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Evaluation of thioamides, thiolactams and thioureas as hydrogen sulfide (H_2S) donors for lowering blood pressure

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ABSTRACT

Hydrogen sulfide (H_2S) is a biologically important gaseous molecule that exhibits promising protective effects against a variety of pathological processes. For example, it was recognized as a blood pressure lowering agent. Aligned with the need for easily modifiable platforms for the H_2S supply, we report here the preparation and the H_2S release kinetics from a series of structurally diversified thioamides, thiolactams and thioureas. Three different thionation methods based on the usage of a phosphorus pentasulfide and Lawesson reagent were applied to prepare the target thioamides and thiolactams. Furthermore, obtained H_2S donors were evaluated both in *in vivo* and *in vitro* studies. The kinetic parameters of the liberating H_2S was determined and compared with NaHS and GYY4137 using two different detection technics i.e.; fluorescence labeling 7-azido-4-methyl-2*H*-chromen-2-one and 5,5'-dithiobis (2-nitrobenzoic acid), sulfhydryl probe, also known as the Ellman's reagent. We have proved that the amount of releasing H_2S from these compounds is controllable through structural modifications. Finally, the present study shows a hypotensive response to an intravenous administration of the developed donors in the anesthetized rats.

1. Introduction

Hydrogen sulfide (H_2S) at high concentrations is an offensive-smelling and toxic gas with a high solubility in water. However, pioneering work of Kimura's group revealed that at very low concentrations H_2S plays a role of biological mediator [1]. It is believed that H_2S in living organisms is responsible for the regulation of several physiologically relevant processes e.g. vasorelaxation, hormone secretion, apoptosis and multicellular events like neuro modulation or inflammatory responses [2]. Due to this reason a number of H_2S releasing agents have been developed and validated in various biochemical studies [3]. Among them, those related to H_2S-mediated vasodilator effects have been paid a great attention [4]. In nature, H_2S is formed during an anaerobic bacterial digestion of the organic compounds. Moreover, H_2S is produced in the colon by the commensal bacteria by the reduction of alimentary sulfates [5]. Under the biological conditions hydrogen sulfide is delivered enzymatically in metabolic pathways from L-cysteine by cystathionine β-synthase and cystathionine γ-lyase, from D-cysteine *via* mercaptopyruvate sulfurtransferase (MPST) with D-amino acid oxidase, and from 3-mercaptopyruvate by MPST with cysteine aminotransferase [6]. Naturally occuring and rich source of the sulfur-based organic compounds is garlic [7]. Recent studies suggest that at least some beneficial effects of garlic are due to H_2S production. So far, the best characterized compound from garlic is allicin (diallyl thiosulfinate), which quickly decomposes into several compounds such as diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS). Although natural H_2S donors may be attractive options for *in vivo* studies due to lack of toxicity, they unveil problems that accompany many synthetic donors [8,9]. However, there are some relevant limitations to the use of these compounds: no susceptibility to chemical transformations, poor water solubility, creation of various byproducts after H_2S release [10]. The most common class of H_2S donors employed in biological studies are the inorganic sulfide salts such as sodium hydrosulfide (NaHS) and sodium sulfide (Na_2S). Generally, sulfide salts are considered as a short-lasting H_2S donors as they release H_2S rapidly [11–13]. Such uncontrollable and fast H_2S release may exert toxic actions in living systems. Moreover, these compounds release H_2S spontaneously already in time of solution preparation. Such H_2S loss from aqueous solutions makes substantial problems with precisely controlled H_2S dose administration what finally may affect biological outcome.

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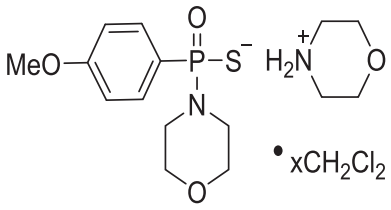


Fig. 1. GYY4137 dichloromethane complex.

Additionally the fact that sulfide salts are hygroscopic may be a source of error in calculations the exact H_2S concentration of dose to be applied to the biologic system [14,15]. Another problem often raised with commercial sulfide salts, especially NaHS is that almost always they contain some amount of undefined impurities [16]. Due to these inconveniences organic molecules which act as H_2S agents were developed and used. Thus far various types of organic donors like; dithiolthiones, thiobenzamides, thiocarbamates, dithioesters, and thionoesters, which release H_2S *via* different mechanisms have been reported [17], e.g. hydrolysis [18], cellular thiol activation [19], and photolysis [20], The most widely studied and characterized synthetic H_2S donor is GYY4137 (morpholin-4-ium 4-methoxyphenyl(morpholino) phosphinodithioate) complex (Fig. 1). Although, GYY4137 is a water-soluble derivative of Lawessons reagent (LR), which releases H_2S *via* hydrolysis, the rate of H_2S generation firmly depends on pH and turn to be very slow at physiological pH [21], Recently, we have shown that the amount of the released H_2S from GYY4137 (140 μM) at pH 7.4 in aqueous solution is < 2 μM /2,3 μmol/90 min/13 h [22], Additionally, the GYY4137 suffers from several other inconveniences. First, it is often prepared as a dichloromethane complex (ratio 3:1), which is residual from crystallization. Dichloromethane is metabolized to carbon oxide which has biological impact similar to H_2S, therefore some effects attributed to GYY4137-derived H_2S may come from CO [23]. Finally, it is most likely that reported biological effects observed after administration of GYY4137 are not H_2S-dependent but can be caused by GYY4137 itself or products of its metabolism after H_2S release (Zheng et al., 2015) [24]. Despite the number of literature data regarding H_2S donors only a few show the impact on the vascular system provided from the *in vivo* studies.

Regardless of the significant progress in the field of H_2S donor chemistry, there is still a lack of compounds that would meet all the requirements for ideal H_2S donor e.g. water-solubility and generation only innocuous (if any/or not at all) byproducts. Last but not least, it should also have well-defined release mechanism. Among of the organic H_2S donors, thio-analogs of amides and urea have long attracted more and more attention due to their practical application as the precursors for the synthesis of various organic compounds [25–28]. They are also an important class of bioactive molecules in the field of pharmaceutical industry [29–34].

The fact that thioacetamide, one of the simplest organosulfur compound release H_2S by hydrolysis has been known for over a century [35,36]. As so far, multiple methods for thioamide and thiourea derivatives synthesis have been reported, using various reagents and diverse reaction conditions [37–44]. Encouraged by the studies revealing biological activity of thioamides and thioureas [28–33], we envisioned those compounds as a valuable substructures for the design of sufficient H_2S donors. We assumed also that the type of substituents on the nitrogen atom of amino group of target compounds may provide donors with the desired properties in controlled H_2S release. In this work we designed and synthesized the series of thioamides, thiolactames and thioures derivatives. Then, the newly prepared compounds were evaluated for their H_2S-releasing properties using two independent approaches i.e. fluorescence and DTNB methods. Finally, the selected compounds were submitted to experiments aimed to evaluate their hemodynamic effect on normotensive in rats.

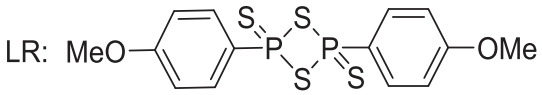


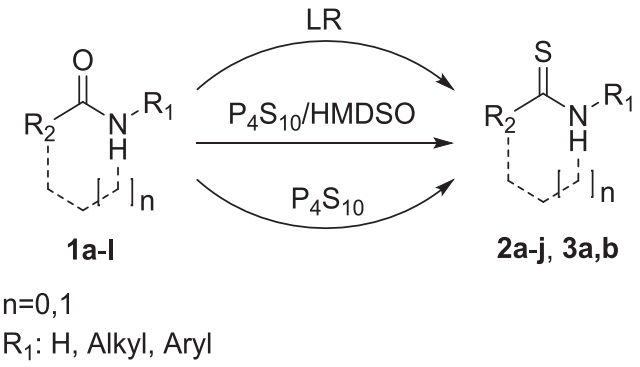
Fig. 2. Lawesson's reagent (LR).

2. Results

The concept of thionation, i.e. the conversion of the carbonyl group into thiocarbonyl by exchange of an oxygen atom for a sulfur atom is the most commonly used procedure for the preparation of organosulfur compounds. This transformation can be carried out with several commercially available reagents [45]. Among them, a phosphorus pentasulfide (P_4S_{10}) and the 2,4-bis(4-methoxyphenyl)-1,3-dithiaphosphetane 2,4-disulfide, also known as the Lawesson's reagent, are the most popular (Fig. 2) [46–51]. Despite the many advantages of using P_4S_{10} as a thionating reagent, LR is the common choice in organic synthesis. As long as a number of reported protocols regarding LR demonstrate the utility of this reagent, only several of them concomitantly highlighted its limitations. The major difficulties of LR application are byproducts derived from the reagent itself, which cannot be removed by any extractive procedure and must be separated by the time consuming chromatographic techniques. Such tedious separation protocol of the desired product from the LR byproducts can be cumbersome or even impossible, what limits the reagent utility [52,53].

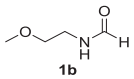
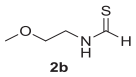
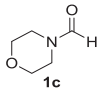
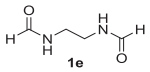
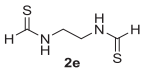
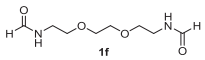
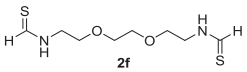
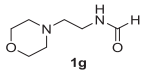
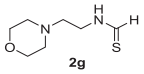
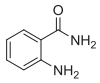
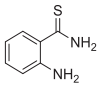
Regardless of the high popularity of the Lawesson's reagent as a thionating agent there are several reports refining applicability of the phosphorus pentasulfide by the addition of hexamethyldisiloxane (HMDSO). It was shown that supplementation of P_4S_{10} with HMDSO dramatically increases thionation rates. Moreover, the application of P_4S_{10}/HMDSO system requires simple extractive workup to remove derived byproducts from the final reaction mixture [54,55]. Due to the lack of generality and potential problems with the product isolation, both LR and P_4S_{10} were engaged in the synthesis of the target thioamides **2** and thiolactams **3** (Scheme 1). A comparative results obtained under different reaction conditions were shown in Table 1.

Thioformamide (**2a**), *N*-(2-methoxyethyl)methanethioamide (**2b**) 4-thioformylmorpholine (**2c**) and *N*-methylthioformamide (**2d**) were provided from the corresponding formamide derivatives **1a-j** using P_4S_{10} in diethyl ether with the yields ranging from 75 to 86% [56], In addition, the thionation of selected amides **1f-g** was conducted under different conditions using P_4S_{10} alone, P_4S_{10}/HMDSO system and LR in three different organic solvents; diethyl ether, DCM and THF (Table 1, entries 5–7). In all cases the application of P_4S_{10}/HMDSO or LR provided corresponding thioamides **2f-g** with higher yield than with P_4S_{10} alone what remains in agreement with the literature data [57]. The reaction of acetamide **1h** with LR gave desired product **2h** in 93% yield within three hours (Table 1, entry 8). The influence of the used solvent on the reaction performance was negligible. As shown in Table 1 the reaction of aromatic amides **1i** and **1j** (entries 9–10) with LR afforded



Scheme 1. Synthesis of thioamides **2a-j** and thiolactams **3a-b**.

Table 1
Thionation of amides and lactams under various conditions.

Entry	Amide/Lactam	Thionation reagent	Solvent	T [°C]	Time [h]	Product	Yield [%][a]	log *P*[b]
1	 **1a**	P_4S_{10}	Et_2O	0 → 20	3	 **2a**	86	−0.33
2	 **1b**	P_4S_{10}	Et_2O	0 → 20	3	 **2b**	75	0.02
3	 **1c**	P_4S_{10}	Et_2O	0 → 20	3	 **2c**	80	0.81
4	 **1d**	P_4S_{10}	Et_2O	0 → 20	3	 **2d**	82	0.01
5	 **1e**	P_4S_{10}	Et_2O	0 → 20	24	 **2e**	75	0.10
		P_4S_{10}/HMDSO	DCM	60	24		86	
		LR	THF	20	24		87	
6	 **1f**	P_4S_{10}	Et_2O	0 → 20	24	 **2f**	70	−0.25
		P_4S_{10}/HMDSO	DCM	60	24		84	
		LR	THF	20	24		80	
7	 **1g**	P_4S_{10}	Et_2O	0 → 20	24	 **2g**	78	−0.23
		P_4S_{10}/HMDSO	DCM	60	24		90	
		LR	THF	20	24		85	
8	 **1h**	LR	THF	20	3	 **2h**	93	−0.26
			DCM				89	
9	 **1i**	LR	THF	20	24	 **2i**	90	1.76
			DCM				88	
10	 **1j**	LR	THF	20	24	 **2j**	89	0.99
			DCM				85	
11	 **1k**	LR	DCM	20	2	 **3a**	93	−0.58
12	 **1l**	LR	DCM	20	2	 **3b**	93	−0.02

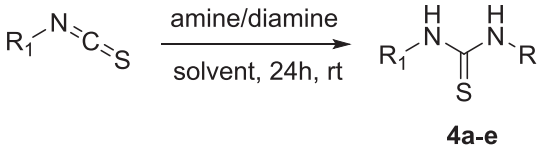
[a] Isolated yield.
[b] Calculated using ChemBioDraw ultimate software.

the corresponding target thioamides **2i** and **2j** in high yields up to 90%. The impact of the used solvent on the thionation was examined. There was no evident correlation between used medium and thionation efficiency, products **2i** and **2j** were obtained with comparable yields (Table 1, entries 9–10). Subtly enhanced yields of the amides **2i** and **2j** in THF may be explained by the higher solubility of the LR reagent in this solvent. Additionally, two different size-chain thiolactams were synthesized (Table 1, entries 11 and 12) [58]. Pyrrolidin-2-tione (**3a**) and piperidine-2-thione (**3b**) were provided from the corresponding lactams; 2-pyrrolidinone (**1k**) and 2-piperidinone (**1l**), using LR reagent in DCM (Scheme 1).

The desired products were isolated in high yields up to 93% within two hours. The effect of the lactam ring size on the reaction yield was not observed.

Thoughtful screening of the literature regarding potential H_2S agents provides basis that the thiourea could act as a H_2S donor [29–34]. Therefore, we designed and synthesized a series of compound based on thiourea scaffold (Scheme 2).

The variously substituted thiourea derivatives **4a-e** were obtained by the reaction of corresponding isothiocyanate with appropriate amine or diamine (Scheme 2) [59–61]. Three different commercially available isothiocyanate were used: methyl isothiocyanate, phenyl isothiocyanate and benzyl isothiocyanate. As shown in the Table 2 the reaction of aromatic isothiocyanate with a structurally diversified amines possessing in their structure hydrophilic groups afforded the corresponding



Scheme 2. Synthesis of thiourea derivatives from the corresponding isothiocyanates.

thiourea derivatives **4c-e** in high yields up to 87% (Table 2, entry 3). The reactions also proceeded efficiently with both aliphatic and heterocyclic amines. Application of methyl isothiocyanate resulted in the corresponding thiourea derivatives **4a-b** with the yields up to 92% (Table 2, entries 1 and 2). For diamines the appropriate excess of isothiocyanate (2 equiv.) was used (Table 2, entries 2 and 4).

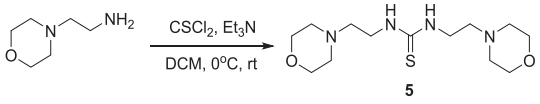
The solvents such as an acetone, toluene and DCM were used. The reaction performed in DCM using methyl isothiocyanate resulted in higher yield of the corresponding thiourea derivatives **4a** and **4b** than analogous one conducted in toluene or acetone (Table 2, entries 1 and 2). In the case of isothiocyanates with aromatic ring, toluene revealed to be the most suitable solvent (Table 2, entries 3–5). The higher yield may be due to a higher solubility of the used reagents in given solvent. Additionally, we have developed protocol for the synthesis of symmetrically substituted urea derivative **5** (Scheme 3) [62].

The 1,3-bis(2-morpholinoethyl)-thiourea (**5**) was obtained *via*

Table 2
Synthesis of thiourea derivatives **4a-e.**

Entry	R_1	Amine/Diamine	Molar ratio Isothiocyjanate/amine	Solvent	Product **4**	Yield [%][a]	log *P*[b]
1	H_3C	H_2N O	1:1	DCM acetone toluene	**4a**	92 89 83	0.34
2		H_2N NH_2	2:1	DCM acetone toluene	**4b**	89 85 81	−0.60
3		H_2N O	1:1	DCM toluene	**4c**	80 87	0.90
4		H_2N H N NH_2	2:1	DCM toluene	**4d**	75 80	1.53
5		H_2N N O	1:1	DCM toluene	**4e**	79 83	1.11

[a] Isolated yield.
[b] Calculated using ChemBioDraw ultimate sofware.



Scheme 3. Synthesis of 1,3-bis(2-morpholin-4-ylethyl)thiourea (**5**).

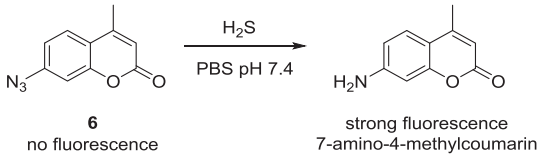
Table 3
In vitro H_2S release evaluation from compounds **2a-j**, **3a-b**, **4a-f** and **5** using DTNB probe.[a]

Entry	Donor	Conc. [μM]
1	**2a**	254
2	**2b**	271
3	**2c**	407
4	**2d**	335
5	**2e**	736
6	**2f**	180
7	**2g**	95
8	**2h**	434
9	**2i**	106
10	**2j**	115
11	**3a**	190
12	**3b**	267
13	**4a**	129
14	**4b**	102
15	**4c**	100
16	**4d**	85
17	**4e**	95
18	**5**	189

[a] 10 mM Ellman's Reagent Solution in sodium phosphate buffer (pH 8.0, 50 mM), room temperature, spectra recorded 15 min after addition of H_2S donor at 412 nm. For each donors the absorbance was measured at least three times. Background values were subtracted from the sample values.

reaction of 2-morpholinoethan-1-amine with thiophosgene in DCM with 85% yield. The structures of all obtained compounds (**2a-j**, **3a-b**, **4a-e** and **5**) were confirmed by ^{1}H-, ^{13}C NMR spectroscopy, mass spectra analysis and elemental analysis (supplementary materials). Spectroscopic data for the known compounds correspond with the published one. Further, we have evaluated the potential H_2S-releasing capability of the synthesized compounds. Initially, we have validated the newly prepared compounds toward H_2S liberation using recently established and commonly used procedure based on DTNB probe also known as Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid). In general, DTNB method is used for the determination of the free sulfhydryl group concentration [63–65]. DTNB assay for 1 mM solutions of H_2S donors was performed at pH 8.0 according to the protocol provided by the manufacturer (supplementary materials). As shown in Table 3 all prepared compounds liberate H_2S. The highest amount of the released H_2S was observed for **2e** and **2h** thio-derivatives (Table 3, entries 5 and 8), while the lowest one was recorded for compounds **2g**, **4d** and **4e** (Table 3, entries 7, 16 and 17).

Since the DTNB operates at basic conditions which does not meet physiological one (pH 7.4) and can affect the amount of the liberaitng H_2S, we turn our attention toward alternative technique which allows us to measure the reactive sulfur species in physiological environment. Fortunately, the rapid progress drives the emergence of the assay for H_2S detection, which possesses the biotarget sensitivity and synthetic convenience. Up to date, a variety of fluorescent probes for tracking the H_2S in biological samples is available [66]. Due to the fast catabolism and low stability of H_2S we required a probe that would react rapidly and selectively with low levels of hydrogen sulfide and produce a robust fluorescent signal. In addition, the convenient probe should be characterized by the facile synthesis with an efficient and fast protocol for purification. For these reasons a 7-azido-4-methyl-2H-chromen-2-one (**6**) the non-fluorescence derivative of 7-amino-4-methylcoumarin (Scheme 4) have been carefully selected [67]. It should be emphasized that probe **6** in contrast to DTNB sensor demonstrates high selectivity and sensitivity toward H_2S over other relevant reactive sulfur species *in vitro*, as well as identified exogenous H_2S in living cells [68]. The target probe **6** was synthesized in 88% yield from *m*-hydroxyaniline in accordance with the previously described procedure [68]. As reflecting diverse state-of-the-art OFF/ON fluorescent mechanism, including the release of fluorophore triggered by reduction of an azide to the amine was showed in Scheme 4. In our experiments, 1 mM of each donor was dissolved in a mixed MeCN/sodium phosphate buffer solution (2:8 v/v,



Scheme 4. Mechanism of H_2S detection for the probe **6**.

Table 4
In vitro H_2S release evaluation from compounds **2a-j, 3a-b, 4a-e** and **5** determined in fluorometric assay.

Fluorescence method[a]		
Entry	Donor	Conc. [μM][b]
1	NaHS	260
2	GYY4137	14
3	**2a**	228
4	**2b**	146
5	**2c**	137
6	**2d**	134
7	**2e**	101
8	**2f**	98
9	**2g**	96
10	**2h**	94
11	**2i**	92
12	**2j**	82
13	**3a**	120
14	**3b**	182
15	**4a**	93
16	**4b**	107
17	**4c**	100
18	**4d**	100
19	**4e**	95
20	**5**	191

[a] 1 mM of probe **6** in a mixed MeCN/sodium phosphate buffer solution (2:8 v/v, pH 7.4); a room temperature, the fluorescence response was monitored in range time from 1 to 60 min after addition of the H_2S donor. For each donors fluorescence intense was measured three times.

[b] The H_2S amount determined after 15 min. For calculation were used the mean obtained from three measurements. Background values were subtracted from the sample values.

pH 7.4) containing probe **6** (1 mM). The changes in the fluorescence emission spectra at 430 nm (λ_{ex} = 365 nm) were recorded for one hour at room temperature.

Finally, the fluorescence signals were converted to H_2S concentrations based on a reference curve obtained with a series of NaHS standard solutions. In each case H_2S concentrations were determined after 15 min. In order to evaluate the release properties of H_2S by studied agents, we carried out independent verification of H_2S release profiles from known H_2S donors; NaHS and GYY4137 under the same experimental conditions (Table 4, entries 1 and 2). Alike previously for DTNB assay, also in the fluorescence method with probe **6** all prepared compounds showed H_2S-releasing capabilities. As can be expected the H_2S-release from all tested compounds is lower than for NaHS. In turn amounts of H_2S release from each of studied donors **2a-j**, **3a-b**, **4a-e** and **5** were higher than for well recognized GYY4137. For compound **2a**, **3b** and **5** the highest H_2S-releasing was observed (Table 4, entries 3, 14 and 20). The compound **2j** released the smallest amounts of H_2S, but still on the higher level than GYY 4137 (Table 4, entry 12). All other compounds were similarly found to generate H_2S under studied conditions. Additionally, for arbitrary selected donors **2a**, **3b** and **5**, fluorescence measurements were performed in assay buffer with addition of rat blood plasma (20% v/v). The obtained results were similar to those obtained in buffer alone, what implies that the intracellular environment does not affect H_2S-release capability of selected donors. Differences in the H_2S-realising values can be associated with the solubility of these compounds in an aqueous medium (for compound with the higher value of log *P* the amount of H_2S released were lower). We assume that hydrophobic properties of a given compounds can reduce the hydrolysis rate of the studied donors and consequently decrease the generation of H_2S. As can be seen the H_2S release data for thioamide **2a** and thiourea derivatives **4a-e** correspond to those reordered using DTNB sensor. One the other hand the higher values observed in case of using Ellman's reagent may arise from it varied selectivity toward sulfur species.

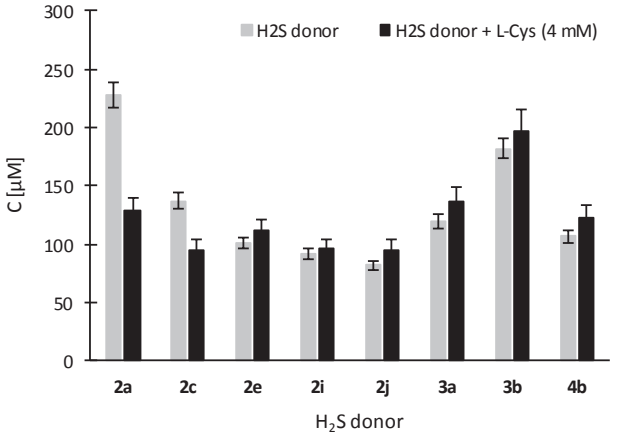


Fig. 3. Comparison of the H_2S amount released from the selected H_2S donors (1 mM) in the presence of L-cysteine (grey bars) and in the assay buffer alone (black bars).

Cellular nucleophiles play crucial roles in biological systems. The thiols that occur in cells in the large amount and characterized by the strongest potent reactivity are cysteine (Cys) and reduced glutathione (GSH). According to the literature data, release of H_2S from donors based on thioamide structural motif may be modulated by the organic thiols such as reduced glutathione or/and cysteine [69–71]. Therefore, additional experiments were pursued in the presence of L-cysteine for selected donors **2a**, **2c**, **2e**, **2i**, **2j**, **3a, 3b** and **4b**. Due to the lack of selectivity of the DTNB method towards thiol groups further measurements with cysteine were carried out with 7-azido-4-methyl-2*H*-chromen-2-one (**6**). Regardless of the literature data regarding selectivity of the used probe **6**, we have validated this compound using two different reactive sulfur species. These studies proved that probe **6** exhibited high selectivity towards H_2S over cysteine and reduced glutathione (GSH) under studied conditions. As shown in Fig. 3, cysteine (4 mM) successfully triggered H_2S release, at a relatively higher level for **2e**, **2i**, **2j**, **3a**, **3b** and **4b** donors (Table 5, entries 3–8). While the release of H_2S from **2a** and **2c** in the presence of 4 mM cysteine was lower than observed in the assay buffer alone (Table 5, entries 1–2).

Obtained results demonstrate the capability of the thioamides, thiolactams and thioureas derivatives to release H_2S in complex biological systems and also show that cysteine is the regulator of this type

Table 5
In vitro H_2S release evaluation in the presence of L-Cysteine (4 mM) determined in fluorometric assay.

Fluorescence method with probe **6**[a]		
		PBS/L-cysteine [4 mM]
Entry	Donor	Conc. [μM][b]
1	**2a**	128
2	**2c**	95
3	**2e**	111
4	**2i**	96
5	**2j**	95
6	**3a**	136
7	**3b**	197
8	**4b**	123

[a] 1 mM of probe **6** in sodium phosphate buffer (pH 7.4), 4 mM L-cysteine, room temperature, the fluorescence response was monitored in a range time from 1 to 60 min after addition of the H_2S donor. For each donors the fluorescence intense was measured three times.

[b] The H_2S amount was determined after 15 min. For calculation were used the mean obtained from three measurements. Background values were subtracted from the sample values.

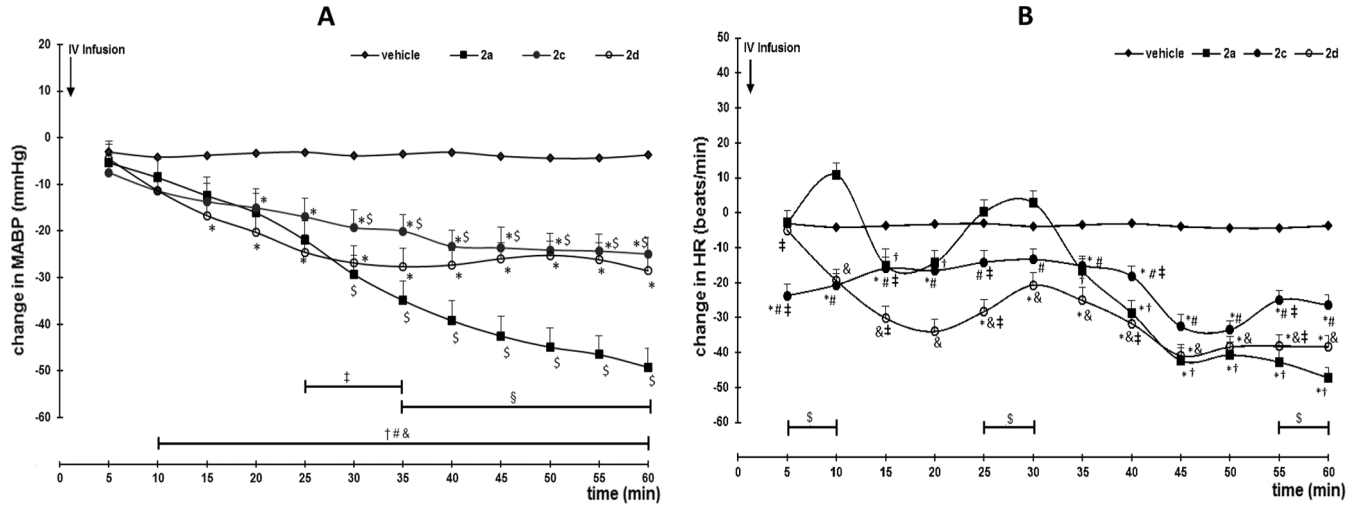


Fig. 4. Change in (**A**) mean arterial blood pressure (MABP, mmHg) and (**B**) heart rate (HR, beats/min) after the intravenous administration of vehicle (20% v/v of DMSO in saline, n = 5) or H_2S donors [**2a** (n = 5), **2c** (n = 5) and **2d** (n = 5) at a dose of 0.8 mmol/kg/BW] in normotensive Sprague Dawley rats. *p < 0.05 – vs. baseline, †p < 0.05 – vehicle vs. **2a** series., v $^{\#}$p < 0.05 – vehicle vs. **2c** series, $^{\&}$p < 0.05 – vehicle vs. **2d** series $^{\$}$p < 0.05 – **2a** vs. **2c** series, §p < 0.05 – **2a** vs. **2d** series. ‡p < 0.05 – **2c** vs. **2d** series.

of donors. The observed differences in H_2S liberation between the results obtained in both used analytical methods can be associated with way of sample processing and conditions of measurements which are substantially different for the used methods. In comparison to fluorescent method, DTNB method requires alkaline conditions (pH 8.0) The above factors can lead to falsely elevated or decreased H_2S amounts in samples and may be the cause of the observed differences in the results obtained.

2.1. Haemodynamic effects of investigated H_2S donors

Mean arterial blood pressure (MABP) and heart rate (HR) were measured at baseline and after intravenous administration of a vehicle and newly synthesized H_2S donors. The compounds **2a**, **2c**, **2d**, **2j**, **3a**, **4b** and **5** were selected as representatives due to their adequate solubility in the vehicle and proved H_2S release at physiological pH (the fluorescence method). Additionally, we conducted experiments in which hemodynamic effects of thiourea were investigated. The impact of selected H_2S donors on MABP and HR in the normotensive rats are shown in Figs. 4–8. There were no significant differences in MABP and HR at baseline between the experimental series. The vehicle did not affect MABP and HR. All investigated H_2S donors produced hemodynamic effects, however, there were differences in the onset and the size of the responses. Specifically, the greatest decrease in MABP was present after administration of **2a** whereas the lowest drop in MABP was present after administration of **3a**. Simultaneously, a significant reduction in the heart rate was observed. It is important to mention that similar effect has not been observed for the parent lactam 2-pyrrolidinon under similar physiological conditions (Supplementary Materials). The H_2S donors **2a**, **2c**, **2d** produced immediate response. In contrast thiourea and the donor **2j** produced decrease in MABP 45 min and 30 min after the administration, respectively. In general, decrease in MABP was accompanied by a decrease in HR for H_2S donors **2a**, **2c**, **2d**, **2j** and **3a**. However, donor **5** produced a decrease in MABP which was associated with an unstable increase in HR.

3. Conclusions

By preparing and directly comparing thioamide, thiolactam and thiourea donor platforms, we demonstrate access to a wide array of

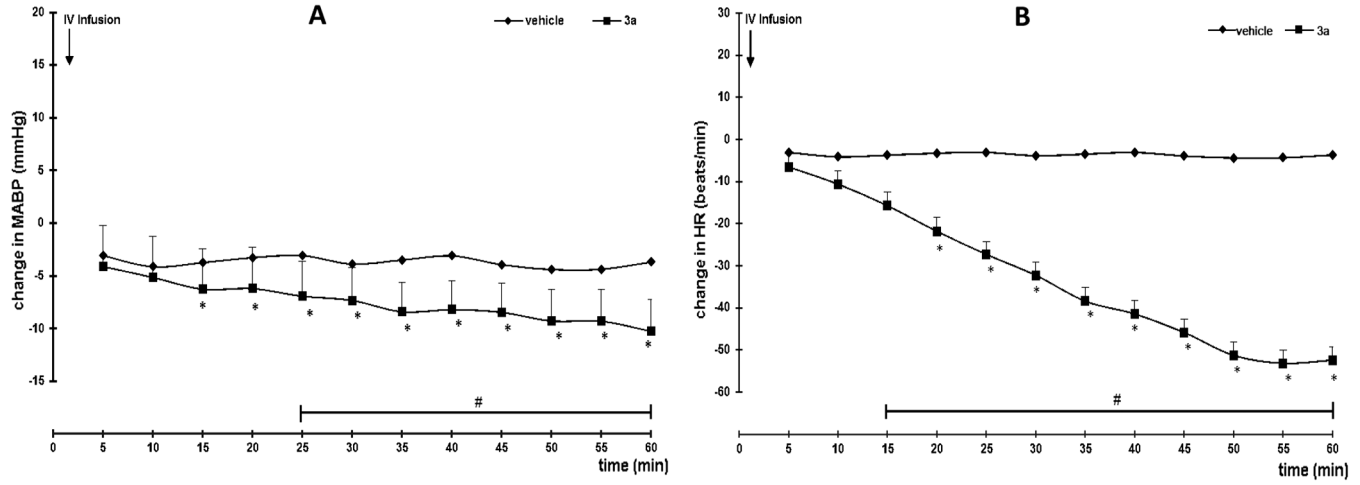


Fig. 5. Change in (**A**) mean arterial blood pressure (MABP, mmHg) and (**B**) heart rate (HR, beats/min) after the intravenous administration of vehicle (20% v/v of DMSO in saline, n = 5) or H_2S donor [**3a** (n = 5) at a dose of 0.8 mmol/kg/BW] in normotensive Sprague Dawley rats. *p < 0.05 – vs. baseline, $^{\#}$p < 0.05 – vehicle vs. **3a** series.

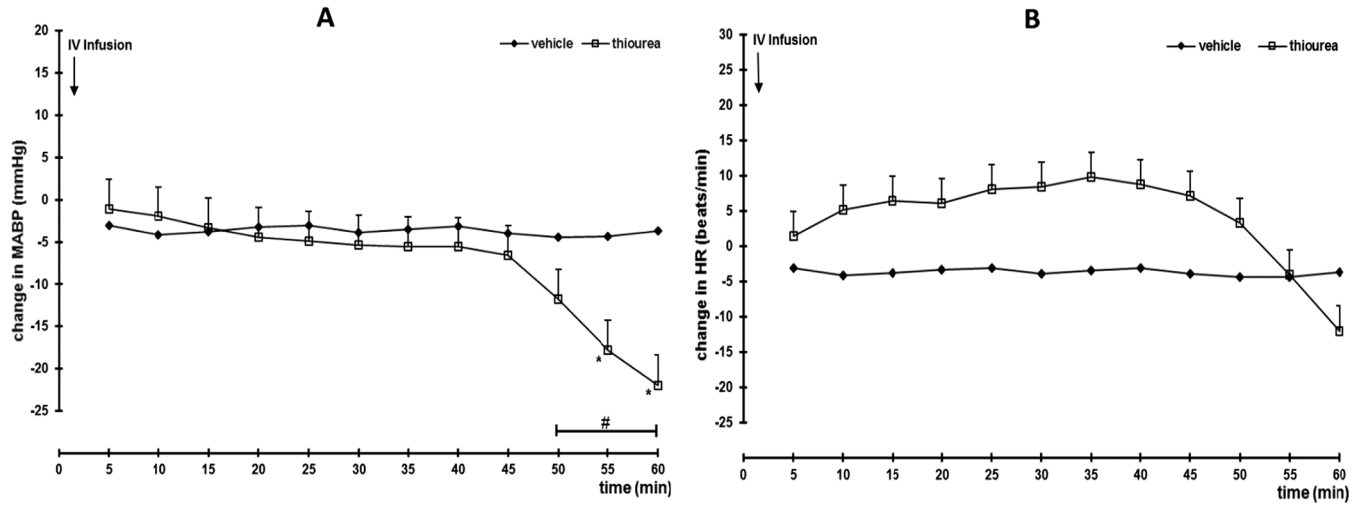


Fig. 6. Change in (**A**) mean arterial blood pressure (MABP, mmHg) and (**B**) heart rate (HR, beats/min) after the intravenous administration of vehicle (20% v/v of DMSO in saline, n = 5) or H_2S donor [**thiourea** (n = 5) at a dose of 0.8 mmol/kg/BW] in normotensive Sprague Dawley rats. *p < 0.05 – vs. baseline, #p < 0.05 – vehicle vs. **thiourea** series.

structural motifs that function as controllable hydrolysis-based H_2S donors. The liberating H_2S ability of these compounds was determined and compared with NaHS and GYY4137. Obtained results proved that controllable H_2S release can be achieved by structural modifications of the donors. We also found that thiolactam pyrrolidin-2-thione (**3a**) reduces heart rate with a moderate effect on blood pressure in normotensive Sprague Dawley rats. Further studies evaluating mechanisms of the thiolactams action on the circulatory system are needed. Altogether obtained results highlighted the potential of investigated compounds as the H_2S-donors for basic studies and for the rational design of pharmacotherapeutic agents to treat cardiovascular disorders.

4. Materials and methods

General methods. All the chemicals were obtained from commercial sources and the solvents were of analytical grade. ^{1}H- and ^{13}C NMR spectra were recorded in $CDCl_3$, DMSO-d or acetone-d_6. Chemical shifts are expressed in parts per million. The coupling constants (J) are given in hertz (Hz). The elemental analyses were performed on CHN Perkin-Elmer 240 apparatus. TLC analyses were done on Kieselgel 60 F_{254} aluminum sheets. Column chromatography was performed on Merck silica gel 60/230-400 mesh. SiliCycle 60 F_{254} silica gel sheets were used for analytical thin layer chromatography (TLC) and visualized by fluorescence quenching under UV light. Commercial reagents for quantitating sulfhydryl groups by DTNB method were purchased from Thermo Fisher Scientific (Waltham, Mass., USA). Buffer reagents were purchased from Sigma Aldrich (Saint Louis, Mo., USA) and were used without purification. All spectroscopic measurements were performed in 0.1 mM sodium phosphate buffer (pH 7.4) or 0.1 M sodium phosphate buffer (pH 8.0). UV/Vis spectra were recorded at ambient temperature using a U-1900 spectrophotometer (Hitachi, Chiyoda, Tokyo, Japan) and quartz cuvettes. Fluorescence spectra were recorded at 20 °C in quartz cuvettes using a F7000 spectrofluorometer (Hitachi).

General procedure for thionation reaction using P_4S_{10} (Method A). To a cooled (0–5 °C) solution of appropriate amide **1** (1.0 mmol) in diethyl ether (3.0 mL) was added tetraphosporus decasulfide (0.25 mmol) in small portions. The reaction mixture was allowed to reach the ambient temperature and stirred for appropriate time. After the completion, the reaction mixture was filtered and concentrated under vacuum. To the resulting mixture was added silica gel, concentrated in vacuum and purified by silica gel column chromatography using hexanes/ethyl acetate as an eluent to give the title compound **2**.

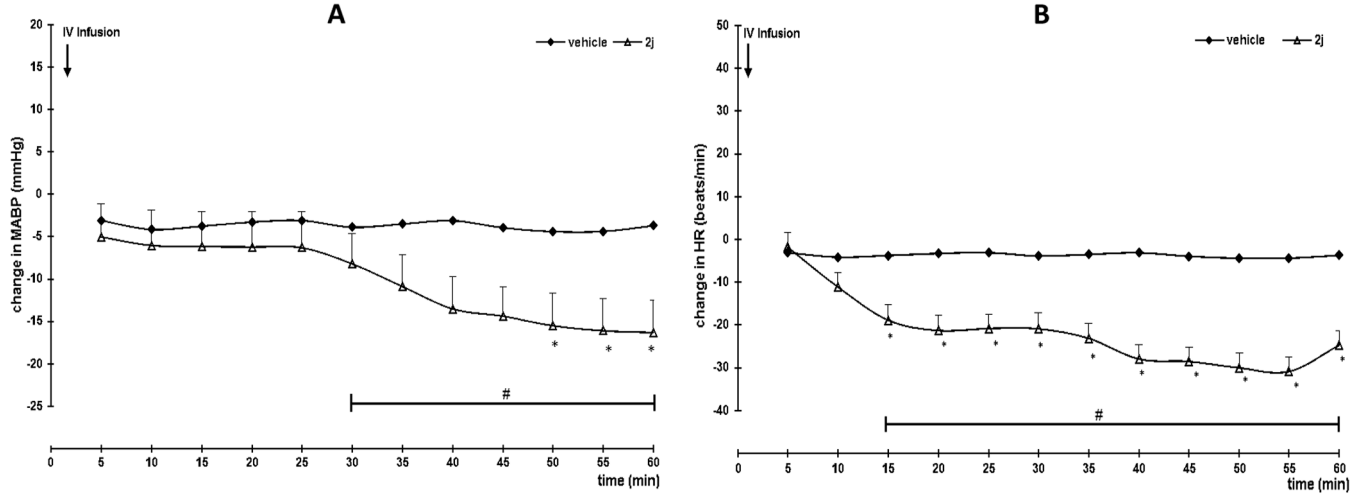


Fig. 7. Change in (**A**) mean arterial blood pressure (MABP, mmHg) and (**B**) heart rate (HR, beats/min) after the intravenous administration of vehicle (20% v/v of DMSO in saline, n = 5) or H_2S donor [**2j** (n = 5) at a dose of 0.8 mmol/kg/BW] in normotensive Sprague Dawley rats. *p < 0.05 – vs. baseline, #p < 0.05 – vehicle vs. **2j** series.

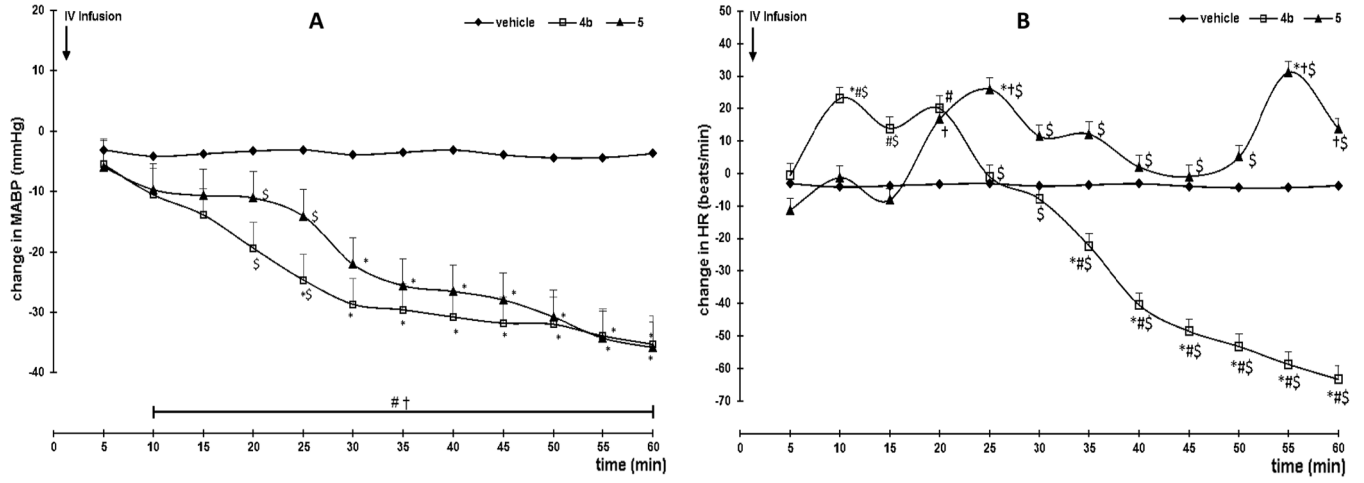


Fig. 8. Change in (**A**) mean arterial blood pressure (MABP, mmHg) and (**B**) heart rate (HR, beats/min) after the intravenous administration of vehicle (20% v/v of DMSO in saline, n = 5) or H_2S donors [**4b** (n = 5) and **5** (n = 5) at a dose of 0.8 mmol/kg/BW] in normotensive Sprague Dawley rats. $^{*}p < 0.05$ – vs. baseline, $^{\#}p < 0.05$ – vehicle vs. **4b** series, $^{\dagger}p < 0.05$ – vehicle vs. **5** series, $^{\$}p < 0.05$ – **4b** vs. **5** series.

***N,N'*-((ethane-1,2-di-yl-bis(oxy))bis(ethane-2,1-diyl))dimethanethio-amide (2f).** The reaction was carried out according to the general Method A with *N,N'*-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))-di-formamide **1f**. The compound **2f** was obtained with 70% yield (165 mg, 0.7 mmol). ^{1}H NMR (400 MHz, DMSO) δ 10.11 (br s, 1H), 9.35–9.13 (m, 1H), 3.70–3.63 (m, 2H), 3.58 (t, J = 5.2 Hz, 2H), 3.54–3.41 (m, 5H), 3.31 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 192.07, 189.06, 70.03, 70.02, 70.01, 69.35, 67.72, 67.66, 48.72, 43.29; Element. Anal. calcd. for $C_8H_{16}N_2O_2S_2$: C: 40.66, H: 6.82, N: 11.85; found C: 40.49, H: 6.75, N: 11.81; HRMS (ESI) calcd for $C_8H_{16}N_2O_2S_2Na$ $(M + Na)^+$ 259.0551; found 259.0549.

***N*-(2-morpholinoethyl)methanethioamide (2g).** The reaction was carried out according to the general Method A with *N*-(2-morpholinoethyl)formamide **1g**. The compound **2g** was obtained with 78% yield (136 mg, 0.78 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.11 (s, 1H), 9.21 (d, J = 6.4 Hz, 1H), 3.55 (q, J = 7.8, 6.2 Hz, 2H), 3.30 (s, 6H), 2.71–2.23 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.86, 66.98, 66.40, 55.17, 53.30, 38.96; Element. Anal. calcd. for $C_7H_{14}N_2OS$: C: 48.25, H: 8.10, N: 16.08; found C: 48.05, H: 8.73, N: 15.92; HRMS (ESI) calcd. for $C_7H_{14}N_2OSNa$ $(M + Na)^+$ 197.0725; found 197.0722.

General procedure for thionation using P_4S_{10}/HMDSO (Method B). Amide **1** (1.0 mmol), P_4S_{10} (0.25 mmol), DCM (1.0 mL) and HMDSO (1.67 mmol) were combined, refluxed and stirred 24 h. After this time the reaction mixture was cooled to 0 °C and aqueous K_2CO_3 solution (1.26 mL of 5.3 M/ mmol of P_4S_{10} taken) was added. Then to the reaction mixture acetone (equal to one-half of the reaction solvent) and water (1 mL/mmol of P_4S_{10} taken) were added. The reaction mixture was stirred vigorously for 30 min at 0 °C. After this time water and an extraction solvent were added, the layers were separated, and the organic phase was washed with aqueous solution of K_2CO_3, water, and brine. The organic extract was dried over $MgSO_4$ and evaporated, and the crude product was purified by column chromatography using hexanes/ethyl acetate as an eluent to give product **2**.

***N,N'*-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))dimethanethio-amide (2f).** The reaction was carried out according to the general Method B with *N,N'*-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))diformamide (**1f**). The compound **2f** was obtained with 84% yield (199 mg, 0.84 mmol). ^{1}H- and ^{13}C NMR data were similar to those obtained previously.

***N*-(2-morpholinoethyl)methanethioamide (2 g).** The reaction was carried out according to the general Method B with *N*-(2-morpholinoethyl)formamide (**1g**). The compound **2g** was obtained with 90% yield (157 mg, 0.9 mmol). ^{1}H- and ^{13}C NMR data were similar to those obtained previously.

General procedure for thionations with LR (Method C). To a THF solution of appropriate amide **1** (1.0 mmol) was added Lawesson's reagent (0.5 mmol) at room temperature and the mixture was stirred for 24 h. To the resulting mixture silica gel was added and concentrated in vacuum. The residue was purified by column chromatography using hexanes/ethyl acetate as an eluent to give product **2**.

***N,N'*-((ethane-1,2-diyl-bis(oxy))bis(ethane-2,1-diyl))dimethanethio-amide (2f).** The reaction was carried out according to the general method A with *N,N'*-((ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))diformamide (**1f**). The compound **2f** was obtained with 80% yield (189 mg, 0.8 mmol). ^{1}H- and ^{13}C NMR data were similar to those obtained previously.

***N*-(2-morpholinoethyl)methanethioamide (2g).** The reaction was carried out according to the general Method A with *N*-(2-morpholinoethyl)formamide (**1g**). The compound **2g** was obtained with 85% yield (148 mg, 0.85 mmol). ^{1}H- and ^{13}C NMR data were similar to those obtained previously.

General procedure for preparation of thiourea derivatives 3. Method D. The phenyl isothiocyanate (135 mg 1.0 mmol) was dissolved in toluene (5 mL) and then appropriate amine or diamine was added (1.0 or 2.0 mmol, respectively). The reaction mixture was stirred for 24 h. The precipitate was collected by filtration, washed with toluene, concentrated in vacuum. The residue was purified by column chromatography using hexanes/ethyl acetate as an eluent to afford **4d**.

1,1′-(azane-di-yl-bis(ethane-2,1-di-yl))bis(3-phenylthiourea) (4d). The reaction was carried out according to the general Method D with 1-(2-aminoethyl)ethane-1,2-diamine. The compound **4d** was obtained with 78% yield (291 mg, 0.78 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.48–7.19 (m, 10*H*), 4.06 (s, 1H), 3.90 (d, J = 7.4 Hz, 1H), 3.53 (d, J = 6.8 Hz, 6H); Element. Anal. calcd. for $C_{18}H_{23}N_5S_2$: C: 57.88, H: 6.21, N: 18.75; found C: 57.71, H: 6.03, N: 18.57; HRMS (ESI) calcd for $C_{18}H_{23}N_5S_2Na$ $(M + Na)^+$ 396.1293; found 396.1291.

General procedure for preparation of thiourea derivatives 3 in DCM. Method E. A solution of appropriate amine or diamine (1.0 or 2.0 mmol, respectively) in dichloromethane (10 mL) was cooled to 0 °C followed by the addition of triethylamine (3.0 mmol). Corresponding phenyl isothiocyanate (1.0 mmol) was added to the reaction mixture and stirred at room temperature for 12 h (monitored by TLC for completion). The reaction mixture was washed with water (3 × 10 mL) followed by brine (3 × 10 mL). Organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using hexanes/ethyl acetate as an eluent to give **4d**.

1,1′-(azanediylbis(ethane-2,1-diyl))bis(3-phenylthiourea) (4d).

The reaction was carried out according to the general Method E with 1-(2-aminoethyl)ethane-1,2-diamine. The compound **4d** was obtained with 75% yield (280 mg, 0.75 mmol).

General procedure for H_2S detection by fluorescence method in sodium phosphate buffer. The solutions of probe **6** (1 mM) and tested H_2S donor (1 mM) were prepared in sodium phosphate buffer at pH 7.4 (20% CH_3CN). For the assay, appropriate donor was added to test tube/sample cuvette solution containing probe **6**. The total volume of the solution being measured was 2000 µL. The final concentration of probe **6** and donor in test tube/sample cuvette were 1 mM. The fluorescence response was monitored over time. Emission spectra were collected between 350 nm and 550 nm. For all measurements, the excitation wavelength was 365 nm. The measurement points covered the time range from 1 to 60 min after addition of donor. For each donors fluorescence intense was measured three times and the results were averaged.

General procedure for H_2S detection by fluorescence method in the presence of cysteine. The solution of probe **6** (1 mM) and tested H_2S donor (1 mM) were prepared in sodium phosphate buffer containing 20% CH_3CN (pH = 7.4). In these experiments appropriate donor (1 mM) was added directly to the test tubes/sample cuvette solution containing probe **6** and cysteine. The final concentration of probe **6** and donor were 1 mM, while concentration of cysteine was 4 mM. The total volume of the solution being measured was 2000 µL. Emission spectra were collected between 350 nm and 550 nm. The fluorescence response of probe **6** was monitored with λ_{ex} = 365 nm. The measurement points covered the time range from 1 to 60 min after addition of donor. For each donor fluorescence intense was measured three times and the results were averaged.

General procedure for H_2S detection using the Ellman's test. For each sample to be tested were prepared test tube/sample cuvette containing 50 µL of Ellman's reagent solution and 2.5 mL of reaction buffer (Ellman's reagent solution and reaction buffer were prepared according to the procedure/ manual attached by Thermo Fisher Scientific (Catalog number: 22582). Then to the separate test tubes/sample cuvette prepared in step 1 was added 250 µL of donor solution (1 mM) (for reference cuvette was added 250 µL of reaction buffer, respectively). 1 mM Solution of donor was prepared using reaction buffer as a solvent. Then test tubes/ sample cuvette were mixed and incubated at room temperature for 15 min. After this time was measured absorbance of each sample at 412 nm. For each donors absorbance was measured three times and the results were averaged. Concentration of sulfhydryl groups in each samples was calculate from the molar extinction coefficient of the TNB anion ($\varepsilon = 14.150\ M^{-1}\ cm^{-1}$) according to the procedure/manual attached by Thermo Fisher Scientific (Catalog number: 22582).

Animal experiments. The experiments were performed according to Directive 2010/63 EU on the protection of animals used for scientific purposes and approved by the 2nd Local Bioethical Committee in Warsaw (permission number: WAW2/127/2018). The animals were obtained from the Animal Breeding Department of the Medical University of Warsaw and housed in the Central Laboratory of Experimental Animals in group cages with access to standard laboratory chow and water, ad libitum. The rats were maintained in a temperature- and humidity- controlled room with a 12/12-hour light–dark cycle. The studies were performed on male, 18–20-weeks-old, normotensive Sprague Dawley rats (SD). All measurements were performed under general anaesthesia with urethane (Sigma-Aldrich) given intraperitoneally (IP) at a dose of 1.5 g/kg of body weight (BW). Before the measurements rats were implanted with a venous catheter and an arterial catheter. The arterial catheter was connected to the Biopac MP 150 (Biopac Systems, Goleta, USA) for haemodynamic recordings. The venous catheter was used for administration of investigated compounds.

Reagents/Drugs. The following drugs were used: compound **2a**, **2c**, **2d**, **2j**, **3a**, **4b**, **5** and thiourea used as a H_2S donor. The solution of H_2S donor was prepared in 0.9% saline containing 20% v/v dimethyl sulfoxide (DMSO).

Haemodynamic measurements. The measurements started 60 min after the induction of anaesthesia and stabilization of haemodynamic parameters. Arterial blood pressure was recorded 20 min at baseline (before treatment) and 60 min after intravenous infusions of either 0.3 mL of the vehicle (20% v/v of DMSO (dimethyl sulfoxide) in saline (aqueous 0.9% NaCl)) or H_2S donors at a dose of 0.8 mmol/kg BW (n = 5).

Data analysis and statistics. The data are expressed as means + SEM (standard error). Mean arterial blood pressure (MABP) and heart rate (HR) were calculated on the arterial blood pressure tracing by AcqKnowledge 4.3.1 Biopac software (Biopac Systems, Goleta, USA). For the evaluation of MABP and HR response within the series, baseline recordings were compared with recordings after administration of evaluated compounds by means of the analysis of variance (ANOVA) for repeated measures. Comparisons between the treatments were performed by ANOVA. If ANOVA showed a significant difference, the post-hoc Tukey's test was performed. A value of two-sided $p < 0.05$ was considered significant. Analyses were conducted using STATISTICA 13.0.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.102941.

References

[1] a) K. Abe, H. Kimura, J. Neurosci. 16 (1996) 1066–1071;
b) D. Mancardi, C. Penna, A. Merlino, P. Del Soldato, D.A. Wink, P. Pagliaro, Biochim. Biophysica Acta (BBA) Bioenerg. 1787 (2009) 864–872;
c) E.M. Bos, H. van Goor, J.A. Joles, M. Whiteman, H.G.D. Leuvenink, Brit. J. Pharmacol. 172 (2015) 1479–1493;
d) H. Li, Y. Ma, O. Escaffre, T. Ivanciuc, N. Komaravelli, J.P. Kelley, C. Coletta, C. Szabo, B. Rockx, R.P. Garofalo, A. Casola, J. Virol. 89 (2015) 5557–5568.
[2] a) E. Łowicka, J. Bełtowski, Pharmacol. Rep. 59 (2007) 4–24;
b) L. Li, P.K. Moore, Trends Pharmacol. Sci. 29 (2008) 84–90;
c) D.J. Elsey, R.C. Fowkes, G.F. Baxter, Cell Biochem. Funct. 28 (2010) 95–106;
d) K. Abe, H. Kimura, J. Neurosci. 16 (1996) 1066–1071;
e) R. Hosoki, N. Matsuki, H. Kimura, Biochem. Biophys. Res. Commun. 237 (1997) 527–531;
f) K. Abe, H. Kimura, J. Neurosci. 16 (1996) 1066–1071;
g) R. Hosoki, N. Matsuki, H. Kimura, Biochem. Biophys. Res. Commun. 237 (1997) 527–531;
h) C.D. Russo, G. Tringali, E. Ragazzoni, N. Maggiano, E. Menini, M. Vairano, P. Preziosi, P.J. Navarra, J. Neuroendocrinol. 12 (2000) 225–233;
i) G. Yang, R. Wang, Acta Physiologica Sinica 59 (2007) 133–140;
j) Y. Kaneko, T. Kimura, S. Taniguchi, M. Souma, Y. Kojima, Y. Kimura, H. Kimura, I. Niki, FEBS Lett. 583 (2009) 377–382;
k) M. Okamoto, T. Ishizaki, T. Kimura, Nitric Oxide 46 (2015) 32–36;
l) E. Distrutti, L. Sediari, A. Mencarelli, B. Renga, S. Orlandi, G. Russo, G. Caliendo, V. Santagada, G. Cirino, J.L. Wallace, S. Fiorucci, J. Pharmacol. Exp. Ther. 319 (2006) 447–458;
m) A. Sparatore, E. Perrino, V. Tazzari, D. Giustarini, R. Rossi, G. Rossoni, K. Erdman, H. Schröder, P. Soldato, Free Rad, Biol. Med. 46 (2009) 586–592;
n) R. Zanardo, V. Branaleone, E. Distrutti, S. Fiorucci, G. Crino, J. Wallace, J. Fed. Am. Soc. Exp. Biol. 20 (2006) 2118–2120.
[3] a) K. Kashfi, K.R. Olson, Biochem. Pharmacol. 85 (2013) 689–703;
b) S. Taniguchi, I. Niki, J. Pharmacol. Sci. 116 (2011) 1–5;
c) M.W. Warenycia, R.J. Reiffenstein, L.R. Goodwin, F.P. Dieken, Toxicol. Lett. 47 (1989) 221–224;
d) L.R. Goodwin, D. Francom, F.P. Dieken, J.D. Taylor, M.W. Warenycia, R.J. Reiffenstein, G. Dowling, J. Anal. Toxicol. 13 (1989) 105–109;
e) S.P. Parajuli, S. Choi, J. Lee, Y.D. Kim, C.G. Park, M.Y. Kim, H. Il Kim, C.H. Yeum, J.Y. Jun, Korean J. Physiol. Pharmacol. 14 (2010) 83–89;
f) L. Li, M. Bhatia, P. Moore, Curr. Opin. Pharmacol. 6 (2006) 125–129;
g) H. Pae, Y. Lee, J. Eun-Kyeong, H. Chung, Arch. Pharm. Res. 32 (2009) 1155–1162;
h) R. Banerjee, Curr. Opin. Chem. Biol. 37 (2017) 115–121;

i) T. Roger, F. Raynaud, F. Bouillaud, C. Ransy, S. Simonet, C. Crespo, M-. Bourguignon, N. Villeneuve, J.-P. Vilaine, I. Artaud, E. Galardon, ChemBioChem 14 (2013) 2268–2271.
[4] a) M. Ali, C. Ping, Y. Mok, L. Ling, M. Whiteman, M. Bhatia, P.K. Moore, Brit. J. Pharmacol. 149 (2006) 625–634;
b) Y. Cheng, J. Nidisang, G. Tang, K. Cao, R. Wang, J. Am, Physiol., Heart Circul. Physiol. 287 (2004) H2316–H2323;
c) C. Coletta, A. Papapetropoulos, K. Erdelyi, G. Olah, K. Modis, P. Panopoulos, A. Asimakopoulou, D. Gero, I. Sharina, E. Martin, C. Szabo, Proc. Natl. Acad. Sci. USA 23 (2012) 801–807;
d) M. Bhatia, Life 57 (2005) 603–606;
e) J. Du, Y. Hui, Y. Cheung, G. Ben, H. Jiang, X. Chen, C. Tang, Heart Vessels 19 (2004) 75–80;
f) B. Geng, J. Yang, Y. Qi, J. Zhao, Y. Pang, J. Du, C.H. Tang, Biochem. Biophys. Res. Comm. 313 (2004) 362–368;
g) G. Tang, L. Wu, W. Liang, R. Wang, Am. Soc. Pharmacol. Exp. Therapeut. 69 (2005) 1757–1764.
[5] a) S. Fiorucci, E. Distrutti, G. Cirino, J.L. Wallace, Gastroenterology 131 (2006) 259–271;
b) D.R. Linden, Antioxid. Redox Signal. 10 (2014) 818–830;
c) L. Tomasova, L. Dobrowolski, H. Jurkowska, M. Wrobel, T. Huc, K. Ondrias, R. Ostaszewski, M. Ufnal, Nitric Oxide 60 (2016) 50–58.
[6] a) K.N. Maclean, J.P. Kraus, in *Signal transduction and the gasotransmitters*, Humana Press, New Jersey, 2004, pp. 275–292;
b) H. Kimura, Amino Acids 41 (2011) 113–121;
c) N. Shibuya, M. Tanaka, M. Yoshida, Y. Ogasawara, T. Togawa, K. Ishii, H. Kimura, Antioxid Redox Signal. 11 (2009) 703–714;
d) N. Nagahara, T. Ito, H. Kitamura, T. Nishino, Histochem Cell. Biol. 110 (1998) 243–250;
e) J. Bełtowski, D. Tokarzewska, Biomed. Rev. 20 (2010) 7–15;
f) T. Chiku, D. Padovani, W. Zhu, S. Singh, V. Vitvitsky, R. Banerjee, J. Biol. Chem. 284 (2009) 11601–11612;
g) N. Shibuya, Y. Mikami, Y. Kimura, N. Nagahara, H. Kimura, J. Biochem. 146 (2009) 623–626;
h) S.E. Bearden, R.S. Beard, J.C. Pfau, Am. J. Physiol. Heart Circul. Physiol. 299 (2010) H1568–H1576.
[7] P.M. Kris-Etherton, K.D. Hecker, A. Bonanome, S.M. Coval, A.E. Binkoski, K.F. Hilpert, A.E. Griel, T.D. Etherton, Am. J. Med. 113 (2002) 71–88.
[8] G.A. Benavides, G.L. Squadrito, R.W. Mills, H.D. Patel, T.S. Isbell, R.P. Patel, V.M. Darley-Usmar, J.E. Doeller, D.W. Kraus, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 17977–17982.
[9] C. Jacob, A. Anwar, T. Burkholz, Planta Med. 74 (2008) 1580–1592.
[10] a) H. Amagase, J. Nutr. 136 (2006) 716S;
b) Y. Zhao, T.D. Biggs, M. Xian, Chem. Commun. 50 (2014) 11788–11805.
[11] a) E.R. DeLeon, G.F. Stoy, K.R. Olson, Anal. Biochem. 421 (2012) 203;
b) D. Yoo, R.C. Jupiter, E.A. Pankey, V.G. Reddy, J.A. Edward, K.W. Swan, T.C. Peak, R. Mostany, P.J. Kadowitz, Am. J. Physiol. Heart. Circ. Physiol. 309 (2015) H605–H614.
[12] R.C. Zanardo, V. Brancaleone, E. Distrutti, S. Fiorucci, G. Cirino, J.L. Wallace, FASEB J. 20 (2006) 2118–2120.
[13] W.M. Zhao, J. Zhang, Y.J. Lu, R. Wang, EMBO J. 20 (2001) 6008–6016.
[14] L. Liu, H. Liu, D. Sun, W. Qiao, Y. Qi, H. Sun, C. Yan, Circ. J. 76 (2012) 1012–1019.
[15] J.W. Calvert, M. Elston, C.K. Nicholson, S. Gundewar, S. Jha, J.W. Elrod, A. Ramachandran, D.J. Lefer, Circulation 122 (2010) 11–19.
[16] R. Greiner, Z. Palinkas, K. Basell, D. Becher, H. Antelmann, P. Nagy, T.P. Dick, Antioxid. Redox Signaling. 19 (2013) 1749–1765.
[17] a) C.R. Powell, K.M. Dillon, J.B. Matson, Biochem. Pharm. 149 (2018) 110–123;
b) C. Szabo, A. Papapetropoulos, Pharmacol. Rev. 69 (2017) 497–564;
c) C. Yang, L. Chen, S. Xu, J.J. Day, X. Li, M. Xian, Front. Pharmacol. 8 (2017) 664;
d) M.M. Cerda, T.D. Newton, Y. Zhao, B.K. Collins, C.H. Hendon, M.D. Pluth, Chem. Sci. 10 (2019) 1773–1779;
e) N. Lougiakis, A. Papapetropoulos, E. Gikas, S. Toumpas, P. Efentakis, R. Wedmann, A. Zoga, Z. Zhou, E.K. Iliodromitis, A.-L. Skaltsounis, M.R. Filipovic, N. Pouli, P. Marakos, I. Andreadou, J. Med. Chem. 59 (2016) 1776–1790;
f) S.D. Zanatta, B. Jarrott, S.J. Williams, Aust. J. Chem. 63 (2010) 946–957;
g) W. Yin, L. Lan, Z. Huang, J. Ji, J. Fang, X. Wang, H. Ji, S. Peng, J. Xu, Y. Zhang, Eur. J. Med. Chem. 115 (2016) 369–380;
h) Y. Zheng, B. Yu, K. Ji, Z. Pan, V. Chittavong, B. Wang, Angew. Chem. Int. Ed. 55 (2016) 4514–4518;
i) Y. Zhao, A.K. Steiger, M.D. Pluth, Chem. Commun. 54 (2018) 4951–4954;
j) Y. Zhao, H.A. Henthorn, M.D. Pluth, J. Am. Chem. Soc. 139 (2017) 16365–16376;
k) A.K. Steiger, S. Pardue, C.G. Kevil, M.D. Pluth, J. Am. Chem. Soc. 138 (2016) 7256–7259;
l) A.K. Steiger, Y. Yang, M. Royzen, M.D. Pluth, Chem. Commun. 53 (2017) 1378–1380;
m) M.M. Cerda, Y. Zhao, M.D. Pluth, J. Am. Chem. Soc. 140 (2018) 12574–12579.
[18] a) C.J. Gargallo, A. Lanas, J. Dig. Dis. 14 (2013) 55;
b) A. Sparetore, G. Santus, D. Giustarini, R. Rossi, P. Del Soldato, Expert Rev. Clin. Pharmacol. 4 (2011) 109–121;
c) J.L. Chan, J.L. Wallace, Am. J. Physiol. 305 (2013) G467–G473.
[19] a) Y. Zhao, H. Wang, M. Xian, J. Am. Chem. Soc. 133 (2011) 15;
b) Y. Zhao, S. Bhushan, C. Yang, H. Otsuka, J.D. Stein, A. Pacheco, B. Peng, N.O. Devarie-Baez, H.C. Aguilar, D.J. Lefer, M. Xian, A.C.S. Chem, Biol. 8 (2013) 1283–1290.
[20] a) Z.M. Voronkov, L. Shagun, L. Ermolyuk, L. Timokhina, J. Sulfur Chem. 25 (2004) 131;
b) Z.M. Lukeman, J.C. Scaiano, J. Am. Chem. Soc. 127 (2005) 7698;
c) Z.N. Fukushima, N. Ieda, K. Sasakura, T. Nagano, K. Hanaoka, T. Suzuki, N. Miyata, H. Nakagawa, Chem. Commun. 50 (2014) 587–589.
[21] L. Li, M. Whiteman, Y.Y. Guan, K.L. Neo, Y. Cheng, S.W. Lee, Y. Zhao, Circulation 117 (2008) 2351–2360.
[22] A. Drapala, D. Koszelewski, L. Tomasova, R. Ostaszewski, M. Grman, K. Ondrias, M. Ufnal, Acta Biochim. Pol. 64 (2017) 561–566.
[23] P.K. Moore, M. Whiteman, Chemistry, biochemistry and pharmacology of hydrogen sulfide, Springer, 2015.
[24] Y. Zheng, X. Ji, K. Ji, B. Wang, Acta Pharm. Sin. B. 5 (2015) 367–377.
[25] a) P. Reynaud, Y. El Hamad, C. Davrinche, E. Nguyen-Tri-Xuong, G. Tran, G.P. Rinjard, J. Heterocycl. Chem. 29 (1992) 991–993;
b) E. Barresi, G. Nesi, V. Citi, E. Piragine, I. Piano, S. Taliani, F. Da Settimo, S. Rapposelli, L. Testai, M.C. Breschi, C. Gargini, V. Calderone, A. Martelli, J. Med. Chem. 60 (2017) 7512–7523.
[26] I. Kraemer, W. Schunack, Arch. Pharm. (Weinheim, Ger.) 319 (1986) 1091–1098.
[27] B.K. Bhattacharya, H.H. Singh, L.D.S. Yadav, G. Hoornaert, Acta Chim. Acad. Sci. Hung. 110 (1982) 133–144.
[28] G.F. Meijs, E. Rizzardo, T.P.T. Le, Y. Chen, Makromol. Chem. 193 (1992) 369–378.
[29] a) D.B. Sherman, A.F. Spatola, W.S. Wire, T.F. Burks, T.M.D. Nguyen, P.W. Schiller, Biochem. Biophys. Res. Commun. 162 (1982) 1126–1132;
b) W. Tan, J. Wei, X. Jiang, Org. Lett. 19 (2017) 2166–2169;
c) A. Shakeel, A.A. Altaf, A.M. Qures, A. Badshah, J. Drug Design Med. Chem. 2 (2016) 10–20;
d) D.C. Schroeder, Chem. Rev. 55 (1955) 181–228;
e) R. Grzywa, Ł. Winiarski, M. Psurski, A. Rudnicka, J. Wietrzyk, T. Gajda, J. Oleksyszyn, Bioorg. Med. Chem. Lett. 26 (2016) 667–671;
f) T. Murai, Top Curr. Chem. 251 (2005) 247–272;
g) R.N. Hurd, G. DeLaMater, Chem. Rev. 61 (1981) 45–86;
h) T.S. Jagodzinski, Chem. Rev. 103 (2003) 197–228;
i) P.A. Bartlett, K.L. Spear, N.E. Jacobsen, Biochemistry 21 (1982) 1608–1611;
j) M.D. Bond, B. Holmquist, B.L. Vallee, J. Inorg. Biochem. 28 (1986) 97–105;
k) K.L. Yu, A.F. Torri, G. Luo, C. Cianci, K. Grant-Young, S. Danetz, N.A. Meanwell, Bioorg. Med. Chem. Lett. 12 (2002) 3379–3382;
l) Q.L. Wei, S.S. Zhang, J. Gao, W.H. Li, L.Z. Xu, Z.G. Yu, Bioorg. Med. Chem. Lett. 14 (2006) 7146–7153;
m) M.K. Gannon, J.J. Holt, S.M. Bennett, B.R. Wetzel, T.W. Loo, M.C. Bartlett, T.J. Raub, J. Med. Chem. 52 (2009) 3328–3341;
n) E.J. Petersson, J.M. Goldberg, R.F. Wissner, Phy. Chem. Chem. Phys. 16 (2014) 6827–6837.
[30] a) A. Albert, H. Knecht, E. Andersen, V. Hungerford, M.H. Schreier, C. Papageorgiou, Bioorg. Med. Chem. 8 (1988) 2203–2205;
b) E. Rodríguez-Fernández, J.L. Manzano, J.J. Benito, R. Hermosa, E. Monte, J.J. Criado, J. Inorg. Biochem. 99 (2005) 1558–1572;
c) O.J. D'Cruz, F.M. Uckun, Curr. HIV Res. 4 (2006) 329–345;
d) D. Manna, G. Roy, G. Mugesh, Acc. Chem. Res. 46 (2013) 2706–2715;
e) M. Weiss, R. Buhl, M. Medve, E.M. Schneider, Crit. Care Med. 25 (1997) 128–134;
f) L. Harper, L. Chin, J. Daykin, A. Allahabadia, J. Heward, S.C. Gough, C.O. Savage, J.A. Franklyn, Clin. Endocrinol. 60 (2004) 671–675;
g) I.V. Van Daele, H. Munier-Lehmann, M. Froeyen, J. Balzarini, S.V. Van Calenbergh, J. Med. Chem. 50 (2007) 5281–5292;
h) Y. Touati-Jallabe, E. Bojnik, B. Legrand, E. Mauchauffée, N.N. Chung, P.W. Schiller, S. Benyhe, M.C. Averlant-Petit, J. Martinez, J.F. Hernandez, J. Med. Chem. 56 (2013) 5964–5973;
i) L.Y. Ma, Y.C. Zheng, S.Q. Wang, B. Wang, Z.R. Wang, L.P. Pang, M. Zhang, J.W. Wang, L. Ding, J. Li, C. Wang, B. Hu, Y. Liu, X.D. Zhang, J.J. Wang, Z.J. Wang, W. Zhao, H.M. Liu, J. Med. Chem. 58 (2015) 1705–1716.
[31] a) P. Jeschke, A. Harder, W. Etzel, W. Dau, G. Thielking, G. Bonse, K. Linuma, Pest Manag. Sci. 57 (2001) 1000–1006;
b) T. Lincke, S. Behnken, K. Ishida, M. Roth, C. Hertweck, Angew. Chem. 122 (2010) 2055–2057;
c) P. Angehrn, E. Goetschi, H. Gmuender, P. Hebeisen, M. Hennig, B. Kuhn, T. Luebbers, P. Reindl, F. Ricklin, A. Schmitt-Hoffmann, J. Med. Chem. 54 (2011) 2207–12024;
d) A. Bach, J.N.N. Eildal, N. Stuhr-Hansen, R. Deeskamp, M. Gottschalk, S.W. Pedersen, A.S. Kristensen, K. Strømgaard, J. Med. Chem. 54 (2011) 1333–1346.
[32] M.H. Cynamon, R. Gimi, F. Gyenes, C.A. Sharpe, K.E. Bergmann, H.J. Han, L.B. Gregor, R. Rapolu, G. Luciano, T. Welch, J. Med. Chem. 38 (1995) 3902–3907.
[33] T.E. Renau, M.S. Ludwig, J.C. Drach, L.B. Townsend, Bioorg. Med. Chem. Lett. 2 (1992) 1755–1760.
[34] a) A.S. Mehanna, J.D. Belani, C.J. Kelley, L.A. Pallansc, Med. Chem. 3 (2007) 513–519;
b) S. Banala, R.D. Süssmuth, ChemBioChem 11 (2010) 1335–1337.
[35] L. Lehrman, P. Schneider, J. Chem. Educ. 32 (1955) 474–475.
[36] X. Wang, B. Wang, Q. Huang, B. Zhang, Z. Hua, Zhonghua Yi Xue Za Zhi 95 (2015) 3176–3179.
[37] a) M.R. Maddani, K.R. Prabhu, J. Org. Chem. 75 (2010) 2327–2332;
b) H. Miyabe, Y. Takemoto, Bull. Chem. Soc. Japan. 81 (2008) 785–795;
c) W. Du, D.P. Curran, Org. Lett. 5 (2003) 1765–1768.
[38] K.A. Mahammed, V.P. Jayashankar, N. Premsai Rai, K. Mohana Raju, P.N. Arunachalam, Synlett 14 (2009) 2338–2340.
[39] M.L. Boys, V.L. Downs, Synth. Commun. 36 (2006) 295–298.
[40] T. Kanbara, K. Okamoto, T. Yamamoto, Synlett (2007) 2687–2690.

[41] C.K. Khatri, A.S. Mali, G.U. Chaturbhuj, Monatsh. Chem. 148 (2017) 1463–1468.
[42] a) A. Manaka, M. Sato, Synth. Commun. 35 (2005) 761–764;
b) Z.D. Wang, G. Feng, M.G. Garcia, T. Wang, Eur. J. Org. Chem. (2018) 598–604.
[43] S. Ray, A. Bhaumik, A. Dutta, R.J. Butcher, C. Mukhopadhyay, Tetrahedron Lett. 54 (2013) 2164–2170.
[44] Z. Yin, B. Zheng, J. Sulfur Chem. 34 (2013) 527–531.
[45] a) T. Ozturk, E. Ertas, O. Mert, Chem. Rev. 110 (2010) 3419–3478;
b) Y.A. Tayade, A.D. Jangale, D.S. Dalal, Chem. Select 3 (2018) 8895–8900.
[46] Z. Kaleta, G. Tarkanyi, A. Gormory, F. Kalman, T. Nagy, T. Soos, Org. Lett. 8 (2006) 1093–1095.
[47] U. Pathak, L. Pandey, S. Mathur, M. Suryanarayana, Synthesis 44 (2011) 377–379.
[48] C.H. Yang, G.-J. Li, C.-J. Gong, Y.-M. Li, Tetrahedron 71 (2015) 637–642.
[49] H.Z. Lecher, R.A. Greenwood, K.C. Whitehouse, T.H. Chao, J. Am. Chem. Soc. 78 (1956) 5018–5022.
[50] S. Scheibye, J. Kristensen, S.O. Lawesson, Tetrahedron 35 (1979) 1339–1343.
[51] J. Perregaard, S. Scheibye, H.J. Meyer, I. Thomsen, S.O. Lawesson, Bull. Soc. Chim. Belg. 86 (1977) 679–691.
[52] T.J. Curphey, J. Org. Chem. 67 (2002) 6461–6473.
[53] I. Thomsen, K. Clausen, S. Scheibye, S.O. Lawesson, Org. Synth. 62 (1984) 158–164.
[54] T.J. Curphey, Tetrahedron Lett. 43 (2002) 371–373.
[55] T.J. Curphey, Tetrahedron Lett. 41 (2000) 9963–9966.
[56] B. Narayana, K.K. Vijaya Raj, B.V. Ashalatha, N. Suchetha Kumari, B.K. Sarojini, European Journal of Medicinal Chemistry 39 (2004) 867–872.
[57] B. Kaboudin, L. Malekzadeh, Synlett 19 (2011) 2807–2810.
[58] F. Shibahara, R. Sugiura, T. Murai, Org. Lett. 11 (2009) 3064–3067.
[59] M. Sarkis, D.-N. Tran, M.C.D. Lang, C. Garbay, E. Braud, Synlett 25 (2014) 1257–1262.
[60] M. Urbano, M. Guerrero, S. Velaparthi, M. Crisp, P. Chase, P. Hodder, M.T. Schaeffer, S. Brown, H. Rosen, E. Roberts, Bioorg Med Chem Lett. 21 (2011) 6739–6745.
[61] Y. He, L. Zhang, X. Liu, X. Li, M. Zheng, H. Li, K. Yu, K. Chen, X. Shen, H. Jiang, H. Liu, J. Med. Chem. 52 (2009) 2465–2481.
[62] Y. Hagooly, S. Rozen, J. Org. Chem. 73 (2008) 6780–6783.
[63] A.S. Nashet, D.T. Osuga, R.E. Feeney, Anal. Biochem. 79 (1977) 394–405.
[64] G.L. Ellman, Arch. *Biochem. Biophys*. 82 (1959) 70–77.
[65] T. Gura, Nat. Med. 14 (2008) 706–709.
[66] a) H. Peng, Y. Cheng, C. Dai, A.L. King, B.L. Predmore, D.J. Lefer, B. Wang, Angew. Chem. Int. Ed. 50 (2011) 9672–9675;
b) R. Wang, Z. Li, C. Zhang, Y. Li, G. Xu, Q.-Z. Zhang, L.-Y. Li, L. Yi, Z. Xi, ChemBioChem 17 (2016) 962–968;
c) R. Kawagoe, I. Takashima, K. Usui, A. Kanegae, Y. Ozawa, A. Ojida, ChemBioChem 16 (2015) 1608–1615.
[67] a) J.P. Goddard, J.L. Reymond, Trends Biotechnol. 22 (2004) 363–370;
b) H.A. Henthorn, M.D. Pluth, J. Am. Chem. Soc. 137 (2015) 15330–15336.
[68] a) B. Chen, W. Li, C. Lv, M. Zhao, H. Jin, H. Jin, J. Du, L. Zhang, X. Tang, Analyst 138 (2013) 946–951;
b) Z. Wu, Z. Li, L. Yang, J. Han, S. Han, Chem. Commun. 48 (2012) 10120–10122.
[69] A. Martelli, L. Testai, V. Citi, A. Marino, I. Pugliesi, E. Barresi, G. Nesi, S. Rapposelli, S. Taliani, F. Da Settimo, M.C. Breschi, V. Calderone, A.C.S. Med, Chem. Lett. 4 (2013) 904–908.
[70] Y. Zhao, H. Wang, M. Xian, J. Am. Chem. Soc. 133 (2011) 15–17.
[71] Y. Zhao, S. Bhushan, C. Yang, H. Otsuka, J.D. Stein, A. Pacheco, B. Peng, N.O. Devarie-Baez, H.C. Aguilar, D.J. Lefer, M. Xian, ACS Chem. Biol. 8 (2013) 1283–1290.