



**REVIEW ARTICLE**

# Anti-inflammatory and antiviral roles of hydrogen sulfide: Rationale for considering H<sub>2</sub>S donors in COVID-19 therapy

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The COVID-19 pandemic caused by SARS-Cov-2 demands rapid, safe and effective therapeutic options. In the last decades, the endogenous gasotransmitter hydrogen sulfide (H<sub>2</sub>S) has emerged as modulator of several biological functions and its deficiency has been associated with different disorders. Therefore, many H<sub>2</sub>S-releasing agents have been developed as potential therapeutic tools for diseases related with impaired H<sub>2</sub>S production and/or activity. Some of these compounds are in advanced clinical trials. Presently, the pivotal role of H<sub>2</sub>S in modulating the inflammatory response and pro-inflammatory cytokine cascade is well recognized, and the usefulness of some H<sub>2</sub>S-donors for the treatment of acute lung inflammation has been reported. Recent data is elucidating several mechanisms of action, which may account for antiviral effects of H<sub>2</sub>S. Noteworthy, some preliminary clinical results suggest an inverse relationship between endogenous H<sub>2</sub>S levels and severity of COVID-19. Therefore, repurposing of H<sub>2</sub>S-releasing drugs may be a potential therapeutic opportunity for treatment of COVID-19.

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**KEYWORDS**

COVID-1, drug repurposing, hydrogen sulfide, H<sub>2</sub>S-donor, SARS-CoV-2

**Abbreviations:** 3CL<sup>pro</sup>, 3-chymotrypsin-like protease; 3-MST, 3-mercaptopyruvate sulfurtransferase; AnxA1, annexin A1; CAT, cysteine aminotransferase; CBS, cystathionine β-synthase; CMV, cytomegalovirus; COVID-19, coronavirus disease 2019; CSE, cystathionine γ-lyase; DADS, diallyl disulfide; DATS, diallyl trisulfide; DIC, disseminated intravascular coagulation; FPR2, formyl peptide receptor 2; HSV-1, herpes simplex virus type 1; IKKβ, I-kappa-β kinase-β; IRF-3, IFN regulatory factor 3; IκBα, inhibitor of NF-κB; K<sub>ATP</sub>, ATP-sensitive potassium channels; MERS-CoV, Middle East respiratory syndrome coronavirus; MPO, myeloperoxidase; NiV, Nipah virus; Nrf2, nuclear factor erythroid 2-related factor 2; PLAs, platelet-leukocyte aggregates; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFN, sulforaphane; TMPRSS2, transmembrane serine protease 2; TSMT, thiol-S-methyltransferase.

**1 | INTRODUCTION**

The current pandemic caused by the new betacoronavirus SARS-CoV-2 (COVID-19) has placed the global healthcare system in such an unprecedented emergency state that the situation demands the rapid implementation of effective and safe pharmacological strategies to extinguish viral infection or, at least, to mitigate its pathogenic effects and to slow down its contagion. In this perspective, early SARS-CoV-2 clinical studies evaluated the effects of many drugs, both old and new, known for their broad antiviral spectrum, such as [favipiravir](#), [ribavirin](#),

galidesivir, **remdesivir**, and **hydroxychloroquine** (Harrison, 2020; Li & De Clercq, 2020). Moreover, the repurposing of available drugs developed for different therapeutic indications offers a promising strategy to take advantage of the safety and therapeutic window of such drugs. This should lead to a more rapid evaluation of their use against SARS-CoV-2, considering the current health emergency and the lack of therapeutic solutions.

The "cytokine storm" syndrome, with subsequent acute lung injury (and other systemic disorders), is a frequent feature of many infectious diseases, including those caused by many viral pathogens (Tisoncik et al., 2012). Indeed, rapid virus replication, massive infiltration of immune cells and uncontrolled production of pro-inflammatory cytokines were already observed in severe acute respiratory syndromes previously caused by other betacoronaviruses, such as SARS-CoV (which caused the severe acute respiratory syndrome epidemic in 2002–2003) and MERS-CoV (which caused the epidemic of Middle East respiratory syndrome in 2012–2013) (Channappanavar & Perlman, 2017). Noteworthy, a cytokine storm is also observed in the most serious cases of COVID-19 (Mehta et al., 2020). Indeed, patients with severe clinical conditions exhibit pulmonary distress syndrome, lung oedema, and respiratory failure. In addition, liver, heart, and kidney injury, as well as impaired haemostatic regulation, are also frequently observed. In particular, intensive care patients display elevated levels of pro-inflammatory cytokines. Many cytokines detected in these patients derive from a Th17-type immune response (as already reported for MERS-CoV and SARS-CoV patients). The corresponding IL-17-related pathway triggers a wide pro-inflammatory cascade through the induction of specific cytokines, such as **IL-6** and **TNF**, chemokines, and matrix metalloproteinases, the latter responsible for tissue remodelling and damage (Wu & Yang, 2020). The production of **IL-1 $\beta$**  and IL-6 is also induced by direct interaction of SARS-CoV-2 viral components with host **toll-like receptors** (Russell et al., 2020) and indirectly through the activation of the **NF- $\kappa$ B** signalling pathway, as demonstrated by Wang et al. (2007) in murine RAW264.7 cells. The **SARS-CoV-2 spike protein** is associated with increased degradation of the inhibitor of NF- $\kappa$ B (**I $\kappa$ B $\alpha$** ), leading to the activation of the NF- $\kappa$ B signalling pathway. Once activated and translocated into the nucleus, NF- $\kappa$ B promotes the transcription of a large variety of genes encoding stress-response proteins, chemokines and pro-inflammatory cytokines. Notably, the excessive NF- $\kappa$ B activation is involved in the lung inflammatory process induced by respiratory viruses, including SARS-CoV (Catanzaro et al., 2020; Dosch, Mahajan, & Collins, 2009).

To date, the powerful and uncontrolled inflammatory response promoted by the "cytokine storm" is considered the main pathogenic factor of SARS-CoV-2 infections, accounting for the most severe symptomatic features of the disease and fatal outcomes (Mehta et al., 2020). Accordingly, effective strategies able to mitigate the force of this inflammatory storm are considered a valuable option for reducing the severity of the disease. For instance, **tocilizumab**, a monoclonal anti-IL-6 antibody, is taken as a promising candidate for fighting against the "cytokine storm" and elevated levels of **IL-6** in patients who show serious lung inflammation and organ damage (Mehta et al., 2020). Furthermore, blockade of the NF- $\kappa$ B pathway

reduces lung damage and inflammation and significantly increases mouse survival after SARS-CoV infection. These results confirm that the NF- $\kappa$ B signalling pathway can be a major mediator of the inflammation induced by SARS-CoV infection and that NF- $\kappa$ B inhibition may be considered a promising antiviral strategy for SARS-CoV infections and other pathogenic human coronaviruses (DeDiego et al., 2014).

Recent insights point out that the changes in the vascular endothelium are likely to be pivotal elements in facilitating the diffusion of the inflammatory damage in the lung and other organs associated with SARS-CoV-2 infection. Endothelial cells are responsible for the endogenous production of vasoactive factors and for preventing immune cell adhesion to the vascular wall (Citi, Martelli, et al., 2020; Park & Park, 2015). Diffuse endothelial dysfunction is a hallmark of many cardiovascular and metabolic pathologies, such as hypertension, diabetes, and atherosclerosis. Noteworthy, clinical and preclinical studies support the hypothesis that COVID-19 leads to more severe systemic complications in patients with cardiovascular or metabolic pathologies (Sardu et al., 2020). In particular, hypertension and diabetes are the most common co-morbidities associated with a worsening prognosis in COVID-19 patients. Furthermore, dysregulation of the immune response leads to marked increases in permeability of lung endothelium, with subsequent acute respiratory distress syndrome and thrombotic events, especially in late-stage COVID-19 infection.

Recently, inhalation of **NO** gas was investigated as a strategy to prevent COVID-19 (e.g., clinical trials NCT04306393, NCT04312243, NCT04338828, and NCT04305457) and to improve arterial oxygenation against respiratory distress (Teman et al., 2015). NO diffuses into the lungs and bronchi where it promotes vasorelaxant and bronchodilatory effects. NO also promotes ciliary movements, which help to remove viral particles from the airways, and in a pig experimental model, NO inhibits pulmonary viral replication (Xu, Zheng, Dweik, & Erzurum, 2006). In humans, higher levels of exhaled NO correlate with a lower incidence of respiratory illness, suggesting that endogenous NO represents a crucial defence against viruses in the airways (Keyaerts et al., 2004).

Noteworthy, NO is a well-known endothelium-derived factor, and it belongs to the class of endogenous gasotransmitters. Together with NO, **H<sub>2</sub>S** is another important member of this class of endogenous mediators, and while it exhibits many NO-like effects, the mechanisms of action are quite different. Physiological levels of H<sub>2</sub>S have systemic anti-inflammatory effects, prevent endothelial dysfunction in cardiovascular-related pathologies, and act as a scavenger of ROS and peroxynitrite (Calderone, Martelli, Testai, Citi, & Breschi, 2016). Furthermore, it is widely known that exposure to low H<sub>2</sub>S levels significantly improves respiratory function by regulating mucolytic activity (Bazhanov, Ansar, et al., 2017) and through the up-regulation of **endothelial NOS** and increased NO bioavailability, thus indirectly protecting the airways from viral infection disease (King et al., 2014).

In this paper, the pharmacological basis for a potential beneficial role of H<sub>2</sub>S against COVID-19 and the potential repurposing of H<sub>2</sub>S donor drugs in the management of this disease are discussed.

## 2 | ENDOGENOUS H<sub>2</sub>S

Abe and Kimura (1996) first discovered that H<sub>2</sub>S is endogenously produced and is endowed with relevant physiological roles. Thereafter, H<sub>2</sub>S was recognized as the third gasotransmitter, after **carbon monoxide (CO)** and NO, and its biosynthesis and metabolism pathways were widely investigated (Martelli et al., 2012). In mammalian cells, H<sub>2</sub>S is produced by non-enzymic and enzymic routes (Yang et al., 2019). **Cystathionine  $\gamma$ -lyase (CSE)** and **cystathionine  $\beta$ -synthase (CBS)**, which act within the “transsulfuration pathway”, are the main H<sub>2</sub>S biosynthesizing enzymes (Miles & Kraus, 2004; Pan, Liu, Gong, Yang, & Zhu, 2012). A further biosynthetic pathway involves the tandem cooperation of **cysteine aminotransferase (CAT)** and **3-mercaptopyruvate sulfurtransferase (3-MST)** (Beltowski, 2019). A major source of H<sub>2</sub>S production in the brain derives from 3-MST, which is localized into neuronal cytosol and mitochondria. 3-MST produces H<sub>2</sub>S and bound sulfane sulfur more efficiently than CBS, which was previously believed to be the only H<sub>2</sub>S-producing enzyme in the CNS. Furthermore, the 3MST–CAT pathway produces polysulfides as well as H<sub>2</sub>S. In particular, levels of polysulfides measured as bound sulfane sulfur in 3-MST overexpressing cells were more than double those in control cells (Kimura et al., 2017; Shibuya et al., 2009).

Regarding the degradation of H<sub>2</sub>S, several pathways have been described. H<sub>2</sub>S is a reducing agent and thus can be oxidized by several circulating oxidants. However, one of the main catabolic pathways for H<sub>2</sub>S operates in the mitochondria, leading to the formation of thiosulfate, which is then converted by rhodanese enzyme into sulfite and finally sulfate. In addition, H<sub>2</sub>S can be methylated through thiol-S-methyltransferase (TSMT) with the formation of dimethyl sulfide. Finally, H<sub>2</sub>S interacts with Hb to produce sulfhaemoglobin (Guo, Cheng, & Zhu, 2013).

S-sulfhydration, a post-translational modification of cysteine residues of several target proteins, is considered the most plausible molecular mechanism for the pleiotropic effects of H<sub>2</sub>S (Banerjee, 2011; Meng, Zhao, Xie, Han, & Ji, 2018). Noteworthy, H<sub>2</sub>S coexists in biological systems with sulfane sulfur species. They are heterogeneous compounds containing a reactive, labile sulfur atom, which is covalently bonded to other sulfur atoms or to a sulfur and a hydrogen atom to form reactive sulfur compounds, including persulfides, polysulfides, and some forms of elemental sulfur (Iciek, Bilska-Wilkosz, & Gorny, 2019; Toohey, 2011). Sulfane sulfur species can be viewed as a form of “H<sub>2</sub>S storage”, to maintain a low-grade toxicity and to allow gasotransmitter release in response to biological signals (Ishigami et al., 2009; Toohey, 2011). Furthermore, sulfane sulfur species may act themselves through S-sulfhydration and therefore may be largely responsible for those biological activities attributed to H<sub>2</sub>S (Toohey, 2012). Indeed, it has been demonstrated that alterations in sulfane sulfur levels reflect some pathophysiological conditions related to H<sub>2</sub>S-altered biosynthesis, strongly suggesting the close reciprocal relationships.

Although intense research activity has been carried out and many roles of H<sub>2</sub>S in the homeostatic regulation of different systems and in the pathogenesis of several disorders have been elucidated, some mechanisms and pathways of H<sub>2</sub>S signalling are not yet completely

understood. As defective endogenous production of H<sub>2</sub>S has been associated with many systemic disorders, great efforts have been directed to the development of effective pharmacological agents able to increase H<sub>2</sub>S levels. The pharmacological modulation of H<sub>2</sub>S is a dynamic field in recent drug discovery research, which has been well-examined and reported in some seminal reviews (Gojon & Morales, 2020; Szabo & Papapetropoulos, 2017; Zheng et al., 2018). Presently, a large number of natural and synthetic compounds have been recognized as effective H<sub>2</sub>S donors (Keyaerts et al., 2004; Sardu et al., 2020), and some of them are in clinical trials for the treatment of cardiovascular diseases (SG-1002 for heart failure) (Polhemus et al., 2015) and cancer disease (**sulforaphane [SFN]**) (Jiang et al., 2018).

## 3 | H<sub>2</sub>S IN INFLAMMATORY LUNG DISEASES

The pro-inflammatory response and cytokine storm are involved in the most severe cases of SARS-CoV-2 infection disease (Coperchini, Chiovato, Croce, Magri, & Rotondi, 2020), and consistently, high levels of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, and TNF) and chemokines (**CCL2**, **CCL3**, and **CCL5**) have been detected in COVID-19 patients. In addition, haemopoietic growth factors such as **G-CSF** and **GM-CSF** are also elevated in COVID-19 patients (Schett, Sticherling, & Neurath, 2020). Interestingly, the exacerbation of NF- $\kappa$ B activation described above is also peculiar to SARS-CoV infection disease (Dediego et al., 2014).

Very recently, Renieris et al. (2020) investigated the role of H<sub>2</sub>S in COVID-19 respiratory disease, evaluating H<sub>2</sub>S plasma levels during progression of the disease and its association with final outcome, in a cohort of patients with COVID-19 pneumonia. In this study, a correlation between the severity of SARS-CoV-2 infection, cytokine production, and H<sub>2</sub>S plasma levels has been described, suggesting a potential predictive role of H<sub>2</sub>S in the outcome of pneumonia caused by SARS-CoV-2. Indeed, the kinetics of circulating H<sub>2</sub>S revealed that patients with favourable outcome display levels of the gasotransmitter higher than those found in patients with severe COVID-19 pneumonia. This evidence suggests that the reduction of H<sub>2</sub>S bioavailability may be considered as an indicator of enhanced pro-inflammatory response and that the administration of exogenous H<sub>2</sub>S may be viewed as a pharmacological strategy to restore H<sub>2</sub>S plasma levels in order to counteract the severe consequences of COVID-19 infection (Renieris et al., 2020).

IL-6 has been proposed as the main pro-inflammatory mediator involved in the cytokine storm that leads to severe lung injury, respiratory failure, and death in COVID-19 patients (Gubernatorova, Gorshkova, Polinova, & Drutskaya, 2020). Noteworthy, H<sub>2</sub>S is likely to be an effective down-regulator of IL-6 and an inhibitor of the NF- $\kappa$ B pathway (Kodala et al., 2015; Rios, Szczesny, Soriano, Olah, & Szabo, 2015). Generally, the role of H<sub>2</sub>S in inflammatory response in lung function and disease has been widely studied, and recent evidence highlights that H<sub>2</sub>S positively correlates with lung function and improves peak expiratory and inspiratory flow rate (Tian et al., 2012).

Both exogenous and endogenous H<sub>2</sub>S exert beneficial effects in the respiratory system by regulating mucolytic activity. H<sub>2</sub>S is able to make the mucus less viscous, because it promotes the breakage of mucins via interactions with disulfide bonds (Costantino, Lampa, & Nappi, 2006). Furthermore, in human bronchiolar epithelia, H<sub>2</sub>S triggers electrolyte absorption through the activation of **ATP-sensitive potassium channels** (K<sub>ATP</sub>) and inhibition of **Na<sup>+</sup>/K<sup>+</sup>-ATPase** and calcium-sensitive potassium channels (Pouokam & Althaus, 2016). These properties enhance mucociliary clearance, promoting the expulsion of foreign microorganisms.

Disturbances of the endogenous production of H<sub>2</sub>S are related to pathological processes and progression of several diseases, including hypertension, hypoxic pulmonary hypertension, myocardial injury, and viral infection (Calderone et al., 2016; Chen et al., 2020; Citi et al., 2018). The role of exogenous H<sub>2</sub>S in lung disease has been studied by administering H<sub>2</sub>S donor agents. The importance of the “nature” of fast or slow H<sub>2</sub>S-releasing molecules in inflammatory responses was mostly assessed by using molecules able to generate H<sub>2</sub>S with slow and sustained release kinetics. Indeed, the administration of **NaHS**, a “fast releasing” H<sub>2</sub>S donor, induced an important inflammatory reaction in mice, underlined by an increased **myeloperoxidase** (MPO) activity (a marker for tissue leukocyte infiltration) and by the presence of accumulated leukocytes in the lung (Bhatia, 2012). In contrast, the slow H<sub>2</sub>S-releasing compound **GY4137** showed anti-inflammatory activity in vivo and reduced plasma pro-inflammatory cytokines (TNF, IL-1 $\beta$ , and IL-6) in an experimental model of **LPS**-induced pulmonary inflammation in rats. Furthermore, GY4137 treatment was related to a marked antioxidant effect, by restoring the activity of the antioxidant enzymes **catalase** and SOD in lung tissues, and leading to normalization of the balancing between reduced and oxidized GSH (**GSH/GSSG** ratio) (Faller et al., 2018). GY4137 also inhibited the expression of pro-inflammatory genes by modulating the activation of NF- $\kappa$ B and IFN regulatory factor 3 (IRF-3) (Li et al., 2015). Mechanistically, Zhang et al. (2019) revealed that H<sub>2</sub>S blocks activation of the NF- $\kappa$ B pathway in a model of monocrotaline pyrrole-induced inflammatory response in pulmonary artery endothelial cells, through the sulfhydration of IKK $\beta$  at Cys179 residue, thus inhibiting IKK $\beta$  activity. Such a mechanism of action leads to protective effects in vivo against pulmonary vascular inflammation, vascular remodelling, and pulmonary arterial hypertension, suggesting that the post-transcriptional regulation of IKK $\beta$  is a novel target of H<sub>2</sub>S to prevent vascular inflammation (Zhang et al., 2019).

In a mouse model of LPS-induced acute lung injury, GY4137 prevented lung injury and neutrophil transmigration, by reducing chemoattractant signalling molecules in vitro in endothelial cells and in vivo in lung tissue (Faller et al., 2018). Indeed, neutrophil infiltration in lungs is a key event in the exacerbation of pulmonary disease associated with COVID-19. Interestingly, H<sub>2</sub>S modulates the egress of leukocytes, namely, neutrophils, from the bloodstream to inflamed tissues (Zanardo et al., 2006), and this effect is dependent upon activation of **annexin A1** (AnxA1) pro-resolving pathway (Brancaleone, Mitidieri, Flower, Cirino, & Perretti, 2014). Indeed, AnxA1 is exposed to the neutrophil surface and prevents them from transmigration into

the subendothelial space, thus suppressing cytokine production in inflamed tissues (Perretti & D'acquistio, 2009). This mechanism is associated with a reduction of adhesion molecule expression and occurs through the engagement of a specific receptor called **formyl peptide receptor 2 (FPR2)** (Dufton et al., 2010). It is feasible that this overall mechanism could operate in the lung of COVID-19 patients, where the use of H<sub>2</sub>S-releasing molecules would limit the passage of leukocytes into the underlying tissue. On one hand, this effect could down-regulate the release of cytokines by activated neutrophils and macrophages; on the other hand, the production of growth factors (G-CSF and GM-CSF) responsible for increase in circulating neutrophils could be suppressed, thus inhibiting the generation of the “cytokine storm”. It is noteworthy to underline that H<sub>2</sub>S donors impair the stability of thrombi formed into the vessels, thus facilitating thrombolysis (Grambow et al., 2017). This effect has been associated with a reduced formation of platelet-leukocyte aggregates (PLAs), which normally facilitate both leukocyte recruitment and extravasation to sites of inflammation and thrombus formation (Badnya et al., 2014; Diacovo, Roth, Buccola, Bainton, & Springer, 1996; Finsterbusch, Schrottmaier, Kral-Pointner, Salzmann, & Assinger, 2018). In the case of COVID-19 patients, there is a high incidence of coagulative disorders, generating a disseminated intravascular coagulation (DIC), which is lethal in severe conditions (Marietta, Coluccio, & Luppi, 2020). The possibility of modulating PLA formation by H<sub>2</sub>S is therefore of therapeutic interest, as PLAs promote both vascular inflammation and coagulation. Indeed, the inhibition of such aggregates could attenuate, at the same time, both events and achieve amelioration of the patient's conditions.

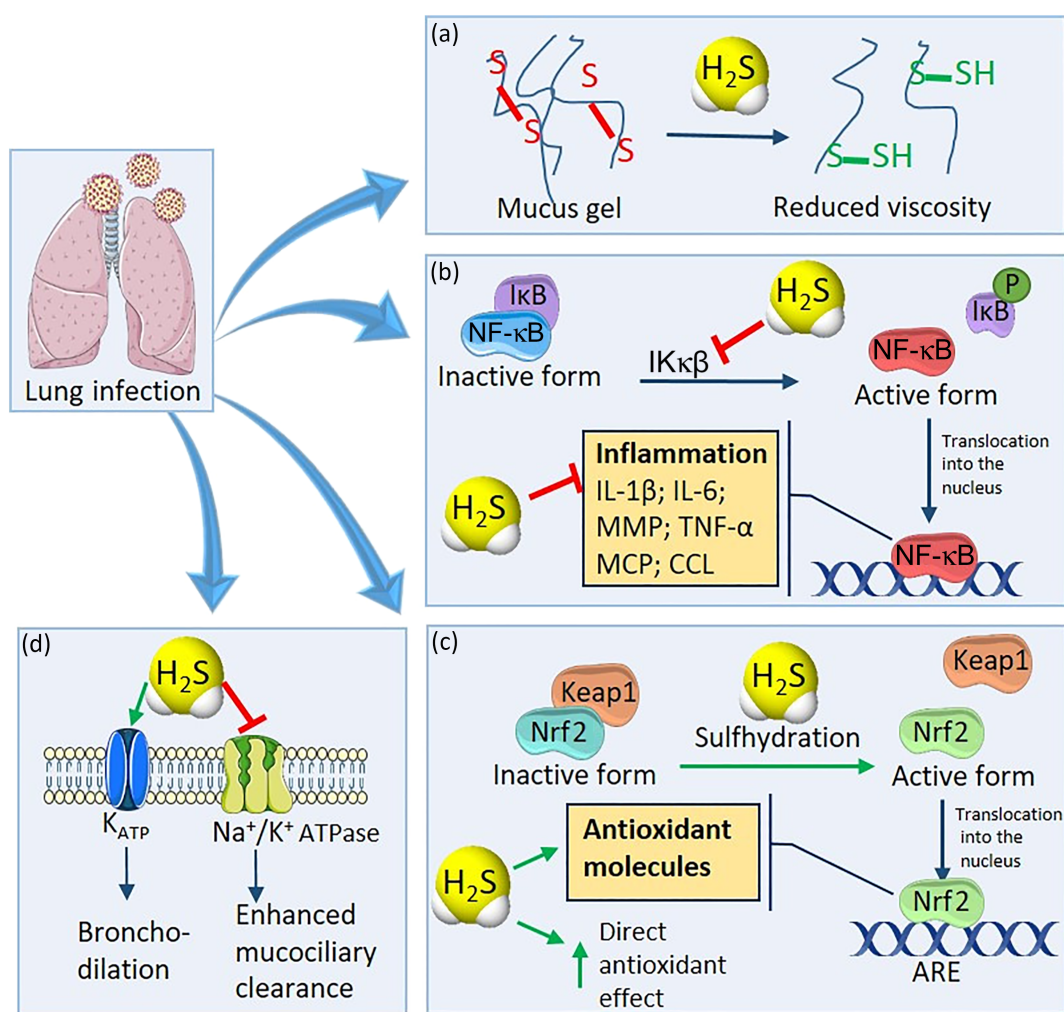
Other studies have shown that administration of H<sub>2</sub>S donors in ovalbumin-treated rats significantly reduced IL-6 and IL-8 and increased anti-inflammatory **IL-10** levels in the lung and plasma. H<sub>2</sub>S directly suppressed the pro-inflammatory response and the production of ROS in neutrophils, underlining the beneficial potential of H<sub>2</sub>S-releasing compounds in the prophylaxis of acute lung injury. Moreover, H<sub>2</sub>S promoted anti-inflammatory effects through epigenetic alterations. In particular, it modulated the acetylation and methylation of histones involved in the regulation of pro-inflammatory factor production and contributed to reduce cytokine release following stimulation with LPS in mice (Faller et al., 2018). The administration of diallyl disulfide (DADS; a natural, slow-releasing, H<sub>2</sub>S donor) promoted a protective effect in naphthalene-induced lung injury (Benavides et al., 2007; Martelli et al., 2013). DADS treatment increased GSH levels in the lung tissue, inhibited TNF, IL-6, and IL-8 release, and was associated with suppression of lung inflammatory cell recruitment, in particular neutrophil infiltration (Liu et al., 2018). Sulforaphane, a naturally occurring isothiocyanate able to release H<sub>2</sub>S (Lucarini et al., 2018), also decreased pro-inflammatory mediator release in a mouse model of LPS-induced acute lung injury. In particular, the transcription factor Nrf2 was involved in SFN-mediated lung protection through the improvement of mitochondrial function and energy metabolism. Nrf2 is a nuclear factor responsible for promoting the expression of multiple antioxidant genes and preventing oxidative damage. Such a



mechanism of action has been described also for synthetic isothiocyanates whose H<sub>2</sub>S donor profile has been widely described (Citi, Corvino, et al., 2020; Citi et al., 2014, 2019; Martelli, Citi, et al., 2020; Martelli, Piragine, et al., 2020; Martelli et al., 2014; Prawn et al., 2009; Sestito, Daniele, et al., 2019; Sestito, Prucoli, et al., 2019; Testai et al., 2016). Anethole dithiolethione, another H<sub>2</sub>S donor, has been widely used as H<sub>2</sub>S donor or as a moiety for the development of H<sub>2</sub>S-releasing hybrid drugs, including non-steroidal anti-inflammatory agents, such as H<sub>2</sub>S-aspirin and H<sub>2</sub>S-diclofenac. These novel hybrid drugs have been shown to have better anti-inflammatory effects than the corresponding “parent drugs” **aspirin** and **diclofenac**, and the administration of H<sub>2</sub>S-diclofenac was shown to be more effective in reducing lung MPO activity in a rat model of LPS-induced septic shock, compared with diclofenac alone (Takayama et al., 2011) (Figure 1).

#### 4 | H<sub>2</sub>S AND VIRAL INFECTIONS

The effect of H<sub>2</sub>S on viral infections represents a promising research field, which is presently still poorly investigated. Many pulmonary viral infections increase ROS levels and impair antioxidant enzymes, including those associated with the Nrf2–ARE pathway, with consequent reduction of antioxidant response. This leads to the suggestion that agents able to activate the Nrf2 system and induce sustained antioxidant activity may represent a useful strategy to counteract the viral infection by respiratory viruses (Komaravelli & Casola, 2014). As reported above, many H<sub>2</sub>S-releasing agents evoke significant protective effects against oxidative stress in the host cells, which are mediated by H<sub>2</sub>S and/or related sulfane sulfur through well-clarified mechanisms, such as increased nuclear translocation of Nrf2 and inhibition of **NADPH oxidase enzymes** (Gojon & Morales, 2020).

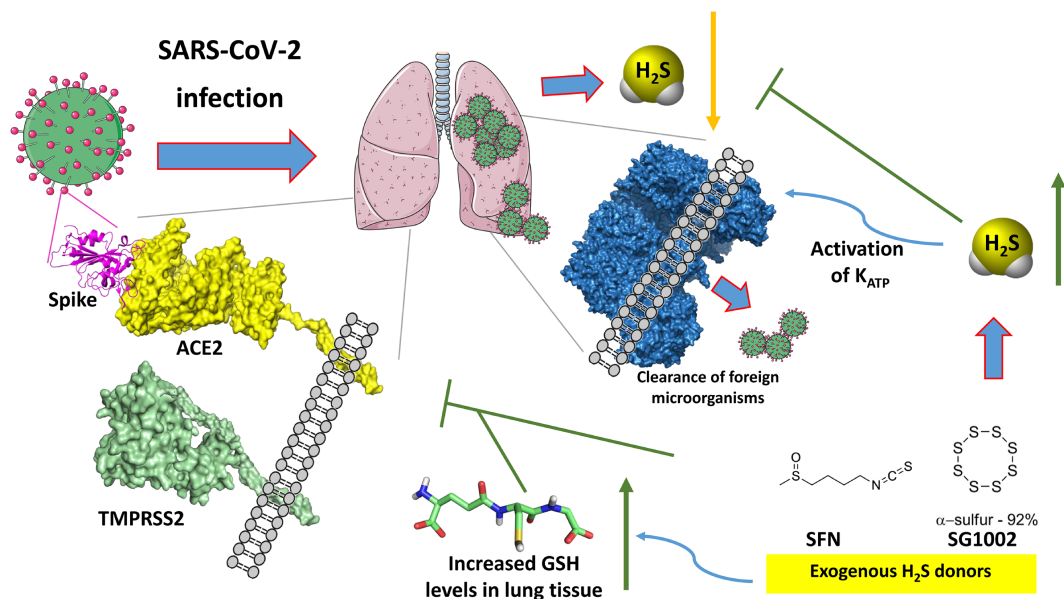


**FIGURE 1** Role of H<sub>2</sub>S in lung inflammation. H<sub>2</sub>S shows many beneficial effects in lung inflammation disease. In particular, (a) H<sub>2</sub>S is able to break mucin disulfide bonds, making the mucus less viscous and easier to be expelled by the respiratory ciliary apparatus, facilitating the elimination of potentially harmful viruses or extraneous particles. (b) H<sub>2</sub>S blocks activation of the NF-κB pathway, through the sulphydration and inhibition of IKKβ enzyme, preventing the translocation of NF-κB into the nucleus, thus preventing generation of the cytokine storm. (c) H<sub>2</sub>S promotes the activation of Nrf2, enhancing the expression of antioxidant molecules and enzymes. Moreover, H<sub>2</sub>S is endowed with direct antioxidant activity, thus protecting the tissues from the oxidative stress. (d) H<sub>2</sub>S activates K<sub>ATP</sub> channels expressed on the cell membrane of bronchial smooth muscle cells, promoting bronchodilation, and blocks the Na<sup>+</sup>/K<sup>+</sup> ATPase pump, triggering electrolyte absorption and enhancing mucociliary clearance

Besides the triggering of the "antioxidant machinery" directly attributable to H<sub>2</sub>S, other "indirect" effects are also involved in antioxidant activity of H<sub>2</sub>S. For instance, H<sub>2</sub>S contributes to the maintenance of elevated levels of GSH, which is one of the main intracellular antioxidant compounds and an effective scavenger of reactive species (Gojon & Morales, 2020). GSH can itself be another player with antiviral effects. Indeed, GSH ability to limit viral infections has been long reported. In particular, GSH levels were dramatically reduced after 24 h of herpes simplex virus type 1 (HSV-1) viral infection and exogenous administration of GSH almost totally abolished HSV-1 replication (Palamara et al., 1995). Experiments carried out on human small airway epithelial cells and on homogenates of BALB/c mouse lung and trachea (following intranasal inoculation of mouse-adapted influenza strain A/X-31 virus) showed that GSH reduced viral replication in the in vitro model and viral titer in organs explanted after the in vivo experiments (Cai et al., 2003). Very recently, GSH was also proposed as a potentially useful agent against SARS-CoV-2, since high-throughput artificial intelligence-based binding affinity prediction indicated a possible GSH interaction with **ACE2** or **transmembrane serine protease 2 (TMPRSS2)**, two human proteins closely involved in the process of viral adhesion and entry into the host cell (Kim et al., 2020).

Some studies reported the potential antiviral effects of organosulfur molecules. However, only subsequent studies have shown that these compounds act as H<sub>2</sub>S donors, letting us hypothesize a posteriori that the antiviral effect can be linked to the release of this gas. For instance, Fang, Li, Cui, and Dong (1999) evaluated the effect of diallyl trisulfide (DATS) in cytomegalovirus (CMV)-induced hepatitis. In their research, the authors reported that the polysulfide DATS, derived from *Allium sativum* L., reduced CMV viral load in infected organs in human and mice (Fang, Li, Cui, & Dong, 1999; Liu et al., 2011; Liu, Fang, Dong, Li, & Zhen, 2004) through the inhibition of gene transcription and promotion of CMV-induced T-regulatory helper cells amplification, with the consequent immunosuppressive effect against CMV observed in murine models (Li et al., 2013; Yi, Feng, Xiang, & Ge, 2005; Zhen et al., 2006). H<sub>2</sub>S accounts (at least in part) for the above antiviral effects, since Benavides et al. (2007) demonstrated that garlic polysulfides (e.g., DADS and DATS) are effective H<sub>2</sub>S donors. Similarly, Lin et al. (2005) studied the antiviral properties of the root extract of *Isatis indigotica* L. (also called *Isatis tinctoria* L.), a plant belonging to the *Brassicaceae* or Crucifers and widely used in Chinese traditional medicine. Among the various compounds contained in the extract, sinigrin (precursor of the H<sub>2</sub>S donor **allyl isothiocyanate**) was the most effective compound in inhibiting the function of **3-chymotrypsin-like protease (3CL<sup>pro</sup>)**, also called main protease or M<sup>pro</sup> of SARS-CoV (responsible for the 2002–2003 epidemic). Notably, SARS-CoV 3CL<sup>pro</sup> is the protease responsible for the proteolytic processing of polypeptides into functional proteins, necessary for viral replication. Thus, sinigrin can affect SARS-CoV infection by inhibiting 3CL<sup>pro</sup> (Lin et al., 2005). The results obtained using the natural isothiocyanate sulforaphane (present in several plants of *Brassicaceae* family) indicate that the

antiviral effect of *Brassicaceae* extracts can be mainly attributed to their isothiocyanate metabolites. As reported above, isothiocyanates have been recently described as smart H<sub>2</sub>S donors by Calderone and colleagues (Calderone et al., 2016; Citi et al., 2014, 2019; Lucarini et al., 2018; Marino et al., 2016; Martelli, Citi, et al., 2020; Martelli, Piragine, et al., 2020; Martelli et al., 2014; Testai et al., 2016). In particular, sulforaphane demonstrated antiviral activity against the influenza A/WSN/33 (H1N1) virus by reducing virus replication in vitro using canine kidney cells at low micromolar concentrations (Zhansheng et al., 2019). These results seem to find a confirmation in a randomized, double-blind, placebo-controlled clinical study. In this trial, healthy volunteers were treated with SFN or placebo and received a standard nasal vaccine dose of live attenuated influenza virus. Their peripheral blood immune cell population was analysed, paying particular attention to NK cells. Blood samples were examined at day 1 (before vaccine dosing) and at days 2 and 21 (after vaccine dosing). The results showed that sulforaphane increased virus-induced **granzyme B** production by NK cells. Granzyme B is a protease released by NK or T cells in order to induce cell apoptosis, and this increase in granzyme B levels was negatively associated with influenza RNA levels in nasal lavage fluid cells, demonstrating again an antiviral role for sulforaphane (Muller et al., 2016). Concerning other H<sub>2</sub>S donors, some studies were focused on the phosphinodithioate GYY4137. GYY4137 is a well-known slow H<sub>2</sub>S donor, and it was used by the Casola group for a thorough investigation on H<sub>2</sub>S and its antiviral activity against respiratory syncytial virus (RSV). Indeed, the researchers observed that the infection with RSV was associated with low levels of CSE mRNA and protein expression in airway epithelial cells, with consequent reduction of H<sub>2</sub>S generation (Li et al., 2015). Moreover, in RSV-infected CSE knockout mice, they recorded increased viral replication and airway inflammation, compared with wild-type mice (Ivanciuc et al., 2016). Based on these observations, they used GYY4137 to evaluate the role of H<sub>2</sub>S on several paramyxovirus infections (RSV, human metapneumovirus, and Nipah virus [NiV]) and recorded a significant reduction of viral replication and of pro-inflammatory mediator production. The mechanism of GYY4137-induced antiviral effect was not linked to genome replication or to the synthesis of viral mRNA or proteins, but to the inhibition of syncytia formation and viral assembly and release. As reported above, GYY4137 showed also anti-inflammatory effects due to regulation of NF-κB and IRF-3 activation after their nuclear translocation (Li et al., 2015). This GYY4137 effect on RSV was also confirmed using in vivo experiments where intranasal administration of GYY4137 to infected mice, within 24 h of infection, inhibited viral replication, reduced pro-inflammatory mediators, and improved airway dysfunction (Ivanciuc et al., 2016). Recent studies showed that GYY4137 also exhibited similar effects (reduced viral replication and decrease of pro-inflammatory mediators) in in vitro models using other types of highly pathogenic RNA viruses, such as influenza A and B, Far Eastern subtype tick-borne flavivirus, Crimean–Congo hemorrhagic fever virus, Rift Valley fever virus, and Ebola virus (Bazhanov,



**FIGURE 2** Potential mechanisms of H<sub>2</sub>S donors as anti-COVID-19 agents

Escaffre, et al., 2017). Other thiol-activated gem-dithiol-based H<sub>2</sub>S donors, while structurally different from GYY4137 yet sharing the ability to release H<sub>2</sub>S, also inhibited viral replication, decreased pro-inflammatory mediators and improved airways dysfunction, both in *in vitro* and *in vivo* models. This provides strong evidence to suggest that H<sub>2</sub>S is the actual player responsible for the observed antiviral effects (Bazhanov et al., 2018).

The antiviral activity of a library of H<sub>2</sub>S-releasing molecules, together with reference H<sub>2</sub>S donors (e.g., GYY4137 and sodium hydrosulfide), was initially tested on pseudotyped NiV. This preliminary screening showed that most sulfur molecules produced significant effect and a disulfide compound (XM-01) was selected for further evaluation on both enveloped and non-enveloped viral strains, such as RSV, influenza virus (A/WSN/33 strain), and rotavirus. The H<sub>2</sub>S-releasing effects of XM-01 (a cysteine-based perthiol derivative) were previously reported in a study aimed at identifying cardioprotective H<sub>2</sub>S donors (Zhao et al., 2013). As observed in previous GYY4137 studies, XM-01 exhibited antiviral effects on enveloped viruses. Noteworthy, no activity was observed on non-enveloped viruses, such as rotavirus. Thus, as the antiviral action may be due to alterations of the viral membrane, GYY4137 and its analogues could be useful against enveloped viruses at the entry phase of host cell infiltration. However, the author hypothesized that the antiviral activity can also rely on mechanisms other than H<sub>2</sub>S (Pacheco & Chemistry WSUDO, 2017). Finally, in a very recent paper, it was hypothesized that H<sub>2</sub>S may also exhibit antiviral activity against SARS-CoV-2 by interfering with ACE2 and TMPRSS2 (Yang, 2020) (Figure 2).

## 5 | CONCLUSIONS

The severity of COVID-19 pandemic is mainly based on two relevant aspects: high degree of contagiousness and high frequency of massive

inflammatory reactions, which lead to serious and life-threatening disease outcomes. In this perspective, the pathophysiological role of the gasotransmitter H<sub>2</sub>S provides opportunities for additional investigation. This endogenous mediator can modulate the inflammatory response in a complex regulation of the cytokine cascade. Moreover, preliminary studies point to a role of H<sub>2</sub>S in regulating the host response to viral infections (Yang, 2020). Such evidence provides a strong case for the potential antiviral benefits offered by different classes and chemotypes of H<sub>2</sub>S donor molecules as therapeutic agents, as recently proposed also by other pioneering hypotheses (Evgen'ev & Frenkel, 2020; Yang, 2020). Several compounds already in clinical development for other therapeutic indications, such as SG1002 or sulforaphane, can be viewed as potential high-value candidates for rapid repurposing for antiviral therapy against COVID-19.

## Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (<http://www.guidetopharmacology.org>), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos, et al., 2019; Alexander, Fabbro, et al., 2019; Alexander et al., 2019a,b; Alexander, Mathie, et al., 2019; Harding et al., 2018).

## CONFLICT OF INTEREST

G.G. is a founder and CSO of Sulfagenix, Inc. G.M. is CEO and president of Sulfagenix, Inc. All the other authors declare no conflict of interest.

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