

Cognitive Vitality Reports[®] are reports written by neuroscientists at the Alzheimer's Drug Discovery Foundation (ADDF). These scientific reports include analysis of drugs, drugs-in-development, drug targets, supplements, nutraceuticals, food/drink, non-pharmacologic interventions, and risk factors. Neuroscientists evaluate the potential benefit (or harm) for brain health, as well as for age-related health concerns that can affect brain health (e.g., cardiovascular diseases, cancers, diabetes/metabolic syndrome). In addition, these reports include evaluation of safety data, from clinical trials if available, and from preclinical models.

Hydrogen sulfide (H₂S) slow-releasing donors

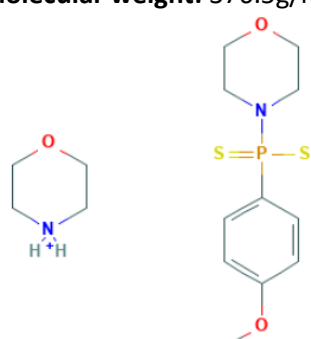
Evidence Summary

Some evidence suggest slow-releasing hydrogen sulfide donors may have beneficial effects in age-related diseases, especially cardiovascular disease.

Neuroprotective Benefit: A rationale exists for the use of H₂S donor compounds in Alzheimer's, though finding the correct dosing strategy may be difficult.

Aging and related health concerns: H₂S may be reduced in age-related diseases, and many preclinical studies suggest benefits on the use of H₂S donor molecules, especially for cardiovascular disease.

Safety: High levels of H₂S may be toxic to cells; however, the evidence does not suggest that slow-releasing H₂S donor molecules would significantly increase plasma levels of H₂S.

<p>Availability: Not currently available, in preclinical development</p>	<p>Dose: Animal studies have generally used ~50 mg/kg/day. However, the dosage could be titrated based on levels of H₂S in the plasma.</p>	<p>Molecular Formula: C₁₅H₂₅N₂O₃PS₂ Molecular weight: 376.5g/mol</p>
<p>Half-life: In cell culture studies, GYY4137 can release low concentration of H₂S over seven days.</p>	<p>BBB: Unknown; though H₂S is a gaseous molecule and could presumably get into the brain</p>	
<p>Clinical trials: One trial ongoing for STEMI</p>	<p>Observational studies: Several studies have been conducted on plasma H₂S levels and different pathologies</p>	<p>GY4137 Source: Pubchem</p>

What is it?

Hydrogen sulfide (H₂S) is a gastrotransmitter, an endogenous gaseous signaling molecule in the same category as nitric oxide and carbon monoxide. It is produced from L-cysteine primarily by the enzymes cystathionine γ -lyase (CSE) and cystathionine β -synthase (CBS) and by 3-mercaptopyruvate transferase (3-MST) in the presence of 3-mercaptopyruvate. CSE is primarily expressed in the cardiovascular system while CBS is primarily expressed in the nervous system. 3-MST has been reported to be expressed in the endothelium of the thoracic aorta. While toxic at high doses, increasing H₂S via slow-releasing H₂S donor drugs may have beneficial effects involved with aging including relaxation of blood vessels, antioxidant and anti-inflammatory effects, activation of SIRT1, and increased expression of Klotho via inhibition of angiotensin II activity ([Predmore et al, 2010](#)).

There are several H₂S donor molecules in use. Sodium hydrogen sulfide (NaHS) is an H₂S tool compound. However, it releases its hydrogen sulfide moiety very fast in a short-lived bolus and may generate toxic concentrations of H₂S in cells. Newer, novel, H₂S donor drugs release hydrogen sulfide more slowly. For instance, in one cell culture study, incubation of GYY4137, a slow-releasing H₂S molecule, led to a sustained generation of low concentrations of H₂S (<20 μ M) over seven days while incubation with NaHS generated a high concentration (up to 400 μ M) that persisted for only one hour ([Lee et al, 2011](#)). This report focuses primarily on slow-releasing H₂S donor molecules (GY4137, AP39, FW1256).

Neuroprotective benefit: A rationale exists for the use of H₂S donor compounds in Alzheimer's, though finding the correct dosing strategy may be difficult.

Types of evidence:

- One study of the association of plasma H₂S and Alzheimer's disease in humans
- One study on H₂S levels in postmortem tissue from Alzheimer's patients
- Seven preclinical cell culture and animal studies in Alzheimer's disease
- One preclinical study in Parkinson's disease

Human research to suggest prevention of dementia, prevention of decline, or improved cognitive function?

None

Human research to suggest benefits to patients with dementia:

[Liu et al \(2008\)](#) (article in Chinese) measured plasma H₂S levels in 31 Alzheimer's patients, 28 vascular dementia (VD) patients, 20 cerebrovascular disease (CVD) patients, and 23 normal controls. They reported a reduction in plasma H₂S levels in AD, VD, and CVD patients compared to controls. Plasma levels of H₂S were negatively correlated with disease severity. Levels of H₂S from postmortem tissue of Alzheimer's patients (AD n=13, control n=6) were reduced compared to control patients. Levels of CBS were not reduced suggesting that the activity of H₂S generating enzymes may decline with age ([Eto et al, 2002](#)).

Mechanisms of action for neuroprotection identified from laboratory and clinical research

Alzheimer's: Potential Benefit

In cell culture studies, a mitochondrial-targeted slow-releasing H₂S donor, AP39, improved cell survival and mitochondrial respiration at low doses (25-100nM) but reduced cell survival and mitochondrial respiration at higher doses (250nM). AP39 also improved mitochondrial respiration at low doses in neurons from an Alzheimer's animal model (APP/PS1). Treatment with AP39 (100nM/kg) in an Alzheimer's animal model starting at six weeks improved cognition, reduced brain atrophy, and reduced levels of amyloid ([Zhao et al, 2015](#)).

In another mouse model of Alzheimer's disease (A β hippocampal injections), intracerebroventricular (i.c.v.) coadministration of A β with the hydrogen sulfide donor, NaHS, reduced hippocampal cell death, reduced amyloid accumulation, and reduced inflammation (astrogliosis, microgliosis, and levels of IL-1 β and TNF- α) ([Xuan et al, 2012](#)). NaHS has also improved cognition, reduced amyloid, prevented

neurodegeneration, suppressed neuroinflammation, and/or increased autophagic flux in several other Alzheimer's or aged animal models ([Liu et al, 2016](#); [Chen et al, 2019](#); [He et al, 2014](#); [Zhan et al, 2018](#); [Liu et al, 2015](#)).

Parkinson's disease

In a mouse model of Parkinson's disease (acute MPTP injection), administration of GYY4137 (50 mg/kg/day) for three days before and two weeks after MPTP improved behavioral performance in mice (i.e. rotarod, balance beam, and grid walking). These effects were less apparent at lower doses (12.5 and 25 mg/kg/day). In addition, GYY4137 attenuated the loss of dopaminergic neurons in the substantia nigra and reduced oxidative stress ([Hou et al, 2017](#)).

APOE4 Interactions:

None reported

Aging and related health concerns: H₂S may be reduced in age-related diseases, and many preclinical studies suggest benefits on the use of H₂S donor molecules, especially for cardiovascular disease.

Types of evidence:

- Five studies on serum levels of H₂S and age, cardiovascular disease, and diabetes
- One study on levels in atherosclerotic plaques
- Six preclinical studies in aging models
- 17 preclinical studies in cardiovascular disease models
- One preclinical study and one review in peripheral neuropathy models
- One preclinical study and one review in diabetes models
- Two preclinical studies in inflammation
- One preclinical study in cancer

Lifespan: Potential Benefit

Levels of serum H₂S did not differ with age in healthy subjects in their 50s, 60s, or 70s (levels were ~35µmol/L in each age group) ([Chen et al, 2005](#)).

[Wu et al \(2017\)](#) reported that levels of H₂S were reduced in the heart, liver, and kidney in a mouse model of aging (D-galactose-induced senescent mice). Treatment with an H₂S donor molecule (NaHS, up to 100µmol/kg/day) over two months increased levels of H₂S in tissues and reversed the aging



phenotype in these mice (including reducing senescent cells, increasing cell proliferation, and increasing levels of antioxidant proteins). NaHS treatment in aged mice also reduced kidney fibrosis and kidney inflammation ([Lee et al, 2018](#)).

In *C. elegans*, genetic reduction of 3-MST (but not CSE) reduced lifespan and healthspan. This effect was reversed after treatment with a slow-releasing H₂S donor, GYY4137. GYY4137 also increased lifespan in wild-type *C. elegans*. It was hypothesized that the lifespan-promoting effects of this molecule were due to its anti-oxidant properties ([Qabazard et al, 2014](#); [Qabazard et al, 2013](#)). Other H₂S donor drugs (such as FW1256) have also extended lifespan in wild type *C. elegans* (up to 51%) and in a genetic *C. elegans* model of caloric restriction (the *eat-2* mutant model) by up to 18.6% though only when treated throughout the worms' lifespan, not when given at the adult stage only. Interestingly, FW1256 increased basal mitochondrial respiration rate, reduced ATP levels, and increased DNA damage suggesting that H₂S donor molecules may have complex effects in animal models ([Ng et al, 2020](#)).

In an endothelial cell culture assay, GYY4137 and AP39 both reduced senescent cells by ~50% compared to control conditions. Neither had an effect on telomere length or the extent of DNA damage. They increased the expression of IL8 but had no effect on the expression of pro-inflammatory SASP factors ([Latorre et al, 2018](#)).

Cardiovascular disease: Potential Benefit

It was reported that plasma levels of H₂S were decreased in patients with acute coronary syndrome compared to patients with stable angina pectoris or control patients ([Lao et al, 2015](#)). Plasma levels of H₂S were also negatively correlated with heart function in patients with chronic kidney disease ([Kuang et al, 2018](#)). In addition, levels of H₂S in human atherosclerotic plaques and in mice with diabetic cardiomyopathy were reduced ([Zhang et al, 2018](#)).

On the other hand, another study reported increased levels of plasma-free H₂S in patients with coronary artery disease or peripheral arterial disease compared to control patients. The authors suggest that these conflicting results could be due to the different assays to measure H₂S ([Peter et al, 2013](#)).

AP39

In a mouse model of ischemia/reperfusion (I/R), treatment with the mitochondrial-targeted slow-releasing H₂S donor AP39 prior to reperfusion (0.01, 0.1, and 1 μmol/kg) dose-dependently reduced infarct size. These effects were independent of PI3K/Akt and eNOS signaling ([Karwi et al, 2016](#)). In anaesthetized wild type and nitric oxide deficient rats, transient treatment with AP39 reduced blood



pressure, decreased heart rate, and improved pulse wave velocity (a measure of arterial stiffness) ([Tomasova et al, 2014](#)). In mice subjected to cardiac arrest and subsequent cardiopulmonary resuscitation (CPR), treatment with AP39 either before or right after CPR improved neurological function and ten-day survival rate ([Ikeda et al, 2015](#)).

GY4137

In a mouse model of myocardial infarction, two- and seven-day treatment with GYY4137 preserved heart size and function. In addition, it prevented cardiac fibrosis and increased blood vessel density in the infarct area ([Lilyanna et al, 2015](#)). In another mouse model of myocardial infarction, GYY4137 was administered two hours after surgery for 28 days in both WT and CSE^{-/-} mice. In both animals, the infarct size was reduced, and cardiac function was improved ([Ellmers et al, 2020](#)).

Rats were treated with GYY4137 (12.5, 25, or 50 mg/kg/day) over seven days and subjected to myocardial I/R injury. Treatment with GYY4137 reduced infarct size and improved cardiac function. It also increased plasma levels of H₂S and CSE activity, reduced markers of oxidative stress (malondialdehyde – MDA – and myeloperoxidase levels) in the serum and reduced the number of apoptotic cells in the heart ([Meng et al, 2015](#)). In another rat model of myocardial I/R, treatment with GYY4137 (266 μmol/kg) ten minutes prior to reperfusion reduced infarct size by 47%. Co-administration with L-NAME or LY294002 attenuated these beneficial effects suggesting that GYY4137 acted through the PI3K/Akt and NO signaling pathways in this model ([Karwi et al, 2016](#)). Similar results were observed with GYY4137 pretreatment in diabetic mice (STZ injection) subjected to I/R. Infarct size and the number of apoptotic cells were reduced (though there was no change in plasma glucose levels). The researchers also observed an increase in antioxidant enzymes (SOD and Nrf2) and a reduction in MDA levels ([Qiu et al, 2018](#)).

In a mouse model of atherosclerosis (ApoE^{-/-}), eight-week treatment with GYY4137 reduced atherosclerotic plaque size and improved plaque stability. It also improved lipid levels (reduced LDL-c and increased HDL-c) and improved levels of inflammation (reduced IL-6, TNF-α; increased IL-10) ([Zheng et al, 2019](#)). Similar results were reported in another mouse model of atherosclerosis with the addition of partial restoration of aortic endothelium-dependent relaxation ([Liu et al, 2013](#)). In a mouse model of diabetes-accelerated atherosclerosis (Ldlr^{-/-} mice with STZ-injection and high fat diet), treatment with GYY4137 reduced aortic plaque formation and the expression of inflammatory molecules (ICAM1, VCAM1, IL-6, TNF-α, IL-1β, and MCP1). The proposed mechanism was through a reduction in NLRP3 expression ([Zheng et al, 2020](#)). Another study of diabetes-induced accelerated atherosclerosis showed similar effects with GYY4137 treatment and suggested that the Nrf2 pathway was required for the

beneficial effects ([Meng et al, 2016](#)). In a mouse model of atherosclerosis with concomitant hyperhomocysteinemia (mice were treated with L-methionine in the drinking water), treatment with GYY4137 (3.6 mg/kg over 16 weeks) reduced plaque size, improved lipid parameters (reduced LDL-c, increased HDL-c), reduced macrophage infiltration into the plaque area, and reduced serum homocysteine levels. In addition, GY4137 increased the catalytic activity of the H₂S enzyme, CSE ([Fan et al, 2019](#)).

In spontaneously hypertensive rats, treatment with GYY4137 (10-50 mg/kg/day) over four weeks reduced systolic blood pressure and cardiac fibrosis. In cell culture studies, it reduced angiotensin II-induced cardiac fibroblast proliferation ([Meng et al, 2014](#)). In another study in spontaneously hypertensive rats, treatment with GYY4137 (133 μM/kg/day) over 14 days normalized blood pressure, increased endothelial-dependent relaxation of blood vessels, and reduced oxidative stress (measured by increased SOD and reduced MDA) ([Zhu et al, 2021](#)). Interestingly, however, in anesthetized rats, Na₂S, but not GYY4137, reduced blood pressure over several minutes. Cell culture studies suggested that GYY4137 was stable at physiological pH but released H₂S at a low pH. The authors suggest this may be the reason for the lack of effect in this particular model ([Drapala et al, 2017](#)). This exhibits the difficulty in the use of H₂S donors as a treatment for particular diseases, as finding the correct doses may be difficult.

In cell culture studies with human valvular interstitial cells, treatment with H₂S donor compounds (including GYY4137) prevented calcification of cells in response to phosphate treatment ([Sikura et al, 2019](#)).

Peripheral neuropathy: Insufficient evidence based on rodent studies

In a streptozotocin (STZ)-induced mouse model of peripheral diabetic neuropathy, treatment with GYY4137 (50mg/kg) over four weeks improved allodynia and mechanical and thermal hyperalgesia. In addition, it reduced microgliosis and inflammation and prevented neuronal loss in the spinal cord. It had no effect on hyperglycemia compared to untreated animals. ([Shayea et al, 2020](#)). Some preclinical studies suggest that H₂S has pronociceptive properties; however, it is unclear whether administration of a slow H₂S donor would have any effects on neuropathic pain ([Velasco-Xolalpa et al, 2013](#)).

Diabetes: Insufficient/Mixed Evidence

Evidence suggests that H₂S synthesis is impaired in diabetic patients, and plasma levels of H₂S are reduced in diabetic patients. Evidence is mixed whether administration of hydrogen sulfide donors (such as NaHS) is able to improve glucose tolerance in diabetes ([Piragine and Calderone, 2020](#)). In mice fed

normal chow, GYY4137 increased insulin resistance, while in an obesity model (mice fed a high fat diet), GYY4137 increased insulin sensitivity ([Geng et al, 2013](#)).

Inflammation: Potential Benefit in rodents

In an LPS mouse model of inflammation, treatment with FW1256 (100mg/kg) one hour prior to LPS injection reduced levels of inflammation in the plasma (IL-1 β , TNF α , PGE $_2$) ([Huang et al, 2016](#)).

In another mouse model of inflammation (injection of monosodium urate crystals into the peritoneum), pretreatment of GYY4137 reduced the levels of inflammatory cytokines (IL-6 and MCP-1). Cell culture studies suggested that GYY4137 could reduce NLRP3 activation in response to monosodium urate crystal exposure ([Castelblanco et al, 2018](#)).

Cancer: Insufficient Evidence

Cell culture studies suggested that GYY4137 reduced survival of seven different cancer cell lines (HeLa, HCT-116, Hep G2, HL-60, MCF-7, MV4-11, and U2OS) in a concentration-dependent manner while having no effect on normal human lung fibroblasts. This effect required the sulfur moiety, as a structural analog (ZYJ1122) lacking sulfur had no effect on the cancer cell lines. Mechanistic studies suggested that GYY4137 possessed both pro-apoptotic effects and caused cell-cycle arrest in cancer cells. *In vivo*, GYY4137 (100-300 mg/kg/day) reduced tumor growth in two mice xenograft models using HL-60 and MV4-11 cells (both leukemia models) over 14 days. Previous studies reported mixed results regarding the use of H $_2$ S donors on cancer cell lines. However, these studies all used sulfide salts (e.g. NaHS) which generate large amounts of H $_2$ S over a short period of time (e.g. one hour) while GYY4137 is capable of a sustained H $_2$ S release (e.g. seven days) ([Lee et al, 2011](#)).

Safety: High levels of H $_2$ S may be toxic to cells; however, the evidence does not suggest that slow-releasing H $_2$ S donor molecules would significantly increase plasma levels of H $_2$ S.

Types of evidence:

- Many studies in preclinical cell culture and animal models

There is no safety data in humans on the use of slow-release H $_2$ S donors. Preclinical studies do not suggest toxicity with the use of H $_2$ S donors, though cell culture studies suggest that at high doses, H $_2$ S donors may be toxic (e.g. [Zhao et al, 2015](#)).



Drug interactions:

Although there is not currently data on drug interactions, presumably H₂S donors would interact with each other and potentially with high sulfur containing foods or supplements (e.g. broccoli or garlic).

Sources and dosing:

None of the slow-releasing H₂S donor molecules are commercially available. Some foods contain sulfur molecules, though it is not clear how much they would increase systemic H₂S. Animal studies have generally used ~50 mg/kg/day for GYY4137. However, the dosage of donor molecules could be titrated based on levels of H₂S in the plasma.

Research underway:

One clinical study is examining the effects of an H₂S donor (sodium thiosulfate) in patients with ST-segment elevation myocardial infarction (STEMI) ([NCT02899364](#)). No studies are ongoing for any of the H₂S donors per NIH Reporter.

Search terms:

- hydrogen sulfide + aging, peripheral neuropathy, alzheimer, cardiovascular, apoe4
- AP39
- GY4137
- FW1256
- RT101
- AP123
- FW1251

Websites visited:

- Clinicaltrials.gov
- Pubmed
- Longecity

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