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RESEARCH ARTICLE

Alleviating cellular oxidative stress through treatment with superoxide-triggered persulfide prodrugs

Yin Wang[#], Kearsley M. Dillon[#], Zhao Li, Ethan W. Winckler, and John B. Matson*

[*] Dr. Y. Wang, K. M. Dillion, Z. Li, E. W. Winckler, Prof. J. B. Matson
Department of of Chemistry, Virginia Tech Center for Drug Discovery, and Macromolecules Innovation Institute
Virginia Tech
Blacksburg, VA 24061, United States
E-mail: jbmatson@vt.edu
[#] These authors contributed to this work equally.

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Abstract: Overproduction of superoxide anion ($O_2^{\bullet-}$), the primary cellular reactive oxygen species (ROS), is implicated in various human diseases. To reduce cellular oxidative stress caused by overproduction of superoxide, we developed a compound that reacts with $O_2^{\bullet-}$ to release a persulfide (RSSH), a type of reactive sulfur species related to the gasotransmitter hydrogen sulfide (H_2S). Termed **SOPD-NAC**, this persulfide donor reacts specifically with $O_2^{\bullet-}$, decomposing to generate *N*-acetyl cysteine (NAC) persulfide. To enhance persulfide delivery to cells, we conjugated the SOPD motif to a short, self-assembling peptide (Bz-CFFE-NH_2) to make a superoxide-responsive, persulfide-donating peptide (**SOPD-Pep**). Both **SOPD-NAC** and **SOPD-Pep** delivered persulfides/H_2S to H9C2 cardiomyocytes and lowered ROS levels as confirmed by quantitative *in vitro* fluorescence imaging studies. Additional *in vitro* studies on RAW 264.7 macrophages showed that **SOPD-Pep** mitigated toxicity induced by phorbol 12-myristate 13-acetate (PMA) more effectively than **SOPD-NAC** and several control compounds, including common H_2S donors.

Introduction

Superoxide anion ($O_2^{\bullet-}$), the product of a one-electron reduction of O_2 generated primarily in mitochondria during cellular respiration, is a reactive oxygen species (ROS) implicated in various diseases related to mitochondrial dysfunction.[1] While a minimum level of $O_2^{\bullet-}$ is essential for cell survival, excessive production leads to damage to proteins, lipids, and nucleic acids.[2] Beyond the harm that superoxide can inflict directly, it is also a key precursor for other even more damaging ROS; for example, it reacts with nitric oxide (NO) to produce peroxynitrite ($ONOO^-$), a powerful and destructive oxidant.[3] Superoxide dismutases, enzymes that deactivate $O_2^{\bullet-}$, exist in nearly all cells and have evolved over 2 billion years to limit the extent of cell damage from $O_2^{\bullet-}$.[4] Despite this cellular defense against $O_2^{\bullet-}$, its overproduction is ascribed to many oxidative stress-induced diseases, including degenerative disorders, ischemia-reperfusion (IR) injury resulting from surgery, cardiovascular disease, and some cancers (e.g., prostate cancer).[5] Given the roles of $O_2^{\bullet-}$ in both cell signaling and disease, turn-on fluorescence probes capable of detecting $O_2^{\bullet-}$ have been developed.[6]-[7] Surprisingly, no compounds exist that react specifically with $O_2^{\bullet-}$ to both reduce $O_2^{\bullet-}$ levels and release a molecule capable of further alleviating oxidative stress.

One signaling species that may be capable of regulating redox balance in cells with high levels of $O_2^{\bullet-}$ is a persulfide (RSSH).[8] Persulfides are related to the signaling gas (gasotransmitter) hydrogen sulfide (H_2S), a reactive sulfur species crucial to many (patho)physiological processes.[9] Because of their potentially dominant roles in H_2S-related signaling pathways, combined with their ability to persulfidate biological targets (conversion of RSH into RSSH) under conditions where H_2S cannot, persulfides have recently gained attention.[10] Key reactivities of persulfides are their nucleophilicity and reducing abilities; both small molecule and protein-bound persulfides regulate redox signaling through direct and indirect routes.[11] For example, glutathione persulfide quickly scavenges the biological oxidant H_2O_2,[12] and persulfidation of p66Shc, a protein involved in mitochondrial redox signaling, inhibits mitochondrial ROS production.[13] Therefore, we envisioned that a persulfide donor that could be triggered by $O_2^{\bullet-}$ might be a powerful tool for reducing its deleterious effects. Beyond decreasing $O_2^{\bullet-}$ levels, a superoxide-triggered persulfide donor might help uncover the roles and connections among $O_2^{\bullet-}$ and persulfides in the reactive species interactome.[4]

The difficulty in developing persulfide donors comes from the instability of persulfides, which react quickly with various species, including themselves, oxidants, and free thiol groups.[14] Therefore, protected persulfides (i.e., prodrugs), in which a specific stimulus activates the removal of the protecting group to release the persulfide, are needed to achieve controlled delivery. Accordingly, persulfide donors exist that respond to biologically relevant stimuli, including enzymes,[15] light,[16] and H_2O_2.[17] We envisioned that a superoxide-triggered persulfide donor could be constructed by linking a diphenylphosphinate ester, which can be oxidized by $O_2^{\bullet-}$ in a bond cleaving reaction,[18] to a 4-($RSSCH_2$)phenoxy group. In a cascade of reactions, $O_2^{\bullet-}$ should

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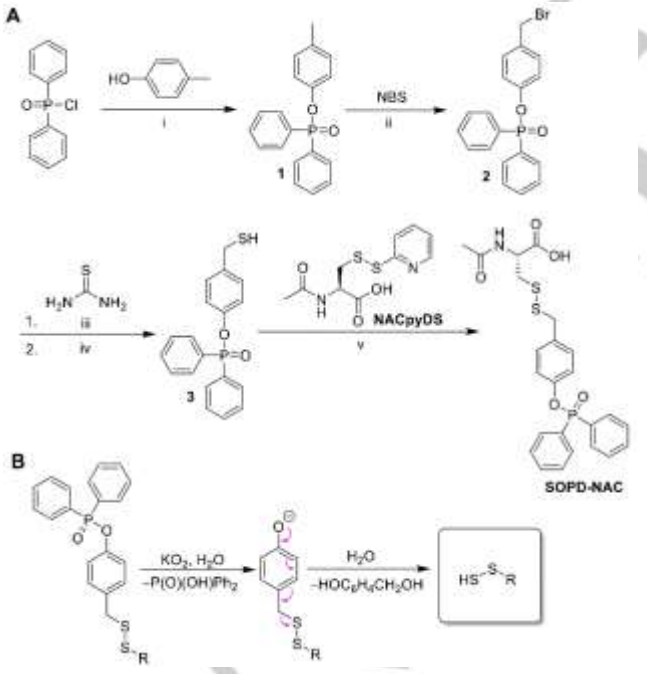
react at phosphorous to cleave the 4-(RSSCH$_2$)phenoxy group, which would then undergo a 1,6-elimination reaction to reveal a persulfide anion (RSS$^-$). Such a persulfide donor would serve two functions—capturing $O_2^{\bullet-}$ and releasing a persulfide. Additionally, we aimed to develop a delivery system for this donor that would enhance its water solubility, bioavailability, and provide a potential mechanism for eventual targeting to specific cells or tissues. Therefore, we detail here our efforts to develop a small molecule superoxide-triggered persulfide donor that could be used on its own and could also be easily conjugated to a self-assembling peptide-based delivery vehicle to improve solubility and bioactivity.

Results and Discussion

We chose the diphenylphosphinate group as the stimuli-responsive unit because of its ability to selectively react with $O_2^{\bullet-}$.[18] We conjugated this group to *N*-acetyl cysteine (NAC), which we selected because of its powerful antioxidant capability[17b, 19] and its free thiol group for conjugation to a thiol-containing diphenylphosphinate. Synthesis was carried out as shown in Scheme 1A. Briefly, diphenylphosphinic chloride was treated with *p*-cresol under basic conditions. The resulting tolyl diphenylphosphinate (**1**) was then brominated by using NBS to generate a benzyl bromide (**2**). Bromide displacement was achieved by treating compound **2** with thiourea followed by hexylamine, resulting in a benzyl thiol (**3**). Finally, we treated thiol **3** with the activated disulfide of NAC (**NACpyDS**) in a disulfide exchange reaction to afford the target persulfide donor termed **SOPD-NAC** (SOPD = **s**uper**o**xide-triggered **p**ersulfide **d**onor). A proposed mechanism for persulfide release is shown in Scheme 1B.



Scheme 1. A) Synthesis of **SOPD-NAC**. Reaction conditions: i) NEt_3, DMAP, THF, 16 h, rt, 82% yield; ii) AIBN, C_6H_6, 16 h, reflux, 91% yield; iii) CH_3OH, 16 h, rt; iv) hexylamine, $CHCl_3$, 24 h, rt, 90% yield; v) CH_2Cl_2, 24 h, rt, 56% yield. B) Proposed mechanism of superoxide-triggered persulfide release from **SOPD-NAC** (R=NAC).

One promising strategy to address limitations in prodrug solubility and bioavailability is appending drugs or prodrugs to self-assembling peptides. Due to their inherent biodegradability and biocompatibility in most contexts, peptides capable of self-assembly into nanostructures and/or hydrogels are powerful materials for biomedical applications.[20] Previously, we have shown that conjugation of H_2S-donating *S*-aroylthiooximes (SATOs) to short peptides not only extended H_2S release profiles, but also modulated release behaviors as a result of their different self-assembled morphologies.[21] In this regard, conjugating SOPD to a self-assembling peptide scaffold would endow the resultant conjugate with new properties, including improved water solubility and the potential for cell targeting.

Therefore, we conjugated 4-(mercaptomethyl)phenyl diphenylphosphinate to a tetrapeptide Bz-Cys-Phe-Phe-Glu (Bz-CFFE) (Bz = benzoyl) through a disulfide linkage (Scheme 2A). In this two-step synthesis, the 2-thiopyridyl-activated disulfide of Bz-CFFE was prepared and then treated with 4-(mercaptomethyl)phenyl diphenylphosphinate to afford the target superoxide responsive peptide (**SOPD-Pep**). We also synthesized a control peptide termed **SOPD-Pep-Cont**, which included the same peptide sequence conjugated to the diphenylphosphinate group through a thioether linkage instead of a disulfide. **SOPD-Pep-Cont** was synthesized in one step by reacting Bz-CFFE with 4-(bromomethyl)phenyl diphenylphosphinate (Scheme 2B). We chose Bz-CFFE not only because of the free thiol group in the sequence, but also because of its propensity to form discrete one-dimensional nanostructures.[22]

Next, we investigated whether **SOPD-NAC** and **SOPD-Pep** could specifically react with $O_2^{\bullet-}$ to release persulfides by 1,6-elimination (self-immolation), a strategy used in several ROS-triggered H_2S donors.[23] Because very few persulfide-specific chemical probes exist, we first evaluated persulfide release by relying on the ability of persulfides to rapidly react with cysteine (Cys) to generate H_2S.[10] To this end, we evaluated the reactivity of **SOPD-NAC** and **SOPD-Pep** with several potential triggers by monitoring the fluorescence increase of an H_2S-selective fluorescent probe WSP-2 reported previously[24] (Figures S10 and 1A). The experiment probed whether persulfide donors react with the triggers by monitoring the fluorescence intensity at λ_{em}=455 nm, the characteristic wavelength of 7-hydroxycoumarin (Figure 1B).

The results revealed that neither **SOPD-NAC** nor **SOPD-Pep** alone showed any fluorescence increase in the absence of a trigger (control groups in Figures 1A (**SOPD-Pep**) and S10 (**SOPD-NAC**)). However, adding excess Cys and $O_2^{\bullet-}$ led to a 16-fold and 12-fold increase in fluorescence intensity for **SOPD-Pep** and **SOPD-NAC**, respectively (red bars, Figures 1A and S10). Incubating **SOPD-NAC** or **SOPD-Pep** with either Cys alone or $O_2^{\bullet-}$ alone generated a limited increase in fluorescence intensity, significantly lower than that of the treatment group containing both triggers. These results implied that H_2S liberation from **SOPD-NAC** or **SOPD-Pep** requires both Cys and $O_2^{\bullet-}$. Consistent with previous reports on superoxide-responsive probes,[6] other common reactive sulfur, oxygen, and nitrogen species (RSONS)—hypochlorite (ClO^-), peroxynitrite ($ONOO^-$), sulfate (SO_4^{2-}), and glutathione (GSH)—failed to trigger release of H_2S. More importantly, H_2O_2 did not enhance the fluorescence intensity, suggesting that **SOPD-NAC** and **SOPD-Pep** are selective to $O_2^{\bullet-}$. In summary, this screening assay validated that **SOPD-NAC** and

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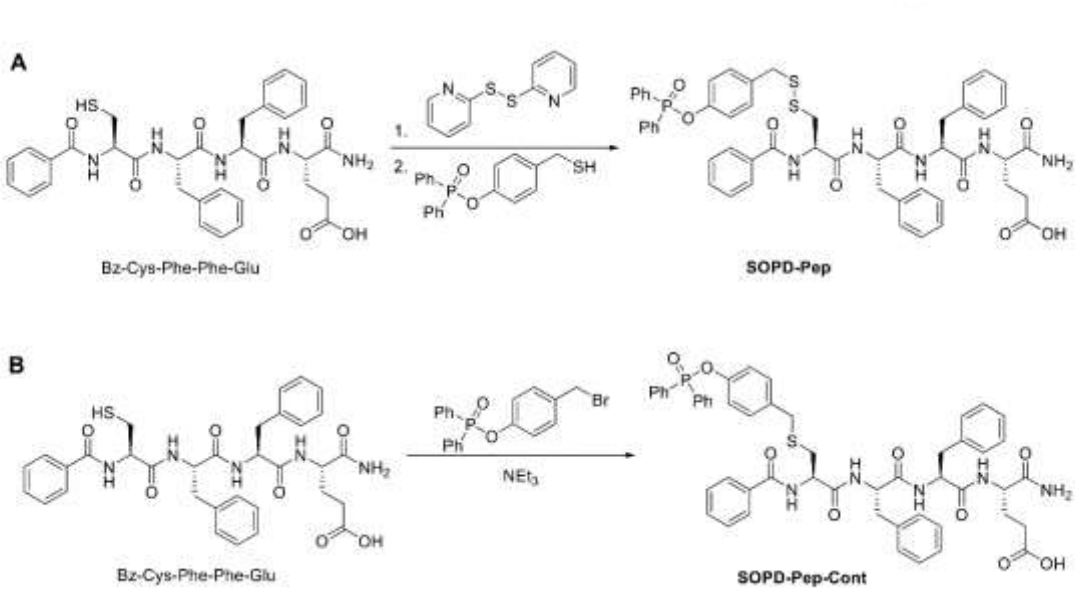
SOPD-Pep possess good selectivity for $O_2^{\bullet-}$ and that both persulfide donors could release H_2S in the presence of Cys (Figure 1B). For direct verification, we also confirmed persulfide release through mass spectrometry (Figure S11).



Scheme 2. A) Synthesis of **SOPD-Pep** and B) **SOPD-Pep-Cont**.

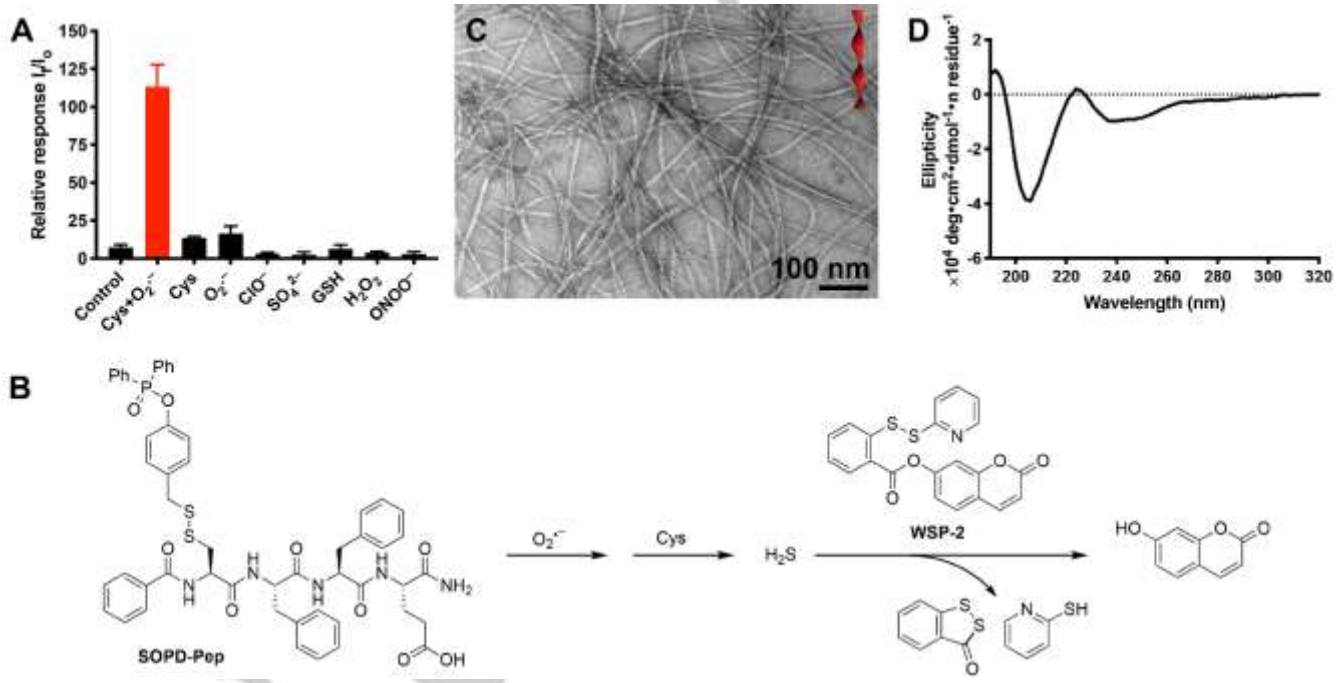


Figure 1. (A) Relative response of 1 mM **SOPD-Pep** and 50 μM WSP-2 to each potential trigger (4 mM) or combination of triggers or control (no trigger added) represented as the ratio of the final fluorescence intensity (I_f) after 30 min to the initial fluorescence intensity (I_0), showing an increased selectivity for Cys + $O_2^{\bullet-}$ over other potential triggers (KO_2 was used as the superoxide source). The results were expressed as the mean ± SD (n = 3). (B) Proposed mechanism for fluorescence turn-on of WSP-2 by H_2S released from **SOPD-Pep** in the presence of Cys and $O_2^{\bullet-}$. (C) Conventional TEM characterization of twisted nanoribbons formed by **SOPD-Pep** in 10 mM phosphate buffer (pH=7.4). The concentration was 100 μM, diluted from 1 mM pre-assembled stock solution. The TEM grid was stained with 2 wt % uranyl acetate prior to imaging. (D) CD spectrum of **SOPD-Pep** in 10 mM phosphate buffer (pH=7.4) at 100 μM.

Given the amphiphilic nature of **SOPD-Pep**, we next studied the morphology of **SOPD-Pep** after dissolution in 10 mM phosphate buffer (pH 7.4) by conventional transmission electron microscopy (TEM). TEM imaging showed that **SOPD-Pep** self-assembled into twisted nanoribbons as indicated by the varying thickness and grayscale intensity (Figure 1C). Average widths were 10 ± 2 nm, and lengths were on the scale of a few micrometers. The molecular packing within these nanoribbons was then assessed by circular dichroism (CD) spectroscopy (Figure 1D). CD revealed that **SOPD-Pep** had a primarily random

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coil structure with some β-sheet contribution. The minimum at 206 nm may result from π−π interactions of the aromatic side chains in nanostructures and the distortion of β-sheets, which have been previously observed in aromatic peptide amphiphile systems.[25] The negative peak near 250 nm was attributed to the absorption of diphenylphosphinate group.

We then focused on biological applications of **SOPD-NAC** and **SOPD-Pep**. Cell viability assays indicated that both **SOPD-NAC** and **SOPD-Pep** were nontoxic to H9C2 cardiomyocytes, a widely used cell line for testing how compounds may affect heart function, at concentrations up to 200 μM (Figure S15). Fluorescence microscopy was then used to investigate whether H_2S could be liberated from **SOPD-NAC** and **SOPD-Pep** and delivered to cells in the presence of $O_2^{\bullet-}$. We used L-buthionine-(*S*,*R*)-sulfoximine (BSO), which induces oxidative stress in cells by depleting glutathione (GSH),[26] to generate superoxide *in vitro*.[27] WSP-5,[24] an H_2S-selective fluorescent probe, was used to monitor H_2S accumulation in H9C2 cells. No additional Cys was added, relying instead on endogenous thiols to convert persulfides into detectable H_2S.

As expected, BSO alone provided a negligible fluorescent signal (Figure 2, first row). Treating cells with **SOPD-NAC** or **SOPD-Pep** without BSO generated a weak fluorescent signal. This most likely resulted from a small amount of H_2S liberated from **SOPD-NAC** or **SOPD-Pep** triggered by endogenous $O_2^{\bullet-}$ within cells (Figure 2, second and third rows). Co-incubation of **SOPD-NAC** with BSO generated a slightly stronger signal compared to **SOPD-NAC** alone (Figure 2, fourth row), indicating some amount of H_2S production from this small molecule persulfide donor. In sharp contrast, co-incubation of **SOPD-Pep** nanoribbons and BSO produced a significant increase in WSP-5 fluorescence (Figure 2, fifth row), demonstrating that **SOPD-Pep** can be successfully activated to release H_2S *in vitro*. Given the identical conditions and equimolar amounts of **SOPD-NAC** and **SOPD-Pep**, the difference in H_2S-release levels likely stems from different cellular accumulations of these two persulfide donors. We speculate that **SOPD-Pep** enters and remains in cells to a greater extent than **SOPD-NAC** due to its nanoscale size, a phenomenon that has been observed for other self-assembled peptide drug-delivery vehicles.[28]

Given that both **SOPD-NAC** and **SOPD-Pep** are triggered by $O_2^{\bullet-}$ *in vitro*, we then asked whether these persulfide donors could decrease ROS production. BSO was used as a superoxide inducer with dihydroethidium (DHE) as an ROS-sensing fluorescent probe.[29] As shown in row 1 of Figure 3, the control group (H9C2 cells without any treatments) showed a dim red fluorescent signal, indicating that a certain amount of ROS was naturally generated within H9C2 cells as a product of normal metabolism of oxygen, consistent with previous reports.[30] However, after treatment with BSO, the red fluorescent signal from DHE increased, implying that ROS had accumulated in cells (Figure 3, second row). We found that H9C2 cells co-incubated with BSO and **SOPD-Pep-Cont** showed a lower red fluorescence compared to those treated only with BSO because **SOPD-Pep-Cont** possesses a diphenylphosphinate group that scavenges $O_2^{\bullet-}$, but it lacks the capacity to release a persulfide (Figure 3, third row). Co-incubation of BSO with persulfide donors **SOPD-NAC** or **SOPD-Pep** decreased the red fluorescence signal (Figure 3 fourth and fifth rows), with **SOPD-Pep** showing a similar fluorescence intensity to the control group. These results agree with the H_2S production studies (Figure 2), indicating that persulfides released from **SOPD-NAC** and **SOPD-Pep** suppress the production of ROS, and that **SOPD-Pep** suppresses more effectively than **SOPD-NAC**.

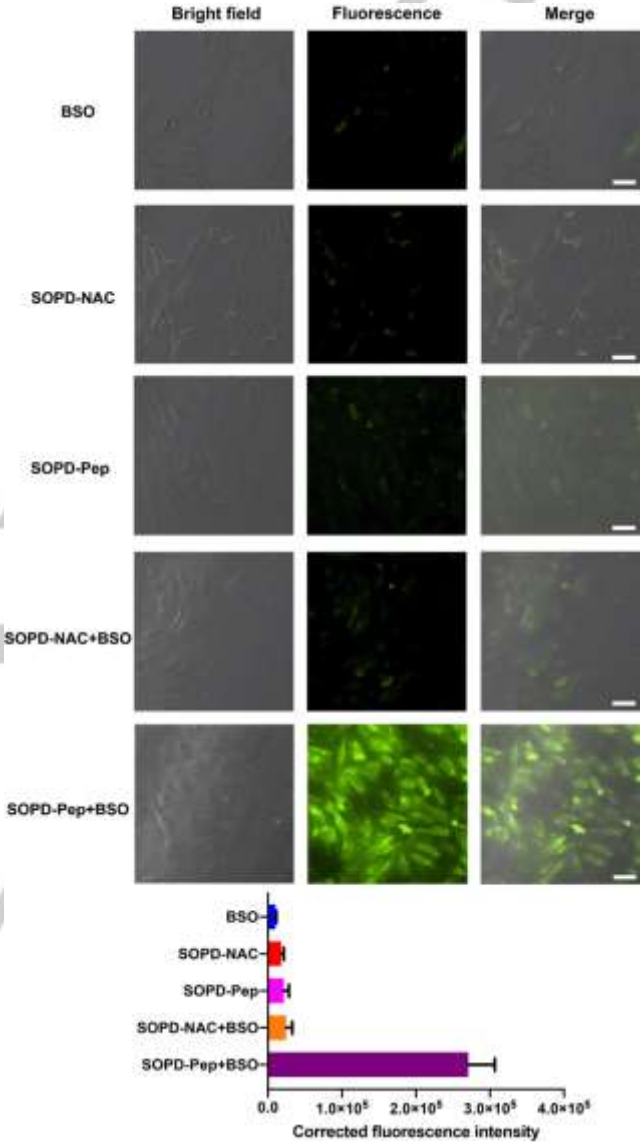

Figure 2. Bright field, fluorescence, and merged images showing fluorescence in H9C2 cells pre-incubated with **SOPD-NAC** or **SOPD-Pep** (200 μM) for 6 h, and subsequently treated with WSP-5 (50 μM) and BSO (5 mM) or an equal volume of PBS for 30 min. Cells were then washed, and fluorescence images were taken in PBS. Scale bars are 50 μm. Averaged fluorescence intensities of these five respective treatment groups were quantified by ImageJ (cell counts are > 30 for each group from three separate wells).

As **SOPD-NAC** and **SOPD-Pep** both deliver persulfides/H_2S into cells and further quench ROS production, we next explored their anti-inflammatory activities on RAW 264.7 macrophages. RAW 264.7 cells were chosen as a model because they generate a considerable amount of $O_2^{\bullet-}$ when incubated with phorbol 12-myristate 13-acetate (PMA).[23d, 31] H_2S alleviates inflammation caused by ROS,[32] but the protective capacity of

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persulfide donors has not been tested in cells treated with PMA. Thus, we envisioned that **SOPD-NAC** and **SOPD-Pep** might rescue RAW 264.7 macrophages from induced oxidative stress.

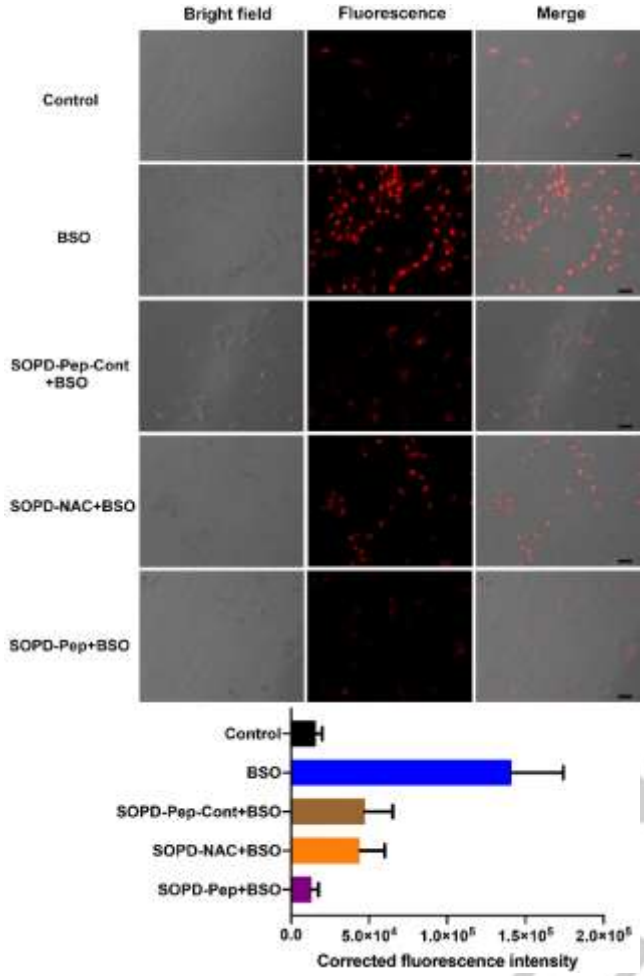

Figure 3. Bright field, fluorescence, and merged images showing fluorescence in H9C2 cells pre-incubated with **SOPD-Pep-Cont** or **SOPD-NAC** or **SOPD-Pep** (200 μM) for 6 h and then treated with DHE (10 μM) and BSO (5 mM) or an equal volume of DMSO for 30 min. Cells were then washed, and fluorescence images were taken in PBS. Scale bars are 50 μm. Averaged fluorescence intensities of these five respective treatment groups were quantified by ImageJ (cell counts are > 30 for each group from three separate wells).

First, we demonstrated that both **SOPD-NAC** and **SOPD-Pep** were nontoxic to RAW 264.7 cells at concentrations up to 200 μM (Figure S16). In contrast, PMA induced significant cytotoxicity at concentrations as low as 1 μg/mL (Figure 4A). In treatment studies, RAW 264.7 cells were pretreated with PMA for 1 h, a period consistent with previous reports,[23d] then **SOPD-Pep** was added without removing PMA solution, and cells were subsequently cultured for another 4 h before analyzing viability. Compared to the PMA-only treatment group, cell viability increased significantly when cells were co-incubated with **SOPD-Pep** and PMA (Figure 4A). For example, exposure of PMA to cells at 2 μg/mL decreased viability to 51% while viability increased to 81% when co-incubated with 100 μM **SOPD-Pep**.

To further ensure that persulfide release was responsible for imparting protection to the macrophages in the presence of PMA, several control studies were carried out (Figure 4B). **SOPD-NAC** showed a moderate ability to rescue cells; this result may be related to a small amount of persulfide/H_2S accumulated within cells, consistent with results shown in Figures 2 and 3. **SOPD-Pep-Cont** showed a similar cell protective ability to **SOPD-NAC**. This small but significant protective capacity likely resulted from the diphenylphosphinate group scavenging $O_2^{\bullet-}$ and, thus, lowering intracellular ROS levels. Under the same conditions, we also compared **SOPD-Pep** to sodium sulfide (Na_2S), a fast-releasing H_2S donor, and GYY4137,[33] a slow-releasing H_2S donor. Na_2S had limited ability to rescue cells, while GYY4137 had no effect. Therefore, **SOPD-Pep** more effectively rescued cells than did Na_2S and GYY4137.

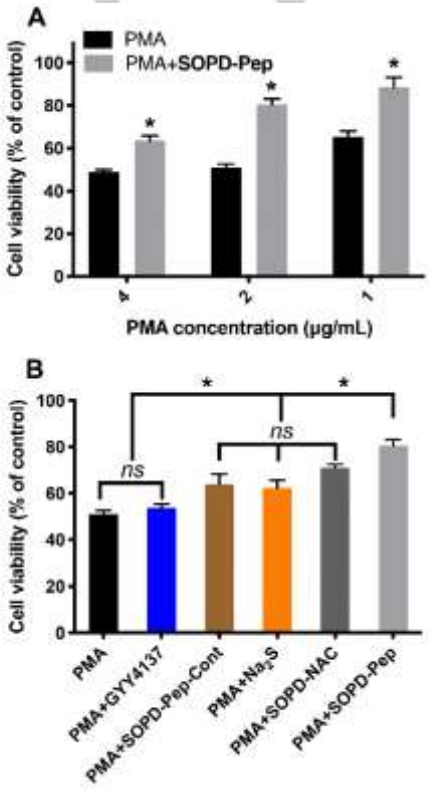

Figure 4. (A) Cell viability of RAW 264.7 macrophage cells pretreated with different concentrations of PMA for 1 h followed by exposure to **SOPD-Pep** (100 μM) for 4 h. * indicates $p < 0.01$ vs PMA-only groups. (B) Cell viability of RAW 264.7 macrophage cells pretreated with PMA (2 μg/mL) for 1 h followed by exposure to different groups (100 μM) for 4 h: GYY4137, **SOPD-Pep-Cont**, Na_2S, **SOPD-NAC**, and **SOPD-Pep**. * indicates $p < 0.01$. Error bars indicate standard deviation of three separate experiments with five replicates per experiment. Group comparisons are indicated as determined by a one-way analysis of variance (ANOVA) with a Student−Newman−Keuls comparisons *post-hoc* test.

Conclusion

In summary, we report a new dual-acting compound that scavenges $O_2^{\bullet-}$ and then decomposes to release a persulfide. This motif, based on a small molecule (**SOPD-NAC)**, was conjugated to a self-assembling peptide (**SOPD-Pep**). In the presence of $O_2^{\bullet-}$, both donors released persulfides, decreasing ROS levels in cells. Further, **SOPD-Pep** showed pronounced anti-inflammatory activity on macrophages, greater than **SOPD-NAC**, **SOPD-Pep-Cont** (a control peptide incapable of persulfide

release), and common H_2S donors. These results highlight the potential of **SOPD-NAC** and **SOPD-Pep** for persulfide-based therapies and demonstrate their power to initiate complex changes in cell behavior. Broadly, this dual superoxide-scavenging–persulfide-donating scaffold could potentially find use to regulate the redox balance within cells, which may alleviate oxidative stress-induced diseases caused by upregulation of $O_2^{\bullet-}$.

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Keywords: Drug Delivery • Oxidative Stress • Peptides • Prodrugs • Stimuli-Responsive

[1] I. Fridovich, *J. Biol. Chem.* **1997**, *272*, 18515-18517.
[2] U. Bandyopadhyay, D. Das, R. K. Banerjee, *Curr. Sci.* **1999**, *77*, 658-666.
[3] C. Szabo, H. Ischiropoulos, R. Radi, *Nat. Rev. Drug Discov.* **2007**, *6*, 662-680.
[4] M. M. Cortese-Krott, A. Koning, G. G. C. Kuhnle, P. Nagy, C. L. Bianco, A. Pasch, D. A. Wink, J. M. Fukuto, A. A. Jackson, H. van Goor, K. R. Olson, M. Feelisch, *Antioxid. Redox Signal.* **2017**, *27*, 684-712.
[5] P. Pacher, J. S. Beckman, L. Liaudet, *Physiol. Rev.* **2007**, *87*, 315-424.
[6] X. Y. Gao, G. X. Feng, P. N. Manghnani, F. Hu, N. Jiang, J. Z. Liu, B. Liu, J. Z. Sun, B. Z. Tang, *Chem. Commun.* **2017**, *53*, 1653-1656.
[7] J. J. Zhang, C. W. Li, R. Zhang, F. Y. Zhang, W. Liu, X. Y. Liu, S. M. Y. Lee, H. X. Zhang, *Chem. Commun.* **2016**, *52*, 2679-2682.
[8] E. Cuevasanta, M. N. Moller, B. Alvarez, *Arch. Biochem. Biophys.* **2017**, *617*, 9-25.
[9] R. Wang, *Physiol. Rev.* **2012**, *92*, 791-896.
[10] M. R. Filipovic, J. Zivanovic, B. Alvarez, R. Banerjee, *Chem. Rev.* **2018**, *118*, 377-461.
[11] S. Kasamatsu, A. Nishimura, M. Morita, T. Matsunaga, H. A. Hamid, T. Akaike, *Molecules* **2016**, *21*, 1721.
[12] T. Ida, T. Sawa, H. Ihara, Y. Tsuchiya, Y. Watanabe, Y. Kumagai, M. Suematsu, H. Motohashi, S. Fujii, T. Matsunaga, M. Yamamoto, K. Ono, N. O. Devarie-Baez, M. Xian, J. M. Fukuto, T. Akaike, *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 7606-7611.
[13] Z. Z. Xie, M. M. Shi, L. Xie, Z. Y. Wu, G. Li, F. Hua, J. S. Bian, *Antioxid. Redox Signal.* **2014**, *21*, 2531-2542.
[14] S. Kawamura, T. Horii, J. Tsurugi, *J. Org. Chem.* **1971**, *36*, 3677-3680.
[15] a)Z. N. Yuan, Y. Q. Zheng, B. C. Yu, S. M. Wang, X. X. Yang, B. H. Wang, *Org Lett* **2018**, *20*, 6364-6367; b)Y. Q. Zheng, B. C. Yu, Z. Li, Z. N. Yuan, C. L. Organ, R. K. Trivedi, S. M. Wang, D. J. Lefer, B. H. Wang, *Angew. Chem. Int. Ed.* **2017**, *56*, 11749-11753; c)K. M. Dillon, R. J. Carrazzone, Y. Wang, C. R. Powell, J. B. Matson, *ACS Macro Lett.* **2020**, *9*, 606-612.
[16] A. Chaudhuri, Y. Venkatesh, J. Das, M. Gangopadhyay, T. K. Maiti, N. D. P. Singh, *J. Org. Chem.* **2019**, *84*, 11441-11449.
[17] a)P. Bora, P. Chauhan, S. Manna, H. Chakrapani, *Org. Lett.* **2018**, *20*, 7916-7920; b)C. R. Powell, K. M. Dillon, Y. Wang, R. J. Carrazzone, J. B. Matson, *Angew. Chem. Int. Ed.* **2018**, *57*, 6324-6328.
[18] S. C. Lim, Y. H. Kim, *Heteroatom Chem.* **1990**, *1*, 261-265.
[19] a)D. Ezerina, Y. Takano, K. Hanaoka, Y. Urano, T. P. Dick, *Cell Chem. Biol.* **2018**, *25*, 447-459; b)M. Zafarullah, W. Q. Li, J. Sylvester, M. Ahmad, *CMLS-Cell. Mol. Life Sci.* **2003**, *60*, 6-20; c)N. P. Murphy, K. J. Lampe, *Biotechnol. Bioeng.* **2018**, *115*, 246-256.
[20] J. B. Matson, S. I. Stupp, *Chem. Commun.* **2012**, *48*, 26-33.
[21] a)J. M. Carter, Y. Qian, J. C. Foster, J. B. Matson, *Chem. Commun.* **2015**, *51*, 13131-13134; b)A. Longchamp, K. Kaur, D. Macabrey, C. Dubuis, J. M. Corpataux, S. Deglise, J. B. Matson, F. Allagnat, *Acta Biomater.* **2019**, *97*, 374-384; c)Y. Qian, K. Kaur, J. C. Foster, J. B. Matson, *Biomacromolecules* **2019**, *20*, 1077-1086; d)Y. Wang, K. Kaur, S. J. Scannelli, R. Bitton, J. B. Matson, *J. Am. Chem. Soc.* **2018**, *140*, 14945-14951; e)Y. Wang, J. B. Matson, *ACS Appl. Bio Mater.* **2019**, *2*, 5093-5098.
[22] a)S. Fleming, R. V. Ulijn, *Chem. Soc. Rev.* **2014**, *43*, 8150-8177; b)M. Reches, E. Gazit, *Science* **2003**, *300*, 625-627.
[23] a)Y. Zhao, M. D. Pluth, *Angew. Chem. Int. Ed.* **2016**, *55*, 14638-14642; b)Y. Zhao, H. A. Henthorn, M. D. Pluth, *J. Am. Chem. Soc.* **2017**, *139*, 16365-16376; c)P. Chauhan, S. Jos, H. Chakrapani, *Org. Lett.* **2018**, *20*, 3766-3770; d)Y. M. Hu, X. Y. Li, Y. Fang, W. Shi, X. H. Li, W. Chen, M. Xian, H. M. Ma, *Chem. Sci.* **2019**, *10*, 7690-7694; e)N. Zhang, P. Hu, Y. F. Wang, Q. Tang, Q. Zheng, Z. L. Wang, Y. He, *ACS Sens.* **2020**, *5*, 319-326.
[24] B. Peng, W. Chen, C. R. Liu, E. W. Rosser, A. Pacheco, Y. Zhao, H. C. Aguilar, M. Xian, *Chem.-Eur. J.* **2014**, *20*, 1010-1016.
[25] a)Y. Hu, R. Lin, P. C. Zhang, J. Fern, A. G. Cheetham, K. Patel, R. Schulman, C. Y. Kan, H. G. Cui, *ACS Nano* **2016**, *10*, 880-888; b)E. L. Bakota, O. Sensoy, B. Ozgur, M. Sayar, J. D. Hartgerink, *Biomacromolecules* **2013**, *14*, 1370-1378.
[26] R. Reliene, R. H. Schiestl, *Carcinogenesis* **2006**, *27*, 240-244.
[27] D. J. Adams, Z. V. Boskovic, J. R. Theriault, A. J. Wang, A. M. Stern, B. K. Wagner, A. F. Shamji, S. L. Schreiber, *ACS Chem. Biol.* **2013**, *8*, 923-929.
[28] a)J. Zhan, Y. B. Cai, S. S. He, L. Wang, Z. M. Yang, *Angew. Chem. Int. Ed.* **2018**, *57*, 1813-1816; b)C. H. Liang, X. R. Yan, R. S. Zhang, T. Y. Xu, D. B. Zheng, Z. Q. Tan, Y. X. Chen, Z. F. Gao, L. Wang, X. Y. Li, Z. M. Yang, *J. Control. Release* **2020**, *317*, 109-117; c)D. Mumcuoglu, M. S. Ekiz, G. Gunay, T. Tekinay, A. B. Tekinay, M. O. Guler, *ACS Appl. Mater. Interfaces* **2016**, *8*, 11280-11287.
[29] K. M. Robinson, M. S. Janes, M. Pehar, J. S. Monette, M. F. Ross, T. M. Hagen, M. P. Murphy, J. S. Beckman, *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 15038-15043.
[30] M. Schieber, N. S. Chandel, *Curr. Biol.* **2014**, *24*, R453-R462.
[31] J. Xu, Y. Zhang, H. Yu, X. D. Gao, S. J. Shao, *Anal. Chem.* **2016**, *88*, 1455-1461.
[32] a)Y. Y. Han, Q. W. Shang, J. Yao, Y. Ji, *Cell Death Dis.* **2019**, *10*, 293; b)J. L. Wallace, *Trends Pharmacol. Sci.* **2007**, *28*, 501-505.
[33] L. Li, M. Whiteman, Y. Y. Guan, K. L. Neo, Y. Cheng, S. W. Lee, Y. Zhao, R. Baskar, C. H. Tan, P. K. Moore, *Circulation* **2008**, *117*, 2351-2360.

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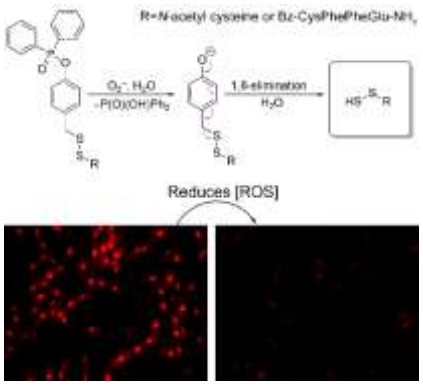

Taming superoxide! Reported here are two dual-acting compounds (**SOPD-NAC** and **SOPD-Pep**) that scavenge superoxide and then decompose to release persulfides (RSSH). Both donors deliver persulfides to cardiomyocytes, lowering intracellular reactive oxygen species levels. Additionally, **SOPD-Pep** shows anti-inflammatory activity in macrophages, greater than **SOPD-NAC** and several H_2S- releasing control compounds.