

Article



Supplementary Material: Novel Thiol Containing Hybrid Antioxidant-Nitric Oxide Donor Small Molecules for Treatment of Glaucoma

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Figure S1: Superoxide scavenging ability of SA compounds in buffer and cells. **a)** The % of superoxide scavenging response from different concentrations of **SA-2**, **SA-9** and **SA-10** in pyrogallol (1 μ M, 10 μ M, 100 μ M and 1,000 μ M) induced superoxide induction assay at 15 minutes time point in buffer solution. The AUC₀₋₁₅ for **SA-2**, **SA-9** and **SA-10** were 11604±815.9, 5972±834.9 and 20433±3128 respectively. **b-d**) The superoxide scavenging ratio (%) after 18h treatment of different concentrations (0.1 μ M, 1 μ M, 10 μ M, 100 μ M and 1,000 μ M) of **SA-2**, **SA-9** and **SA-10** to NTM-5 cell supernatant with or without TBHP (5.5 mM) using pyrogallol induced superoxide induction assay at 3h time point. Control is untreated cells. The cells were previously treated with 5.5 mM of TBHP for 30 minutes followed by SA compounds and TBHP and incubated for 18h. Control is untreated cells. **e**) The AUC_{0-3h} for **SA-2**, **SA-9** and **SA-10** were 18902±2714, 21155±1099 and 21444±1877 respectively. The AUC_{0-3h} for TBHP + **SA-2**, TBHP + **SA-9** and TBHP + **SA-10** were 18285±3960, 14120±2023 and 17893±2714 respectively. Three technical replicates were used for each experiment and all experiments were repeated 2 times. N = 3.



Figure S2: Quantitative measurement of peroxynitrite (ONOO⁻) radical formation after treatment of SA compounds in NTM-5 cells. **a**) NTM-5 cells were labelled with peroxynitrite green sensor followed by treatment with 0.1 μ M or 1 mM of SIN-1, **SA-2**, **SA-9** and **SA-10**. Changes in green fluorescence signal corresponded to level of ONOO⁻ radicals as measured at Ex/Em of 485/530 nm after 2-3h. **b**) NTM-5 cells labelled with peroxynitrite green sensor followed by treatment with 0.1 μ M or 1mM of SIN-1, **SA-2**, **SA-9** and **SA-10** with TBHP (350 μ M). Changes in green fluorescence signal corresponded to level of ONOO⁻ radicals as measured at Ex/Em of 485/530 nm after 2-3h. SIN-1 is used as positive control. N =3.