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Letter

Semi-Synthetic H_2S Releasing Compounds with Antioxidant and Vasorelaxant Properties

Valentina Citi,[▽] Antonino N. Fallica,[▽] Loredana Salerno, Nicola F. Virzì, Valeria Ciaffaglione, Sebastiano Intagliata, Sara Veneziano, Giada Benedetti, Jacopo Spezzini, Alma Martelli, Vincenzo Calderone, and Valeria Pittalà*

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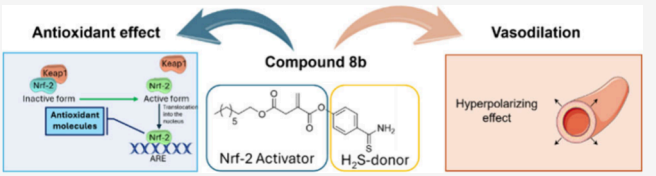
ABSTRACT: Hypertension represents a severe cardiovascular pathology linked to the increase in reactive oxygen species that impair blood vessel function. Herein, we report on the synthesis of hybrid compounds designed to release H_2S and incorporate natural or semisynthetic scaffolds capable of activating the Nrf2 pathway. The molecular hybrids enable a multitarget approach concurrently inducing vasorelaxation upon H_2S release and mitigating oxidative stress through Nrf2-dependent antioxidant responses via the upregulation of cytoprotective proteins, including HO-1. The itaconate derivative **8b** displayed an optimal H_2S release in both amperometric and cellular assays. In human aortic smooth muscle cells, compound **8b** counteracted ROS production and cytotoxicity in H_2O_2-injured cells and led to the activation of potassium channels with consequent cell hyperpolarization and vasorelaxation, which was also observed in isolated rat aortic rings. Overall, our findings indicate that simultaneous Nrf2 activation and H_2S release hold significant potential as a new therapeutic strategy for the treatment of hypertension.



KEYWORDS: *Hydrogen sulfide, oxidative stress, Nrf2, heme oxygenase-1, 4-octylitaconate, hypertension*

Hypertension is a common medical condition in which the blood force against the walls of the arteries is consistently too high. Often called the "silent killer", it may not have noticeable symptoms but can cause serious long-term damage to the heart, kidneys, brain, and other organs.[1] Hypertension is often associated with oxidative stress (OS).[2−4] The relationship between hypertension and OS is bidirectional: OS contributes to the development of hypertension and sustained high blood pressure in turn exacerbates oxidative damage.[5,6] The excessive production of ROS leads to endothelial dysfunction, impairing the production of nitric oxide (NO), a key molecule supporting blood vessels relaxation and dilatation, allowing for smooth blood flow.[7−9] Blood vessels become stiffer and narrower, increasing resistance to blood flow, which can raise blood pressure.[10−12] Developing new therapeutic strategies that target both OS and high blood pressure represents a promising strategy to reduce the risk of cardiovascular diseases. Current antihypertensive treatments effectively reduce blood pressure by modulating vascular tone and cardiac output; however, they are often characterized by side effects and do not directly address the underlying causes that contribute to long-term cardiovascular damage.

Hydrogen sulfide (H_2S) is a gasotransmitter which contributes to cardiovascular health by directly promoting vasodilation, as it activates different classes of potassium channels, for example ATP-sensitive potassium channels (K_{ATP} channels), voltage-gated potassium channels (Kv7 channels) and Ca^{2+}-activated potassium channels (KCa channels).[13−15] Also, H_2S inhibits phosphodiesterase type 5 (PDE5)[16,17] leading to an increase in cGMP levels, which further contributes to vasodilation and blood pressure regulation.[18−20] Natural and synthetic molecules able to donate this gasotransmitter have been described to contribute to lowering blood pressure, improving endothelial function, and protecting the cardiovascular system.[21−25] H_2S also interacts with the ROS-mediated OS response network and plays an important role in the maintenance of stable redox equilibrium neutralizing ROS, thereby preventing oxidative damage in endothelial cells.[26−28] However, nuclear factor erythroid derived 2 (Nrf2) is probably the 'master regulator' of the antioxidant response that should be targeted to fully counteract OS. In an activated state, Nrf2 stimulates the expression of hundreds of genes, most of them encoding for antioxidant/detoxifying enzymes, with heme-oxygenase 1 (HO-1) being one of the most important.[29−31] Thus, the pharmacologic

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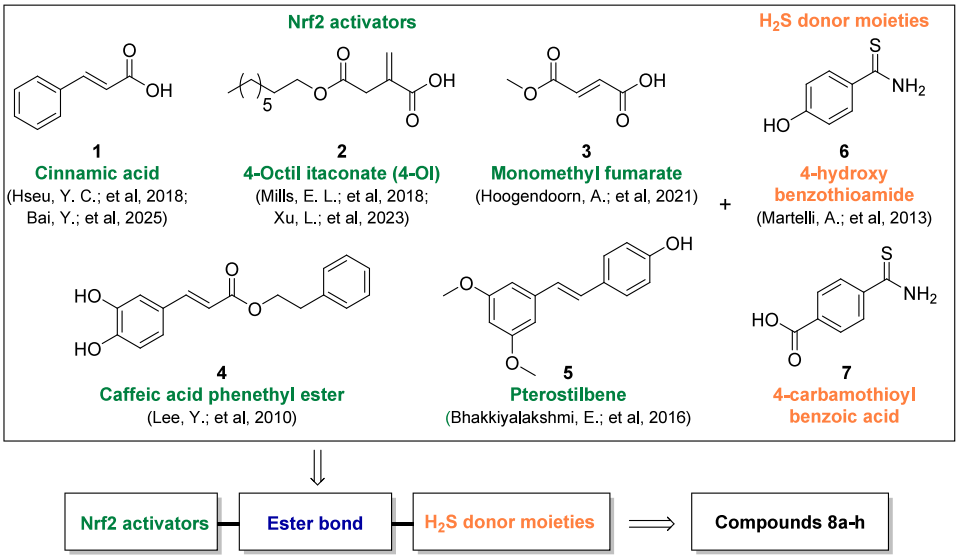
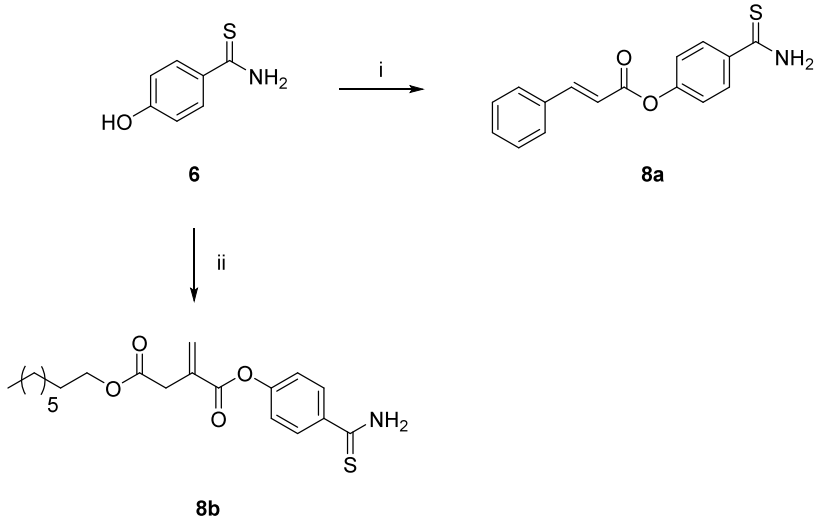


Figure 1. Chemical structures of selected Nrf2 activators, **1**−**5**, and H_2S donor moieties, **6** and **7**, used for the design of new H_2S releasing compounds, **8a**−**8h**.

Scheme 1. Reagents and Conditions: (i) Cinnamic Acid (Compound1), EDC·HCl, DMAP, Dichloromethane, 0 °C, Then Room Temperature Overnight; (ii) 4-OI (Compound2), DCC, DMAP, Dichloromethane, 0 °C, then Room Temperature, under Ar, Overnight



induction of Nrf2 activity is regarded as a good strategy for counteracting the OS.

Drug combination therapy is commonly recognized as an effective method for enhancing the clinical efficacy of medications through either additive or synergistic effects. However, the simultaneous use of multiple drugs can lead to challenges such as reduced patient adherence and an increased risk of drug−drug interactions. As a result, there has been growing interest in the development of multitarget ligands acting simultaneously on various biological targets, offering a potential solution to address the limitations associated with coadministering multiple drugs.

Accordingly, the idea underlying this first exploratory series is to merge in one single molecule a double activity, derived from an H_2S releasing moiety and Nrf2 activators (Figure 1). To this extent we linked together an arylthioamide H_2S donor moiety such as 4-hydroxybenzothioamide (BTA, compound **6**)[32] or 4-carbamothioylbenzoic acid (compound **7**), and natural or synthetic compounds known to activate the Nrf2/HO-1 pathway (compounds **1**−**5**).[32−39]

Activators of the Nrf2/HO-1 pathway were selected based on their potency and on the presence of a suitable anchoring point for the H_2S releasing moiety, such as an esterifiable carboxylic acid moiety or a free hydroxyl group prone to esterification. Selected activators share the common presence of an electrophilic Michael acceptor group, such as the α,β-unsaturated carbonyl system, which enables a Michael addition to cysteine residues of the Kelch-like ECH-associated protein 1 (Keap1), which is the regulator of Nrf2 nuclear translocation.[40,41] HO-1 expression is cascaded by Nrf2 translocation into the nucleus. Selected Nrf2 activator compounds included cinnamic acid (compound **1**), 4-octyl itaconate (4-OI, compound **2**), fumaric acid, monomethyl fumarate (compound **3**), caffeic acid phenethyl ester (CAPE, compound **4**), pterostilbene (compound **5**), and semi-synthetic derivatives previously reported by our research group.[33,40,42−46] Com-

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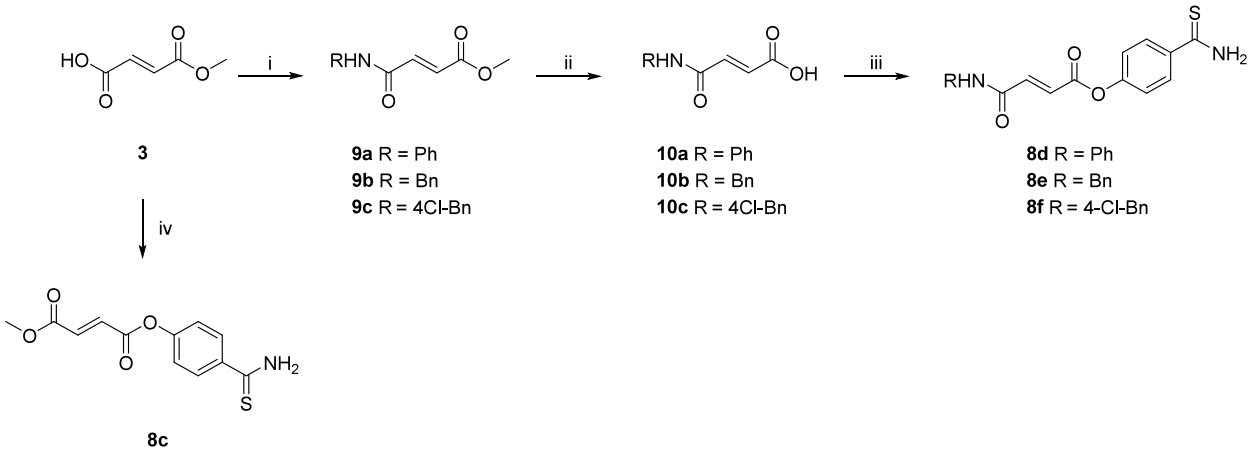
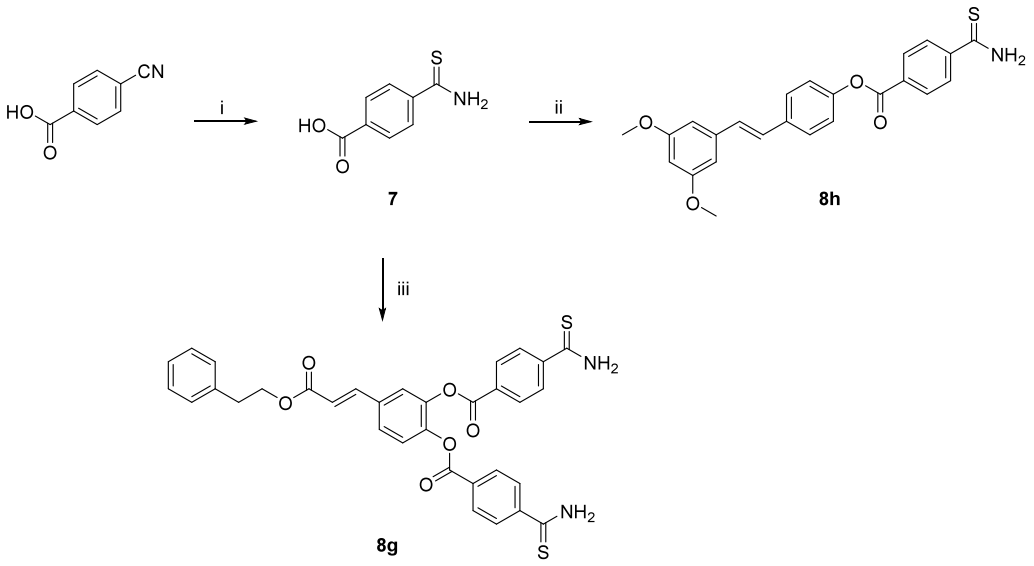
Scheme 2. Reagents and Conditions: (i) Appropriate Amine, EDC·HCl, HOBt, Dry DMF, 0 °C, then Room Temperature, under Ar, 16 h; (ii) LiOH·H_2O, H_2O/CH_3OH, Room Temperature, 2 h; (iii) 4-Hydroxybenzothioamide (Compound6), EDC·HCl, HOBt, Dry DMF, 0 °C, then Room Temperature, under Ar, 16 h; (iv) 4-Hydroxybenzothioamide (Compound6), EDC·HCl, HOBt, DMAP, Dry DMF, 0 °C, then Room Temperature, under Ar, 48 h



3 | **9a** R = Ph, **9b** R = Bn, **9c** R = 4Cl-Bn | **10a** R = Ph, **10b** R = Bn, **10c** R = 4Cl-Bn | **8d** R = Ph, **8e** R = Bn, **8f** R = 4-Cl-Bn

8c

Scheme 3. Reagents and Conditions: (i) P_2S_5, EtOH, Room Temperature, 1 h, then Reflux, 5 h; (ii) Pterostilbene, EDC·HCl, DMAP, Dry Dichloromethane, 0 °C, then Room Temperature, 16 h, under Ar; (iii) CAPE, EDC·HCl, HOBt, Dry DMF, 0 °C, then Room Temperature, 5 h, under Ar



7 | **8h**

8g

pound **6** was previously reported as a long-lasting H_2S releasing agent at low concentrations. Its H_2S production mainly relies on the presence of intracellular thiols; however, the mechanism underlying this process is unknown. Compound **6** also displayed a negligible H_2S release in aqueous environment, pointing out that hydrolytic processes are not primarily responsible for its H_2S generation.[32] Overall, compound **6** is an efficient H_2S releasing agent with a higher hydrolytic stability and sustained temporal H_2S release at controlled concentrations, avoiding toxic effects related to rapid hydrolysis and lower therapeutic efficacy.

Synthesis of cinnamic acid− and 4-OI−H_2S releasing hybrids **8a**, **8b** was achieved by direct condensation of 4-hydroxybenzothioamide with the carboxylic acid function of the proper Nrf2/HO-1 inducer moiety (Scheme 1). Compound **2** (4-OI) was synthesized as reported in the literature.[38]

Compound **8c** was synthesized by direct coupling between monomethyl fumarate **3** and 4-hydroxybenzothioamide **6** in the presence of EDC hydrochloride, HOBt, and DMAP (Scheme 2). Synthesis of fumarate derivatives **8d**−**8f** was achieved in three steps (Scheme 2). Amidation of monomethyl fumarate with an appropriate amine (aniline, benzylamine, or 4-Cl-benzylamine) afforded intermediates **9a**−**9c**. Then, the ester group of **9a**−**c** was hydrolyzed to obtain **10a**−**10c**. Finally, the re-esterification of the carboxylic acid with 4-hydroxybenzothioamide afforded the final products **8d**−**8f**.

Synthesis of CAPE− and pterostilbene−H_2S releasing hybrids is shown in Scheme 3. Thionation of 4-cyanobenzoic acid with P_2S_5 in refluxing ethanol, afforded thioamide **7** that underwent esterification reaction with CAPE (compound **4**) phenolic groups or pterostilbene and EDC hydrochloride as carboxyl activating agent (compound **5**), giving compounds **8g** and **8h**, respectively.

To investigate the overall drug-likeness of our compounds, ADMET molecular studies were conducted using SwissADME (http://swissadme.ch); results are reported in the Supporting

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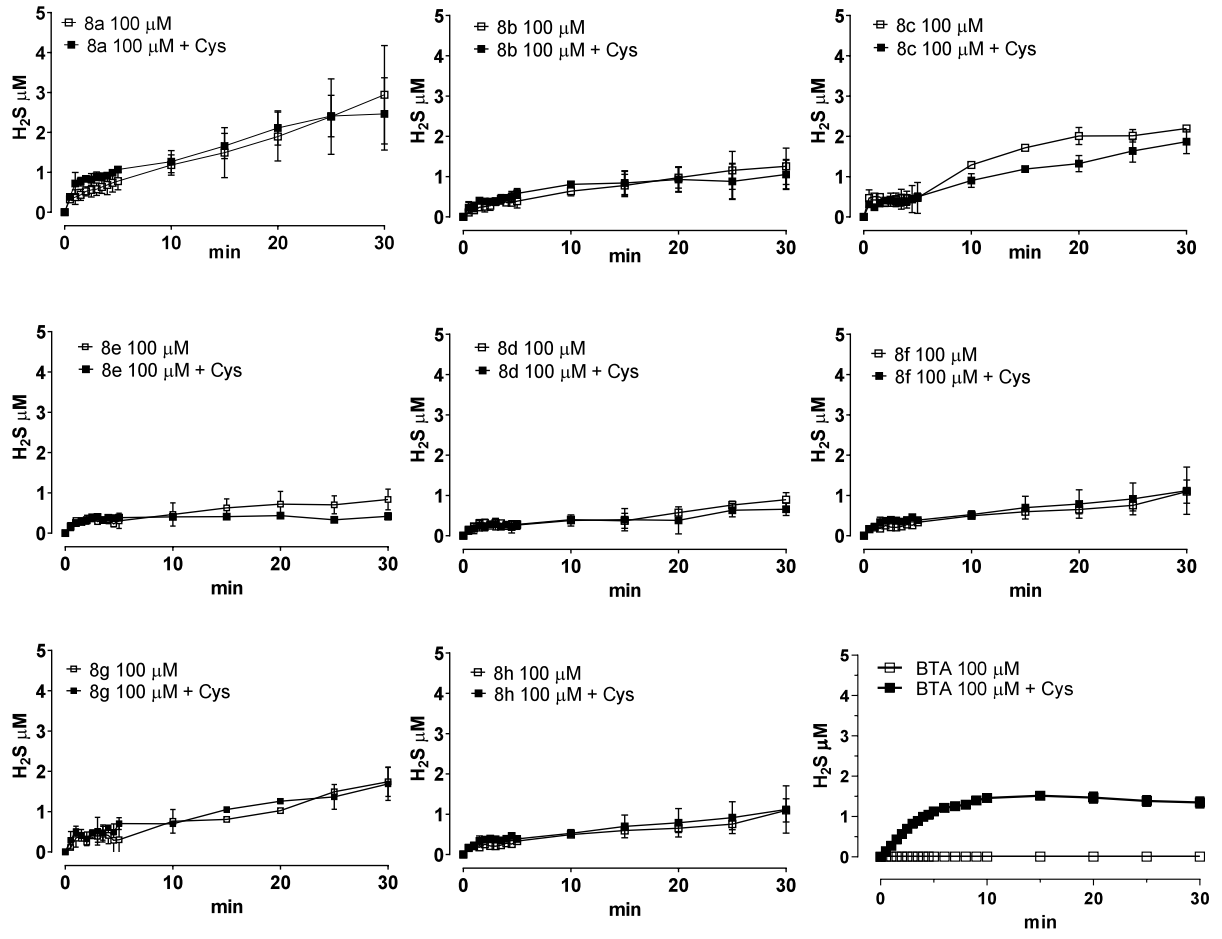

Figure 2. Hydrogen sulfide release with an amperometric approach. The graph shows the H_2S-release kinetics of the tested compounds. The curves represent the slow increase in H_2S formation with respect to time. The data are expressed as mean ± SD (n = 6).

Information (Table S1).[47] Apart from compound **8g**, compounds are predicted to have moderate (compounds **8b** and **8h**) to good water solubility and high human gastrointestinal absorption. The calculated log P values, except for **8g**, showed, for all the compounds, a good hydro/lipophilic balance in a range favorable for absorption across cell membranes but still water-soluble for absorption. All compounds are not predicted to be substrates of the P-glycoprotein nor able to cross the BBB. Most novel H_2S-donors potentially are inhibitors of cytochromes CYP1A2, CYP2C19, and CYP2C9. Interestingly, only compounds **8b**, **8f**, and **8h** are predicted as CYP3A4 inhibitors, while none of them are foreseen as CYP2D6 inhibitors. Apart from **8g**, compounds have no violation to the Lipinski Rule of 5 and also have no violation to other rules (Ghose, Egan, Veber, and Muegge), suggesting a good drug-likeness profile. Overall, all compounds, excluding **8g**, possess a good druglike profile.

We later evaluated the pharmacological profile of the novel synthesized molecules. An amperometric method, performed in the absence of biological substrates, was chosen as it provides a precise measurement of the H_2S-releasing process.[48] The assay was performed in either the absence or presence of L-cysteine (4 mM), used to mimic the endogenous presence of free thiols. All tested compounds at 100 μM exhibited appreciable H_2S production both in the absence and in the presence of L-cysteine, except for BTA which showed a thiol-dependent H_2S release. Most compounds released about 1 μM H_2S, except for **8a**, which generated approximately 4 μM H_2S (Figure 2). This difference could be attributable to the diverse structural and electronic properties of the Nrf2 activator moieties esterified with the H_2S releasing agent, which would indirectly alter the electrophilicity of the carbonyl carbon of the thioamide functional group. Moreover, bulkier or more lipophilic moieties esterified with compounds **6** or **7** would limit the release of H_2S through hydrolytic mechanisms in the aqueous buffer used in the amperometric method, because of steric effects. Considering compound **8a**, the slight electron-withdrawing effect of the conjugated cinnamic acid moiety coupled with the lack of significant steric hindrance and proper hydrophilic balance rationalize the easier nucleophilic attack to the thioamide functional group, with a consequent higher H_2S production in aqueous buffer compared to the other compounds of the series. The H_2S-releasing profiles of the compounds reflect ideal H_2S donors which should provide a sustained, gradual release of H_2S at physiological levels to ensure prolonged therapeutic effects. A slow H_2S releasing kinetic is, indeed, more favorable for therapeutic purposes because it allows to avoid eventual adverse effects due to a massive and fast release of H_2S.[42,49] Recognized slow-release H_2S donors like polysulfides derived from garlic (*Allium sativum* L.) and isothiocyanates derived from *Brassicaceae* family exemplify this desirable profile, making them H_2S-releasing compounds endowed with a plethora of beneficial effects.[50−52]

Human aortic smooth muscle cells (HASMCs) were employed for the detection of intracellular H_2S release, using

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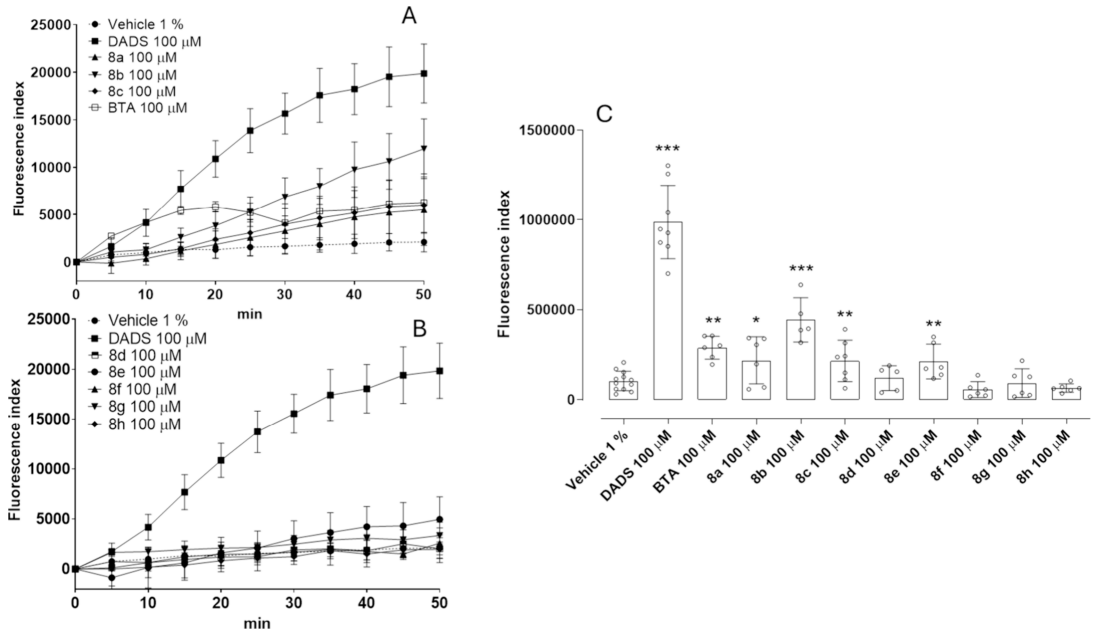

Figure 3. Intracellular H_2S donation. (A) FI over time in the presence of vehicle 1% (DMSO 1%), DADS, **8a**−**8c** and BTA 100 μM. (B) FI over time in the presence of vehicle 1% (DMSO 1%), DADS, **8d**−**8h** 100 μM. (C) Area under the curve (AUC) of the total amount of H_2S released in HASMCs over 50 min. Data are shown as mean ± SD (n = 3 independent experiments, each performed in triplicate). Statistical significance was assessed by one-way ANOVA followed by Bonferroni post test. Asterisks (*) indicates the significant difference vs vehicle: (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$.

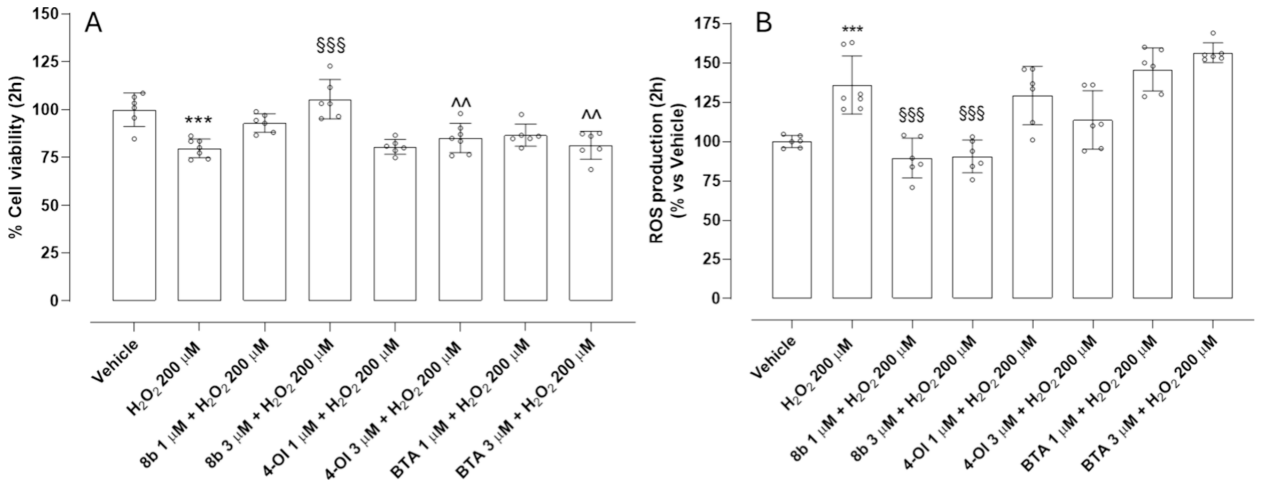

Figure 4. Protective effect of **8b**, 4-OI, and BTA against H_2O_2-induced oxidative stress in HASMCs after 2 h of treatment: (A) the vehicle-treated group is set as 100% of cell viability and (B) the vehicle-treated group is set as 100% of ROS production (data are presented as mean ± SD (n > 6)). Statistical significance was assessed by one-way ANOVA followed by Bonferroni post test. (*) indicates the statistical significance vs vehicle: (***) $p < 0.001$); (§) indicates the statistical significance vs H_2O_2 ((§§) $p < 0.01$); (∧) indicates the statistical significance vs **8b** 3 μM + H_2O_2.

the fluorometric Washington State Probe 1 (WSP-1), which specifically and irreversibly interacts with H_2S.[53,54] The experiment was conducted without adding exogenous thiols, ensuring that the observed effects reflected the ability of the compounds to intracellularly generate H_2S. Vehicle (DMSO 1%) promoted a slight increase in fluorescence index (FI), likely due to the endogenous production of H_2S. Incubation with diallyl disulfide (DADS) 100 μM, used as a reference molecule, resulted in a significant increase in FI, indicating substantial H_2S generation. The thiobenzamide moiety, as expected, significantly released H_2S (Figure 3). Among the hybrid molecules, **8a**−**8c** released H_2S more efficiently than **8d**−**8h**, which showed a negligible increase in intracellular H_2S donation (Figure 3).

The difference in intracellular donation, despite having the same moiety, could be influenced by the physicochemical properties of the molecules, which affect how they cross the cell membrane. Smaller molecules generally diffuse more readily across membranes, while hydrophilic ones may require transporters, as for **8c**. More lipophilic molecules, i.e., compounds with higher log P values, tend to passively diffuse through the lipid bilayer more easily, as we can speculate for compounds **8a** and **8b**. The consensus log P values estimated by SwissADME supported our experimental observation, suggesting that compounds **8a** and **8b** possess an optimal

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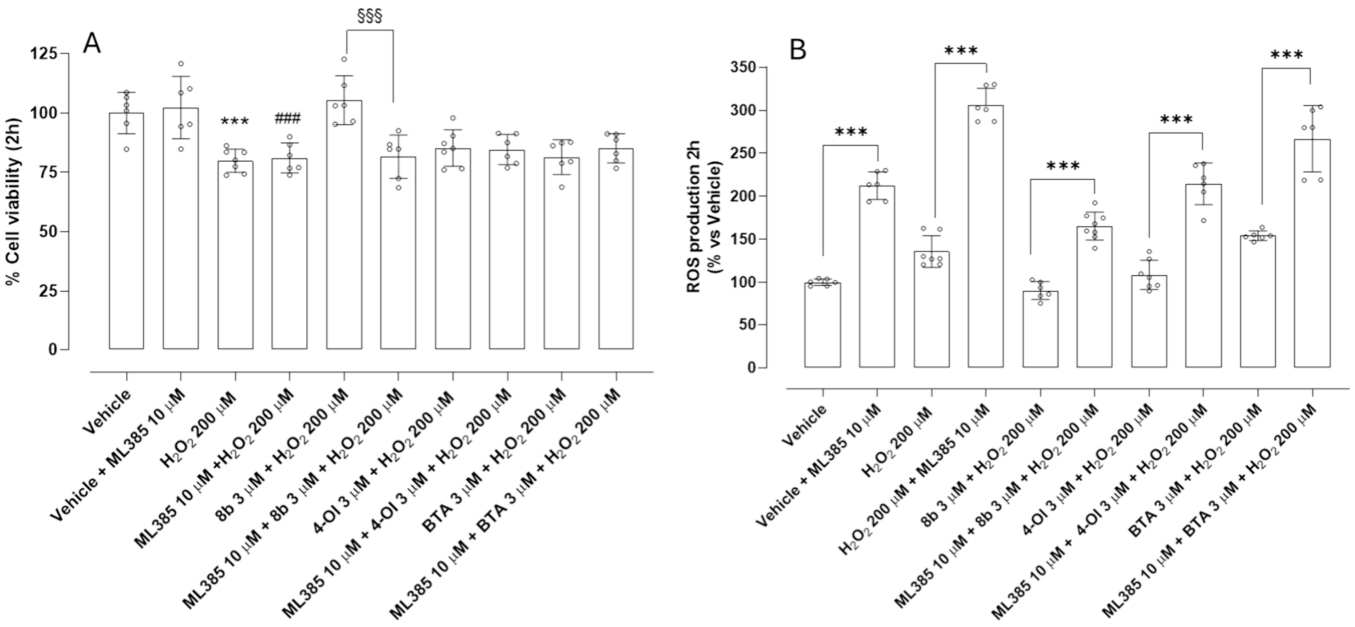

Figure 5. Role of Nrf2 in mediating the protective effect of **8b**, 4-OI and BTA against H_2O_2-induced oxidative stress in HASMCs after 2 h of treatment: (A) the vehicle-treated group is set as 100% of cell viability and (B) the vehicle-treated group is set as 100% of ROS production. Data are presented as mean ± SD ($n > 6$). Statistical significance was assessed by one-way ANOVA followed by Bonferroni post test. (*) indicates the statistical significance vs vehicle ((***) $p < 0.001$); (#) indicates the statistical significance vs vehicle + ML385 ((###) $p < 0.001$); (§) indicates the statistical significance vs **8b** 3 μM + H_2O_2 ((§§§) $p < 0.001$).

hydrophilic/lipophilic balance (log *P* equal to 3.19 and 4.24, respectively; see Table S1) that allows sustained intracellular H_2S release. In addition, the presence of active exporter and importer of 4-OI have been reported,[55] and, accordingly, we can speculate that the higher ability of compound **8b** to generate intracellular H_2S could also depend on this mechanism of cell transport.[56] On the other hand, larger molecules may not permeate cells unless actively transported, as is probably the case for compounds **8g** and **8h**. The higher log *P* values obtained for CAPE derivative **8g** and pterostilbene derivative **8h** (5.37 and 4.60, respectively, Table S1) suggest a reduced intracellular uptake due to higher lipophilicity and a consequent lower H_2S intracellular release. Hence, given its ability to generate intracellular H_2S, compound **8b** was selected for further pharmacological investigations using HASMCs.

This derivative of 4-OI, was evaluated to assess the benefits of incorporating a H_2S-donating moiety in comparison to 4-OI and BTA alone. This modification aims to enhance the protective effects of 4-OI by combining the Nrf2-activating properties of the itaconate derivative with the vasoprotective and vasorelaxant effects of H_2S. 4-OI already activates Nrf2, leading to the expression of antioxidant genes such as HO-1, NQO1, and GCLC, which counteract oxidative damage.[57] H_2S donation from **8b** may further enhance antioxidant defenses by directly scavenging ROS. Exposure to 200 μM H_2O_2 provoked a marked reduction in cell viability and a concomitant massive increase in intracellular ROS production (Figures 4A and 4B). Pretreatment with **8b** at 1 μM elicited only a modest and statistically insignificant improvement in viability. However, increasing the concentration to 3 μM resulted in a statistically significant recovery of cell viability. In parallel, both concentrations of **8b** significantly reduced ROS accumulation, suggesting that H_2S release plays a direct role in mitigating oxidative damage, likely through ROS scavenging. In contrast, treatment with the compounds 4-OI and the H_2S-releasing moiety BTA, each tested at 1 and 3 μM, produced only a slight, insignificant increase in cell viability, and neither compound was effective in reducing intracellular ROS levels. These findings suggest that, while both agents possess intrinsic biological activity, they cannot protect in short-term incubation. Importantly, BTA alone failed to reduce ROS or improve viability. Similarly, the inability of 4-OI alone to suppress ROS suggests that it is not able to rapidly activate Nrf2. **8b** emerges as the most effective in restoring cell viability and reducing ROS level, exerting significant cell protection, compared with 4-OI and BTA. The significant differences observed in the protective effects of **8b**, 4-OI, and BTA on cell viability and ROS production can likely be attributed to the combination of a H_2S-donating moiety BTA with 4-OI, in **8b**. A critical feature of **8b** is its lipophilicity conferred by the octyl chain, which facilitates the rapid diffusion across the cell membrane. This property likely underlies the intracellular accumulation of the compound and the acute onset of its protective effects.

To explore the role of Nrf2 in modulating acute oxidative stress responses, cell viability and ROS production were measured following a 2-h exposure to H_2O_2 200 μM, with or without pretreatment with the Nrf2 inhibitor ML385 10 μM. Interestingly, ML385 did not affect viability, and the coadministration with H_2O_2 did not significantly exacerbate H_2O_2-induced cytotoxicity. However, pretreatment with ML385 significantly enhanced ROS accumulation when incubated with vehicle and a further increase was recorded when incubated with H_2O_2, supporting the importance of Nrf2 in controlling oxidative stress, even in the early phases of exposure (see Figures 5A and 5B). This indicates that, despite the enhanced ROS accumulation upon Nrf2 inhibition, the extent and duration of oxidative stress were not sufficient to induce further cytotoxic effects. These findings underscore that ROS elevation is an early and sensitive indicator of oxidative

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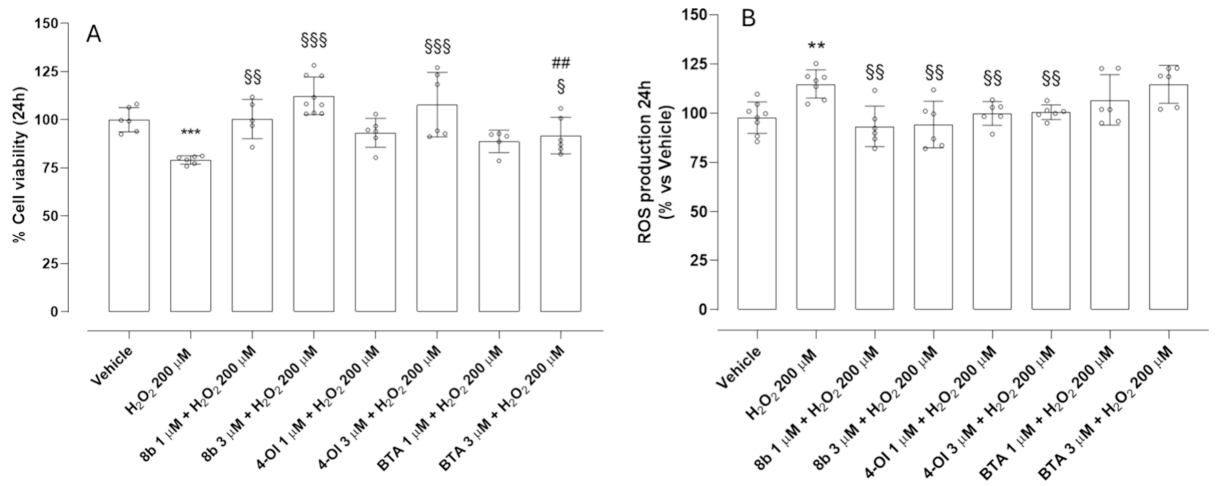

Figure 6. Protective effect of **8b**, 4-OI, and BTA against H_2O_2-induced oxidative stress in HASMCs after 24 h of treatment: (A) the vehicle-treated group is set as 100% of cell viability and (B) the vehicle-treated group is set as 100% of ROS production. Data are presented as mean ± SD ($n > 6$). Statistical significance was assessed by one-way ANOVA, followed by Bonferroni post test. (*) indicates the statistical significance vs vehicle ((***) $p < 0.001$); (§) indicates the statistical significance vs H_2O_2 ((§§) $p < 0.01$; (§§§) $p < 0.001$); (#) indicates the statistical significance vs **8b** 3 μM + H_2O_2 ((##) $p < 0.01$).

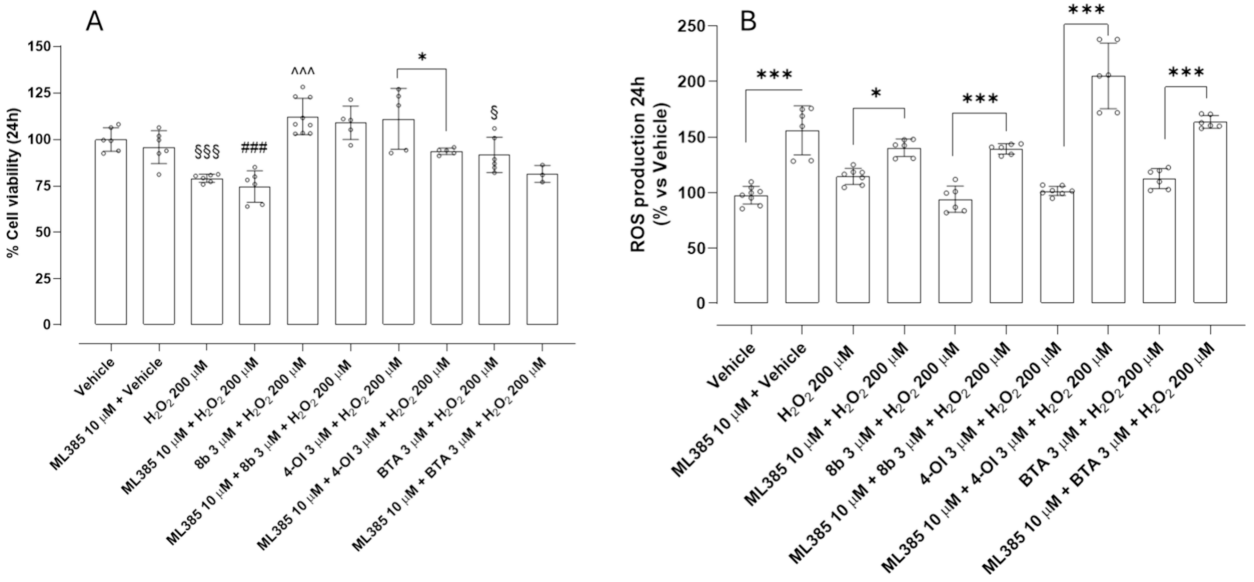

Figure 7. Role of Nrf2 in mediating the protective effect of **8b**, 4-OI, and BTA against H_2O_2-induced oxidative stress in HASMCs after 24 h of treatment: (A) the vehicle-treated group is set as 100% of cell viability and (B) the vehicle-treated group is set as 100% of ROS production. Data are presented as mean ± SD ($n > 6$). Statistical significance was assessed by one-way ANOVA followed by Bonferroni post test. (§) indicates the statistical significance vs vehicle ((§§§) $p < 0.001$); (#) indicates the statistical significance vs vehicle + ML385 ((###) $p < 0.001$); (∧) indicates the statistical significance vs H_2O_2 ((§) $p < 0.05$; (§§§) $p < 0.001$); (*) indicates the statistical significance as reported over the bars ((*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$).

imbalance, while a measurable impact on cell viability likely requires a longer oxidative exposure. Importantly, the protective effect of **8b** was abolished by cotreatment with ML385, indicating that its ROS-lowering activity is largely dependent on Nrf2 activation. Generally, preincubation with ML385 significantly increased the level of ROS production in each treatment, highlighting the central role of Nrf2 in controlling ROS production even after short-term exposure to H_2O_2.

The protective effects of **8b**, 4-OI, and BTA were further assessed following 24 h incubation to evaluate their efficacy under prolonged oxidative stress. The antioxidant and cytoprotective properties observed at early time points were largely preserved after extended exposure, particularly for **8b**, which exhibited significant protection at both 1 and 3 μM. The significant reduction in intracellular ROS levels and preservation of cell viability suggest that **8b** mediates both acute and prolonged protective responses, likely through a combination of H_2S-dependent redox modulation and Nrf2 activation. Similarly, 4-OI significantly reduced ROS at both concentrations and improved cell viability at 3 μM, reflecting a protecting effect upon incubation for a longer period (see Figures 6A and 6B). BTA, which had shown minimal efficacy in the short-term assays, exerted a significant cytoprotective effect at 3 μM after 24 h, though it remained ineffective in reducing ROS at either concentration. The delayed onset of

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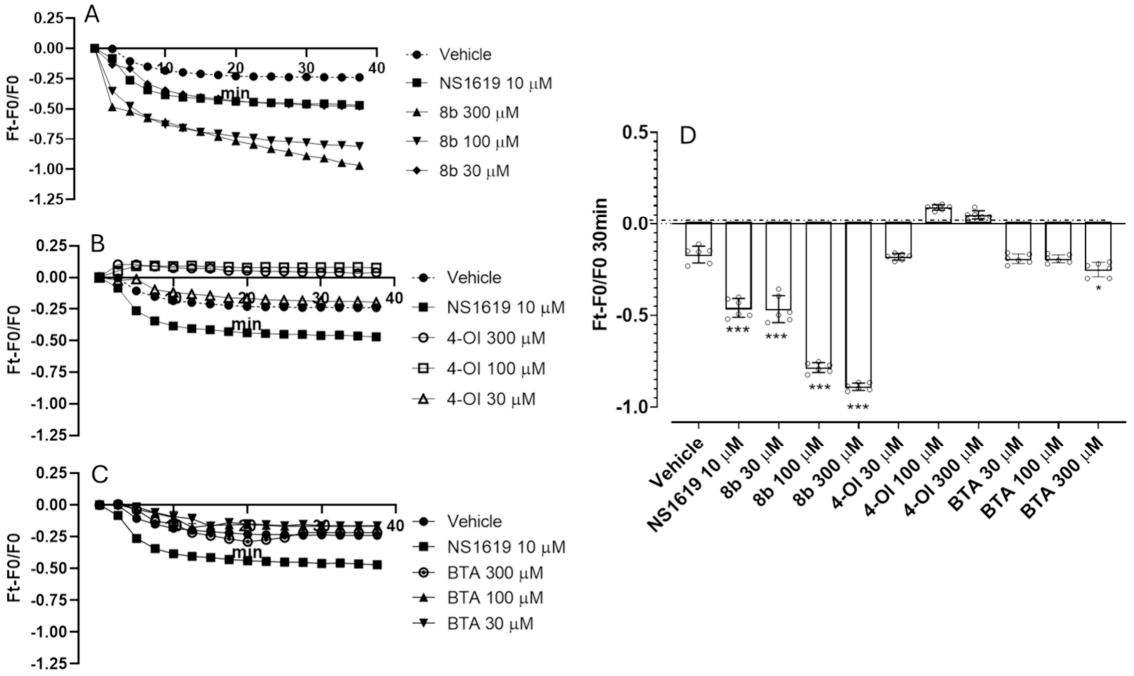

Figure 8. Hyperpolarizing effect of compound **8b**, 4-OI and BTA on HASMCs: (A) hyperpolarization curves over time of vehicle (DMSO 1%), NS1619 10 μM, **8b** 30 μM, 100 μM and 300 μM, (B) 4-OI 30 μM, 100 μM and 300 μM, and (C) BTA 30 μM, 100 μM and 300 μM. (D) Hyperpolarization recorded after 30 min. Data are shown as mean ± SD, (n = 3 independent experiments, each performed in triplicate). Statistical significance was assessed by one-way ANOVA followed by Bonferroni post test. (*): statistical significance vs vehicle ((***) $p < 0.001$).

protection observed with BTA may reflect the time-dependent nature of intracellular H_2S release and downstream signaling, which becomes evident only under conditions of prolonged exposure.

To further elucidate the role of the Nrf2 pathway in mediating the antioxidant and cytoprotective effects of **8b**, 4-OI, and BTA, cell viability and intracellular ROS levels were assessed following 24-h exposure to oxidative stress (200 μM H_2O_2), in the presence or absence of the Nrf2 inhibitor ML385. As shown in the viability data (Figure 7A), pretreatment with ML385 completely abolished the protective effect of 4-OI 3 μM, strongly supporting that its cytoprotective effect is mainly mediated via Nrf2 activation. In contrast, compound **8b** maintained a significant protective effect, even in the presence of ML385. Although a partial reduction in efficacy was observed—suggesting that its activity is partially Nrf2-dependent—the persistence of protection highlights the contribution of additional, Nrf2-independent mechanisms. This duality may be due to the bifunctional design of **8b**: while the 4-OI scaffold confers Nrf2-activating capacity, the incorporation of a thiobenzamide−H_2S-releasing moiety adds a second mechanism of cell protection. H_2S is known to directly scavenge ROS, preserve mitochondrial integrity, and activate prosurvival pathways, including those involving Akt and Nrf2-independent antioxidant enzymes. The effect of BTA 3 μM in cell protection was not affected by Nrf2 inhibition, indicating that its activity is independent of this pathway. However, BTA did not significantly reduce intracellular ROS levels after 24 h. The ROS quantification data (Figure 7B) further reinforced these conclusions. The effect of 4-OI was entirely reversed by the use of ML385. In contrast, **8b** preserved a slight reduction in ROS even with Nrf2 inhibition, suggesting a direct chemical scavenging mechanism via H_2S release.

The vasorelaxant activity of H_2S in HASMCs is mediated by multiple mechanisms, including the ability to activate various classes of potassium (K^+) channels. Activation of these channels leads to membrane hyperpolarization, a process that decreases cell excitability and induces vasodilation. Several studies have demonstrated that H_2S can directly modulate BKCa, K_{ATP}, and Kv7 channels, contributing to smooth muscle relaxation.[13−15] In hypertension, excessive and uncontrolled contraction of the vascular smooth muscle is a key pathophysiological feature. Given the potential therapeutic relevance of membrane hyperpolarization, the effects of **8b**, 4-OI, and BTA on the membrane potential of cultured HASMCs were evaluated (Figure 8). NS1619, a well-established BKCa potassium channel activator, was used as a reference hyperpolarizing agent.[58] Compound **8b** exhibited a potent and concentration-dependent hyperpolarizing effect: **8b** (30 μM) induced a hyperpolarization comparable to the reference drug NS1619. **8b** (100 μM) further increased the hyperpolarizing effect significantly surpassing NS1619. Notably, at 300 μM, **8b** induced an even greater hyperpolarization. The observed hyperpolarization suggests that **8b** may act through the activation of potassium channels, leading to a reduction in the cellular excitability and promoting vasodilation. In contrast, 4-OI did not promote membrane hyperpolarization, even at the highest tested concentration of 300 μM. BTA promoted a significant hyperpolarizing effect only when incubated at 300 μM. The observed superiority of **8b** compared with 4-OI and BTA in promoting membrane hyperpolarization may rely on its hybrid structure, in which the thiobenzamide moiety is conjugated to 4-OI. The choice of using HASMCs was based on the known mechanism of action of H_2S-donors, which exert vasorelaxant effects primarily by activating potassium channels expressed on vascular smooth muscle cells. HASMCs, therefore, provide a mechanistically relevant in vitro model to study the direct cellular effects of H_2S-releasing compounds.

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The vasorelaxant effects of **8b**, 4-OI, and BTA were evaluated in isolated rat aortic rings in the absence of endothelium (A) (Figure 9A). When the endothelium was

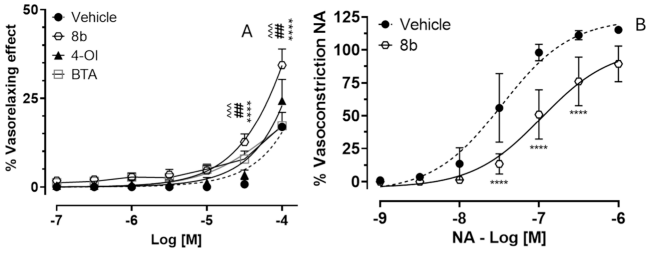

Figure 9. (A) Dose−response curve showing the vasorelaxant effect (%) induced by **8b**, 4-OI and BTA compared to the vehicle in endothelium-denuded isolated rat aortic rings. (B) The inhibitory effect of **8b** in noradrenaline-induced vasoconstriction. (*) indicates significance vs vehicle ((****) $p < 0.0001$). Symbols (#) indicates significance vs 4-OI ((##) $p < 0.01$); (∧) indicates significance vs BTA. Data are expressed as mean ± SD ($n = 6$).

removed, at lower concentrations (ranging between 10^{-9} and 10^{-7} M), **8b**, 4-OI, and BTA exhibited minimal vasorelaxation (data not shown). However, at higher concentrations (10^{-5} and 10^{-4} M), **8b**, 4-OI, and BTA promoted significant vasorelaxation compared with vehicle. Furthermore, **8b** evoked a more pronounced vasorelaxant effect, in comparison with 4-OI and BTA. This is likely due to the additional contribution of H_2S-mediated mechanisms. While both compounds may share a common pharmacophore responsible for their base vasodilatory activity, the enhanced effect of **8b** suggests that its H_2S-releasing moiety provides an additive or synergistic contribution to vascular relaxation. Indeed, H_2S activates K_{ATP} and Kv7 channels in vascular smooth muscle cells, leading to hyperpolarization and reducing smooth muscle contraction and promoting vessel relaxation.[13−15] To date, this is the first time that the vasorelaxant properties of 4-OI are described. Due to the superiority in vasodilatory effect, **8b** was also evaluated for its ability to inhibit the noradrenaline-induced vasoconstriction in denuded rat aortic rings (Figure 9B). As expected, the vehicle group exhibits the strongest vasoconstrictive response, following a sigmoidal dose−response curve with a steep increase in constriction as the noradrenaline concentration rises. In contrast, **8b** significantly attenuated noradrenaline-induced vasoconstriction, as shown by the lower response curve compared to the vehicle.

The findings of this study underscore the pivotal role of H_2S donation in enhancing the cytoprotective properties of **8b**, reinforcing the concept that incorporating H_2S-releasing moieties into drug design represents a valuable strategy for developing novel antioxidant and cytoprotective agents. The ability of **8b** to release H_2S in a slow and controlled manner offers a significant therapeutic advantage, particularly in conditions where oxidative stress plays a central role such as hypertension. Moreover, the observed hyperpolarization suggests a mechanism of action involving the activation of potassium channels, which leads to a reduction in cellular excitability and promotes vasodilation. This effect is particularly relevant in the context of vascular health, in regulating vascular tone and reducing blood pressure. The potential of **8b** to modulate these pathways highlights its broader pharmacological relevance, not only as an antioxidant but also as a regulator of vascular function.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.5c00624.

Experimental procedures, ^{1}H NMR and ^{13}C NMR spectra of final compounds **8a−8h**; in silico ADMET assessment of final compounds **8a−8h** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Valeria Pittalà − *Department of Drug and Health Sciences, University of Catania, 95125 Catania, Italy;* orcid.org/0000-0003-1856-0308; Email: valeria.pittala@unict.it

Authors

Valentina Citi − *Department of Pharmacy, University of Pisa, 56126 Pisa, Italy; Interdepartmental Research Center, "Biology and Pathology of Ageing", University of Pisa, 56126 Pisa, Italy*

Antonino N. Fallica − *Department of Drug and Health Sciences, University of Catania, 95125 Catania, Italy;* Present Address: For Antonino N. Fallica: Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, 08028, Barcelona, Spain; and Barcelona Institute for Global Health (ISGlobal), Hospital Clínic−Universitat de Barcelona, 08036 Barcelona, Spain; orcid.org/0000-0003-1899-8414

Loredana Salerno − *Department of Drug and Health Sciences, University of Catania, 95125 Catania, Italy;* orcid.org/0000-0001-6458-3717

Nicola F. Virzì − *Department of Drug and Health Sciences, University of Catania, 95125 Catania, Italy*

Valeria Ciaffaglione − *Department of Drug and Health Sciences, University of Catania, 95125 Catania, Italy;* Present Address: For Valeria Ciaffaglione: Institute of Crystallography, National Council of Research (CNR), P. Gaifami 18, 95126 Catania, Italy

Sebastiano Intagliata − *Department of Drug and Health Sciences, University of Catania, 95125 Catania, Italy;* orcid.org/0000-0002-0201-1745

Sara Veneziano − *Department of Pharmacy, University of Pisa, 56126 Pisa, Italy*

Giada Benedetti − *Department of Pharmacy, University of Pisa, 56126 Pisa, Italy*

Jacopo Spezzini − *Department of Pharmacy, University of Pisa, 56126 Pisa, Italy*

Alma Martelli − *Department of Pharmacy, University of Pisa, 56126 Pisa, Italy; Interdepartmental Research Center, "Biology and Pathology of Ageing", University of Pisa, 56126 Pisa, Italy;* orcid.org/0000-0002-2090-7107

Vincenzo Calderone − *Department of Pharmacy, University of Pisa, 56126 Pisa, Italy; Interdepartmental Research Center, "Biology and Pathology of Ageing", University of Pisa, 56126 Pisa, Italy*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.5c00624

Author Contributions

[▽]Valentina Citi and A.N.F. contributed equally to this work and should be considered cofirst authors. Conceptualization: V. Citi, V. Calderone, A.M., and V.P.; Data curation: V.

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Notes

No unexpected or unusually high safety hazards were encountered.

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■ ABBREVIATIONS

4-OI, 4-octyl itaconate; ADMET, absorption, distribution, metabolism, excretion, and toxicity; AUC, area under the curve; CAPE, caffeic acid phenethyl ester; DADS, diallyl disulfide; DCC, N,N′-Dicyclohexylcarbodiimide; DCM, dichloromethane; DHE, dihydroethidium; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; EDC·HCl, 1-Ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride; EtOAc, ethyl acetate; EtOH, ethanol; FI, fluorescence index; GCLC, Glutamate−cysteine ligase catalytic subunit; HASMCs, human aortic smooth muscle cells; HO-1, heme oxygenase-1; HOBt, hydroxybenzotriazole, K_{ATP}, ATP-sensitive potassium channels; KCa, Ca^{2+}-activated potassium channels; Keap1, Kelch-like ECH-associated protein 1; Kv7, voltage-gated potassium channels; NQO1, NAD(P)H:quinone oxidoreductase 1; Nrf2, Nuclear factor erythroid 2-related factor 2; OS, oxidative stress; PDE5, phosphodiesterase type 5; ROS, reactive oxygen species; TLC, thin-layer chromatography; WSP-1, Washington State Probe 1

■ REFERENCES

(1) Kalehoff, J. P.; Oparil, S. The Story of the Silent Killer: A History of Hypertension: Its Discovery, Diagnosis, Treatment, and Debates. *Curr. Hypertens Rep* **2020**, *22* (9), 72.

(2) Petrie, J. R.; Guzik, T. J.; Touyz, R. M. Diabetes, Hypertension, and Cardiovascular Disease: Clinical Insights and Vascular Mechanisms. *Can. J. Cardiol* **2018**, *34* (5), 575−584.

(3) Bourgonje, A. R.; Bourgonje, M. F.; Post, A.; la Bastide-van Gemert, S.; Kieneker, L. M.; Bulthuis, M. L. C.; Gordijn, S. J.; Gansevoort, R. T.; Bakker, S. J. L.; Mulder, D. J.; et al. Systemic oxidative stress associates with new-onset hypertension in the general population. *Free Radic Biol. Med.* **2022**, *187*, 123−131.

(4) Camargo, L. L.; Wang, Y.; Rios, F. J.; McBride, M.; Montezano, A. C.; Touyz, R. M. Oxidative Stress and Endoplasmic Reticular Stress Interplay in the Vasculopathy of Hypertension. *Can. J. Cardiol* **2023**, *39* (12), 1874−1887.

(5) Franco, C.; Sciatti, E.; Favero, G.; Bonomini, F.; Vizzardi, E.; Rezzani, R. Essential Hypertension and Oxidative Stress: Novel Future Perspectives. *Int. J. Mol. Sci.* **2022**, *23* (22), 14489.

(6) Zhang, Z.; Zhao, L.; Zhou, X.; Meng, X.; Zhou, X. Role of inflammation, immunity, and oxidative stress in hypertension: New insights and potential therapeutic targets. *Front. Immunol.* **2023**, *13*, No. 1098725.

(7) Incalza, M. A.; D'Oria, R.; Natalicchio, A.; Perrini, S.; Laviola, L.; Giorgino, F. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vascul Pharmacol* **2018**, *100*, 1−19.

(8) Jia, G.; Aroor, A. R.; Jia, C.; Sowers, J. R. Endothelial cell senescence in aging-related vascular dysfunction. *Biochim Biophys Acta Mol. Basis Dis* **2019**, *1865* (7), 1802−1809.

(9) Shaito, A.; Aramouni, K.; Assaf, R.; Parenti, A.; Orekhov, A.; Yazbi, A. E.; Pintus, G.; Eid, A. H. Oxidative Stress-Induced Endothelial Dysfunction in Cardiovascular Diseases. *Front. Biosci. (Landmark Ed.)* **2022**, *27* (3), 105.

(10) Simko, F.; Pechanova, O. Remodelling of the heart and vessels in experimental hypertension: advances in protection. *J. Hypertens.* **2010**, *28* (Suppl 1), S1−S6.

(11) Sunano, S.; Sekiguchi, F. Endothelium-derived factors in hypertensive blood vessels, especially nitric oxide and hypertension]. *Yakugaku Zasshi* **2003**, *123* (7), 495−515.

(12) Yang, S.; Gong, X.; Qi, Y.; Jiang, Z. Comparative study of variations in mechanical stress and strain of human blood vessels: mechanical reference for vascular cell mechano-biology. *Biomech Model Mechanobiol* **2020**, *19* (2), 519−531.

(13) Jiang, B.; Tang, G.; Cao, K.; Wu, L.; Wang, R. Molecular mechanism for H(2)S-induced activation of K(ATP) channels. *Antioxid Redox Signal* **2010**, *12* (10), 1167−1178.

(14) Liang, G. H.; Xi, Q.; Leffler, C. W.; Jaggar, J. H. Hydrogen sulfide activates Ca(2)(+) sparks to induce cerebral arteriole dilatation. *J. Physiol* **2012**, *590* (11), 2709−2720.

(15) Martelli, A.; Testai, L.; Breschi, M. C.; Lawson, K.; McKay, N. G.; Miceli, F.; Taglialatela, M.; Calderone, V. Vasorelaxation by hydrogen sulphide involves activation of Kv7 potassium channels. *Pharmacol. Res.* **2013**, *70* (1), 27−34.

(16) Smimmo, M.; Casale, V.; Casillo, G. M.; Mitidieri, E.; d'Emmanuele di Villa Bianca, R.; Bello, I.; Schettino, A.; Montanaro, R.; Brancaleone, V.; Indolfi, C.; et al. Hydrogen sulfide dysfunction in metabolic syndrome-associated vascular complications involves cGMP regulation through soluble guanylyl cyclase persulfidation. *Biomed Pharmacother* **2024**, *174*, No. 116466.

(17) Szabo, C. Hydrogen sulfide, an enhancer of vascular nitric oxide signaling: mechanisms and implications. *Am. J. Physiol Cell Physiol* **2017**, *312* (1), C3−C15.

(18) Coletta, C.; Papapetropoulos, A.; Erdelyi, K.; Olah, G.; Modis, K.; Panopoulos, P.; Asimakopoulou, A.; Gero, D.; Sharina, I.; Martin, E.; et al. Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (23), 9161−9166.

(19) La Fuente, J. M.; Fernandez, A.; Pepe-Cardoso, A. J.; Martinez-Salamanca, J. I.; Louro, N.; Angulo, J. L-cysteine/hydrogen sulfide pathway induces cGMP-dependent relaxation of corpus cavernosum and penile arteries from patients with erectile dysfunction and improves arterial vasodilation induced by PDE5 inhibition. *Eur. J. Pharmacol.* **2019**, *863*, No. 172675.

(20) Testai, L.; D'Antongiovanni, V.; Piano, I.; Martelli, A.; Citi, V.; Duranti, E.; Virdis, A.; Blandizzi, C.; Gargini, C.; Breschi, M. C.; et al. Different patterns of H2S/NO activity and cross-talk in the control of the coronary vascular bed under normotensive or hypertensive conditions. *Nitric Oxide* **2015**, *47*, 25−33.

(21) Barresi, E.; Nesi, G.; Citi, V.; Piragine, E.; Piano, I.; Taliani, S.; Da Settimo, F.; Rapposelli, S.; Testai, L.; Breschi, M. C.; et al.

Iminothioethers as Hydrogen Sulfide Donors: From the Gasotransmitter Release to the Vascular Effects. *J. Med. Chem.* **2017**, *60* (17), 7512−7523.
(22) Citi, V.; Martelli, A.; Bucci, M.; Piragine, E.; Testai, L.; Vellecco, V.; Cirino, G.; Calderone, V. Searching for novel hydrogen sulfide donors: The vascular effects of two thiourea derivatives. *Pharmacol. Res.* **2020**, *159*, No. 105039.
(23) Martelli, A.; Piragine, E.; Citi, V.; Testai, L.; Pagnotta, E.; Ugolini, L.; Lazzeri, L.; Di Cesare Mannelli, L.; Manzo, O. L.; Bucci, M.; et al. Erucin exhibits vasorelaxing effects and antihypertensive activity by H(2) S-releasing properties. *Br. J. Pharmacol.* **2020**, *177* (4), 824−835.
(24) Martelli, A.; Testai, L.; Citi, V.; Marino, A.; Bellagambi, F. G.; Ghimenti, S.; Breschi, M. C.; Calderone, V. Pharmacological characterization of the vascular effects of aryl isothiocyanates: is hydrogen sulfide the real player? *Vascul Pharmacol* **2014**, *60* (1), 32−41.
(25) Mitidieri, E.; Tramontano, T.; Gurgone, D.; Citi, V.; Calderone, V.; Brancaleone, V.; Katsouda, A.; Nagahara, N.; Papapetropoulos, A.; Cirino, G.; et al. Mercaptopyruvate acts as endogenous vasodilator independently of 3-mercaptopyruvate sulfurtransferase activity. *Nitric Oxide* **2018**, *75*, 53−59.
(26) Ali, F. F.; Mohammed, H. H.; Elroby Ali, D. M. Protective effect of hydrogen sulfide against stress-induced lung injury: involvement of Nrf2, NFκB/iNOS, and HIF-1α signaling pathways. *Cell Stress Chaperones* **2022**, *27* (1), 55−70.
(27) Citi, V.; Martelli, A.; Gorica, E.; Brogi, S.; Testai, L.; Calderone, V. Role of hydrogen sulfide in endothelial dysfunction: Pathophysiology and therapeutic approaches. *J. Adv. Res.* **2021**, *27*, 99−113.
(28) Shen, Y.; Shen, Z.; Luo, S.; Guo, W.; Zhu, Y. Z. The Cardioprotective Effects of Hydrogen Sulfide in Heart Diseases: From Molecular Mechanisms to Therapeutic Potential. *Oxid. Med. Cell. Longev.* **2015**, *2015*, No. 925167.
(29) Hybertson, B. M.; Gao, B.; Bose, S. K.; McCord, J. M. Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. *Mol. Aspects Med.* **2011**, *32* (4−6), 234−246.
(30) Vomund, S.; Schafer, A.; Parnham, M. J.; Brune, B.; von Knethen, A. Nrf2, the Master Regulator of Anti-Oxidative Responses. *Int. J. Mol. Sci.* **2017**, *18* (12), 2772.
(31) Zhang, H.; Davies, K. J. A.; Forman, H. J. Oxidative stress response and Nrf2 signaling in aging. *Free Radical Biol. Med.* **2015**, *88* (Pt B), 314−336.
(32) Martelli, A.; Testai, L.; Citi, V.; Marino, A.; Pugliesi, I.; Barresi, E.; Nesi, G.; Rapposelli, S.; Taliani, S.; Da Settimo, F.; et al. Arylthioamides as H2S Donors: l-Cysteine-Activated Releasing Properties and Vascular Effects in Vitro and in Vivo. *ACS Med. Chem. Lett.* **2013**, *4* (10), 904−908.
(33) Bai, Y.; Tan, D.; Deng, Q.; Miao, L.; Wang, Y.; Zhou, Y.; Yang, Y.; Wang, S.; Vong, C. T.; Cheang, W. S. Cinnamic acid alleviates endothelial dysfunction and oxidative stress by targeting PPARδ in obesity and diabetes. *Chin. Med.* **2025**, *20* (1), 13.
(34) Bhakkiyalakshmi, E.; Dineshkumar, K.; Karthik, S.; Sireesh, D.; Hopper, W.; Paulmurugan, R.; Ramkumar, K. M. Pterostilbene-mediated Nrf2 activation: Mechanistic insights on Keap1:Nrf2 interface. *Bioorg. Med. Chem.* **2016**, *24* (16), 3378−3386.
(35) Hoogendoorn, A.; Avery, T. D.; Li, J.; Bursill, C.; Abell, A.; Grace, P. M. Emerging Therapeutic Applications for Fumarates. *Trends Pharmacol. Sci.* **2021**, *42* (4), 239−254.
(36) Hseu, Y. C.; Korivi, M.; Lin, F. Y.; Li, M. L.; Lin, R. W.; Wu, J. J.; Yang, H. L. Trans-cinnamic acid attenuates UVA-induced photoaging through inhibition of AP-1 activation and induction of Nrf2-mediated antioxidant genes in human skin fibroblasts. *J. Dermatol Sci.* **2018**, *90* (2), 123−134.
(37) Lee, Y.; Shin, D. H.; Kim, J. H.; Hong, S.; Choi, D.; Kim, Y. J.; Kwak, M. K.; Jung, Y. Caffeic acid phenethyl ester-mediated Nrf2 activation and IkappaB kinase inhibition are involved in NFkappaB inhibitory effect: structural analysis for NFkappaB inhibition. *Eur. J. Pharmacol.* **2010**, *643* (1), 21−28.
(38) Mills, E. L.; Ryan, D. G.; Prag, H. A.; Dikovskaya, D.; Menon, D.; Zaslona, Z.; Jedrychowski, M. P.; Costa, A. S. H.; Higgins, M.; Hams, E.; et al. Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. *Nature* **2018**, *556* (7699), 113−117.
(39) Xu, L.; Cai, J.; Li, C.; Yang, M.; Duan, T.; Zhao, Q.; Xi, Y.; Sun, L.; He, L.; Tang, C.; Sun, L. 4-Octyl itaconate attenuates LPS-induced acute kidney injury by activating Nrf2 and inhibiting STAT3 signaling. *Mol. Med.* **2023**, *29* (1), 58.
(40) Giurdanella, G.; Longo, A.; Salerno, L.; Romeo, G.; Intagliata, S.; Lupo, G.; Distefano, A.; Platania, C. B. M.; Bucolo, C.; Li Volti, G.; Anfuso, C. D.; Pittala, V. Glucose-impaired Corneal Re-epithelialization Is Promoted by a Novel Derivate of Dimethyl Fumarate. *Antioxidants (Basel)* **2021**, *10* (6), 831.
(41) Pittalà, V.; Vanella, L.; Maria Platania, C. B.; Salerno, L.; Raffaele, M.; Amata, E.; Marrazzo, A.; Floresta, G.; Romeo, G.; Greish, K.; et al. Synthesis, In Vitro and In Silico Studies of HO-1 Inducers and Lung Antifibrotic Agents. *Future Medicinal Chemistry* **2019**, *11* (13), 1523−1536.
(42) Powell, C. R.; Dillon, K. M.; Matson, J. B. A review of hydrogen sulfide (H(2)S) donors: Chemistry and potential therapeutic applications. *Biochem. Pharmacol.* **2018**, *149*, 110−123.
(43) Sorrenti, V.; Vanella, L.; Platania, C. B. M.; Greish, K.; Bucolo, C.; Pittala, V.; Salerno, L. Novel Heme Oxygenase-1 (HO-1) Inducers Based on Dimethyl Fumarate Structure. *Int. J. Mol. Sci.* **2020**, *21* (24), 9541.
(44) Pittala, V.; Vanella, L.; Salerno, L.; Di Giacomo, C.; Acquaviva, R.; Raffaele, M.; Romeo, G.; Modica, M. N.; Prezzavento, O.; Sorrenti, V. Novel Caffeic Acid Phenethyl Ester (Cape) Analogues as Inducers of Heme Oxygenase-1. *Curr. Pharm. Des.* **2017**, *23* (18), 2657−2664.
(45) Pittala, V.; Vanella, L.; Salerno, L.; Romeo, G.; Marrazzo, A.; Di Giacomo, C.; Sorrenti, V. Effects of Polyphenolic Derivatives on Heme Oxygenase-System in Metabolic Dysfunctions. *Curr. Med. Chem.* **2018**, *25* (13), 1577−1595.
(46) Zheng, Y.; Chen, Z.; She, C.; Lin, Y.; Hong, Y.; Shi, L.; Zhang, Y.; Cao, P.; Xu, X. Four-octyl itaconate activates Nrf2 cascade to protect osteoblasts from hydrogen peroxide-induced oxidative injury. *Cell Death Dis.* **2020**, *11* (9), 772.
(47) Daina, A.; Michielin, O.; Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.* **2017**, *7* (1), No. 42717.
(48) Mhatre, S.; Rai, A.; Ali, H.; Patil, A.; Singh, N.; Verma, R.; Auden, J.; Chandler, C.; Dash, A.; Opere, C.; et al. Comparison of colorimetric, spectroscopic and electrochemical techniques for quantification of hydrogen sulfide. *Biotechniques* **2024**, *76* (2), 71−80.
(49) Calderone, V.; Martelli, A.; Testai, L.; Citi, V.; Breschi, M. C. Using hydrogen sulfide to design and develop drugs. *Expert Opin Drug Discov* **2016**, *11* (2), 163−175.
(50) Benavides, G. A.; Squadrito, G. L.; Mills, R. W.; Patel, H. D.; Isbell, T. S.; Patel, R. P.; Darley-Usmar, V. M.; Doeller, J. E.; Kraus, D. W. Hydrogen sulfide mediates the vasoactivity of garlic. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104* (46), 17977−17982.
(51) Citi, V.; Martelli, A.; Testai, L.; Marino, A.; Breschi, M. C.; Calderone, V. Hydrogen sulfide releasing capacity of natural isothiocyanates: is it a reliable explanation for the multiple biological effects of Brassicaceae? *Planta Med.* **2014**, *80* (8−9), 610−613.
(52) Rose, P.; Dymock, B. W.; Moore, P. K. GYY4137, a novel water-soluble, H2S-releasing molecule. *Methods Enzymol* **2015**, *554*, 143−167.
(53) Peng, B.; Chen, W.; Liu, C.; Rosser, E. W.; Pacheco, A.; Zhao, Y.; Aguilar, H. C.; Xian, M. Fluorescent probes based on nucleophilic substitution-cyclization for hydrogen sulfide detection and bioimaging. *Chemistry* **2014**, *20* (4), 1010−1016.
(54) Zhou, Y.; Mazur, F.; Liang, K.; Chandrawati, R. Sensitivity and Selectivity Analysis of Fluorescent Probes for Hydrogen Sulfide Detection. *Chem.-Asian J.* **2022**, *17* (5), No. e202101399.

https://doi.org/10.1021/acsmedchemlett.5c00624
ACS Med. Chem. Lett. 2026, 17, 199−210

(55) Chen, C.; Liu, C.; Sun, P.; Zhang, Z.; Wang, Z.; Liu, P.; Li, X. Itaconate uptake via SLC13A3 improves hepatic antibacterial innate immunity. *Dev. Cell* **2024**, *59* (21), 2807−2817e2808.
(56) Chen, C.; Li, X. The cell autonomous and non-autonomous roles of itaconate in immune response. *Cell Insight* **2025**, *4* (1), No. 100224.
(57) Dong, Q.; Liu, F.; Zhu, J.; Li, M.; Chen, A.; Feng, L.; Lan, Z.; Ye, Y.; Lu, L.; Liang, Q.; et al. 4-Octyl itaconate inhibits vascular calcification partially via modulation of HMOX-1 signaling. *Eur. J. Pharmacol.* **2024**, *985*, No. 177122.
(58) Edwards, G.; Niederste-Hollenberg, A.; Schneider, J.; Noack, T.; Weston, A. H. Ion channel modulation by NS 1619, the putative BKCa channel opener, in vascular smooth muscle. *Br. J. Pharmacol.* **1994**, *113* (4), 1538−1547.

https://doi.org/10.1021/acsmedchemlett.5c00624
ACS Med. Chem. Lett. 2026, 17, 199−210