## Article

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

Charles E Amankwa ${ }^{1,2}$, Sudershan R Gondi ${ }^{1,2}$, Adnan Dibas ${ }^{1,2}$, Courtney Weston ${ }^{1,2}$, Arlene Funk ${ }^{1,2}$, Tam Nguyen ${ }^{3}$, Kytai T Nguyen ${ }^{3}$, Dorette Z Ellis ${ }^{2,4}$ and Suchismita Acharya ${ }^{1,2,4, *}$

Citation: Amankwa, C.E.; Gondi, S.R.; Dibas, A.; Weston, C.; Funk, A.; Nguyen, T.; Nguyen, K.T.; Ellis, D.Z.; Acharya, S. Novel Thiol Containing Hybrid AntioxidantNitric Oxide Donor Small Molecules for Treatment of Glaucoma.

Antioxidants 2021, 10, 575. https://doi.org/10.3390/antiox10040575

Academic Editor: Urara Hasegawa

Received: 28 February 2021
Accepted: 5 April 2021
Published: 8 April 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.


Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

1 Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, TX 76107, USA; CharlesAmankwa@my.unthsc.edu (C.E.A.); sudershan.gondi@unthsc.edu (S.R.G.); dibasa@yahoo.com (A.D.); courtney.weston@outlook.com (C.W.); Arlene.Funk@my.unthsc.edu (A.F.);
2 North Texas Eye Research Institute, University of North Texas Health Science Center, Fort Worth, TX 76107, USA;
3 Department of Bioengineering, University of Texas at Arlington, Arlington, TX 76010, USA; tam.nguyen12@mavs.uta.edu (T.N.); knguyen@uta.edu (K.T.N.)
4 Department of Pharmaceutical Sciences, College of Pharmacy, University of North Texas Health Science Center, Fort Worth, TX 76107, USA, dorette.ellis@unthsc.edu

* Correspondence: suchismita.acharya@unthsc.edu


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 ( $1 \mu \mathrm{M}, 10 \mu \mathrm{M}$, $100 \mu \mathrm{M}$ and $1,000 \mu \mathrm{M})$ induced superoxide induction assay at 15 minutes time point in buffer solution. The AUC $_{0-15}$ for SA-2, SA-9 and SA-10 were $11604 \pm 815.9,5972 \pm 834.9$ and $20433 \pm 3128$ respectively. b-d) The superoxide scavenging ratio (\%) after 18 h treatment of different concentrations ( $0.1 \mu \mathrm{M}, 1 \mu \mathrm{M}, 10 \mu \mathrm{M}, 100$ $\mu \mathrm{M}$ and $1,000 \mu \mathrm{M}$ ) 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 3 h 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 18 h . Control is untreated cells. e) The AUC ${ }_{0-3}$ for $\mathbf{S A - 2 , S A - 9}$ and $\mathbf{S A}-10$ were $18902 \pm 2714$, $21155 \pm 1099$ and $21444 \pm 1877$ respectively. The AUC $_{0-3 \mathrm{~h}}$ for TBHP $+\mathbf{S A - 2}$, TBHP + SA- 9 and TBHP + SA-10 were $18285 \pm 3960,14120 \pm 2023$ and $17893 \pm 2714$ respectively. Three technical replicates were used for each experiment and all experiments were repeated 2 times. $\mathrm{N}=3$.


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 $0.1 \mu \mathrm{M}$ 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 $485 / 530 \mathrm{~nm}$ after 2-3h. b) NTM-5 cells labelled with peroxynitrite green sensor followed by treatment with $0.1 \mu \mathrm{M}$ or 1 mM of SIN-1, SA-2, SA-9 and SA-10 with TBHP ( $350 \mu \mathrm{M}$ ). Changes in green fluorescence signal corresponded to level of ONOO- radicals as measured at $\mathrm{Ex} / \mathrm{Em}$ of $485 / 530 \mathrm{~nm}$ after 2-3h. $\mathrm{SIN}-1$ is used as positive control. $\mathrm{N}=3$.

