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The dichotomous role of H₂S in cancer cell biology? Déjà vu all over again

Khosrow Kashfi^{1,*}

¹Department of Molecular, Cellular and Biomedical Sciences, Sophie Davis School of Biomedical Education, City University of New York School of Medicine, New York, USA

Abstract

Nitric oxide (NO) a gaseous free radical is one of the ten smallest molecules found in nature, while hydrogen sulfide (H₂S) is a gas that bears the pungent smell of rotten eggs. Both are toxic yet they are gasotransmitters of physiological relevance. There appears to be an uncanny resemblance between the general actions of these two gasotransmitters in health and disease. The role of NO and H₂S in cancer has been quite perplexing, as both tumor promotion and inflammatory activities as well as anti-tumor and antiinflammatory properties have been described. These paradoxes have been explained for both gasotransmitters in terms of each having a dual or biphasic effect that is dependent on the local flux of each gas. In this review/commentary, I have discussed the major roles of NO and H₂S in carcinogenesis, evaluating their dual nature, focusing on the enzymes that contribute to this paradox and evaluate the pros and cons of inhibiting or inducing each of these enzymes.

Graphical Abstract

*Correspondence: Khosrow Kashfi, PhD, FRCS, Department of Molecular, Cellular, and Biomedical Sciences, City University of New York Medical School, 138th Street and Convent Avenue, New York, NY 10031, Tel: (212) 650-6641, Fax: (212) 650-7692, kashfi@med.cuny.edu.

Conflict of interest

KK holds an equity position in Avicenna Pharmaceuticals, Inc. to which NOSH-NSAIDs briefly described in this review have been licensed.

Authorship Contributions

KK – formulated the general concept of this review, researched the literature and wrote the manuscript.

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Key Works

Nitric oxide; hydrogen sulfide; carcinogenesis; iNOS; CBS; CSE

1. Introduction

Nitric oxide (NO) a gaseous free radical is one of the ten smallest molecules found in nature, while hydrogen sulfide (H₂S) is a gas that bears the pungent smell of rotten eggs. Both are toxic yet are recognized as gasotransmitters that have multiple roles in normal physiology. For example, NO regulates vascular relaxation [1], controls inflammation by inhibiting NF- κ B activation [2, 3], and suppresses expression of pro-inflammatory mediators in mast cells, macrophages and vascular smooth muscles [4–6]. NO regulates blood flow [1, 7], modulates platelet and leukocyte activation, adhesion, and aggregation [8]. The important role of NO in human biology was recognized in 1998 when the Nobel Prize in Physiology and Medicine was awarded to Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad for establishing NO as a messenger molecule. Abe and Kimura established the physiological role of H₂S in a landmark study in 1996 showing it to be a neuromodulator [9]. However, it was Rui Wang in 2002 who proposed it to be the third gasotransmitter [10]; the first and second being NO and carbon monoxide, respectively. A quick inspection of the literature regarding the various aspects of NO and H₂S in health and disease leads one to conclude that there appears to be an uncanny resemblance between the general actions of these two gasotransmitters. For example, H₂S has also been shown to have vasorelaxant effects [11], and be protective in an animal model of myocardial ischemia-reperfusion injury [12]. Similarly to NO, H₂S also inhibits leukocytes adherence to blood vessel walls [13] and appears to be intimately involved in reducing and promoting the resolution of inflammation [14].

The role of NO in cancer has been quite perplexing, as both tumor promotion and inflammatory activities as well as anti-tumor and antiinflammatory properties have been described [15–18]. Much like NO, the role of H₂S in inflammation, immunity, and cancer biology is also controversial, with some studies suggesting anti-inflammatory, anti-cancer effects, whereas others suggest a contribution to immune-mediated tissue injury and tumor promotion [19–25]. These paradoxes and controversies have been explained for both gasotransmitters in terms of each having a dual or biphasic effect that is dependent on the local flux of each gas [16, 18, 24]. In this commentary, I have discussed the major roles of NO and H₂S in carcinogenesis, evaluating their dual nature, focusing on the enzymes that contribute to this paradox and evaluate the pros and cons of inhibiting or inducing each of these enzymes. Although I do recognize that there is a contribution to be made by CO, which can also play a role in cancer development/inhibition. Very briefly, CO is a colorless, odorless gas that is often referred to, as a “silent killer” because it is hard to detect and it’s intoxication can be fatal. Despite its toxicity, CO has many physiological roles, for example, at low concentrations it relaxes smooth muscles and lowers the blood pressure [26]. The major endogenous source of CO in the body is breakdown of haem. Physiological degradation of haem occurs in a tightly controlled manner involving the enzyme haem oxygenase (HO) [27], of which there are three isozymes. HO-1, which is inducible and is stimulated in response to cellular stress [28–30]; HO-2, is constitutively expressed and its

function is associated with neurotransmission and regulation of vascular tone [28, 31]; and HO-3, which is also constitutive but its function is unknown as it does not possess any heme degrading activity [32, 33]. Physiologically, similar to NO, CO activates guanylyl cyclase to produce cGMP but it is only about 1/80th as effective as NO [32]. Low concentrations of CO also activate K_{ATP} channels and affect MAPK signaling pathways [34]. Similar to NO and H_2S , CO also exhibits both pro and anti tumor activities, reviewed in [34]. Although not reviewed here, there is interaction between all three gasotransmitters. For example, H_2S may stimulate the expression of HO-1 through a signaling pathway depending on ERK and also through phosphorylation MAPK. The HO-1 then inhibits iNOS expression thus reducing NO production [35].

2. NO and H_2S biosynthesis and metabolism

NO is synthesized and released when L-arginine is metabolized by the enzyme nitric oxide synthase (NOS) [36, 37] of which there are three isoforms (Fig 1A). Neuronal (nNOS or NOS1) and endothelial (eNOS or NOS3) are constitutive (cNOS) and are calcium-dependent forms of the enzyme that are regulated by negative feedback [38], phosphorylation [39], and interaction with regulatory molecules [40]. cNOS produce low levels of NO over a short period of time, nanomolar concentrations for seconds or minutes, to regulate neural and vascular function respectively [41, 42]. In leukocytes, NO derived from NOS may diffuse across the plasma membrane and cytoplasm. NO reacts with the active site of soluble guanylate cyclase (sGC) and produces cyclic GMP (cGMP). cGMP activates cGMP-dependent Protein Kinase G (PKG), which phosphorylates multiple substrates [43]. In platelets, activation of the cGMP dependent kinase phosphorylates a variety of substrates, and is involved in platelet adhesion and aggregation [44]. In general, an increase in cGMP leads to smooth muscle relaxation, vasorelaxation and decrease of platelet aggregation [44, 45]. The third isoform (iNOS or NOS2), is calcium-independent, is regulated transcriptionally and is induced by inflammatory cytokines, oxidative stress, hypoxia, and various endotoxins [46, 47]. iNOS produces micromolar levels of NO which may be for hours or days [48–50] and is involved in immune surveillance. In most cancers including colon, breast, gastric, glioma, leukemia, melanoma, ovarian, prostate, renal, and squamous carcinoma, it is this isoform that is highly expressed [34, 51–57]. However, nNOS has also been reported to be induced in glioma, melanoma, and myeloma; whereas eNOS is induced in pancreatic cancer, sarcoma, and renal cell carcinoma [34, 51, 53].

NO can also be produced through reduction of nitrite/nitrate under low oxygen conditions. In a paper published in 2003, it was shown that nitrite infusion in the human forearm increased forearm blood flow before and during exercise, in a NOS independent manner [58]. The mechanism for this increase in blood flow was suggested to be due to increases in NO levels as a result of nitrite reduction by deoxygenated Hb (deoxyHb). These results suggested that nitrite was indeed a bioavailable pool for NO, and thus described a new physiological function for hemoglobin as a nitrite reductase, potentially contributing to hypoxic vasodilation. The biggest conceptual challenge to the notion that deoxyHb plays a major role in bioactivation of nitrite is that NO produced in the red blood cell would be rapidly scavenged by the dioxygenation reaction where NO reacts with oxygenated Hb (OxyHb) to form methemoglobin (MetHb) and nitrate [59]. Considering the kinetics of this

reaction, it becomes evident that very little NO that is made in the red blood cell can get out [60]. Several proposed mechanisms have been constructed to explain how nitrite-derived bioactivity can be exported from the red blood cell and these generally involve either a compartment model with submembrane localization of the reaction allowing for inefficient free NO escape, or the formation of another nitrogen oxide such as a nitrosothiol or N_2O_3 that can get out of the red blood cell and carry NO activity [61–65], (reviewed in [59]). The mechanism by which myoglobin (Mb) would lead to nitrite bioactivation is the same as for Hb but the reaction was found to be substantially faster due to its lower heme redox potential [66]. Mb-mediated nitrite reduction has been reported to be important in both cardiomyocytes [67] and smooth muscle [68].

In general the oxidative pathway leads to modification of proteins by S-nitrosylation of cysteine residues [69–71] (Fig 1A). Such post translational modifications also affects transcriptional activity by alteration of DNA binding of the protein, for example as that seen for the transcription factor NF- κ B which loses ability to bind DNA [72, 73]. Hypoxia-inducible factor-1 (HIF-1), estrogen receptor and NF- κ B are also redox sensitive transcription factors that are regulated by S-nitrosylation [74]. S-nitrosylation may lead to either progression or inhibition of cancer [53]. S-nitrosylation of NF- κ B and matrix metalloproteinase 9 (MMP9) promotes cell death whereas S-nitrosylation of caspase-3, caspase-9, and c-Jun N-terminal kinase prevents activity and inhibits apoptosis [73]. Death receptors were also found to be regulated in this way, for example nitrosylation of death receptor DR4 and Fas promotes apoptosis via the death-inducing signal complex [75, 76].

Peroxynitrite which is a strong oxidative and nitrosative agent is produced when NO reacts with oxygen radicals [53], this results in direct nitrosylation or nitrosation or nitration of signaling proteins. Nitrosative signaling in cancer cells is known to contribute to more proliferation, metastasis, and resistance to therapy and therefore, poor treatment outcomes [77]. On the other hand, apoptotic and necrotic cell death can occur by peroxynitrite via lipid peroxidation, cysteine oxidation, and by protein nitrosylation [78, 79]. Physiologically, endogenously generated NO diffuses through lumen of blood vessels and intracellular compartments and reacts with hemoglobin. This elimination pathway leads to the formation of nitrates and is most likely the major mechanism for NO catabolism [80].

Most, if not all of the biosynthesis of H_2S (Fig 1B) has been attributed to three enzymes [81], cystathionine β -synthase (CBS, EC 4.2.1.22), cystathionine γ -lyase (CSE aka CGL, EC 4.4.1.1) and the tandem enzymes cysteine aminotransferase (CAT, EC 2.6.1.3) and 3-mercaptopyruvate sulfurtransferase (3-MST, EC 2.8.1.2). CBS, CSE, and CAT require pyridoxal 5'-phosphate (PLP) as a cofactor and use L-cysteine as substrate [82, 83], 3-MST produces H_2S using 3-mercaptopyruvate (3-MP), a metabolite of L-cysteine, which is produced by PLP-dependent CAT [84]. CBS, CSE and MST are constitutive enzymes and have different expression levels in different tissues and organs [34], although there is some recent evidence suggesting that CSE may be inducible [85]. Expression of CSE is modulated in diverse conditions such as inflammation mediated by tumor necrosis factor- α (TNF- α) and lipopolysaccharides (LPS), metabolites such as glucocorticoids and glucose, as well as dietary restriction and endoplasmic reticulum (ER) stress [85–89]. H_2S can also be generated endogenously through nonenzymatic reduction of elemental sulfur in the blood

[90] using reducing equivalents supplied through the glycolytic pathway [91] or from various storage forms of sulfur such as thiosulfate, thiocysteine and sulfite [92], (reviewed in [81] and also by Olson in this Special Issue, [93]).

Mammalian enzymes generally metabolize L-amino acids, however, H₂S can also be synthesized from D-cysteine [94, 95]. D-Cysteine is metabolized by the peroxisomal enzyme D-amino acid oxidase (DAO) [96] to 3-MP, which is a substrate for the mitochondrial enzyme 3-MST [84] in the production of H₂S. The DAO/3-MST pathway is only active in the brain and the kidney because that is where DAO is localized. In the kidney, the production of H₂S from D-cysteine is 60 times higher than that from L-cysteine [97].

CBS expression levels have been reported to be higher in colon, breast, prostate, biliary tract, myeloma, renal, human malignant hyperthermia, and ovarian cancers compared to adjacent normal tissue or non-transformed cells [34, 98–103]. CSE has also been reported to have a high expression in colon, melanoma, lung, and prostate cancers [104–106]; while MST is reported to be high in glioma and melanoma [34, 104, 107].

Many cellular effects of H₂S are mediated by its interactions with membrane ion channels. Notably the ATP-sensitive potassium (K_{ATP}) channel is targeted/opened by H₂S leading to vasorelaxation in vascular smooth muscle [11] and enhancing cardiovascular function [108]. Apart from K_{ATP} channel, voltage-dependent calcium channels are also important targets of H₂S, albeit there appears to be tissue-specificity [109]. In isolated mouse pancreatic β -cells [110] and rat cardiomyocytes [111], H₂S inhibited L-type VDCC currents; whereas in neurons, the activities of Cav3.2 T-type VDCCs and Cav1.2 L-type VDCCs were enhanced by endogenous and/or exogenous H₂S [112, 113].

Solubility of H₂S in water is about 117 mmol/L and when dissolved acts as a weak acid with the equilibrium: $\text{H}_2\text{S} \leftrightarrow \text{HS}^- + \text{H}^+ \leftrightarrow \text{S}^{2-} + \text{H}^+$. Only the first reaction is physiology relevant as the pK_{a1} is around 6.9, which is approximately equivalent to the intracellular pH in many tissues and it is not far from the pH of mammalian blood (7.4) [114]. In the presence of oxygen and metal catalysts such as iron (III), H₂S is spontaneously oxidized, thus significantly reducing its concentration in a matter of hours [115]. Mitochondrial oxidation via three consecutive reactions is how much of the H₂S is metabolized [116]. In fact, H₂S is the only gasotransmitter that is enzymatically metabolized and the only inorganic compound that can be used by mammalian mitochondria to generate ATP [117, 118]. This oxidation requires one mole of oxygen for every mole of H₂S oxidized along the electron transport chain.

3. Interaction between NO and H₂S

NO and H₂S share many similar actions, including modulation of leukocyte adherence to the vascular endothelium, both are “gasotransmitters”, and both bind avidly to hemoglobin [10]. The interaction of hemoglobin with NO leads to the formation nitrosyl hemoglobin and with H₂S to the formation of green sulfhemoglobin [119]. Hemoglobin may also act as the common sink for CO, forming scarlet carboxyhemoglobin [120]. The competition for the common hemoglobin sink can potentiate the biological activity of the other. NO can act as a

reactive oxygen species (ROS) thus altering the reduced/oxidized glutathione balance; it can also inhibit enzymes and ion channels through S-nitrosylation [10]. It has also been suggested that H₂S may also be involved in the reduction of thiols [10, 121]. Recently, it was shown that the activity of myeloperoxidase (MPO), a haem protein that has been associated with many inflammatory events and cardiovascular diseases [122], was inhibited by H₂S reduction of its thiols [123]. There is considerable body of evidence strongly suggesting that NO and H₂S interact with each other, affecting each other's bioavailability and reactivity [124–126]. Most of the research on NO and H₂S cross-talk has been conducted in the cardiovascular system and the nature of this cross-talk in cancer is still unclear. Below I have presented an overview of this cross-talk in the cardiovascular system, however, I believe that these principles and mechanisms could essentially be applied to most cancers if not all.

In a cell-free system, it has been shown that NO inhibits CBS activity by binding to the heme group of the enzyme [127]. In a similar manner, that is the formation of a five co-ordinate ferrous nitrosyl complex impairing the binding of CBS to cysteine of homocysteine [126], NaSH has also been shown to directly inhibit a recombinant form of bovine eNOS most likely by an interaction between co-factors such as NADPH or tetrahydrobiopterin [128, 129]. On the other hand, it has also been shown that NO can increase H₂S biosynthesis through increases in expression of CBS and CSE enzymes in vascular smooth cells [11, 130]. While H₂S has been shown to increase NO levels by increasing IL-1 β -induced iNOS expression again in vascular smooth cells [131] through NF- κ B activation by a mechanism involving the ERK1/2 signaling cascade. In bovine arterial endothelial cells, H₂S has been shown to increase eNOS activation either indirectly by inducing its phosphorylation through an Akt-dependent mechanism [132] or directly by inducing Ca²⁺ release from the intracellular storage in the endoplasmic reticulum [133], thus providing yet another mechanism for its vasodilatory effects. These observations have been confirmed in human umbilical vein endothelial cells [134], where it was also found that L-cysteine supplementation stimulated NO production, while inhibition of CSE blocked it. Also, CSE knockdown inhibited, but CSE overexpression increased, NO production as well as endothelial cell proliferation. In these studies, LY294002 (Akt/PI3-K inhibitor) or SB203580 (p38 MAPK inhibitor) abolished the effects of H₂S on eNOS phosphorylation, NO production, and cell proliferation. In endothelial cells, H₂S increases cGMP in a NO-dependent manner while inhibition of eNOS reduces both H₂S-mediated angiogenesis and vasorelaxation [135].

NO and H₂S affect each other's synthesis as outlined above, however, they can also act directly to form novel molecular entities, which in turn exert further biological effects. For example, incubation of NaSH (releases H₂S) with a range of NO donors and NO gas in vitro lead to the formation of a nitrosothiol molecule. The formation of this nitrosothiol was also shown *in vivo* using liver homogenates from LPS treated rats. The authors suggest that this nitrosothiol may regulate the physiological effects of both NO and H₂S [136]. The interaction between SNP (sodium nitroprusside, releases NO) or 2-(N,N-diethylamino)-diazene-2-oxide (releases NO) and NaSH produced an active intermediate species identified as HNO (nitroxyl, Angeli's salt), which induced an increase in the strength of myocardial contractility in rat hearts, which was opposite to the effects of either agent alone [137]. H₂S also reacts with S-nitrosothiols to form thionitrous acid (HSNO), the smallest S-

nitrosothiol [138]. At the cellular level, HSNO can be metabolized to afford species such as NO^+ , NO, and NO^- , all of which have distinct physiological consequences of their own. Thus HSNO can act a signaling molecule that may play a key role in cellular redox regulation [138]. Emerging data shows that the interaction between H_2S and NO can also generate polysulfides (H_2S_n ; H_2S_2 and H_2S_3) [139]. In this study it was shown that H_2S_n generation was a fast reaction, and it activated transient receptor potential ankyrin 1 (TRPA1) channels in astrocytes and dorsal root ganglion (DRG) neurons, which can modify synaptic activity [140, 141]. H_2S_n has various other physiological effects. It facilitates the translocation of nuclear factor-like 2 (Nrf2) to the nucleus through modification of its binding partner, kelch-like ECH-associated protein 1 (Keap1), to up-regulate the transcription of antioxidant genes [142]. It also regulates the activity of a tumor suppressor, phosphatase and tensin homolog (PTEN) [143], activates protein kinase G1 α (PKG1 α) to relax vascular smooth muscle [144], and suppresses the activity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [145].

Recently, it was reported that incubation of RSNOs with excess Na_2S resulted in the formation of SSNO^- (perthionitrite or nitrosopersulfide), which decomposed to NO and polysulfides at physiological pH [146, 147]. Since RSNOs can be formed from the interaction of H_2S and S-nitrosothiols [148], this strongly suggests that H_2S could regulate not only NO production from one of its own metabolites (RSNOs), but it could also affect and sustain RSNOs' reactivity and signaling. The main features of NO- H_2S cross-talk are shown in Figure 2.

4. The yin – yang of NO and H_2S in carcinogenesis

NO has a dichotomous role in cancer biology, with some reports suggesting that NO possesses anti-tumor and antiinflammatory properties, while others implicate NO in tumor promotion and inflammation. For example, the production of endogenous NO is associated with apoptosis in cancer cells [149] characterized by upregulation of tumor suppressor p53, changes in expression of pro- and anti-apoptotic Bcl-2 family members, activation of caspases, chromatin condensation, and DNA fragmentation [150]. On the other hand, a large body of experimental and clinical data suggests a promoting role of NO in tumor progression and metastasis. Expression of iNOS has been reported in malignancies of the breast [77, 151, 152], prostate [153], lung [154], brain [155] and colon [156, 157]. It appears that, low concentrations of NO of less than 100 nM prevents certain cell types from apoptosis, and thereby favor tumorigenesis and progression [158, 159]. A sustained low efflux of NO derived from eNOS stimulates the proliferation of the endothelial cells [160]. Whereas high concentrations of NO, more than 500 nM, may be pro-apoptotic, producing cytotoxicity and antitumor activity [161, 162]. High NO levels that produced cytotoxic effects on tumor cells are derived from macrophages, neutrophils, endothelial cells, hepatocytes, cardiac myocytes and chondrocytes [163–166]. Thus NO displays a dual nature with respect to carcinogenesis, which may be attributed to low and high fluxes of NO [15–18, 53, 167, 168].

The major sources of human exposure to NO are nitrovasodilators, which include nitroglycerine (glycerol trinitrate), amyl nitrite, isosorbide mono- and dinitrate, erythrityl

tetranitrate and sodium nitroprusside [25]. These medications are taken sublingually, orally or subcutaneously for the treatment of angina pectoris and other coronary artery diseases. Nitroglycerine has been used effectively for over 100 years, and the other organic medicinal nitrates have been available since the 1930s. In theory, these medications can inhibit or promote the development of cancer. For example, glyceryl trinitrate induced apoptosis through activation of caspases in three different colon cancer cell lines [169] and inhibited tumor genesis in murine skin [170]. Further, isosorbide mononitrate and isosorbide dinitrate were shown to inhibit angiogenesis, tumor growth and metastasis in mice [171]. In contrast, feeding glyceryl trinitrate to F344 rats induced hepatocellular carcinomas that were characterized by K-ras point mutations [172]. Consistent with this finding is data showing elevated blood levels of nitrates and nitrites in patients with hepatocellular carcinoma [173].

Given the clinical importance and extensive use of NO-releasing vasodilators, the association between the commonly used NO-releasing nitrates and colorectal cancer risk was undertaken by using the data from the Framingham Heart and Offspring studies [174]. The Framingham Heart study is a long-term, multigenerational study, designed to identify genetic and environmental factors influencing the development of cardiovascular and other diseases [175]. Examination and testing of 5,209 residents of Framingham, Massachusetts, was initiated in 1948 and with approximately 200 members of the original cohort currently alive and under follow-up. An offspring cohort and a third generation cohort were subsequently added, followed more recently by two minority cohorts (Omni Group 1 and Omni Group 2) [176]. Using these data, it was concluded that nitrovasodilators do not increase the risk of colorectal cancer [174], reviewed in [25].

Statins are cholesterol-lowering drugs that are widely used in both men and women. As a class, they competitively and reversibly inhibit the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which is the rate-limiting enzyme for cholesterol biosynthesis in the liver; this leads to decreases in serum low-density lipoprotein cholesterol (LDL-C), triglyceride, and cholesterol levels [177]. It has been proposed that statins exert both LDL-C-dependent and LDL-C-independent or pleiotropic effects [178, 179]. One of the pleiotropic effects of statins is upregulation of eNOS resulting in higher production endothelial NO, which is important for vasodilation, platelet aggregation, vascular smooth muscle proliferation, and endothelial-leukocyte interactions [179, 180]. Statins upregulate eNOS activity through multiple mechanisms. One pathway involves Rho/ROCK signaling. In vitro studies show that Rho inhibition increases eNOS expression [181]. Statins also increase eNOS activity by post-translational activation of the phosphatidylinositol 3-kinase/protein kinase Akt pathway as eNOS is phosphorylated by Akt [39]. Other pleiotropic effects of statins, which increase eNOS activity, include effects on caveolin 1; transcription factor kruppel-like factor-2; and mobilization of endothelial progenitor cells [179].

Emerging research suggests that statins may prevent various types of cancers, including colon [182–184] and breast [185–187] as well as ovarian, pancreatic, lung, and lymphomas, reviewed in [188]. Statins inhibit cancer cell proliferation by arresting the cell cycle at the G₁-S phase and inducing apoptosis [189, 190]. Fluvastatin inhibits epidermal growth factor-induced invasion of pancreatic cancer cells by inhibiting the translocation of RhoA from the cytosol to the membrane [191]. Furthermore, statins have been shown to inhibit *in vivo*

tumor growth at clinically relevant doses and to diminish metastasis in animal models [192, 193]. Of interest, simvastatin and fluvastatin have been shown to enhance NO levels through increases in iNOS mRNA and protein expression. Statin induced NO and tumor cell cytotoxicity was inhibited by 1400W [N-(3-aminomethyl)benzylacetamidine], a relatively specific iNOS inhibitor [194].

It is important to note that these agents have been used for more than 30 years. They induce iNOS and/or eNOS, yet there is no data to suggest that people who have been using these drugs essentially for long period of times, are at a greater risk of developing any kind of cancer.

Much like NO, H₂S also has a dichotomous role in inflammation, immunity, and cancer biology, with some studies suggesting anti-inflammatory, anti-cancer effects, whereas others suggest a contribution to immune-mediated tissue injury and tumor promotion. For example, CSE and myeloperoxidase (MPO) activity were increased in the carrageenan-induced rat hindpaw model of inflammation. Pretreatment with DL-propargylglycine which is a CSE inhibitor reduced this edema [195]. Endotoxin administration to mice increased CSE gene expression in liver and kidney and increased H₂S levels in the plasma, liver, and kidney with histological evidence of lung, liver, and kidney tissue inflammatory damage [196]. NaSH administration resulted in histological signs of lung inflammation, increased lung and liver MPO activity, and increased plasma TNF- α levels [196]. Elevated H₂S levels have also been observed in the plasma of septic shock patients [196]. Interestingly, when NO-releasing flurbiprofen was administered to LPS treated rats, there was a dose-dependent inhibition of liver H₂S synthesis and CSE mRNA levels [197, 198]. Taken together, these observations suggest a proinflammatory role for H₂S. Proinflammatory actions of H₂S have also been reported in an animal model of pancreatitis [22, 23].

Anti-inflammatory effects of H₂S have also been reported. For example, administration of carrageenan into a rat air pouch resulted in leukocyte and neutrophil infiltration. Pretreatment with various H₂S donors, (NaSH, N-acetylcysteine, Lawesson's reagent) reduced the number of leukocytes in a dose-dependent manner [13]. This reduction was comparable to that seen by using the NSAID, diclofenac; the NOS inhibitor, L-NAME (N $^{\omega}$ -Nitro-L-arginine methyl ester); or dexamethasone. Pretreatment with β -cyanoalanine, a CSE inhibitor reversed the inhibitory effects of N-acetylcysteine. Pretreatment with diclofenac, NaSH, or Na₂S similarly reduced carrageenan-induced rat paw edema while β -cyanoalanine showed a significant increase in paw swelling in response to carrageenan [13].

Through peroxynitrite scavenging, H₂S may inhibit tissue oxidative damage [199, 200]. H₂S has been shown to induce apoptosis in neutrophils [201], which could contribute to resolution of inflammation [202]. In LPS-stimulated microglia and astrocytes, H₂S donors inhibited TNF- α inflammatory effect, due to inhibition of iNOS and p38 MAPK signaling [203]. In the neuroblastoma cell line SH-SY5Y, H₂S inhibited rotenone-induced cell apoptosis via regulation of p38- and JNK-MAPK pathway [204]. Thus it appears that just like NO, physiological concentrations of H₂S produce anti-inflammatory effects, whereas higher concentrations may be pro-inflammatory [19, 20].

5. Modulation of NO levels as a strategy to treat cancer

Different approaches have been used in utilizing NO as an anticancer target. These have included using NO as a radiotherapy and chemotherapy sensitizer, NO-releasing drugs, and NOS inhibitors [205]. Since tumors in general do not have well developed vasculature, administered drugs may not reach their intended target. NO donors can enhance tumor blood flow and oxygen supply, thereby resensitizing the tumor to chemotherapeutic agents and/or radiation therapy. While NO-releasing agents and NOS inhibitors have opposing effects, they both can serve as anticancer agents, as it has often been said, “the devil is in the details”. In the following sections, I have visited each of these areas; some briefly with appropriate references to in-depth reviews and some in more detail.

5.1. Using NO as a radiotherapy and chemotherapy sensitizer

Radiation is used in some instances to decrease tumor size before surgery. Unfortunately, many tumors are radio resistant mainly due to tumor hypoxia, with the degree of hypoxia correlating well with the response to radiation [206–208]. After O₂, NO is the second most potent chemical radio sensitizer [209, 210]. In a xenograft model of colorectal cancer, NO and ionizing radiation synergistically promoted apoptosis and growth inhibition by activating p53, further iNOS gene transfer enhanced tumor radio responsiveness [211].

Resistance of cancer cells to drug induced apoptosis is a hallmark mechanism of resistance. Tumor cells develop various mechanisms to escape the cytotoxic and antiproliferative effects of chemotherapeutic drugs. Understanding the underlying mechanisms of resistance may lead to the development of novel therapeutics. While chemotherapy remains one of the most common treatment options for various cancers, whether used alone as a single agent or in combination, its clinical effectiveness is limited due to the resistant phenotype of cancers in general. Both innate and adaptive chemoresistance have been characterized. The innate or intrinsic resistance is the resistance before treatment due to the presence of a subset of resistant cancer stem cells (CSCs) and of various resistant factors in the tumor cells that make them resistant to chemotherapy. The adaptive or acquired resistance develops following treatment as a result of various mechanisms such as mutations, overexpression of the therapeutic targets, and alternative compensatory signaling pathways [212–214]. Several mechanisms have been reported to overcome chemotherapy resistance [213], these have been thoroughly reviewed recently [212]. Another problem encountered in chemotherapy is the development multidrug resistance (MDR). This is a phenomenon by which tumor cells that have been exposed to a cytotoxic agent develop cross-resistance to a range of structurally and functionally unrelated compounds. MDR is one of the major obstacles to the successful pharmacologic treatment of tumors [215–217].

When MCF-7 human breast cancer cells were exposed to an aqueous solution of NO delivered as a bolus 30 min prior to doxorubicin, the cytotoxic effect of the combination was greater than that of doxorubicin alone. The NO donor diethylamine nitric oxide, but not inactivated donor, also increased doxorubicin cytotoxicity. Of note, the treatment sequence was important since the simultaneous application of NO with doxorubicin was not all that effective, and the exposure of the cells to doxorubicin prior to the NO obliterated the augmentation [218]. Treatment of the doxorubicin-resistant epithelial colon cancer cell line

(HT29-dx) with a variety of diverse NO donors (SNAP, GSNO, and SNP) or the induction of iNOS by a cytokine mixture (IFN- γ , TNF- α , and IL-1 β) or the statin, atorvastatin resulted in the reversal of resistance [215]. This effect was mediated, in part; by tyrosine nitration of MDR associated protein and the inhibition of drug efflux. NO donors also increased drug accumulation in two other doxorubicin-resistant cell populations that is of lung epithelial cells A549 and the human myelogenous leukemic cells K562 [215]. Treatment of cisplatin-resistant prostate cancer cells by the NO donor DETANONOate sensitized the resistant tumor cells to apoptosis by CDDP (cisplatin-diamine-dichloro platinum) and the combination was synergistic [219]. Treatment with DETANONOate inhibited the constitutively activated prosurvival/antiapoptotic signaling pathway mediated by NF- κ B, which was shown to be as a result of S-nitrosylation of both p50 and p65 subunits [212, 220, 221]. *meta*-NO-releasing aspirin (NCX-4016) [222] was evaluated against cisplatin-sensitive and cisplatin-resistant (CR) human ovarian cancer cells (HOCCs) [223]. NCX-4016 significantly reduced the surviving fractions of cisplatin-sensitive and CR HOCCs. Treatment of cells with NCX-4016 followed by cisplatin showed a significantly greater extent of toxicity when compared with treatment of cells with either agent alone. NCX-4016 inhibited EGFR and STAT-3 signaling and, hence, increased the levels of p53 and Bax and decreased the levels of Bcl-xL, survivin, and cyclin D1, resulting in the inhibition of proliferation and the induction of apoptosis by cisplatin. Using colon cancer xenografts, simultaneous treatment with oxaliplatin and *para*-NO-aspirin (NCX-4040) or oxaliplatin followed by NCX-4040 showed comparable additive effects. However, treatment with NCX-4040 followed by oxaliplatin showed synergistic interactions possibly by sensitizing the cancer cells to the cytotoxic effects of oxaliplatin [224]. RRx-001 (see the following section) is a novel aerospace-derived compound under active investigation as a chemo-, immuno- and radiosensitizer in Phase II clinical trials [225]. Results from a Phase-I clinical trial of RRx-001 in heavily pretreated patients with advanced/refractory solid tumors showed evidence of anticancer efficacy, with 15 of 25 patients enrolled in the study exhibiting partial response or stable disease [226]. Furthermore, after progression on RRx-001 treatment, 4 patients were re-challenged with drugs to which they had previously acquired resistance, and all 4 exhibited re-sensitization. In a recent case report [227], a patient with platinum-resistant small-cell lung cancer (SCLC) was successfully rechallenged with cisplatin/etoposide after being treated with RRx-001. The authors conclude that “the prolonged partial response of this platinum-resistant SCLC to reintroduced carboplatin/etoposide after RRx-001 belies and contradicts the prevailing orthodoxy in oncology that rechallenge with chemotherapy after the emergence of resistance is an exercise in futility and risk, which potentially exposes patients to toxicity without benefit”.

5.2. “Designer” NO-releasing compounds as chemotherapeutic agents

There are many synthetic “designer” NO-releasing compounds that are at various stages of development; some are preclinical, some are in different phases of clinical trials and some have been abandoned for various reasons [18, 25]. Below, I have briefly discussed some of these promising NO-releasing compounds that have utility against various cancers and have provided some recent in-depth reviews of the subject matter.

5.2.1. Nitric oxide-releasing NSAIDs (NO-NSAIDs)—NO-NSAIDs were developed to reduce the potential side effects associated with traditional NSAIDs [228, 229]. The rationale for their development was largely based on the observations that in the gastrointestinal (GI) system NO can enhance the local mucosal defense mechanisms thus increasing blood flow, mucus and bicarbonate secretions [7], reviewed in [25, 230]. NO-NSAIDs are traditional NSAIDs linked to an NO-releasing moiety via an aliphatic or an aromatic spacer (Fig 3A). All NO-NSAIDs tested were more potent in inhibiting the growth of various human cancer cell lines compared to their traditional counterparts [231]. Aromatic spacers generated more potent NSAID derivatives than aliphatic ones [222, 232], and positional isomerism greatly influenced all cell kinetic parameters that influence cellular mass. It should be noted that although structure-activity studies with NO-aspirin had indicated that NO was pivotal for its anticancer effects [233] it also became apparent that in cases where the spacer was aromatic, the biological actions may have been due to formation of a putative quinone methide (QM) intermediate [234–236]. NO-NSAIDs have shown efficacy in various animal models of cancer, including a rat model of colonic adenocarcinoma [237], *Min* (APC^{Min/+}) mouse [238], F344 rats treated with the carcinogen, azoxymethane [239], pancreatic cancer [240], urinary bladder cancer [241], and estrogen receptor negative breast cancer [242]. Molecular targets of NO-NSAIDs in cancer have been thoroughly reviewed elsewhere [25, 230].

5.2.2. Diazeniumdiolate based NO-releasing compounds and HNO-NSAIDs—

Decomposition of diazeniumdiolates or NONOates can lead to the formation of nitroxyl (HNO) and/or NO [243, 244] (Fig 3B). HNO has beneficial effects in overcoming heart failure [245], preconditioning against myocardial infarction [246], and treating alcohol abuse [247]. The first anticancer activity of HNO which was generated using Angeli's salt (Na₂N₂O₃) was reported in 2008 [248] where it was shown that it suppressed proliferation of human breast cancer cell lines under both hypoxic and normoxic conditions and in xenografts.

IPA/NO-aspirin and DEA/NO-aspirin (Fig 3C) are two relatively new NONO-NSAIDs [249] with enhanced GI safety profiles, strong anti-inflammatory properties, and significant cytotoxicity compared to either aspirin or the parent diazeniumdiolate toward nonsmall cell lung carcinoma cells (A549) [250]. Both IPA/NO-aspirin and DEA/NO-aspirin were equally effective against hormone sensitive and insensitive breast cancer cell lines with no appreciable cytotoxicity towards a related nontumorigenic cell line.

5.2.3. JS-K and PABA/NO—JS-K (Fig 3D) and PABA/NO (Fig 3E) are members of the diazeniumdiolate class of NO-releasing prodrugs that were designed to be activated by glutathione-*S*-transferase (GST) [251]. The rationale for this was based on the observation that GST- π , one of the phase II detoxification metabolizing enzymes is frequently over-expressed in most cancers. JS-K [252, 253] and PABA/NO [254, 255] have shown promise as anti-cancer agents. In brief, JS-K has shown efficacy against leukemia, renal, prostate, and brain cancer cells, both ER-positive and ER-negative breast cancer cell lines without affecting normal mammary epithelial cells, primary glioblastoma cells, and lung adenocarcinoma cells [252, 256–259]. Mechanistically, dearylation of the diazeniumdiolate

by GSH causes release of two equivalents of NO [260] and rapid depletion of GSH, resulting in alterations in the redox potential of the cellular environment, and activation of the MAPK stress signaling pathways, and induction of apoptosis. In Acute Lymphoblastic Leukemia (ALL) cell lines, JS-K inhibited growth, reduced proliferation, caused cell cycle arrest, increased apoptosis, and reduced the transcriptional activity of β -catenin [261, 262].

5.2.4. RRx-001 an endogenously NO generating compound: The aerospace connection—RRx-001 (Fig 3F) also known as ABDNAZ (1-bromoacetyl-3,3-dinitroazetidine) is a novel aerospace-derived compound that differs from other NO-donating compounds in that the molecule induces local, endogenous and biphasic production or release of NO, rather than fragmenting to release NO systemically, reviewed in [18]. This compound contains a unique high energetic organic nitro functional group, a gem dinitroazetidine that has not been used to date for medical and pharmaceutical applications. In an aerospace setting, compounds containing this energetic functionality, such as 1,3,3-trinitroazetidine, are designed to fragment explosively to propel rockets [263]. Modification of this structure by removing one of the nitro groups and substituting it with a bromoacetate group resulted in RRx-001, a nonexplosive that may be used to treat cancer [25, 264]. On infusion, the compound rapidly, irreversibly and selectively binds to hemoglobin [265], and with glutathione [266, 267] causing increases in oxidative stress [268]. While the RRx-001 glutathione adduct is rapidly excreted, RRx-001-bound hemoglobin remains in circulation for the duration of the lifetime of the red blood cell [269]. RRx-001 effectively inhibited the growth of eleven human cancer cell lines of varying tissue origins [270] by inducing intracellular ROS and RNS in a dose- and time-dependent manner. In some studies, DNA damage (γ H2AX expression) was observed the implication of which is that RRx-001 may be used as a chemotherapeutic agent but has no potential utility as a chemopreventive one.

5.3. Inhibition of iNOS/eNOS as strategies to treat cancer

All NOS isoforms metabolize L-arginine to L-citrulline and NO via two consecutive NADPH-dependent monooxygenation reactions. Two identical monomers constitute the active NOS, each having a C-terminal reductase and an N-terminal oxygenase domain. In mammals, all three isoforms contain highly conserved catalytic cores, which historically was an obstacle when attempting to find a specific inhibitor of a particular isoform [271]. The X-ray crystal structures of iNOS [272, 273] and eNOS [274] became available in the late 1990s and that of nNOS in 2002 [275]. These allowed for computer-based drug design of each isoform [276]. Potential therapeutic and challenges in developing NOS inhibitors have been reviewed in [277, 278].

In many clinical cancer studies, high iNOS expression has correlated relatively well with poor patient survival. These include colon, breast, gastric, hepatocellular carcinoma, melanoma, ovarian, leukemia, gastric, prostate, esophageal, and cervical [279, 280]. It has thus been suggested that iNOS expression may be used as a biomarker of poor patient prognosis and perhaps survival [281, 282]. By contrast, favorable prognosis has been associated with high iNOS expression in ovarian (10-year prognosis) [283] and non-small cell lung cancers [284].

Treatment of triple-negative breast cancer (TNBC) cell lines, which express high levels of iNOS with 1400W, which is a highly selective iNOS specific inhibitor, L-NAME which is a relatively selective eNOS inhibitor, and L-NMMA, which is pan-NOS inhibitor reduced cell proliferation, migration, and mammosphere formation [57]. In the same study, using a xenograft model of TNBC, treatment of mice with L-NAME, and L-NMMA significantly reduced tumor growth. Clinically, L-NMMA causes an acute elevation in blood pressure through inhibition of eNOS, which is what was seen in this study. To counteract this, the anti-hypertensive agent amlodipine was also incorporated. Administration of AG, another iNOS specific inhibitor instead of 1400W which has shown toxicity at high doses [285] to athymic nude mice bearing TNBC xenografts, abated tumor growth and metastatic burden [55]. Further, growth of glioma [286] or melanoma [287] cells in xenografts was significantly reduced when iNOS was silenced in these cells before they were implanted. The overarching data from all of these studies is the observation that the enhanced growth of the iNOS-overexpressing tumors appears to be due to enhanced angiogenesis.

6. Modulation of H₂S levels as a strategy to treat cancer

The therapeutic potential of H₂S-donating compounds have been described in the cardiovascular system [288, 289], urology [290], neuroinflammation [291], and ophthalmology [292]. These general areas have been thoroughly reviewed elsewhere [34, 109, 293]. Here I have focused on modulation of H₂S levels and the role of H₂S-donating compounds that are in different stages of development as agents that may have utility against cancer, although where appropriate, I have also summarized some novel compounds that have other indications.

It should be noted that in the literature the terms H₂S-donating and H₂S-releasing are used almost synonymously, but actually they represent two very different phenomena. H₂S-releasing compounds can be classified as sulfide salts such as NaSH, Na₂S and CaS, which form HS⁻ and H₂S immediately upon solvation in physiological buffers. These compounds are extensively used in studying the biological actions of H₂S, however, they are of limited therapeutic value since their effective residence time in tissues is relatively short [18]. H₂S-donating compounds are either naturally occurring compounds (mainly in foods) or synthetic hybrids, which can donate/deliver their H₂S “payload” either slowly or relatively rapidly depending on the inherent nature of the molecules.

6.1. H₂S as a radio /chemo -sensitizer

There are only a few reports addressing the potential use of H₂S as an adjuvant in radiotherapy or as a chemosensitizer. When HepG2 cells that were maintained under hypoxic conditions were treated with PPG and AOAA, which putatively are specific inhibitor of CSE and CBS respectively, radiation responses including cell killing, micronuclei MN formation, and caspase-3 activity were significantly enhanced [294]. Actually, AOAA is not very selective, as it also inhibits CSE [295]. However, treatment of these cells with low concentrations of NaSH (100 μM) had a protective effect against radiation damages but higher concentrations increased cytotoxicity. Thus, H₂S may confer intrinsic radioresistant properties to these cancer cells. The beneficial effects of H₂S as co-

treatment to radiotherapy were evaluated *in vivo* using xenograft models of cervix and ER(-) breast cancers [296]. Using electron paramagnetic resonance (EPR) oximetry, it was shown that a single NaSH administration decreased oxygen consumption and increased tumor perfusion. Tumors irradiated 15 minutes after NaSH administration were more sensitive to irradiation compared with those that received irradiation alone. As eluded above, tumor hypoxia, arising from an imbalance between oxygen consumption and blood supply, is a major cause of resistance to radiotherapy. Thus this study underscores the importance of H₂S in radiosensitization that were due to improved tumor oxygenation.

Effects of H₂S in combination with conventional chemotherapeutic agents appear to be controversial. Cisplatin (CP) is a potent chemotherapeutic agent for the treatment of many solid- organ cancers. However, nephrotoxicity is a major side effect of its use, affecting 20–30% of patients. Several lines of evidence indicate that CBS plays an important role in regulating the redox balance and metabolism of the ovarian cancer cells promoting tumor growth and metastasis [99]. Of note, *in vitro* effects of CBS silencing was reversed by addition of Na₂S in three different ovarian cancer cell lines. Silencing CBS in a CP-resistant orthotopic model *in vivo* by nanoliposomal delivery of CBS siRNA inhibited tumor growth, reduced nodule formation and sensitized ovarian cancer cells to CP. Thus in this study, CBS promoted ovarian tumor growth and maintained drug resistant phenotype by controlling cellular redox behavior and mitochondrial bioenergetics. In another study, CP-treated rats presented with increases in plasma creatinine and BUN levels, urinary volume and sodium and potassium fractional excretions [297]. Treatment with PAG an inhibitor of CSE reduced all these alterations. Thus, the animals treated with PAG + CP presented less renal damage compared with those injected with CP alone. This protective effect appeared to be due to diminished inflammation.

In a recent study evaluating the effects of H₂S on CP-induced nephrotoxicity in rats [298], it was reported that administration of NaSH reversed CP-induced changes and prevented the progression of CP nephrotoxicity, potentially through its cytoprotective/antioxidant properties. To the contrary, administration of GYY4137, a slow H₂S-donating compound to mice, exacerbated CP-induced nephrotoxicity [299]. Mice were pretreated with GYY4137 for 72 h prior to CP injection. CP treated mice developed renal dysfunction and kidney injury, with elevated BUN, histological damage and increases in TNF- α , IL-6, and IL-1 β , caspase-3, Bak, and Bax. All these markers were significantly increased by GYY4137 pretreatment. Thus this study suggested that GYY4137 exacerbated CP-induced nephrotoxicity in mice potentially through increases in oxidative stress and inflammation.

6.2. Synthetic H₂S-donating compounds as chemotherapeutic agents

6.2.1. GYY4137 and its structural analogs—The first water-soluble slow H₂S-donating molecule to be described was GYY4137 (morpholin-4-ium 4 methoxyphenyl(morpholino) phosphinodithioate [300] (Fig 4A, Table 1). GYY4137 inhibited growth of different human cancer cell lines including cervical carcinoma (HeLa), colorectal carcinoma (HCT-116), hepatocellular carcinoma (HepG2), osteosarcoma (U2OS), breast adenocarcinoma (MCF-7), acute promyelocytic leukemia cells (HL-60), and myelomonocytic leukemia (MV4-11) [301] but did not affect survival of non-cancer human

diploid lung fibroblasts (IMR90 and WI-38). In xenograft models of leukemia, GYY4137 (100–300 mg/kg) decreased tumor growth [301] and in HepG2 xenografts it inhibited STAT3 activation and its target gene expression [302]. GYY4137 has been shown to increase glycolysis, leading to overproduction of lactate, inducing intracellular acidification and cancer cell death [303]. It should be noted that commercial preparations of GYY4137 are sold as a dichloromethane complex, and it has been reported that dichloromethane is metabolized to carbon monoxide (CO) [304, 305] an important gasotransmitter. Since CO is reported to have similar biological effects as H₂S [306–308], it is possible that some of the observed effects of GYY4137 could also be due, at least in part, to a contribution by CO [289].

The concentrations of GYY4137 needed to see any biological/pharmacological effects are rather high. To improve H₂S generation, modifications to the phosphorodithioate core were made resulting in agents, which generate H₂S at different rates (AP67, AP72, AP105, AP106) [289]. Potential anti-cancer activity of these agents is yet to be reported.

6.2.2. H₂S-donating NSAIDs—H₂S-donating NSAIDs are a keen to the NO-releasing NSAIDs described above. ADT-OH, (5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione) the main metabolite of ADT (anethole dithiolethione (Fig 4B) has been used extensively as a H₂S donor with many therapeutic agents including a good number of NSAIDs, reviewed in [81, 309]. H₂S-donating NSAIDs, (HS-aspirin, HS-sulindac, HS-ibuprofen, HS-naproxen, Fig 4C) were shown to have utility as anti-cancer agents [310–315]. Human safety data on some H₂S-donating NSAIDs are beginning to emerge although not yet published (pharmaceutical press releases), underscoring the great potential of these agents against many diseases including cancer. H₂S-donating NSAIDs in general inhibit cell proliferation, enhance cell death by inducing apoptosis, and they block transitions through the cell cycle, predominantly that between G₀/G₁ [310, 311]. The net result being a significant inhibition of cell growth, which presumably, accounts for the ultimate inhibition of carcinogenesis that is observed *in vivo*. HS-aspirin, HS-sulindac, HS-naproxen, and HS-ibuprofen inhibited growth of eleven different cancer cell lines of six different histological subtypes [311].

Another H₂S-donating moiety that has been employed with NSAIDs to produce HS-NSAIDs hybrids is 4-hydroxy benzothiazamide (TBZ, Fig 4D). This moiety has been conjugated with naproxen (ATB-346, Fig 4E) and aspirin (NBS-1121, Fig 4H). In an animal model of synovitis, ATB-346 reduced edema and pain score suggesting possible use in treatment of patients with arthritis [316]. ATB-346 has also shown efficacy against azoxymethane induced colon cancer [313] and was also more effective at preventing intestinal polyp formation than naproxen in the APC^{Min+} mice. In this model, ATB-346 but not naproxen, significantly decreased expression of intestinal cancer-associated signaling molecules, cMyc and β -catenin [315]. ATB-346 also reduced proliferation and increased apoptosis of human melanoma cells, an effect that was modulated by inhibition of NF- κ B activation and inhibition of Akt signaling pathway. Moreover, daily oral dosing of ATB-346 significantly reduced melanoma development *in vivo* [314]. ATB-346 (Table 1) is being developed for the treatment of osteoarthritis, and has completed Phase-I clinical trials in healthy volunteers and is now under Phase-II clinical evaluation.

Recently, a series of H₂S-donating NSAIDs have been reported that utilize not only ADT-OH, or TBZ, but also 1,2-dithiolane (Fig 4J) [317]. Some of these HS-NSAIDs are based on traditional NSAIDs that are nonselective COX inhibitors that have not been reported earlier, some are COX-2 selective, and some are based on acetaminophen. There is limited cell growth inhibition data available for these compounds and none have been evaluated in any animal models of cancer.

In a recent review [105], it was suggested that the H₂S released from H₂S-donating NSAIDs was unlikely to contribute to the overall activity of these class of compounds and that in the studies reported no information was presented on the relative contribution of H₂S *versus* the parent (NSAID) molecule to the anticancer effects. A close examination of the published work shows that indeed such information was available [81, 311, 318]. Structure-activity and reconstitution studies of the individual components of HS-NSAIDs; the parent NSAID and the ADT-OH moiety on the growth properties of HT-29 human colon cancer cells showed that the combination of these two molecules did not reconstitute the potency of the intact HS-NSAIDs. Furthermore, when H₂S release from HS-aspirin was blocked when evaluating the effects of HS-aspirin on cell growth in MDA-MB-231 breast cancer line, the IC₅₀ for cell growth inhibition was increased by about 60-fold, clearly demonstrating a significant contribution by H₂S to cell growth inhibitory effects of HS-aspirin [310].

6.2.2.1. Molecular targets of H₂S-donating NSAIDs in cancer—Apart from effects on cell kinetics (proliferation, cell cycle arrest, apoptosis), which ultimately determine cellular mass, HS-NSAIDs also modulate a number of molecular targets that are important in cancer. These have been thoroughly reviewed elsewhere [18, 81, 309]. These include, NF- κ B [310, 319–322]; ROS [310]; thioredoxin/thioredoxin reductase-1 (Trx/TrxR) [310, 322]; wnt/ β -catenin signaling [323]; and xenobiotic metabolizing enzymes [312].

6.2.3. NOSH-NSAIDs: Dual NO-H₂S-donating hybrids—Based on the chemistry of NO and H₂S, and the structural components of NO-NSAIDs and HS-NSAIDs, my lab postulated that an NSAID possessing moieties that could donate both of these gasotransmitters might be more potent and effective than either one alone [324]. This led to the synthesis and characterization of a new class of anti-inflammatory and anti-cancer compounds, that I have coined NOSH-NSAIDs, of which six have been reported thus far [262, 318, 324]. The structural components of three of these compounds are shown in Fig 4G-I. In terms of GI safety, NBS-1120 [325], AVT-219 [326], and NOSH-sulindac (AVT-18A) [327] were essentially devoid of any GI toxicity. All three compounds exhibited comparable pharmacological properties to their respective parent compound but unlike their native counterparts were GI safe. The antinociceptive activity of NBS-1120 was compared with aspirin in different models of inflammatory pain [328]. Mechanistically, NBS-1120, but not aspirin, was able to reduce the production/release of IL-1 β during carrageenan-induced paw inflammation. Furthermore, NBS-1120, but not aspirin, reduced prostaglandin E₂-induced hyperalgesia, which was prevented by treatment with a ATP-sensitive K_{ATP} blocker (glibenclamide). The enhanced effects of NBS-1120 appear to be due to its ability to reduce the production of pronociceptive cytokines such as IL-1 β and directly block hyperalgesia

caused by a directly acting hyperalgesic mediator in a mechanism dependent on modulation of K_{ATP} channels.

The cell growth inhibitory properties of NBS-1120, NBS-1121, AVT-219, and AVT-18A were evaluated in eleven different human cancer cell lines of six different histological subtypes [325–327]. All four NOSH compounds were extremely effective in inhibiting the growth of these cell lines, however, NBS-1120 was the most potent, with IC_{50} s for cell growth inhibition ranging from 48–280 nM at 24h [324, 326, 327].

Treatment of athymic nude bearing a human colon cancer xenograft with NBS-1120 at a dose of 100mg/kg caused an 85% reduction in tumor growth after 18 days of treatment [262]. Further, in a xenograft mouse model of colon cancer chemoprevention, aspirin at a dose of 50 mg/kg and NBS-1120 at different doses (25–100 mg/kg) inhibited tumor growth and tumor mass with NBS-1120 being ~5-fold more potent than aspirin [325]. Of note was the observation that the stomachs of the aspirin-treated mice exhibited erosions consistent with pre-ulcer development [329]. Thus as a chemo-preventive agent, NBS-1120 appears to be superior to aspirin both in terms of efficacy and safety. In another study comparing the chemotherapeutic efficacy of AVT-219 to that of naproxen in a xenograft mouse model of colon cancer, it was reported that all the mice treated with naproxen died within 2 weeks from what appeared to be severe GI bleeding and damage to the spleen and liver. However, the AVT-219 treated mice did not show any overt signs of toxicity and after 30 days of treatment the AVT-219-treated mice showed a considerable reduction in tumor growth compared with untreated mice which was as a result of increased apoptosis and reduced cell proliferation [326].

6.3. Other H_2S -donating agents of therapeutic interest

Within the mitochondria H_2S appears to have antioxidant properties, modulating mitochondrial cell death pathways and cellular bioenergetics [121, 330, 331]. Mitochondria targeted H_2S donors have been developed by coupling DTT with a phosphonium salt. One of such donors, AP39 ([10-oxo-10-(4-(3-thioxo-3H-1,2-dithiol-5yl) phenoxy)decyl) triphenyl phosphonium bromide], (Fig 5A, Table 1), has shown promising activities [332]. Compared to standard and non-mitochondria-targeted donors such as GYY4137 and ADT-OH, AP39 significantly inhibited the oxidative stress-induced toxicity. It also attenuated the loss of cellular bioenergetics during oxidative stress and protected against oxidative mitochondrial DNA damage. These results suggest that mitochondria targeting is important.

Since H_2S appears to reduce visceral pain [333–335], an H_2S -releasing salt of trimebutine has been developed (GIC-1001, Fig 5B, Table 1). Trimebutine is an opioid antispasmodic that has been used to treat a range of gastrointestinal conditions, including irritable bowel syndrome, for over 40 years. Shown to be safe in a Phase I clinical trial (ClinicalTrials.gov identifier: NCT01738425), this agent is now in Phase II clinical trials as a pre-colonoscopy analgesic (ClinicalTrials.gov identifier: NCT01926444 and NCT02276768).

Several H_2S -based therapeutics target disorders that are characterized by oxidative stress and associated tissue injury [12]. Based on these observations, zerovalent sulfur (SG-1002 Fig 5C, Table 1) is being developed as a medicinal food, with the initial aim of targeting heart

failure [109]. Preclinical studies in relevant animal models of heart failure have confirmed that SG-1002 is effective in decreasing infarct size, improving cardiac function, increasing angiogenesis, decreasing inflammation and downregulating oxidative stress after infarction. A Phase I trial of SG-1002 (ClinicalTrials.gov identifier: NCT02278276) evaluated doses of 200 mg, 400 mg and 800 mg per day in healthy volunteers and reported dose-dependent increases in plasma H₂S levels, and only minor adverse effects. A second study has been designed (ClinicalTrials.gov identifier: NCT01989208) to evaluate the ability of SG-1002 to elevate plasma H₂S levels (in effect, reversing the defects in circulating H₂S) and to reduce markers of oxidative stress and heart failure.

ATB-429 (Fig 4F, Table 1) is an H₂S-releasing derivative of mesalamine and was developed with the notion that the H₂S released from the compound would provide additional anti-inflammatory and ulcer-healing effects to those of mesalamine [336], which is used for treatment of Crohn's disease and ulcerative colitis. In one study, mice with hapten-induced colitis were treated orally twice daily with vehicle, mesalamine or ATB-429 [337]. After 3 days of treatment, there was a significant reduction in the colitis disease activity score and in tissue granulocyte numbers (myeloperoxidase activity), which was not seen with mesalamine.

7. Inhibition CBS or CSE as a strategy to treat cancer

In recent studies it has been shown that CBS was selectively overexpressed in colon cancer, and that H₂S produced by it served to maintain the tumor's cellular bioenergetics and to promote tumor angiogenesis [98, 105]. In these studies the expression of the other two H₂S-producing enzymes (CSE and 3-MST) was not up-regulated in the colorectal cancer cells or tumor tissue. In vitro, shRNA-mediated gene silencing of *CBS* resulted in a significant inhibition of colon cancer cell proliferation; whereas *CSE* silencing did not. Also in colon cancer tumor xenografts, shRNA-mediated knockdown of *CBS* reduced the growth rate [98, 105]. Pharmacological inhibition of CBS with aminooxyacetic acid (AOAA) produced results very similar to gene silencing [98, 105]. In vivo, AOAA inhibited growth of colon can cell xenografts and the growth of patient-derived tumor xenografts [98, 105].

When CBS in an ovarian cancer cell line was silenced by siRNA; that led to inhibition of cell proliferation and metastatic nodule formation [99]. Silencing *CBS* reduced cellular GSH levels, impaired H₂S production, activated p53 and inhibited NF- κ B. There was decreased mitochondrial oxygen consumption and increased ROS production. Collectively these results indicated that CBS was important in regulating the redox balance and metabolism of the ovarian cancer cells promoting tumor growth and metastasis. Human breast cancer (HBC) patient-derived tissue arrays and immunoblot analysis of HBC cells exhibited significantly increased levels of CBS when compared with their normal counterparts [101]. This was associated with increased levels of H₂S and cystathionine (CTH). Silencing of *CBS* in HBC cells caused a significant decrease in the levels of H₂S and CTH. *CBS*-silenced cells exhibited significantly reduced growth in the presence of activated macrophages and in xenograft models. It has also been shown that human prostate cancer tissues and the androgen-dependent prostate cancer cell line LNCaP express high levels of CBS and CSE [100] which were down-regulated by dihydrotestosterone. It should be noted that normal

prostatic epithelial cells (RWPE-1) did not show any CBS/CSE expression and androgen-independent prostate cancer cells (PC-3) expressed very low levels of CSE and no CBS levels [100].

There is very limited data on the functional and differential role of CSE and MST/ACT in cancer. Nevertheless there are some studies that have documented up-regulation of CSE and MST in several tumors. In a recent study it was shown that CSE was overexpressed in both human breast cancer tissues and breast cancer cell lines [338]. CSE knockdown suppressed the proliferation and migration of breast cancer cells while CSE overexpression promoted them. Furthermore, STAT3 expression positively correlated with CSE expression strongly suggesting that STAT3 could regulate CSE transcription. In human melanoma samples CSE expression was highest in primary tumors, decreased in the metastatic lesions and was almost silent in non-lymph node metastases [106]. Overexpression of CSE induced spontaneous apoptosis of human melanoma cells as did diallyl trisulfide a putative H₂S donor; mechanistically this was due to suppression of NF- κ B activity and inhibition of AKT and extracellular signal-regulated kinase pathways. In one report, using human glioblastoma-astrocytoma U-87 MG and neuroblastoma SHSY5Y cell lines, MST expression and activity was reported to be higher compared to CBS and CSE [339], however, no functional determinations were made. Recently it was shown that the Wnt pathway at the transcriptional level regulated CSE gene expression [340] in colon cancer cells. Activating the Wnt pathway increased CSE mRNA and protein levels, while siRNA-mediated silencing of β -catenin decreased CSE mRNA and protein levels. Knockdown of CSE by shRNA or its inhibition by PAG decreased SW480 cell (colon cancer cells that express high levels of β -catenin) proliferation, migration, and tumor growth in a xenograft model.

It should be noted that not all of these enzymatic silencing leads to tumor regression. For example, CBS silencing increased tumor growth in a glioma model. shRNA silencing of *CBS* in U87-MG human glioma cells decreased the latency time to rapid tumor xenograft growth and increased the incidence and volume of intracranial tumors [341]. CBS knockdown increased the expression of HIF2 α and target genes encoding angiopoietin-like 4 (ANGPTL4) and VEGF. HIF2 α knockdown counteracted the stimulatory effect of CBS knockdown on ANGPTL4 and VEGF expression. Furthermore, in human glioma samples, decreased CBS expression was associated with increased ANGPTL4 and VEGF expression. Thus, decreased CBS expression in gliomas may promote tumorigenesis by increasing HIF2 α expression.

8. Induction of iNOS and/or CBS/CSE as a strategy to treat cancer

In some circles this might be viewed as heresy, however, let us examine the data a little more closely. There are reports that L-arginine supplementation can reduce tumor occurrence and development in some tumor models, including chemically induced solid tumors, transplantable solid tumors, and human tumors. For example, colorectal cancer was induced in rats by weekly subcutaneous injections of the colonic procarcinogen 1,2-dimethylhydrazine (DMH) [342]. One group received L-arginine in their of drinking water. The results show that tumor incidence and tumor burden were significantly reduced in

arginine treated rats compared to DMH controls. In an *in vitro* study utilizing SW480 human colon cancer cells, L-arginine administration at similar or higher than physiological concentrations reduced the progression of colon cancer, with iNOS having a role in reducing angiogenesis [343]. Xenograft studies with the same cell line showed that L-arginine supplementation significantly lowered MMP-2, MMP-9 and VEGF receptor levels in tumors whereas tumor NO levels and spleen natural killer (NK) cell activities were higher compared to the control untreated group. Thus L-arginine treatment inhibited the progression of colon cancer in this model by increasing NO levels and consequently enhancing NK cell activity.

In a randomized, double-blind, placebo-controlled protocol consisting of 69 males and 51 females divided into two arms each of 60 patients with colorectal carcinoma (CRC) and 60 patients with colorectal adenoma (CRA), the subjects underwent colonoscopy with biopsy specimens taken from the tumor, paratumor mucosa (within 5 cm of the tumor), and normal mucosa (>10 cm distance from the tumor). Each group was randomly divided further into two groups of 30 patients each, a test group which was administered 30 g (120 mL) of freshly prepared L-arginine orally once a day for 3 days and a control group where the L-arginine was substituted with 5% glucose (120 mL). All subjects with CRC underwent radical surgical resection 3 days after treatment, whereas those with CRA underwent tumor resection by colonoscopy; tissue samples were obtained during the procedures [344]. The results showed that eNOS and nNOS were expressed in the normal and paratumor mucosa; but not in the tumor cells, whereas, the glandular cells in the normal and paratumor mucosa did not express iNOS. The tumor cells showed moderate expression for iNOS, which was significantly increased after treatment. Before treatment, serum NO levels in the patients with CRC were similar to those in the patients with CRA; however, after treatment, the values significantly increased in the CRC group with no significant changes in the CRA group. Thus L-arginine treatment blocked the formation and development of colorectal tumors, this effect correlated with the increased serum NO levels through iNOS induction.

High iNOS expression is associated with favorable prognoses in ovarian [242] and lung cancers [284]. Overexpression of iNOS by gene transfer in prostate and colon cancer cells produced enhanced amounts of NO increasing the sensitivity of these cells to cisplatin-induced cell death [345] or to radiation therapy [346]. In a mouse model of thyroid cancer, iNOS overexpression inhibited tumorigenesis [347]. iNOS expressing pancreatic cancer cells did not produce tumors or metastases in xenograft mouse models due to NO upregulation and apoptosis [348]. Overexpressing iNOS by other means has also produced anti-cancer effects. Microencapsulation of iNOS expressing cells and delivering them to human colon and ovarian cancer xenografts led to increases in apoptosis [15]. Retroviral delivery of iNOS in mice produced slowly progressing tumors and fewer lung metastases than control mice transfected with iNOS-negative retrovirus [349]. Thus, therapeutic overexpression of iNOS within the tumor through gene therapy or other means may be a fruitful approach in treating some cancers.

To the best of my knowledge, I am not aware of any attempts or efforts in trying to induce any of the H₂S-generating enzymes as has been done with iNOS. The focus has been on developing inhibitors of these enzymes. Perhaps at some point in the near future it would be

worthwhile to see whether as in the case of iNOS, induction of CBS/CSE would inhibit tumorigenesis or not, at least in some cases?

8. Concluding remarks and future directions

Given the enormous potential of NO in various aspects of medicine, it is quite surprising that not many NO-releasing agents have reached the marketplace. In the cancer arena, the reasons for this apparent lack of progress are multifactorial but primarily are due to the focus that the scientific community has placed towards chemoprevention rather than chemotherapy. While chemoprevention is ideal, its achievement with drugs is an arduous task, as safety concerns are extremely important and testing for this via clinical trials may prove to be an impossibility. However, chemotherapy of an already developed cancer takes a different dimension with much more options. Case in point is the Phase-I clinical trials with NO-aspirin (NCX-4016) developed by NicOx (Sophia Antipolis, France) which confirmed its GI safety compared to aspirin and its efficacy to inhibit platelet aggregation [350–352]. However, its development as a chemopreventive drug in patients who are at high risk of developing colorectal cancer was terminated due to the putative genotoxicity of a potential metabolite [353]. Although the genotoxicity of this metabolite has not been confirmed by others or for that matter for the intact molecule, nevertheless the development of NCX-4016 as a chemopreventive agent was halted. Had the focus been on chemotreatment as a single agent alone or in combination with established chemotherapeutic agents, the outcome may have been quite different. Another NO-releasing NSAID, NO-naproxen (naproxcinod), which was being evaluated for treatment of knee and hip osteoarthritis [354–358] has completed Phase-III clinical trials. However, concerns by the FDA about its GI and cardiovascular safety for long-term use, did not lead to approval of the NDA application in 2010 without further studies [359].

NO and H₂S appear to have major roles in modulating cancer therapy resistance, and this in itself holds a great deal of promise in improving patient outcomes, thus targeted cancer therapy with NO- and H₂S-based compounds is a feasible proposition. Organ-specific delivery of NO and H₂S is something that appears to be quite achievable in the near future and with that, the possibility of disease-specific NO and H₂S donors. Selective inducers/inhibitors of the various H₂S generating enzymes, such as CBS/CSE, and/or iNOS offers yet another avenue for therapeutic applications and development. However, all of these scenarios/caveats have pros and cons. For example, selective inhibitors of CBS could potentially increase homocysteine levels and thus increase cardiovascular risks. iNOS specific inhibitors could induce immunosuppression. What is abundantly clear is that one size does not fit all and individual cases have to be assessed and evaluated on their own merits, what type of tumor, what is the immune status of the patient, etc.

Lately, most of the science in this arena appears to be directed towards silencing and/or inhibiting the key enzymes that generate NO and H₂S with some dismissing any potential value of inducing these enzymes that may prove useful in certain circumstances. This is reminiscent of the COX-2 era where its expression was deemed central to the carcinogenesis process and hence the inhibition of the eicosanoid pathway by traditional NSAIDs and coxibs was the driving force/mechanism by which these agents prevented tumorigenesis.

Little attention if any was given to the non-COX targets affected by these agents. It took the withdrawal of Vioxx for the scientific community to pause and appreciate a new era beyond COX-2 and its inhibition in cancer [233, 360], déjà vu all over again.

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Abbreviations

1400 W	N-[[3-(aminomethyl)phenyl]methyl]-ethanimidamide
3-MP	3-Mercaptopyruvate
3-MST	3-Mercaptopyruvate sulfurtransferase
ADT-OH	5-(4-hydroxyphenyl)-3H-1, 2-dithiole-3-thione
AG	Aminoguanidine
AOAA	Aminooxyacetic acid
CAT	Cysteine aminotransferase
CBS	Cystathionine β -synthase
cGMP	Cyclic guanosine monophosphate
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CSE	Cystathionine γ -lyase
DAO	D-amino acid oxidase
DEA/NO-aspirin	O ² -(acetylsalicyloyloxymethyl)-1-(N,N-diethylamino)- diazene-1,2-diolate
DeoxyHb	Deoxygenated Hb
DTT	1,2-Dithiole-3-thione
eNOS	Endothelial nitric oxide synthase
ERK	Extracellular signal-regulated kinase
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GSNO	S-nitrosoglutathione
H₂S	Hydrogen sulfide
Hb	Hemoglobin

HIF-1	Hypoxia-inducible factor-1
iNOS	Inducible nitric oxide synthase
IPA/NO-aspirin	O ² -(acetylsalicyloyloxymethyl)-1-(N-isopropylamino)-diazene-1-ium-1,2-diolate
JS-K	O ² -(2,4-Dinitrophenyl)1-[(4-thoxycarbonyl)piperazin-1-yl]diazene-1-ium-1,2-diolate
Keap1	Kelch-like ECH-associated protein 1
L-NAME	N5-[imino(nitroamino)methyl]-L-ornithine methyl ester
L-NMMA	Nitroarginine (N ^G -Monomethyl-L-arginine)
MAPK	Mitogen-activated protein kinase
Mb	Myoglobin
MetHb	Methemoglobin
MPO	Myeloperoxidase
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
Nrf2	Nuclear factor-like 2
NSAIDs	Nonsteroidal anti-inflammatory drugs
PDE5A	Phosphodiesterase 5A
PKG1α	Protein kinase G1 α
PLP	Pyridoxal 5'-phosphate
PPG or PAG	DL-propargylglycine
PTEN	Phosphatase and tensin homolog
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RSNO	S-nitrosothiols
RSSH	Persulfides
sGC	Soluble guanylate cyclase

SNAP	<i>S</i> -Nitroso- <i>N</i> -acetyl-penicillamine
SNP	Sodium nitroprusside
STAT3	Signal transducer and activator of transcription 3
TBZ	4-hydroxy benzothiazamide
TRPA1	Transient receptor potential ankyrin 1
VDCC	Voltage-dependent Ca ²⁺ channels
VEGF	Vascular endothelial growth factor

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Figure 1.

Biosynthesis of nitric oxide and hydrogen sulfide. NO is produced by three nitric oxide synthase (NOS) isoforms: neuronal, endothelial, and inducible (nNOS, eNOS, and iNOS) that catalyze the oxidation of L-arginine to L-citrulline. NO is also produced through reduction of nitrite/nitrate under low oxygen conditions. H₂S is generated from oxidation of the substrates L- homocysteine, cystathionine, L-cysteine and 3-mercaptopyruvate through the enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST). α-ketobutyrate, lanthionine, L-serine and pyruvate are the secondary products formed. Alternatively, production of H₂S occurs non-enzymatically from various storage forms of sulfur like thiosulfate, thiocystine and sulfite. NO reacts with the active site of soluble guanylate cyclase (sGC) and produces cyclic GMP leading to vasorelaxation. Whereas H₂S raises cGMP levels through inhibition of phosphodiesterase 5A (PDE5A) an enzyme that catabolizes it. NO can affect cellular proteins by producing peroxynitrite which in turn can interact with cysteine residues to form S-nitrosothiols (RSNO). H₂S can also interact with sulfhydryl group of cysteines and proteins to form persulfides (R-SSH).

Figure 2.

NO-H₂S cross-talk in the vascular tissue. H₂S decreases the expression level of NO-synthase (eNOS) in a cell-free system [128], while NO can either inhibit CBS activity in a cell free system [127] or increases the expression levels of CSE and CBS [11] to increase H₂S synthesis. H₂S activates eNOS by an Akt-dependent phosphorylation of the protein [132]. NO can interact with H₂S to form HSNO [138] and H₂S_n [139]; H₂S can interact with NO₂⁻ [124] or with RSNO [147, 148] to produce NO. H₂S decreases the sensitivity of the cGMP pathways and also it modifies K_{Ca} channels to decrease their sensitivity to NO. Solid arrows (blue) indicate stimulatory, while T-shaped arrows (red) indicate inhibitory inputs.

Figure 3.

Chemical structures of selected NO-donating compounds. (A) NO-NSAIDs. (B) Activation of diazeniumdiolate prodrugs to release NO or HNO. (C) Structures of IPA/NO-aspirin and DEA/NO-aspirin. (D) JS-K; (E) PABA/NO and (F) RRx-001.

Figure 4.

Synthetic H₂S-donating compounds. (A) GYY4137 a slow H₂S generating compound. (B) ADT (anethole dithiolethione); ADT-OH, (5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione, releases H₂S); (C) various H₂S-donating NSAIDs; (D) TBZ (4-hydroxy benzothiazamide, releases H₂S); (E) ATB-346, H₂S-donating naproxen; (F) ATB-429, H₂S-donating mesalamine; (G and H) NBS-1120 and NBS-1121, NOSH-aspirin, dual NO- and H₂S-donating hybrids of aspirin; (I) AVT-219, NOSH-naproxen, dual NO- and H₂S-donating hybrid of naproxen; (J) 3-butyl-1,2-dithiolane, releases H₂S.

Figure 5.
(A) AP39, a mitochondrial-targeting H₂S-donor; (B) GIC-1001, trimebutine salt; (C) SG-1002, polyvalent sulfur.

Table 1**H₂S-based therapeutics in development**

Compound	Clinical indication / Derivative	Status
ATB-346	Osteoarthritis / Naproxen	Phase-II
ATB-352	Acute pain / Ketoprofen	Preclinical
ATB-340	Thrombosis / Aspirin	Preclinical
ATB-429	Ulcerative colitis / Mesalamine	Preclinical
NBS-1120	Cancer, inflammation, pain / Aspirin	Preclinical
AVT-219	Osteoarthritis, pain / Naproxen	Preclinical
GIC-1001	Colonic pain / Trimebutine salt	Phase-II [*]
SG-1001	Oxidative stress, heart failure / Polyvalent sulfur	Phase-II [†]
GY4137	Cancer, inflammation, hypertension	Unknown
AP39	Inflammation, oxidative stress	Preclinical

^{*} ClinicalTrials.gov identifiers: NCT01926444 and NCT02276768.

[†] ClinicalTrials.gov identifier: NCT01989208.