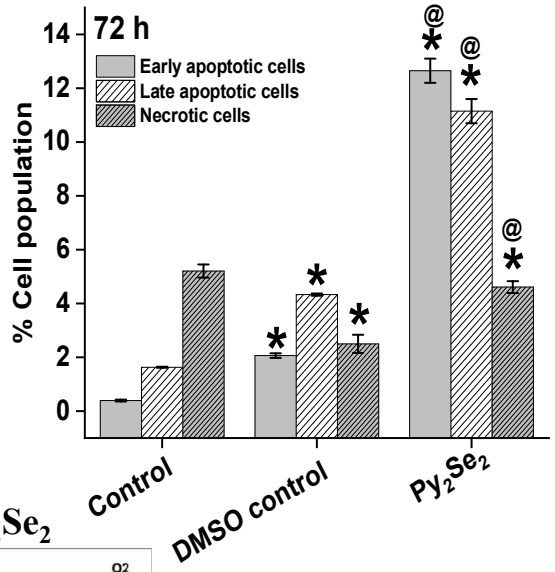


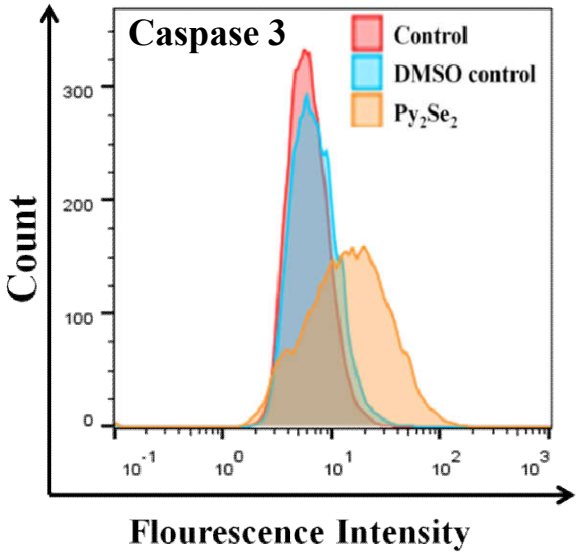
✓ Py_2Se_2 treatment inhibits the activity of thiol and selenoproteins in A549 cells

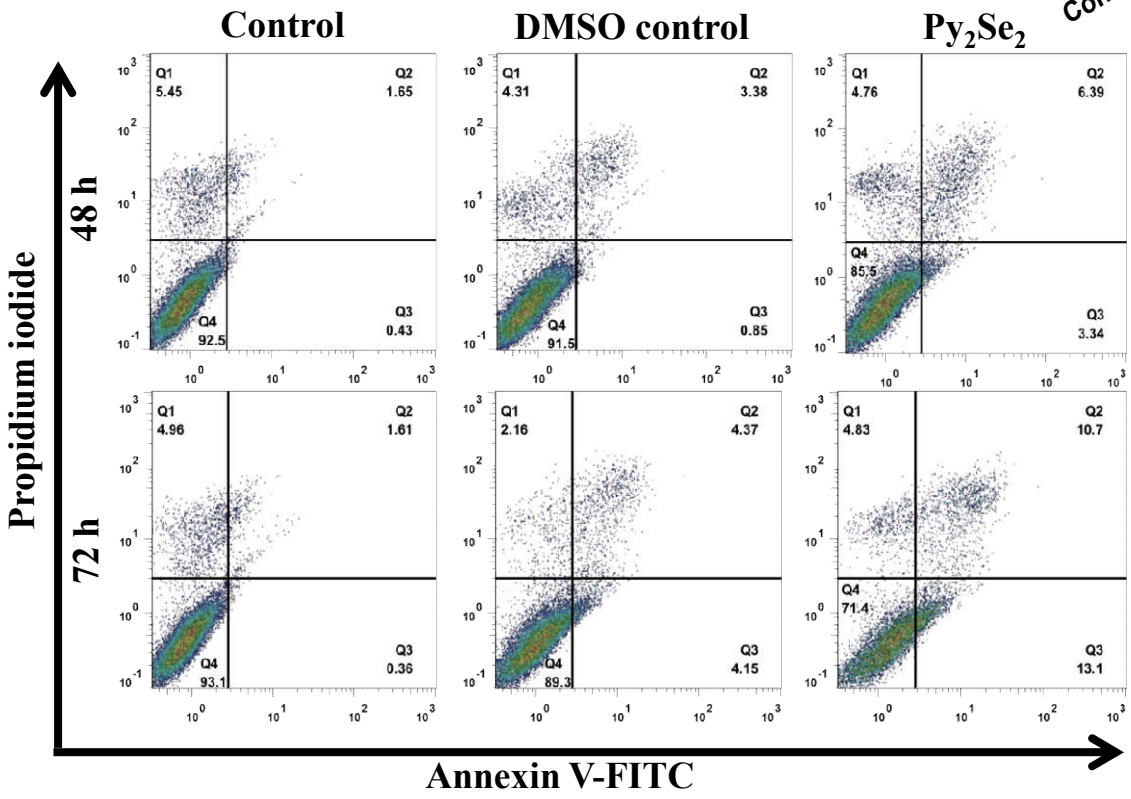
(*Metallomics* 2020, 12, 1253-1266)

Effect of Py_2Se_2 treatment on the DNA damage on apoptosis in A549 cells







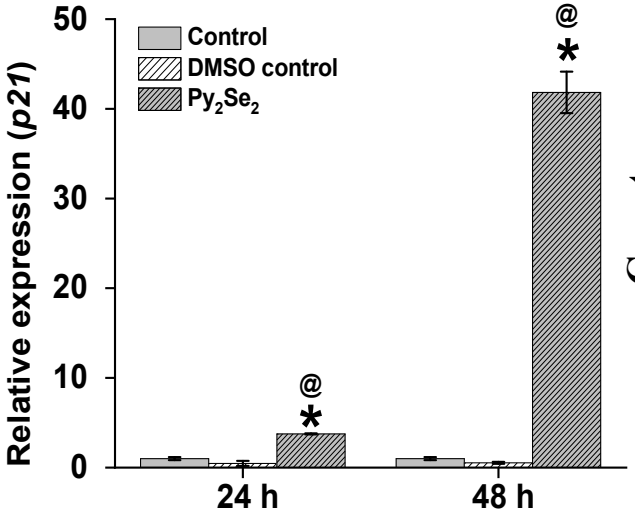


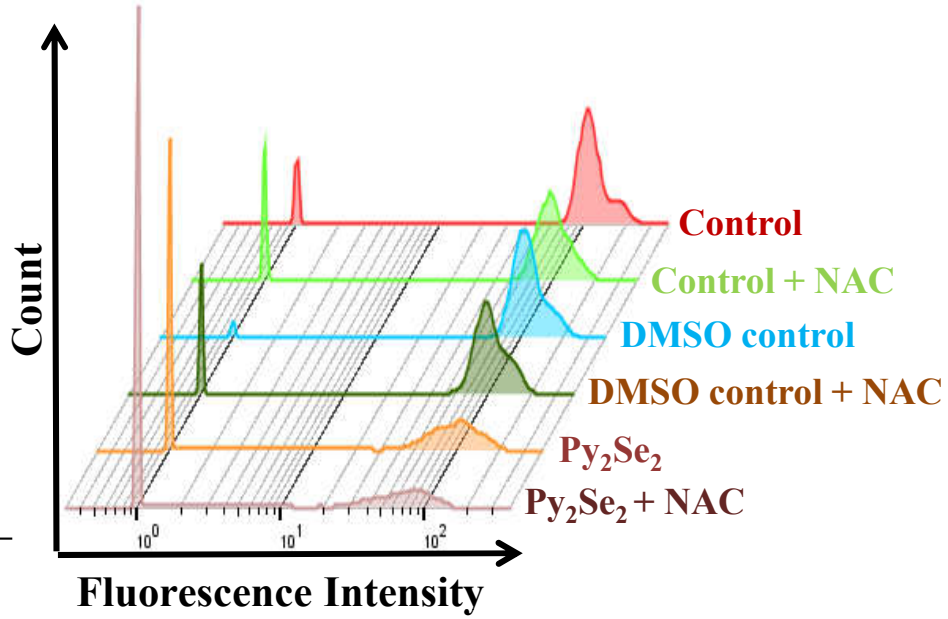
Method - γ-H2AX assay, Annexin V-PI assay, antibody staining

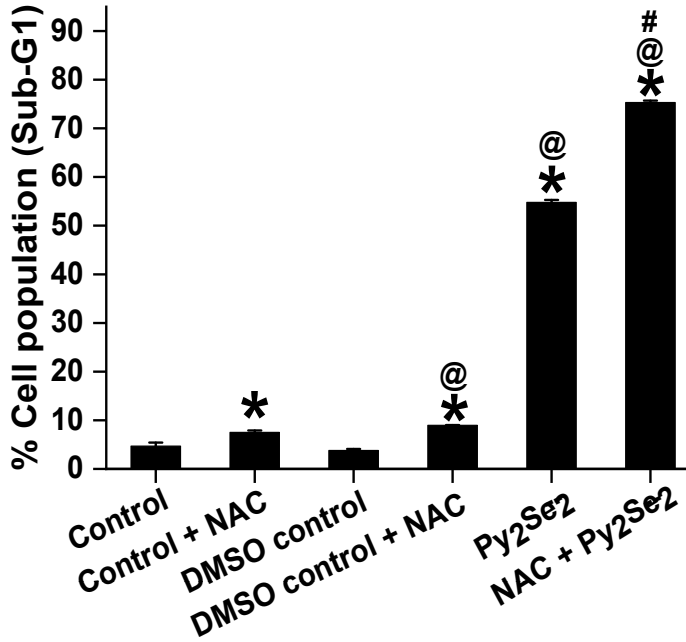
✓ **Py_2Se_2 treatment induces DNA damage and apoptosis in A549 cells.**

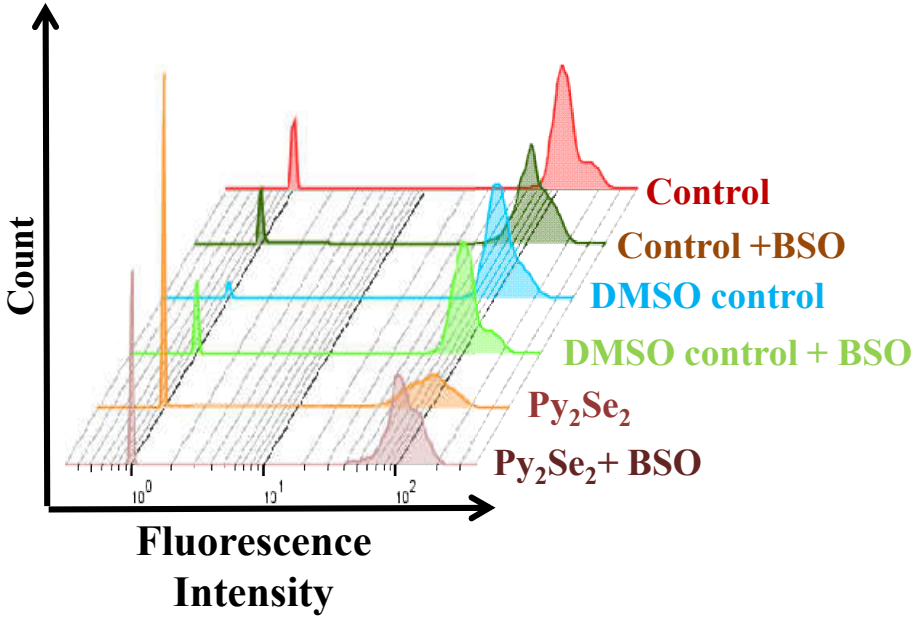
(*Metallomics* 2020, 12, 1253-1266)

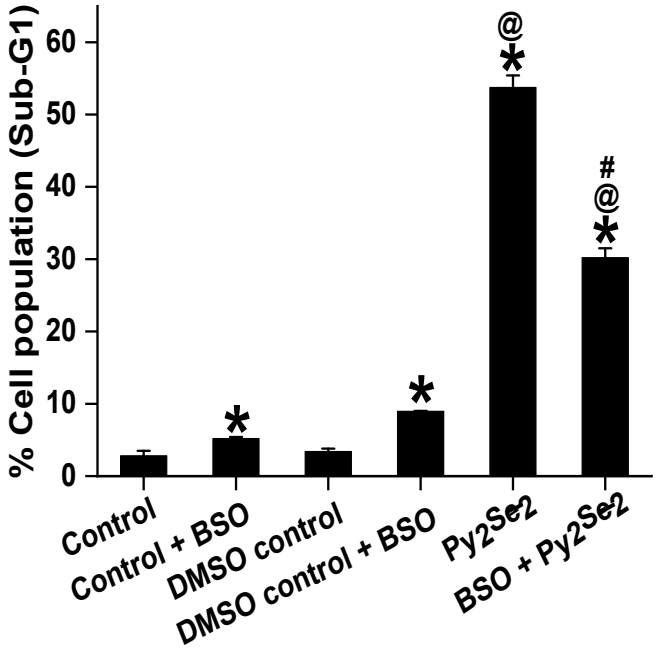
Effect of pharmacological modulation on Py_2Se_2 induced apoptosis in A549 cells









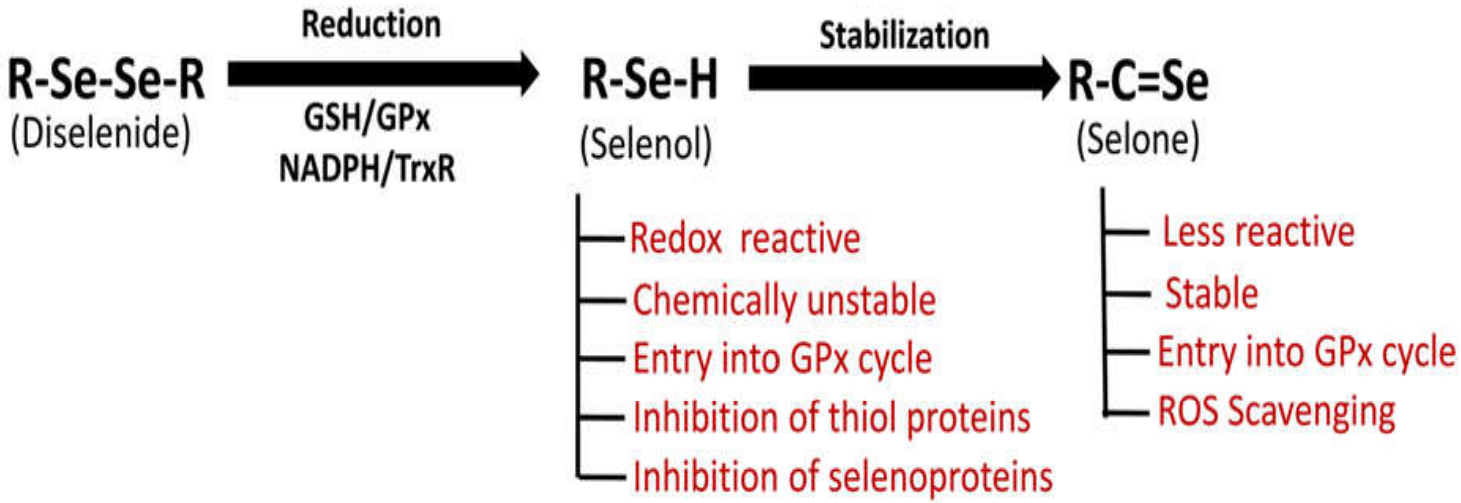


- ✓ Py_2Se_2 treatment induces replicative stress in A549 cells by increasing p21
- ✓ Combinatorial treatment of NAC augments Py_2Se_2 mediated apoptosis
- ✓ Combinatorial treatment of NAC abrogates Py_2Se_2 mediated apoptosis

(*Metallomics* 2020, 12, 1253-1266)

Conclusions

R - Aryl group with pyridine ring



✓Aryl diselenides containing pyridine ring modulates intracellular redox state towards reduction (antioxidant) rather than oxidation (pro-oxidant) side in both normal and cancer cells

✓ The reductive stress mediated by such compounds leads to cytotoxic or apoptotic effect in cancer cells

✓ Cellular redox state, level of TrxR and reductive intermediates (selenol versus selone) appear to be the major determinants of the toxicity of pyridine diselenides

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Miss P. Verma
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