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Discovery of small molecules with vasodilating characteristics and adjustable hydrolytic behavior

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ABSTRACT

In this contribution the development of a new class of vasodilating compounds obtained by lead structure optimization is described. Three groups of compounds were synthesized and tested for their activity on various smooth muscle preparations of the guinea pig. Beside the lead compound **3a**, the most interesting derivative was 1*H*-imidazole-1-carbothioic acid *O*-cyclohexyl ester hydrochloride (**5b**) with a good selective vasodilating potential on aorta and pulmonary artery rings (EC_{50} 14 μM and 24 μM, respectively). Due to the properties of small molecules the hydrolysis behavior of the compounds can be easily adapted hence opening a new route in terms of duration of the agent's effect. With the aid of structure–activity relationship studies, structural motifs influencing the biological activity on isolated smooth muscle cell preparations of the synthesized compounds were proposed. The presented compounds offer good tools in identifying promising molecules as emergency therapy in myocardial infarction.

1. Introduction

In developed countries, the leading cause of death and source of disability are myocardial infarction and arterial thrombosis (stroke). The main determinant of clinical outcome is well established as the fast and complete restoration of blood flow to the blocked artery[1] either by thrombolysis or by acute percutaneous coronary interventions and reopening of the occluded vessel using a stent.[2] As a consequence anti-platelet drugs such as aspirin, clopidogrel and its recently developed follow-up drugs are used to preserve the open vessel.[3] Additionally, vasoactive drugs like nitrates are used to improve coronary blood flow and the hunt for the development of new nitric oxide (NO) releasing compounds still continues.[4] Whereas there have been a couple of new anti-platelet drugs promoted on the market, no new vasodilators have been clinically introduced.[5]

In this contribution, a unique collection of small sulfur-containing compounds was synthesized and tested for vasodilating activity, with their characteristics designed to act as emergency drugs in the treatment of ischemia. The main idea is that the presented compounds are supposed to act immediately as vasoactive compounds, which can for instance be applied in the status of acute ischemia. In this case, after controlling the initial phase the compounds should facilitate the body's duty to get rid of the xenobiotics via degrading themselves through hydrolysis into more polar compounds that can be excreted more easily. This seems contradictory to the general aim of medicinal chemists which is to prolong the duration of a compound's activity within the body by making it more metabolically stable.[6] We present compounds offering the possibility to adjust their hydrolysis properties but still keep the desired biological activity. Thinking ahead, this is—to the best of our knowledge—a new concept, which may open the way to the application of a specific compound/medication depending on how long the patient needs the agent's action.

Previous studies in which we showed that dithio- and thiobenzanilides act as potent spasmolytic agents compared to their benzanilide derivatives[7,8] and that the sulfur present in their scaffold was responsible for the increased activity[9] led us to investigate the biological activity of the small molecule **3a**. The base was commercially available and its short synthesis (conversion to the hydrochloride salt) as well as biological profile on smooth muscle cell preparations made it an attractive candidate for lead structure optimization. Conversion of the compound into its hydrochloride salt resulted in a change of the aggregation state from fluid to solid thus providing a better accessibility to the biological investigation (scaling, solubility). In order to obtain compounds showing the ability to widen selectively blood vessels we modified the structure around the thiocarbonyl group, which was synthetically achieved by 'growing' of compound **3a**. Thus we gained three different groups of compounds with partly high vasodilating activity on aorta and pulmonary artery rings of the guinea pig as assessed

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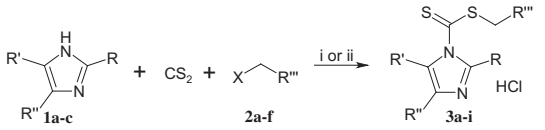


1a R,R',R'': H
1b R: CH_3; R'-R'': CH=CH-CH=CH
1c R: CH_3; R',R'': H

2a X: I; R''': H
2b X: Cl; R''': C_6H_5
2c X: Cl; R''': $(4\text{-}OCH_3)C_6H_4$
2d X: Cl; R''': $(3,4,5\text{-}(OCH_3)_3)C_6H_2$
2e X: Br; R''': $C_2H_4COOC_2H_5$
2f X: Br; R''': $C_2H_4OCOCH_3$

3a R, R', R'', R''': H
3b R, R', R'': H; R''': C_6H_5
3c R, R', R'': H; R''': $(4\text{-}OCH_3)C_6H_4$
3d R, R', R'': H; R''': $(3,4,5\text{-}(OCH_3)_3)C_6H_2$
3e R, R', R'': H; R''': $CH_2COOC_2H_5$
3f R, R', R'': H; R''': CH_2OCOCH_3
3g R: CH_3; R'-R'': CH=CH-CH=CH; R'''=H
3h R: CH_3; R', R'', R''': H
3i R: CH_3; R',R'':H; R''': C_6H_5

Scheme 1. Conditions: (i) NaH, THF, 0 °C; diethylether, 1 M HCl; (ii) K_3PO_4, acetone, room temp; diethylether, 1 M HCl.

by isometric tension recording. Hydrolysis studies, studies concerning their mechanisms of action as well as information of the chemical space covered by the compounds using ChemGPS-NP[10–12] are presented.

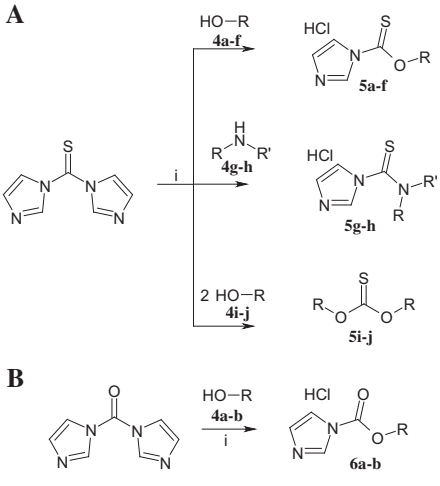
2. Results and discussion

2.1. Chemistry

Applying the approach of lead structure optimization to obtain selectively vasodilating small molecules, we forwarded our studies and designed three different groups of compounds: dithiocarbamide derivatives, *O*-thiocarbamate/carbamide derivatives as well as diesters of thiocarbonic acid. The synthetic routes used to prepare the dithiocarbamide derivatives are summarized in Scheme 1. In general, two routes were available to obtain the desired compounds. The first protocol includes the conversion of imidazole or an imidazole derivative to the corresponding imidazolide under the influence of sodium hydride in tetrahydrofuran (THF) under argon atmosphere at 0 °C followed by adding carbonyl disulfide (CS_2). The end products were obtained by addition of the appropriate alkyl halide at room temperature.[13] This procedure was applied for the preparation of compounds **3a–d** and **3h**. The preparation of compounds **3e–g** and **3i** required the use of potassium phosphate in acetone as described by Wang et al.[14] An excess of CS_2 was added at room temperature followed by the corresponding alkyl halide. In both cases, the compounds were further converted to their hydrochloric salt by using 1 M hydrochloric acid in diethylether to improve water solubility, which represented an advantage in the following biological assays in terms of reproducibility. The compound **3d** was not able to be converted into the salt form and therefore was tested as its free base. The *O*-thiocarbamates **5a–f** as well as the thiocarbamides **5g** and **5h** were synthesized by reaction of 1,1′-thiocarbonyldiimidazole (TCDI) and the corresponding alcohol and amine component, respectively, in dry THF. The reaction worked equally well for both the thiocarbamates and -carbamides with yields above 60%. For the synthesis of the symmetric diesters **5i** and **5j** the same synthetic procedure was applied but with 2 equiv of the corresponding alcohol component. The oxygen analogs of **5a** and **5b**, **6a** and **6b**, were obtained by the same procedure but with 1,1′-carbonyldiimidazole as starting material (Scheme 2). The synthesis of the compounds **8a–h** required replacing the imidazolide moiety against an alkyloxy and -amino substituent, respectively, thus obtaining either symmetrical or asymmetrical diester/-amides of the thiocarbonic acid (Scheme 3). Elemental analysis was used for characterization as well as purity confirmation of the compounds. For the compounds **5a**, **6a**, **8c**, **8d** and **8g** the elemental analysis provided unconvincing data and therefore the bases of these compounds were used and characterized by HRMS.

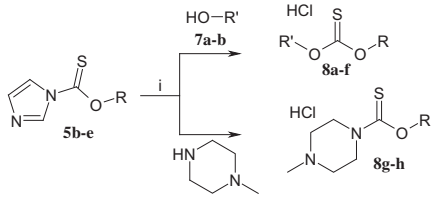
2.2. Activities on smooth and heart muscle preparations and SAR

The vasodilating potency of the compounds was assessed on isolated aorta and pulmonary artery rings of the guinea pig precontracted by 90 mM potassium chloride. A possible spasmolytic activity was studied on terminal ileum preparations



a R: $CH(CH_3)_2$
b R: cyclohexyl
c R: CH_2CH_3
d R: $(CH_2)_2CH_3$
e R: $(CH_2)_3CH_3$
f R: cyclopentyl
g R-R': $(CH_2)_5$
h R, R': $CH_2CH(CH_3)_2$
i R: $(CH_2)_2N(CH_3)_2$; HCl
j R: $(CH_2)_2N(CH_3)_3{}^+Cl^-$

Scheme 2. Conditions: (i) THF, reflux; diethylether, 1 M HCl.



7a R': $(CH_2)_3N(CH_3)_2$
7b R': $(CH_2)_2N(CH_3)_2$

8a R: hexyl; R': $(CH_2)_3N(CH_3)_2$
8b R: CH_2CH_3; R': $(CH_2)_2N(CH_3)_2$
8c R: CH_2CH_3; R': $(CH_2)_3N(CH_3)_2$
8d R: $(CH_2)_2CH_3$; R': $(CH_2)_2N(CH_3)_2$
8e R: $(CH_2)_2CH_3$; R': $(CH_2)_3N(CH_3)_2$
8f R: $(CH_2)_3CH_3$; R': $(CH_2)_3N(CH_3)_2$
8g R: CH_2CH_3
8h R: $(CH_2)_2CH_3$

Scheme 3. Conditions: (i) THF, reflux; diethylether, 1 M HCl.

pre-contracted by 60 mM potassium chloride and inotropic and chronotropic activity, respectively, was investigated on electrically stimulated papillary muscles and isolated right atria. Except for compound **5g** and the two oxygen-analogs **6a** and **6b**, all the dithiocarbamide as well as *O*-thiocarbamate/carbamide derivatives caused relaxation of the pre-contracted vessels in a concentration-dependent manner. Diesters of the thiocarbonic acid proved to be unable to dilate KCl-pre-contracted vessel strips (Table 1). Within the most active group of compounds, the dithiocarbamides, which also contains the lead compound **3a**, every congener showed activity on vascular smooth muscle preparations. Structural extension of the lead scaffold through insertion of an unsubstituted phenyl ring (**3b**) resulted in a potency decrease on aorta, pulmonary artery as well as terminal ileum preparations. Concerning its inotropic activity the compound showed about two-fold increased potential compared to that of **3a**. Considering the activity of the compounds **3c** and **3d**, the vasodilating as well as spasmolytic activity correlates with the number of methoxy groups on the phenyl ring. The trimethoxy benzyl derivative **3d** elucidated improved vasodilating properties on the one hand but on the other hand the terminal ileum as well as papillary muscle preparations were also affected by this compound (15 and 27 μM, respectively). Conversely, the para monomethoxy compound **3c** had less impact on the vasculature than **3a** (13 vs 7.8 μM on aorta preparations and 24 vs 6.9 μM on pulmonary artery rings) but showed selectivity towards vascular tissue up to more than 100 μM giving this compound a highly interesting biological profile.

Replacing the benzyl group by a propionic acid ethyl ester unit (**3e**) slightly reduced activity on the vascular rings and terminal ileum preparations compared to **3a**. Compound **3f** comprising an ethylacetate motif showed a decreased activity on pulmonary artery rings on the one hand, but on the other hand the biological activity of the lead compound on aortic rings as well as papillary muscles was retained (5.8 and 77 μM, respectively). In case of compound **3h**, insertion of a 2-methyl unit on the imidazole core area of **3a** provided similar biological activity on aorta, pulmonary artery rings and terminal ileum preparations. But, compared to the lead **3a**, **3h** did not affect the papillary muscle up to a concentration of 100 μM. Conversely, insertion of a 2-methyl group together with a phenyl motif in the imidazole unit led to decreased activity on the vasculature and ileum, abolished inotropic potency and suddenly to a moderately negative chronotropic activity (56 μM). This phenomenon was also observed in the case of compound **3i** comprising a 2-methyl group on the imidazole ring as well as a benzyl motif linked to the dithiocarbamide unit with a negative chronotropic potential of 15.5 μM. Furthermore, this compound shows the highest vasodilating potential on aortic rings within its group and slightly decreased activity was observed on pulmonary artery and terminal ileum tissue.

In a next step we examined the effect of *O*-thiocarbamate/carbamide derivatives comprising an imidazole on the *N*-side of the carbamic acid as well as a variety of aliphatic alcohols as esters and amines to finally obtain the carbamide derivatives. Interestingly, we observed a steric effect correlating with the vasodilating and spasmolytic activity of the *O*-thiocarbamate compounds. The vasodilating potential of the compounds on aortic rings increased with the number of carbon atoms in the ester motif whereas the antispasmodic effect of the compounds seemed to be in inverse ratio to the size of the ester unit. Compounds **5c** and **5a** representing an ethyl and isopropyl carbamic acid ester showed moderate activity on aorta preparations (53 μM and 46 μM, respectively). **5c** elucidated a high spasmolytic potential (4.8 μM) whereas **5a** showed only modest antispasmodic activity (21 μM). Both compounds did not influence heart muscle preparations up to 100 μM but interestingly, in the case of **5c** a vasodilating effect on pulmonary artery preparations was observed (19 μM). Elongation of the ester motif by one or two methylene groups, respectively, resulted in compounds **5d** and **5e**. Compound **5d** displayed a slight enhancement of the vasodilating activity on aortic strips whereas **5e** showed a two-fold increase in the vasodilating potency compared to **5a** and activity was also present on pulmonary artery rings. The elongation of the alkyl chain did not show any influence on the antispasmodic properties of the compounds. The cyclopentyl ester **5f** showed vasodilating activity similar to that of **5a** but was able to slightly improve the spasmodic potential. Conversely, the cyclohexyl ester **5b** lost the antispasmodic properties up to 100 μM and therefore showed selective activity on the vessel preparations of the aorta and the pulmonary artery with EC_{50} values of 14 and 24 μM. Because of these data it could be suggested that the number of carbons at this area of the molecule plays a significant role in the selective vasodilating action of *O*-thiocarbamates. Moreover, evaluation of the oxygen analogs **6a** and **6b** revealed that the sp^2 sulfur atom (C=S) is crucial for biological activity (Table 1). Concerning the spasmolytic characteristic the activity enhancement of this type of replacement was also proven for thio- and dithiobenzanilide derivatives previously. The sulfur compounds showed higher biological activity than their corresponding amide congeners.[8,9] The thiocarbamide **5g** bearing a piperidine motif did not show any biological activity up to the highest concentration tested (100 μM). Differently behaved was the thiocarbamide derivative **5h** with two *iso*-butyl units at the carbamide nitrogen. On the one hand, this compound exhibited vasodilating activity on aorta (14 μM) as well as pulmonary artery (26 μM) rings and spasmolytic potential on isolated terminal ileum preparations (17 μM) and on the other hand the compound acted as negative chronotropic (48 μM) agent with a distinct negative inotropic behavior (7 μM).

Within the diesters of the thiocarbonic acid, the symmetrical compound **5i** as well as the di-ion **5j** did not show any biological activity on the tested isolated tissue preparations up to a concentration of 100 μM. These data suggest—compared to the compounds **8a** and **8d**–**f**—that on one side of the molecule electron-rich substituents are contraindicated and that lipophilic aryl moieties are preferred. Considering compounds **8a**–**f**, the inotropic activity of the diesters on isolated papillary muscles is increasing in the order ethyl (>100 μM) < propyl (37 μM) < cyclohexyl (5.9 μM) and butyl (4.3 μM). The cyclohexyl derivative **8a** was the only representative of this set of compounds demonstrating activity also on isolated right atria. These data suggest a fairly narrow chemical space for this class of compounds targeting the chronotropic activity. Regarding the spasmolytic activity of **8a** on terminal ileum preparations it had almost similar activity as compound **8f**. Replacement of one ester unit by a piperidine to obtain a thiocarbamate scaffold plus retention of the ethyl and propyl ester function, respectively, revealed compounds **8g** and **8h** with biological activity higher than 100 μM. Hence, the diesters of the thiocarbonic acid can be regarded as the biologically preferred scaffold concerning their potential on heart muscle preparations throughout all groups of compounds presented herein.

2.3. Analysis of the chemical space

To substantiate the SAR studies and to get an overview of the chemical space occupied by the compounds, they were mapped using ChemGPS-NP.[10–12] Using this web-based tool, it is possible to describe the compounds by means of structure-derived physico-chemical properties (e.g., size, shape, aromaticity, flexibility, lipophilicity and polarity). Based on eight principle components (PC) describing relevant descriptors compounds are able to be mapped into the chemical space using interpolation according to PC analysis score prediction.[15] In this study, the first four dimensions accounting for 77% of data variance were used for

Table 1
EC_{50} values in μM of the compounds on different smooth and heart muscle preparations of the guinea pig as well as information about the aromatic nature of the compounds

Compd	C=S present	Aromaticity present	Aorta	Pulmonary artery	Terminal ileum	Papillary muscle	Right atrium
3a	Yes	Yes	7.8	6.9	5.7	78	>100
3b	Yes	Yes	20	63	40	30	>100
3c	Yes	Yes	13	24	>100	>100	>100
3d	Yes	Yes	3.0	7.9	15	27	>100
3e	Yes	Yes	16	20	32	>100	>100
3f	Yes	Yes	5.8	24	>100	77	>100
3g	Yes	Yes	23	25	26	56	>100
3h	Yes	Yes	6.3	11	9.5	>100	>100
3i	Yes	Yes	3.4	13	16	>100	16
5a	Yes	Yes	46	>100	21	>100	>100
5b	Yes	Yes	14	24	>100	>100	>100
5c	Yes	Yes	53	19	4.8	>100	>100
5d	Yes	Yes	30	100	21	>100	>100
5e	Yes	Yes	13	23	21	>100	>100
5f	Yes	Yes	36	>100	16	>100	>100
5g	Yes	Yes	>100	>100	>100	>100	>100
5h	Yes	Yes	14	26	17	6.5	48
5i	Yes	No	>100	>100	>100	>100	>100
5j	Yes	No	>100	>100	>100	>100	>100
6a	No	Yes	>100	>100	>100	>100	>100
6b	No	Yes	>100	>100	>100	>100	>100
8a	Yes	No	>100	>100	16	5.9	35
8b	Yes	No	>100	>100	>100	>100	>100
8c	Yes	No	>100	>100	>100	>100	>100
8d	Yes	No	>100	>100	>100	58	>100
8e	Yes	No	>100	>100	>100	37	>100
8f	Yes	No	>100	>100	17	4.3	>100
8g	Yes	No	>100	>100	>100	>100	>100
8h	Yes	No	>100	>100	>100	>100	>100

interpretation.[12] The synthesized chemical collection contains 29 compounds in total including dithiocarbamide derivatives, *O*-thiocarbamate/carbamide derivatives as well as diesters of the thiocarbonic acid. For the analysis of their chemical space, it was necessary to use the basic form of the compounds as well as the dicationic form of compound **5j** since there is not more than one structure in one SMILES allowed. As can be seen from Figure 1, the set of compounds covers completely the range of low size molecules as all of the compounds (spheres) are located at the negative side of the PC1-axis. PC2 describes the aromatic status of the compounds and based on this dimension the data set presents three volumes of chemical space and therefore allows to classify the compounds into derivatives with high, middle and low aromaticity/conjugational properties. The classification correlates with the found bioactivity of the new compounds and indicates aromaticity as key requirement, especially concerning the biological activity on vessel preparations (Table 1). Polarity issues of the presented compounds are described by PC3. The compound library shows a fairly similar amount of polar molecules, which are located in the negative direction of PC3, and lipophilic compounds, found in its positive direction. Mostly all of the compounds have a moderate degree of structural flexibility as indicated by their

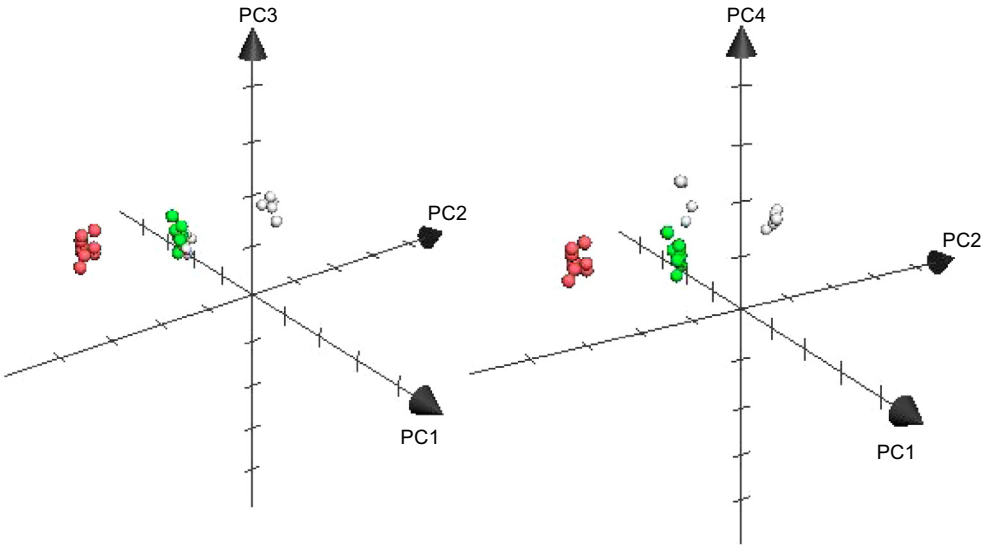


Figure 1. Three-dimensional representations of the chemical space occupied by the presented compounds using ChemGPS-NP.[10,12] The first four dimensions of ChemGPS-NP were used. The data are interpreted as follows: molecule size increases in the positive direction of PC1, aromaticity/conjugation-related properties increase in the positive direction of PC2, compounds are gradually more lipophilic in the positive direction of PC3 and they show higher flexibility in the positive direction of PC4. Color code: gray: dithiocarbamide derivatives, green: *O*-thiocarbamate/carbamide derivatives, red: diesters of the thiocarbonic acid.

location on the positive site of PC4. The clusters of compounds obtained by adding PC4 into the analysis did not show any correlation with the biological activity of the compounds.

2.4. Hydrolysis studies

Beside the evaluation of the physico-chemical properties we went on to validate the therapeutic potential of the compounds as emergency treatment options in ischemia and studied the hydrolytic behavior of three representatives in an aqueous solution. Compounds **3a**, **5b** and **5c** were chosen because the results were evaluated by the compound: degradation product ratio, which was clearly obtained by the ^{1}H NMR spectra. The three representatives were dissolved in D_2O at room temperature and measured by using ^{1}H NMR spectroscopy over a period of 24 hours. Time points were measured in triplicates after 1, 3, 5, 7 and 24 h. In case of **3a**, a dithiocarbamide derivative, we did not detect any degradation products in the reaction solution and thus we consider this compound as stable over a period of 24 h. Compounds **5b** and **5c**, both *O*-thiocarbamates, showed time-dependent hydrolysis properties (Fig. 2) and presented a degradation behavior in releasing their alcohol unit (cyclohexanol and ethanol). After 24 h, the compound: degradation product ratio for compound **5b** was 0.09:1 and for derivative **5c** 0.35:1 representing 8% and 26% left of the original compound, respectively. Their half-lives ($t_{1/2}$) were determined from the equation obtained by fitting the data (Fig. S1) and were 12.3 and 6.5 h for **5b** and **5c**, respectively (Fig. 2). Another chemically conceivable degradation product might be the small molecule COS. However, in this experimental setting, it was not possible to prove the release of this gaseous compound.

In a further set of experiments we wanted to know whether the compounds themselves or their degradation product, the alcohol component, was responsible for biological activity. Therefore, we dissolved compound **5b** and **5c**, respectively, in distilled water and kept the sealed solution at room temperature. After 24 h, we tested the dissolved compound in a concentration according to its EC_{50} value (15 μM for **5b** and 55 μM for **5c**, respectively) on isolated aortic rings in the same experimental setup (pre-contracted by 90 mM KCl) as described for isometric contraction measurements. As demonstrated in Figure 3, both compounds did not show significant vasodilating activity after 24 h dissolved in water at room temperature. This indicates, that the primary compound acts as active agent and that the possibly split-off alcohol unit does not influence the contraction status of the vessel preparation.

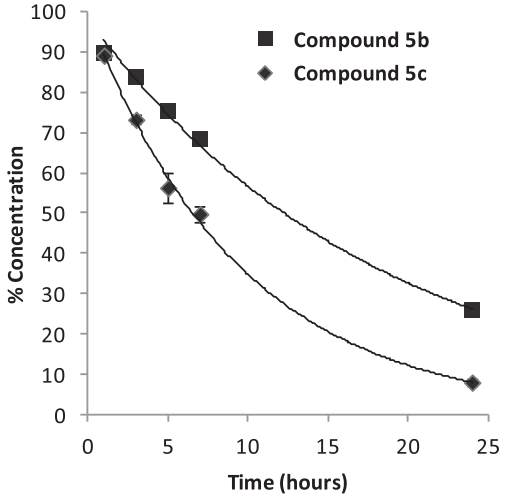


Figure 2. Time-course of the hydrolysis of compound **5b** and **5c** after 1, 3, 5, 7 and 24 h incubation time in D_2O at 20 °C. Values represent the mean of three independent experiments ± SEM.

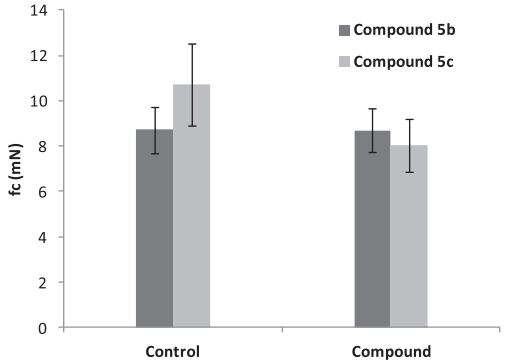


Figure 3. Activity of the compounds **5b** and **5c** on isolated aortic rings. The compounds were dissolved in distilled water 24 h before the experiment and kept at room temperature. Tested concentration was 15 and 55 μM for compound **5b** and **5c**, respectively; $n = 3$. Significance level: $^*P < 0.05$.

To conclude, the latter experiments serve as a first proof of concept that we developed compounds with adjustable hydrolysis behavior. This may be of special interest in the acute treatment of myocardial infarction as the administered compound concentration as well as the time of action open a way to be controlled more easily.

2.5. Mode of action studies

To get a deeper insight into a possible mode of action, two compounds, **3a** as a representative of the dithiocarbamide class, and **5b** representing the *O*-thiocarbamates, were investigated. Since ATP-dependent potassium channels (K_{ATP}-channels) are regarded as possible key players in smooth muscle relaxation mediated by hydrogen sulfide[16,17] and are suggested as an endogenous cardioprotective mechanism[18,19] we focused on studying the involvement of these channels in the biological activity of our compounds. To identify a possible participation of K_{ATP}-channels we tested the effect of compound **3a** and **5b** in presence of 30 and 100 μM glibenclamide, a selective K_{ATP}-channel inhibitor. Compounds were tested at concentrations similar to their EC_{50} values, which means for compound **3a** 8 μM and for compound **5b** 20 μM. However, as can be seen from Figure 4A, compound **3a** still exhibited a vasodilating potential of almost 50% in 30 as well as 100 μM glibenclamide-treated aortic rings indicating no influence of this type of channels in the activity of the dithiocarbamide **3a**. On the other hand, the *O*-thiocarbamate representative **5b** was not able to significantly dilate glibenclamide-pretreated vessel rings anymore suggesting an involvement of the K_{ATP}-channels in the compound's dilating potential. The vasodilation evoked by activation of K_{ATP}-channels can also be NO-mediated in the way that NO, produced by endothelial nitric oxide synthase (eNOS), leads to an increase in cGMP and hence may activate K_{ATP}-channels which results in a relaxation of vascular smooth muscles.[20–22] Therefore, we studied the effect of the most interesting compound, **5b**, together with the eNOS inhibitor nitro-L-arginine. The test compound did not show any significant vasodilating behavior at 20 μM in presence of 100 μM eNOS inhibitor but showed a similar activity profile as on K_{ATP}-channels (Fig. 4B). These data support the hypothesis, that compound **5b** acts through NO-mediated activation of K_{ATP}-channels initiated by stimulation of eNOS. Recently, Suvorava et al. stated the importance of therapeutically used compounds, which are able to improve the endothelial NO generation in cardiovascular disease.[23]

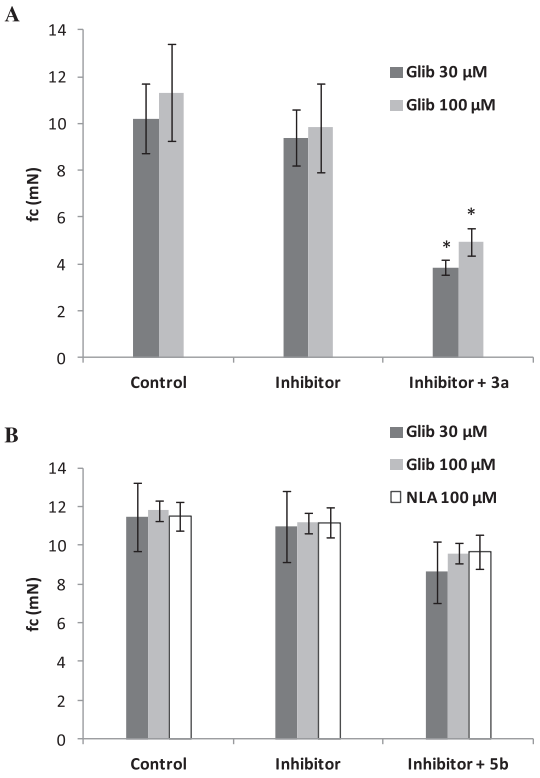


Figure 4. Mode of action of compound **3a** (A) and **5b** (B) on aortic rings precontracted by 90 mM in presence of glibenclamide (Glib) and nitro-L-arginine (NLA), respectively. The change in contraction force (f_c) in mN is plotted on the ordinate. Control represents the f_c before adding the inhibitor (glibenclamide and nitro-L-arginine, respectively) and 'inhibitor' characterizes the changes of f_c evoked by the inhibitor without test compound. The bars symbolize the arithmetic means ± SEM of three to four independent experiments. Significance level: $^{*}P$ <0.05.

3. Conclusion

By applying the idea of fragment-based drug discovery we were able to develop a new type of vasodilating agents presenting characteristics of being of interest in the treatment of acute myocardial infarction. The compounds were supposed to exhibit on the one side a high vasodilating potential and on the other side, they were thought to degrade within the body. The latter referring to a new concept that is giving the compounds a certain amount of instability in aqueous solution ($t_{1/2}$ around 10 h) thus obtaining substances which, once administered, degrade nonenzymatically into more hydrophilic components after a definite time and thus help the body to get rid of the xenobiotic more easily. Three different sets of compounds were synthesized. The biological investigations on isolated organ preparations together with the analysis of the chemical space occupied by the compounds revealed that an sp^2 sulfur atom as well as aromatic features present in the molecule have to be regarded as key requirements for good vasodilating properties. Investigations concerning the mode of action clearly demonstrated a difference in biological activity between the dithiocarbamide (**3a**) and *O*-thiocarbamate (**5b**) derivatives. Compound **3a** did not show any involvement of K_{ATP}-channels. On the contrary, in the case of compound **5b**, NO-mediated activation of K_{ATP}-channels initiated by stimulation of eNOS might be the reason for the compound's observed biological activity. This fact is regarded as an advantage in the development of compounds intended for treatment of myocardial infarction since K_{ATP}-channels as well as endothelial-generated NO are known as endogenous cardioprotective mechanisms.

Considering all these data, this new drug class of self-degrading compounds might represent a safe adjunct to the current treatment of for instance myocardial infarction.

4. Experimental section

4.1. Chemistry

All chemicals were purchased from Sigma–Aldrich Chemie GmbH (Schnelldorf, Germany), Merck (Darmstadt, Germany) or Apollo Scientific Ltd (Bredbury, UK) at analytical grade and used without further purification. Dry THF was freshly distilled over metal sodium and benzophenone. For synthesis of the hydrochloride salts dry diethylether stored over metal sodium was used as solvent. Purification was performed using preparative separation column chromatography (Merck silica gel 60, 230–400 mesh). Analytical TLC (thin layer chromatography) was carried out on pre-coated aluminum plates (Merck silica gel 60, F_{254}) and was used for monitoring the reactions. Melting points were determined on a Kofler melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker Advance DPx200 (200 and 50 MHz). Chemical shifts are reported in δ values (ppm) relative tetramethylsilane (TMS) as internal standard for ^{1}H NMR spectra and the solvent peak for ^{13}C NMR. Peak splitting patterns in the ^{1}H NMR are reported as follows: s, singlet; d, doublet; t, triplet; sept, septet; m, multiplet; br, broad. *J* values are reported in Hertz. Mass spectra (MS) were obtained with a Shimadzu (GC-17A; MS-QP5050A) spectrometer. Purity of the compounds was established by combustion analysis with a Perkin–Elmer 2400 CHN elemental analyzer confirming purity ⩾95%. HRMS was performed on a Bruker micrOTOF-Q II 10240.

4.1.1. General procedure of the preparation of 3a–d and 3h

To a solution of imidazole or its appropriate derivative (2.5 mmol) in dry THF (2 mL), stirred at 0 °C under argon atmosphere, was added a suspension of sodium hydride (NaH) (3 mmol). After 20 min stirring at 0 °C, 3 mmol carbonyl sulfide (CS_2) were added and the reaction mixture was allowed to reach room temperature. After additional 60 min, the corresponding alkyl halide (2.5 mmol) was added and stirred at room temperature till the end of the reaction (monitored by TLC). Then, the reaction mixture was filtered under reduced pressure. The solvent of the filtrate was removed in vacuo and the crude product was purified by column chromatography.[13] Next, the product was dissolved in diethylether, followed by addition of 1 M hydrochloric acid in diethylether. Finally, the so formed desired hydrochloride of the product was filtered off. Except for compound **3d**, all compounds were transformed into their corresponding hydrochloride.

4.1.1.1. 1*H*-Imidazole-1-carbodithioic acid methyl ester hydrochloride (3a)[24]. Yellow crystals; yield 95% (base: purchased from Sigma–Aldrich 694029); mp 108–114 °C. ^{1}H NMR (D_2O, 200 MHz) δ 9.59–9.40 (m, 1H), 8.20–8.02 (m, 1H), 7.54–7.49 (m, 1H), 2.76 (s, 3H). ^{13}C NMR (D_2O, 50 MHz) δ 197.7, 121.2, 119.9, 21.1.

4.1.1.2. 1*H*-Imidazole-1-carbodithioic acid benzylic ester hydrochloride (3b). Yellow crystals; yield 44%. Mp 90–110 °C. ^{1}H NMR (D_2O, 200 MHz) δ 9.43 (s, 1H), 8.05 (s, 1H), 7.42 (s, 1H), 7.38–7.00 (m, 5H), 4.58 (s, 2H). ^{13}C NMR (d_6-DMSO, 50 MHz) δ 197.4, 135.8, 133.9, 129.8, 129.0, 128.3, 127.1, 119.3, 41.6.

4.1.1.3. 1*H*-Imidazole-1-carbodithioic acid 4-methoxybenzylic ester hydrochloride (3c). Yellow crystals; yield 27%. Mp 84–98 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 13.68 (s, 1H), 9.70 (s, 1H), 7.99 (s, 1H), 7.60 (s, 1H), 7.40–7.28 (m, 2H), 6.98–6.81 (m, 2H), 4.63 (s, 2H), 3.81

(s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 193.6, 159.9, 132.9, 130.8 (2C), 123.3, 121.6, 118.5, 114.4 (2C), 55.3, 43.2.

4.1.1.4. 1*H*-Imidazole-1-carbodithioic acid 3,4,5-trimethoxybenzylic ester (3d)[24]. Yellow crystals; yield 39%. Mp 110–113 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.49 (s, 1H), 7.80–7.75 (m, 1H), 7.12–7.09 (m, 1H), 6.61 (s, 2H), 4.56 (s, 2H), 3.87 (s, 6H), 3.85 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 197.3, 153.4 (2C), 137.9, 131.6 (2C), 128.8, 117.6, 106.4 (2C), 60.8, 56.1 (2C), 42.3. MS *m*/*z* 324 (2%, M^+), 181 (100%), 148 (9%), 136 (5%).

4.1.1.5. 2-Methylimidazole-1-carbodithioic acid methyl ester hydrochloride (3h). Yellow crystals; yield: 87%. Mp: 146 °C. ^{1}H NMR (D_2O, 200 MHz) δ 7.73 (d, *J* = 2.3 Hz, 1H), 7.28 (d, *J* = 2.3 Hz, 1H), 2.74 (s, 3H), 2.67 (s, 3H). ^{13}C NMR (D_2O, 50 MHz) δ 199.6, 145.6, 121.9, 118.2, 21.9, 13.1.

4.1.2. General procedure of the preparation of 3e–g and 3i

To a solution of imidazole or its appropriate derivative (2.5 mmol) in acetone (5 mL), potassium phosphate (2.5 mmol) was added. After 15 min stirring at room temperature, 3 mmol carbonyl sulfide (CS_2) were added and the reaction mixture was stirred for additional 15 min. Then, the corresponding alkyl halide (2.5 mmol) was added and stirred at room temperature till the end of the reaction (monitored by TLC). After that, the reaction mixture was filtered under reduced pressure. The solvent of the filtrate was removed in vacuo and the crude product was purified by column chromatography.[14] Next, the product was dissolved in over sodium dried diethylether, followed by addition of 1 M hydrochloric acid in diethylether. Finally, the so formed desired hydrochloride of the product was filtered off.

4.1.2.1. 1*H*-Imidazole-1-carbodithioic acid (propionic acid ethyl ester) ester hydrochloride (3e). Yellow crystals; yield 17%. Mp 68–79 °C. ^{1}H NMR (D_2O, 200 MHz) δ 9.53–9.42 (m, 1H), 8.13–8.05 (m, 1H), 7.51–7.41 (m, 1H), 4.04 (q, *J* = 7.2 Hz, 2H), 3.62 (t, *J* = 6.8 Hz, 2H), 2.83 (t, *J* = 6.8 Hz, 2H), 1.10 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (D_2O, 50 MHz) δ 195.9, 174.0, 134.4, 121.2, 119.9, 62.6, 33.0, 31.8, 13.7.

4.1.2.2. 1*H*-Imidazole-1-carbodithioic acid ethylacetate ester hydrochloride (3f)[24]. Yellow crystals; yield 15%. Mp 70–87 °C. ^{1}H NMR (D_2O, 200 MHz) δ 9.62–9.50 (m, 1H), 8.23–8.14 (m, 1H), 7.58–7.50 (m, 1H), 4.40 (t, *J* = 5.9 Hz, 2H), 3.77 (t, *J* = 5.9 Hz, 2H), 2.01 (s, 3H). ^{13}C NMR (D_2O, 50 MHz) δ 196.7, 174.9, 135.3, 122.2, 120.7, 62.0, 37.5, 21.4.

4.1.2.3. 2-Methylbenzimidazole-1-carbodithioic acid methyl ester hydrochloride (3g). Orange crystals; yield 25%. Mp 90–114 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.09–7.94 (m, 1H), 7.80–7.67 (m, 1H), 7.66–7.50 (m, 2H), 3.12 (s, 3H), 3.04 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 197.2, 149.1, 131.0, 129.9, 127.1, 127.0, 115.7, 112.5, 22.3, 12.8.

4.1.2.4. 2-Methylimidazole-1-carbodithioic acid benzylic ester hydrochloride (3i). Yellow crystals; yield 7%. Mp 102–110 °C. ^{1}H NMR (D_2O, 200 MHz) δ 7.72–7.58 (m, 1H), 7.38–7.14 (m, 6H), 4.49 (s, 2H), 2.58 (s, 3H). ^{13}C NMR (D_2O, 50 MHz) δ 197.0, 145.6, 133.8, 129.8 (2C), 129.3 (2C), 128.8, 121.8, 118.3, 43.1, 13.2.

4.1.3. General procedure of the preparation of 5a–h

To a solution of 5 mmol 1,1′-thiocarbonyldiimidazole (0.89 g) in dry THF, 4.5 mmol of the corresponding alcohol and amine derivative, respectively, were added under argon atmosphere and stirred under reflux. After completion of the reaction (monitored by TLC) the reaction mixture was purified by column chromatography. The so-obtained product was dissolved in dry diethylether and 1 M HCl in diethylether was added to yield the hydrochloride salt of the compound.

4.1.3.1. 1*H*-Imidazole-1-carbothioic acid *O*-isopropyl ester hydrochloride (5a). White crystals; yield 65%. Mp 77–80 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 13.94 (s, br, 1H), 9.63 (s, 1H), 8.01–7.86 (m, 1H), 7.59–7.46 (m, 1H), 5.74 (sept, *J* = 6.2 Hz, 1H), 1.59 (d, *J* = 6.2 Hz, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 178.6, 134.0, 120.9, 119.2, 82.3, 20.9 (2C).

4.1.3.2. 1*H*-Imidazole-1-carbothioic acid *O*-cyclohexyl ester hydrochloride (5b). White crystals; yield 73%. Mp 93–95 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 10.26 (s, br, 1H), 9.55 (s, 1H), 8.00–7.88 (m, 1H), 7.60–7.48 (m, 1H), 5.67–5.39 (m, 1H), 2.22–1.27 (m, 10H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 178.5, 133.8, 121.2, 119.2, 86.7, 30.4 (2C), 24.7, 23.4.

4.1.3.3. 1*H*-Imidazole-1-carbothioic acid *O*-ethyl ester hydrochloride (5c). White crystals; yield 65%. Mp 102–105 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 13.35 (s, br, 1H), 9.71 (s, 1H), 7.95 (s, 1H), 7.54 (s, 1H), 4.87 (q, *J* = 7.1 Hz, 2H), 1.62 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 179.6, 126.7, 121.6, 119.1, 72.6, 13.5.

4.1.3.4. 1*H*-Imidazole-1-carbothioic acid *O*-propyl ester hydrochloride (5d). White crystals; yield 77%. Mp 66–70 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 11.87 (s, br, 1H), 9.65 (s, 1H), 7.95 (s, 1H), 7.57 (s, 1H), 4.75 (t, *J* = 7.1 Hz, 2H), 2.20–1.83 (m, 3H), 1.10 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 179.6, 134.1, 121.4, 119.1, 78.0, 21.2, 10.2.

4.1.3.5. 1*H*-Imidazole-1-carbothioic acid *O*-butyl ester hydrochloride (5e)[24]. White crystals; yield 70%. Mp 74–77 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 12.08, (s, br, 1H), 9.59 (s, 1H), 7.94 (s, 1H), 7.57 (s, 1H), 4.79 (t, *J* = 6.7 Hz, 2H), 2.13–1.80 (m, 3H), 1.62–1.40 (m, 3H), 1.02 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 179.5, 133.9, 121.3, 119.2, 76.5, 29.7, 19.0, 13.5.

4.1.3.6. 1*H*-Imidazole-1-carbothioic acid *O*-cyclopentyl ester hydrochloride (5f). White crystals; yield 73%. Mp 79–82 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 12.58 (s, br, 1H), 9.60 (s, 1H), 7.92 (s, 1H), 7.53 (s, 1H), 5.98–5.76 (m, 1H), 2.22–1.53 (m, 8H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 178.9, 133.9, 121.2, 119.2, 91.2, 32.4 (2C), 23.8 (2C).

4.1.3.7. 1*H*-Imidazole-1-yl-piperidine-1-yl-methanthione hydrochloride (5g). White crystals; yield 95%. Mp 140–156 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 9.76 (s, 1H), 7.63–7.46 (m, 2H), 4.68–3.30 (m, 4H), 2.38–1.52 (m, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 172.0, 134.9, 120.8, 120.1, 53.6 (2C), 23.5 (3C).

4.1.3.8. *N*,*N*-Diisobutyl-1*H*-imidazole-carbothioamide hydrochloride (5h). White crystals; yield 66%. Mp 110–113 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 9.65 (s, 1H), 7.63–7.38 (m, 2H), 4.27–3.04 (m, 4H), 2.78–1.66 (m, 2H), 1.52–0.34 (m, 12H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 174.1, 134.3, 120.9, 120.4, 61.8 (2C), 20.0 (4C), 2C not detected.

4.1.4. General procedure of the preparation of 5i and 5j

The compounds **5i** and **5j** were prepared as described for the compounds **5a**–**h**, but using 11 mmol of the corresponding amine derivative as starting material. For compound **5j**, it was not necessary to prepare the hydrochloride.

4.1.4.1. Di-[2-(dimethylamino)ethyl]-thiocarbonate hydrochloride (5i)[24]. White crystals; yield 69%. Mp 135–137 °C. ^{1}H NMR (d_6-DMSO, 200 MHz) δ 11.19 (s, 2H), 5.02–4.63 (m, 4H), 3.80–3.21 (m, 4H), 2.97–2.1 (m, 12H). ^{13}C NMR (d_6-DMSO, 50 MHz) δ 193.3, 67.3 (2C), 54.1 (2C), 42.4 (4C).

4.1.4.2. Di-[2-*N*,*N*,*N*-trimethylammonium]ethylthiocarbonate dichloride (5j). White crystals; yield 38%. Mp >350 °C. ^{1}H NMR (D_2O, 200 MHz) δ 3.93–3.73 (m, 4H), 5.07–4.87 (m, 4H), 3.22 (s, 18H). ^{13}C NMR (D_2O, 50 MHz) δ 193.8, 66.8 (2C), 64.5, 62.3, 54.2 (6C).

4.1.5. General procedure of the preparation of 6a and 6b

The compounds **6a** and **6b** were prepared as described for the compounds **5a**–**h**, but using 1,1′-carbonyldiimidazole as starting material.

4.1.5.1. 1-Isopropoxycarbonylimidazole hydrochloride (6a). White crystals; yield 72%. Mp 84–86 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 11.37 (s, br, 1H), 9.58 (s, 1H), 7.73 (s, 1H), 7.61 (s, 1H), 5.38 (sept, J = 6.3, 1H), 1.54 (d, J = 6.3 Hz, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 145.1, 135.9, 121.4, 118.7, 77.7, 21.5 (2C).

4.1.5.2. 1-Cyclohexylcarbonylimidazole hydrochloride (6b). White crystals; yield 73%. Mp 111–113 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 11.49 (s, br, 1H), 9.51 (s, 1H), 7.72 (s, 1H), 7.59 (s, 1H), 5.27–4.29 (m, 1H), 2.28–1.04 (m, 10H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 145.1, 135.8, 121.5, 118.7, 82.0, 31.0 (2C), 24.7, 23.3 (2C).

4.1.6. General procedure of the preparation of 8a–h

To a solution of 1.5 mmol **5b**–**e** in dry THF, 1.8 mmol of the corresponding alcohol **7a**–**b** and *N*-methyl piperazine, respectively, were added under argon atmosphere and stirred under reflux for three hours. The so-obtained crude products were then dissolved in dry diethylether and 1 M HCl in diethylether was added to yield the hydrochloride of the compounds.

4.1.6.1. Carbonothioic acid *O*-cyclohexyl *O*-(dimethylamino) propyl diester hydrochloride (8a). White crystals; yield 86%. Mp 131–132 °C. ^{1}H NMR (d_6-DMSO, 200 MHz) δ 10.95 (s, br, 1H), 5.27–5.01 (m, 1H), 4.59–4.34 (m, 2H), 3.21–3.00 (m, 2H), 2.71 (s, 6H), 2.27–1.83 (m, 4H), 1.78–1.16 (m, 8H). ^{13}C NMR (d_6-DMSO, 50 MHz) δ 193.8, 81.8, 69.8, 53.4, 41.9 (2C), 30.3 (2C), 24.6, 23.1, 23.0 (2C).

4.1.6.2. Carbonothioic acid *O*-(dimethylamino)ethyl *O*-ethyl diester hydrochloride (8b). White crystals; yield 91%. Mp 103–108 °C. ^{1}H NMR (d_6-DMSO, 200 MHz) δ 11.18 (s, br, 1H), 4.92–4.65 (m, 2H), 4.62–4.32 (m, 2H), 2.74 (s, br, 6H), 1.47–1.11 (m, br, 3H). ^{13}C NMR (d_6-DMSO, 50 MHz) δ 193.4, 69.5, 66.4, 54.2, 42.5 (2C), 13.4.

4.1.6.3. Carbonothioic acid *O*-(dimethylamino)propyl *O*-ethyl diester hydrochloride (8c). White crystals; yield 70%. Mp 103–106 °C. ^{1}H NMR (d_6-DMSO, 200 MHz) δ 10.89 (s, br, 1H), 4.68–4.24 (m, 4H), 3.17–2.96 (m, 2H), 2.70 (s, br, 6H), 2.82–2.59 (m, 2H), 1.50–1.11 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 194.6, 69.9, 69.2, 53.3, 41.9 (2C), 22.9, 13.5.

4.1.6.4. Carbonothioic acid *O*-(dimethylamino)ethyl *O*-propyl diester hydrochloride (8d). White crystals; yield 73%. Mp 70–78 °C. ^{1}H NMR (d_6-DMSO, 200 MHz) δ 11.19 (s, br, 1H), 4.94–4.63 (m, 2H), 4.52–4.20 (m, 2H), 3.62–3.25 (m, 2H), 2.73 (s, 6H), 1.90–1.45 (m, 2H), 1.10–0.65 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 194.0, 74.9, 66.9, 54.1, 42.5 (2C), 20.8, 9.9.

4.1.6.5. Carbonothioic acid *O*-(dimethylamino)propyl *O*-propyl diester hydrochloride (8e). White crystals; yield 70%. Mp 102–104 °C. ^{1}H NMR (d_6-DMSO, 200 MHz) δ 11.10 (s, br, 1H), 4.63–4.26 (m, 4H), 3.20–3.00 (m, 2H), 2.72 (s, 6H), 2.30–2.03 (m, 2H), 1.85–1.56 (m, 2H), 0.92 (t, J = 6.3 Hz, 3H). ^{13}C NMR (d_6-DMSO, 50 MHz) δ 194.8, 74.7, 70.1, 53.3, 41.8 (2C), 23.0, 21.1, 10.1.

4.1.6.6. Carbonothioic acid *O*-butyl *O*-(dimethylamino)propyl diester hydrochloride (8f). White crystals; yield 65%. Mp 116–118 °C. ^{1}H NMR (d_6-DMSO, 200 MHz) δ 10.93 (s, br, 1H), 4.56–4.31 (m, 4H), 3.20–3.00 (m, 2H), 2.72 (s, 6H), 2.26–2.03 (m, 2H), 1.78–1.56 (m, 2H), 1.47–1.24 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR (d_6-DMSO, 50 MHz) δ 194.9, 73.0, 70.0, 53.4, 41.9 (2C), 29.6, 23.0, 18.5, 13.5.

4.1.6.7. Ethyl 4-methyl-1-piperazinethiocarboxylate hydrochloride (8g). White crystals; yield 81%. Mp 169–173 °C. ^{1}H NMR (d_6-DMSO, 200 MHz) δ 11.66 (s, br, 1H), 4.64–4.20 (m, 2H), 3.83–2.91 (m, 8H), 2.72 (s, br, 3H), 1.28 (s, br, 3H). ^{13}C NMR (d_6-DMSO, 50 MHz) δ 186.9, 67.4, 51.1 (2C), 45.3 (2C), 41.6, 13.8.

4.1.6.8. Propyl 4-methyl-1-piperazinethiocarboxylate hydrochloride (8h). White crystals; yield 70%. Mp 150–162 °C. ^{1}H NMR (d_6-DMSO, 200 MHz) δ 12.03 (s, br, 1H), 4.40–4.29 (m, 2H), 4.13–2.19 (m, 8H), 2.74 (s, 3H), 1.82–1.55 (m, 2H), 0.92 (t, J = 7.4 Hz, 3H). ^{13}C NMR (d_6-DMSO, 50 MHz) δ 187.3, 73.1, 51.3 (2H), 45.6, 41.6, 41.4, 21.5, 10.3.

4.2. Experiments on smooth and heart muscle preparations

In general, experiments were performed according to procedures described previously.[25,7–9] Guinea pigs of both sexes (340–480 g) were killed with a blow on the neck. After excision of the heart, the aorta and the ileum were dissected. The aorta was cut into rings of 2 mm width. The terminal portion of the ileum was removed and the 10 cm nearest to the caecum were discarded. The intestine was placed in a nutrient solution and cut into pieces of 1–2 cm length. The pulmonary artery was dissected close to the heart, cleaned and cut into rings of 2 mm width. Papillary muscles were dissected from the right ventricle for contractility measurements. Purkinje fibers were carefully removed to prevent spontaneous activity. Chronotropic activity was tested on guinea pig isolated right atria. After isolation, all the preparations were stored at room temperature in gassed (95% O_2 and 5% CO_2) Krebs–Henseleit solution with the following composition (in mM): NaCl 114.9, KCl 4.73, $CaCl_2$ 3.2, $MgSO_4$ 1.18, $NaHCO_3$, 24.9, KH_2PO_4 1.18, glucose 10; pH 7.2–7.4. For the experiments, the preparations were placed in a continuously oxygenated (95% O_2 and 5% CO_2) bath of 25 mL nutrient solution at 37 ± 1 °C. The smooth and heart muscle preparations were connected with one end to a tissue holder and the other to a force transducer (Transbridge™, 4-Channel Transducer Amplifier, World Precision Instruments, Sarasota, FL, USA). Aortic and pulmonary artery rings were precontracted by 90 mM KCl and terminal ileum preparations by 60 mM KCl. Papillary muscles were electrically driven with an Anapulse Stimulator Model 301-T and an Isolation UnitModel 305-1 (WPI, Hamden, CT, USA) at a frequency of 1 Hz and a pulse duration of 3 ms. Amplitude of stimulation pulse was adjusted to 10% above threshold level. Resting tension of either 3.92 mN (papillary muscles), 4.9 mN (terminal ileum), 10.37 mN (right atria) or 19.6 mN (aortic and pulmonary artery rings) was kept constant throughout the experiments. After a control period different concentrations of the test compounds were added cumulatively every 45 min. Signals were recorded with a chart recorder (BD 112 Dual Channel, Kipp and Zonen) and evaluated. For statistical analyses the arithmetic means and standard error of the mean (SEM) of

3–5 experiments were calculated. Statistical significance of the results was evaluated by Student's *t*-test for paired observations (Sigma Plot version 9.0). Stock solutions of the test compounds were dissolved preferred in distilled water or, if required, in dimethylsulfoxide (DMSO). To exclude the solvent effect experiments with the solvent only were performed and the effect was subtracted from the results of the compounds. To study the inotropic and chronotropic activity, after a control period of 30 min the different concentrations of the compounds were added to the bathing solution cumulatively, until a steady state was reached. For statistical analyses the arithmetic means and standard error of the mean (SEM) of 4–5 experiments were calculated. Statistical significance of the results was evaluated by the Student's *t*-test for paired observations (Sigma Plot version 9.0).

4.3. Analysis of the chemical space of the compounds

Exploration of the chemical space occupied by the synthesized and tested compounds was studied using the Principal Component Analysis (PCA)-based chemical space navigation tool ChemGPS-NP.[10,12] This analysis is generated by 2D descriptors (total of 35 molecular descriptors) that explain physico-chemical properties of the compounds and are calculated from SMILES. SMILES for all compounds were obtained using the NCI online SMILES translator (http://cactus.nci.nih.gov/translate/). All salts and counter-ions were excluded from the SMILES annotation. Analysis was focused on the first four dimensions of ChemGPS-NP (PC1–PC4). The obtained coordinates were plotted using the software Grapher 2.1 distributed together with MacOS X.

4.4. Hydrolysis studies

20 mg/mL of compound **3a**, **5b** and **5c**, respectively, were dissolved in D_2O and kept well closed at room temperature over 24 h. ^1H NMR was measured after 1, 3, 5, 7 and 24 h and the data were processed using the program 1D WIN-NMR (version 961210). Half-lives of the compounds were calculated in MS Excel 2007 and were obtained by using the compound: degradation product ratio in percentage.

Experiments on isolated aortic rings of the guinea pig were performed as described under Section 4.2. Compounds were dissolved in distilled water 24 h prior to the experiment and kept well closed at room temperature. 15 μM of **5b** and 55 μM **5c**, respectively, were added to the aortic strips pre-contracted by 90 mM KCl. A possible change in the force of contraction (f_c) was recorded with a chart recorder (BD 112 Dual Channel, Kipp and Zonen) for 45 min and evaluated. For statistical analyses the arithmetic means and standard error of the mean (SEM) of three experiments were calculated. Statistical significance of the results was evaluated by the Student's *t*-test for paired observations (Sigma Plot version 9.0).

4.5. Mode of action studies

The antagonist of K_{ATP}-channels, glibenclamide, and the eNOS inhibitor nitro-L-arginine were used to elucidate a possible mode of action in isolated aortic strips of the guinea pig. The vessel preparation was performed as described in Section 4.2. After pre-contraction of the aortic rings by 90 mM KCl, 30 μM and 100 μM glibenclamide, respectively, or 100 μM nitro-L-arginine were added to the bathing solution. After 45 min, the test compounds were administered in a concentration similar to their EC_{50} values and the change in contraction force (f_c) was recorded for another 45 min. For statistical analyses the arithmetic means and standard error of the mean (SEM) of 3–4 experiments were calculated. Statistical significance of the results was evaluated by the Student's *t*-test for paired observations (Sigma Plot version 9.0).

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Supplementary data

Supplementary data (the elemental analyses and HRMS data for the tested compounds and time-course of the hydrolysis together with the equation used to calculate the $t_{1/2}$ as well as the R^2 values) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.05.049.

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