Bioorganic & Medicinal Chemistry 19 (2011) 994–1001

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

A journey from benzanilides to dithiobenzanilides: Synthesis of selective spasmolytic compounds

Gerda Brunhofer [a,†], Walter H. Granig [a,†], Christian R. Studenik [b], Thomas Erker [a,*]

[a] Department of Medicinal Chemistry, University of Vienna, A-1090 Vienna, Althanstrasse 14, Austria
[b] Department of Pharmacology and Toxicology, University of Vienna, A-1090 Vienna, Althanstrasse 14, Austria

ARTICLE INFO

Article history:
Received 27 September 2010
Revised 15 November 2010
Accepted 18 November 2010
Available online 25 November 2010

Keywords:
Thiobenzanilides
Spasmolytic activity
Structure–activity relationship
Benzanilides

ABSTRACT

A series of dithiobenzanilide derivatives was synthesized and each compound was evaluated for its ability to reduce KCl-induced contractions of smooth muscle preparations of the guinea pig. Starting from a recent publication describing benzanilide derivatives as antispasmodic agents, structure–activity guided synthesis was performed to obtain compounds with improved spasmolytic activity. First, compounds with two amide bonds were designed and second, both amide oxygens were replaced by two sp^2 sulfur atoms resulting in dithiobenzanilide derivatives. The most potent antispasmodic dithiobenzanilide **19** showed improved activity with an IC_{50} value of 0.4 μM. Moreover, the study also demonstrated that these active compounds were able to antagonize the effect of spasmogens like acetylcholine and phenylephrine and that the activity is not mediated by activation of ATP-dependent potassium channels (K_{ATP}-channels) or inhibition of endothelial nitric oxide synthase (eNOS).

1. Introduction

In an attempt to improve recently described compounds exerting selective antispasmodic activity, our group initially focused on the benzanilide scaffold as starting point.[1] This biological activity can be beneficial in the treatment of for example the irritable bowel syndrome which manifests, among others, overaction of the intestine as well as gastrointestinal hypermotility.[2,3] However, there has been little study on this topic in the literature. Navarrete-Vázquez et al., reported the spasmolytic activity of substituted benzimidazole derivatives as stilbene bioisosteres with the most active compound showing an IC_{50} value of 1.19 μM in the isolated rat ileum test.[4] In the present study we are going to further develop the class of benzanilides. We synthesized four sets of compounds representing the general structural features shown in Table 1. First, we introduced a second amide bond to obtain dibenzanilide derivatives bearing aliphatic and aromatic units. Doubling of the active principle within a molecule represents a very useful method in the search for biologically active compounds. In a next step we replaced the oxygen of both amide linkers of the dibenzanilide compounds by a sp^2 sulfur atom which is frequently employed in medicinal chemistry as amide bond isostere. But the exchange of the amide bond against a thioxo unit represents an isosteric replacement of the amide linker with slightly modified electron distribution in the ground state. Moreover, the C=S bond is 37% longer than the C=O bond and the van der Waals radius is 32% larger than that of oxygen. We envisaged this conservative modification to improve bioactivity and stability of the compounds.[5–10] Furthermore, we varied the substitution pattern of the phenyl rings of the dithiobenzanilide derivatives. So, we obtained information about active and non-active features in the molecule concerning a selective spasmolytic activity. The most active dithiobenzanilide compound is eightfold more active than simple benzanilide derivatives. Therefore, in the present study we describe the development of new substances with selective antispasmodic activity, which could, indeed, be beneficial in the treatment of gastrointestinal dysmotility.

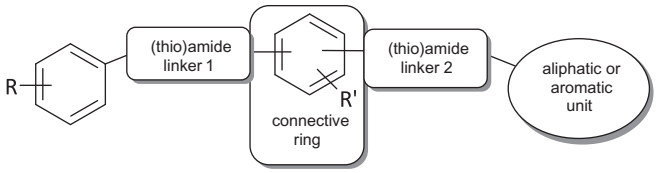
2. Chemistry

The general synthetic procedures of the compounds presented in Table 2 are shown in Scheme 1. Four sets of different substituted dithiobenzanilide derivatives were synthesized. Set I was prepared in a three-step reaction. The first step was a nucleophilic substitution between the appropriate benzoyl chloride and the corresponding 2-nitrophenylamine derivative using tetrahydrofuran (THF) as solvent and triethylamine (TEA) as base. This was followed by the reduction of the nitro group using H_2/Pd. The final step was again a nucleophilic substitution using acetoxyacetyl chloride or pivaloyl chloride. Compound **9** as well as set II–IV were synthesized by a two-step procedure starting from para (compound **9** and set I), meta (set III) or ortho (set IV) phenylenediamine which reacted with two equivalents of the appropriate benzoyl chloride.

* Corresponding author. Tel.: +43 1 4277 55003; fax: +43 1 4277 9551.
E-mail address: thomas.erker@univie.ac.at (T. Erker).
† These authors contributed equally to this study.

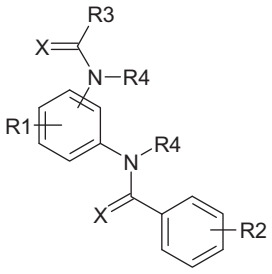
0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.11.043

Table 1
Overview of the general structural features of the compounds of set I–IV and compound **9**



	Linker 1	Substitution pattern of linker 2 at connective ring	Linker 2	Unit
Set I	Amide	Ortho	Amide	Aliphatic
Compound **9**	Amide	Para	Amide	Subst. phenyl
Set II	Thioamide	Para	Thioamide	Subst. phenyl
Set III	Thioamide	Meta	Thioamide	Subst. phenyl
Set IV	Thioamide	Ortho	Thioamide	Subst. phenyl

Table 2
Synthesized compounds

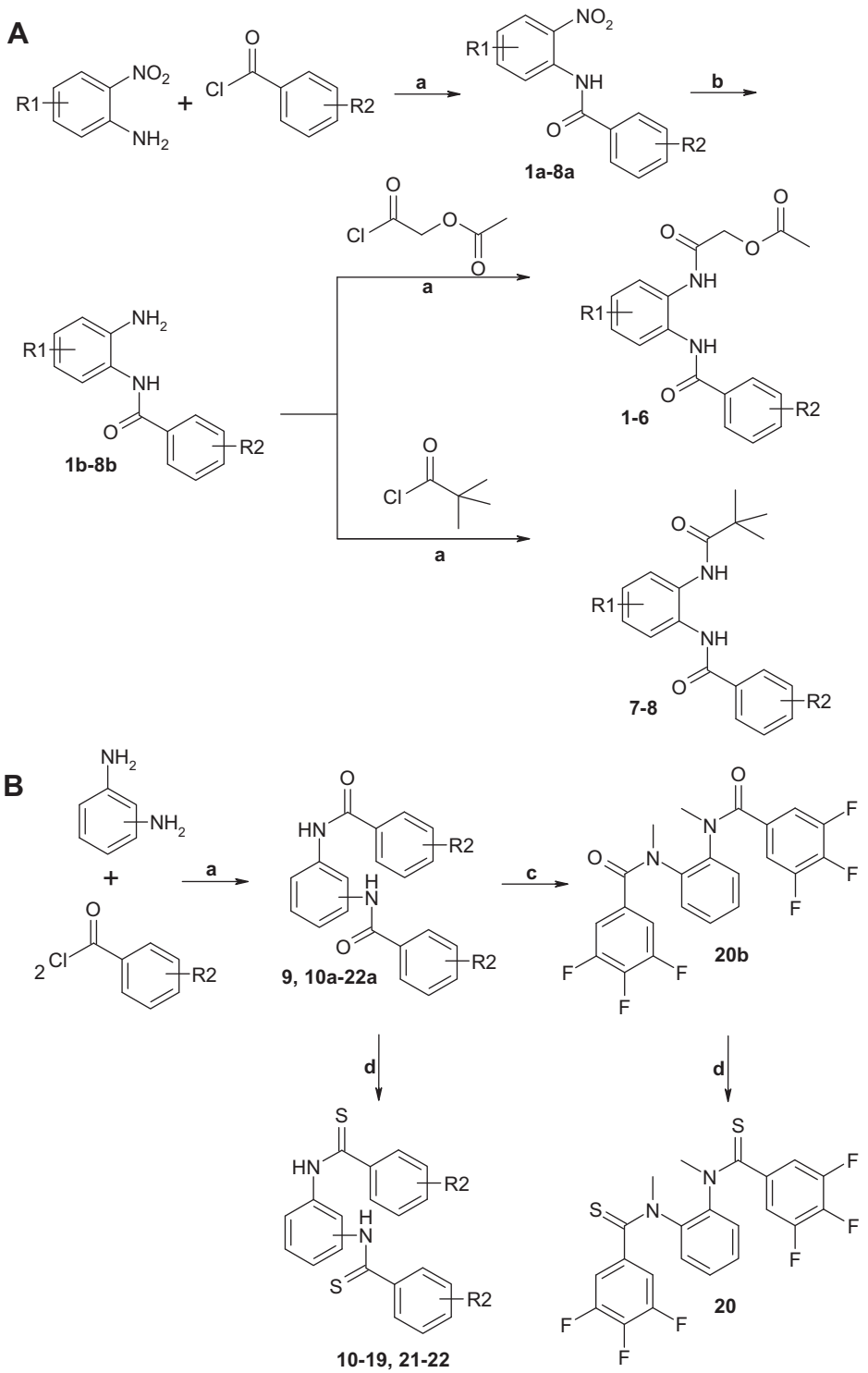


	Compd	Subst.	X	R1	R2	R3	R4
Set I	**1**	Ortho	O	-H	4-OCH_3	$-CH_2OCOCH_3$	-H
	2	Ortho	O	-H	3,4,5-$(OCH_3)_3$	$-C(CH_3)_3$	-H
	3	Ortho	O	4,5-$(CH_3)_2$	4-OCH_3	$-CH_2OCOCH_3$	-H
	4	Ortho	O	4,5-$(CH_3)_2$	3,4,5-$(OCH_3)_3$	$-CH_2OCOCH_3$	-H
	5	Ortho	O	4-OCH_3	4-OCH_3	$-CH_2OCOCH_3$	-H
	6	Ortho	O	4-F	4-OCH_3	$-CH_2OCOCH_3$	-H
	7	Ortho	O	-H	3,4,5-$(OCH_3)_3$	$-C(CH_3)_3$	-H
	8	Ortho	O	4-OCH_3	3,4,5-$(OCH_3)_3$	$-C(CH_3)_3$	-H
Compd	**9**	Para	O	-H	3,4,5-F_3	-(3,4,5-F_3)C_6H_2	-H
Set II	**10**	Para	S	-H	4-F	-(4-F)C_6H_4	-H
	11	Para	S	-H	3,4,5-F_3	-(3,4,5-F_3)C_6H_2	-H
	12	Para	S	-H	3,4,5-$(OCH_3)_3$	-(3,4,5-$(OCH_3)_3$)C_6H_2	-H
Set III	**13**	Meta	S	-H	4-F	-(4-F)C_6H_4	-H
	14	Meta	S	-H	3,4,5-F_3	-(3,4,5-F_3)C_6H_2	-H
	15	Meta	S	-H	4-OCH_3	-(4-OCH_3)C_6H_4	-H
	16	Meta	S	-H	3,4,5-$(OCH_3)_3$	-(3,4,5-$(OCH_3)_3$)C_6H_2	-H
Set IV	**17**	Ortho	S	-H	-F	-(4-F)C_6H_4	-H
	18	Ortho	S	-H	4-OCF_3	-(4-OCF_3)C_6H_4	-H
	19	Ortho	S	-H	3,4,5-F_3	-(3,4,5-F_3)C_6H_2	-H
	20	Ortho	S	-H	3,4,5-F_3	-(3,4,5-F_3)C_6H_2	$-CH_3$
	21	Ortho	S	-H	4-OCH_3	-(4-OCH_3)C_6H_4	-H
	22	Ortho	S	-H	3,4,5-$(OCH_3)_3$	-(3,4,5-$(OCH_3)_3$)C_6H_2	-H

In the case of compound **20** we did an additional step using sodium hydride and methyl iodide to obtain two tertiary amide linkers. Finally, the last step in the synthesis of set II–IV was characterized by a replacement of both amide oxygens by two sulfur atoms which was performed by using Lawesson's reagent. All compounds were obtained in good yields.

3. Pharmacological results and discussion

From our previous investigations we knew that the amide bond is beneficial for inhibiting KCl-induced contractions of smooth muscle preparations.[1] Therefore we first started with doubling of the amide function in the molecule to obtain compounds bearing an acetoxyacetyl or pivaloyl group via a second amide bond (set I, compounds **1**–**8**). To obtain the structural feature shown in Table 1 it was necessary to introduce a connective ring which linked both amide linkers. In set I both linkers are arranged in ortho position. Some of the compounds still possessed selective spasmolytic activity but there was no increase in activity compared to simple benzanilide derivatives.[1] There was also no difference in activity noticeable if the connective ring was either non-substituted or substituted by one or two methyl groups, one methoxy or one fluoro group. In compound **9** we replaced the aliphatic unit against an aromatic structure but there was also no increase in activity detectable (compare Table 3). Hence, we exchanged both amide oxygens against two sp^2 sulfur atoms to obtain dithiobenzanilides. In medicinal chemistry thioamide moieties are considered as isosteres for amide bonds possessing more rigidity and stability towards proteases.[5–10] So, set II–IV represent compounds with a connective ring linking two symmetrically substituted thiobenzanilides. As next step, we varied the substitution pattern of the second thioamide linker at the connective ring



Scheme 1. General synthetic routes for set I (A) and set II–IV (B). Reagents and conditions: (a) THF, TEA; (b) H_2/Pd, MeOH; (c) THF, NaH, CH_3I; (d) Lawesson's reagents, THF, reflux, 2–5 h.

systematically to obtain para (set II), meta (set III) and ortho (set IV) derivatives. The spasmolytic and vasodilating activity showed a clear structure–activity relationship depending on the one hand on the substitution at the connective ring and on the other hand on the substituents on both thioamide-linked phenyl rings. In set II compound **11** bearing a 3,4,5-trifluoro substituent on both terminal rings was the only derivative showing good selective antispasmodic activity with an IC_{50} of 5.9 μM. Mono-fluoro and 3,4,5-trimethoxy substituents caused a complete loss of activity. Meta substitution on the connective ring showed a slight increase in activity. Even the mono-fluoro and mono-methoxy compounds **13** and **15** were active, but mono-fluoro also exhibited vasodilating activity (IC_{50} on aortic rings 33 μM). The 3,4,5-trifluoro-substituted derivative **14** was the most potent compound in this set but unfortunately unselective with IC_{50} values of 14 μM on aortic, 32.2 μM on arteria pulmonalis rings and 3.1 μM on terminal ileum

Table 3
IC_{50} values in μM of the compounds on different smooth muscle preparations of the guinea pig

	Compd	IC_{50} (μM)		
		Aortic rings	Arteria pulmonalis rings	Terminal ileum preparations
Set I	**1**	>100	>100	>100
	2	>100	>100	>100
	3	>100	>100	85
	4	>100	>100	85
	5	>100	>100	>100
	6	>100	>100	>100
	7	>100	>100	53
	8	>100	>100	47
Compound	**9**	>100	>100	>100
Set II	**10**	>100	>100	>100
	11	>100	>100	5.9
	12	>100	>100	>100
Set III	**13**	33	>100	11
	14	14	32.2	3.1
	15	>100	>100	12.4
	16	>100	>100	>100
Set IV	**17**	43.5	4.9	1.83
	18	>100	>100	4.8
	19	>100	34.5	0.4
	20	>100	>100	11.5
	21	>100	>100	2.6
	22	>100	>100	>100

Vessel rings were precontracted by 90 mM KCl solution, terminal ileum preparations by 60 mM KCl solution. $n = 5$.

preparations, respectively. In line with this observation, compounds **17** and **19** (mono-fluoro and 3,4,5-trifluoro derivatives) of set IV showed high but unselective spasmolytic potential. However, compound **19** had an IC_{50} of 0.4 μM on the terminal ileum which is about a factor 10 higher than the activity of simple benzanilide derivatives[1] and a factor 100 higher than on arteria pulmonalis rings (IC_{50} 34.5 μM). It showed no activity on aortic rings. Thus, these data suggest that compound **19** has the potential for a favorable therapeutic range with preferentially acting on terminal ileum tissue than on the vasculature (Fig. 1). Exchange of the 4-fluoro substituent of compound **17** against a 4-trifluoromethoxy or a 4-methoxy group resulted in the selective spasmolytic compounds **18** and **21** with IC_{50} values of 4.8 μM and 2.6 μM. 3,4,5-Trimethoxy substitution led to a loss of activity in all three sets (set II–IV). Compounds **12**, **16**, and **22** showed IC_{50} values >100 μM. Conversion of both secondary thioamide moieties of the most active compound **19** to two tertiary thioamide bonds via insertion of a methyl group resulting in compound **20** led on the one hand to a loss of action on vascular smooth muscle preparations but on the other hand to only a 30-fold decrease of the spasmolytic activity. Therefore, compound **20** also showed a selective antispasmodic potential (compare Fig. 2). Papaverine hydrochloride was used as spasmolytic control in the ileum assay and showed an IC_{50} value of 4 μM which is in accordance to the literature (data not shown).[11] So, we identified at least four compounds (**14**, **17**, **18**, and **21**) with IC_{50} values comparable to papaverine hydrochloride (1.8–4.8 μM) and one compound (**19**) which was even ten times more active (0.4 μM).

These data collectively demonstrate that (1) dithiobenzanilides act concentration dependently as spasmolytic agents and have more potential than simple benzanilides. (2) The activity of the compounds seems to be dependent on the ortho-substitution of both thioamide linker and the substituents of the terminal aromatic rings. (3) Considering the 3,4,5-trifluoro substituted dithiobenzanilides it is obvious that this substitution pattern is beneficial for antispasmodic activity. Trifluoro substituted derivatives showed in set II (compound **11**), set III (compound **14**), set IV (compound **19**) and as compound **9** significant spasmolytic activity. However, the potential of these compounds and the resulting IC_{50} values are dependent on the one hand on the exchange of both amide oxygens against two sp^2 sulfur atoms and on the other hand on the substitution pattern of substituted phenyl rings at the connective ring.

To assess the structural properties of the most interested compound (**19**) we used Lipinski's rule of 5, which has become a

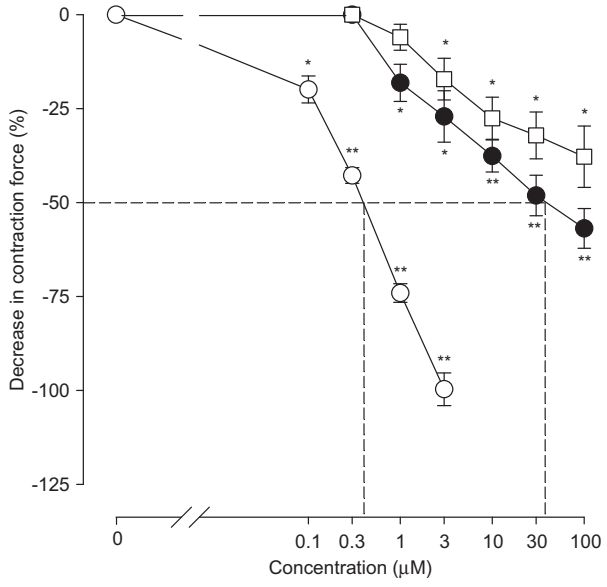


Figure 1. Concentration-dependent effect of compound **19** on terminal ileum preparations (○) ($n = 5$) precontracted by 60 mmol/l KCl, on arteria pulmonalis rings (●) ($n = 7$) and on aortic rings (□) ($n = 5$) precontracted by 90 mmol/l KCl. The decrease in percent of contraction force is semilogarithmically plotted on the ordinate against the concentration of the compounds on the abscissa. Symbols represent the arithmetic means ± SEM and the dashed lines the IC_{50} values. Significance level: $^{*}P < 0.05$; $^{**}P < 0.01$.

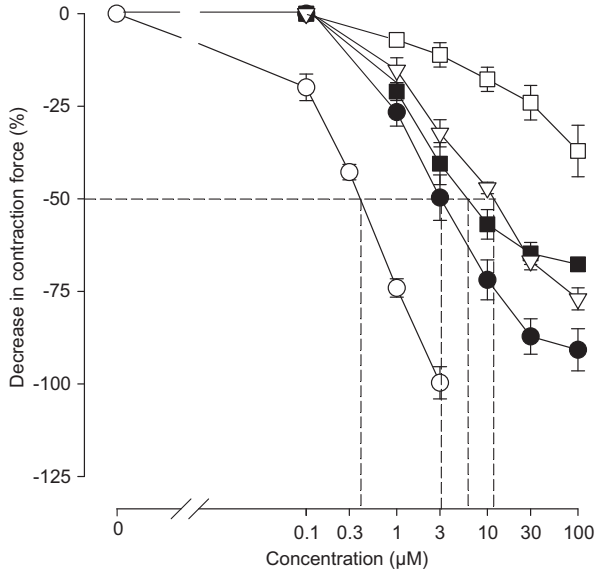


Figure 2. Concentration-dependent effect of compounds **9** (□), **11** (■), **14** (●), **19** (○), and **20** (▽) on terminal ileum preparations precontracted by 60 mmol/l KCl. The decrease in percent of contraction force is semilogarithmically plotted on the ordinate against the concentration of the compounds on the abscissa. Symbols represent the arithmetic means ± SEM from four to six experiments. The dashed lines represent the IC_{50} values. Significance level: $^{*}P < 0.05$; $^{**}P < 0.01$. Due to clearness, significance levels of the compounds are the following: compound **9**: 3–100 μM*; compound **11**: 3 μM* and 10–100 μM**; compound **14**: 3 μM* and 10–100 μM**; compound **19**: 0.1 μM* and 0.3–3 μM**; compound **20**: 3 μM* and 10–100 μM**.

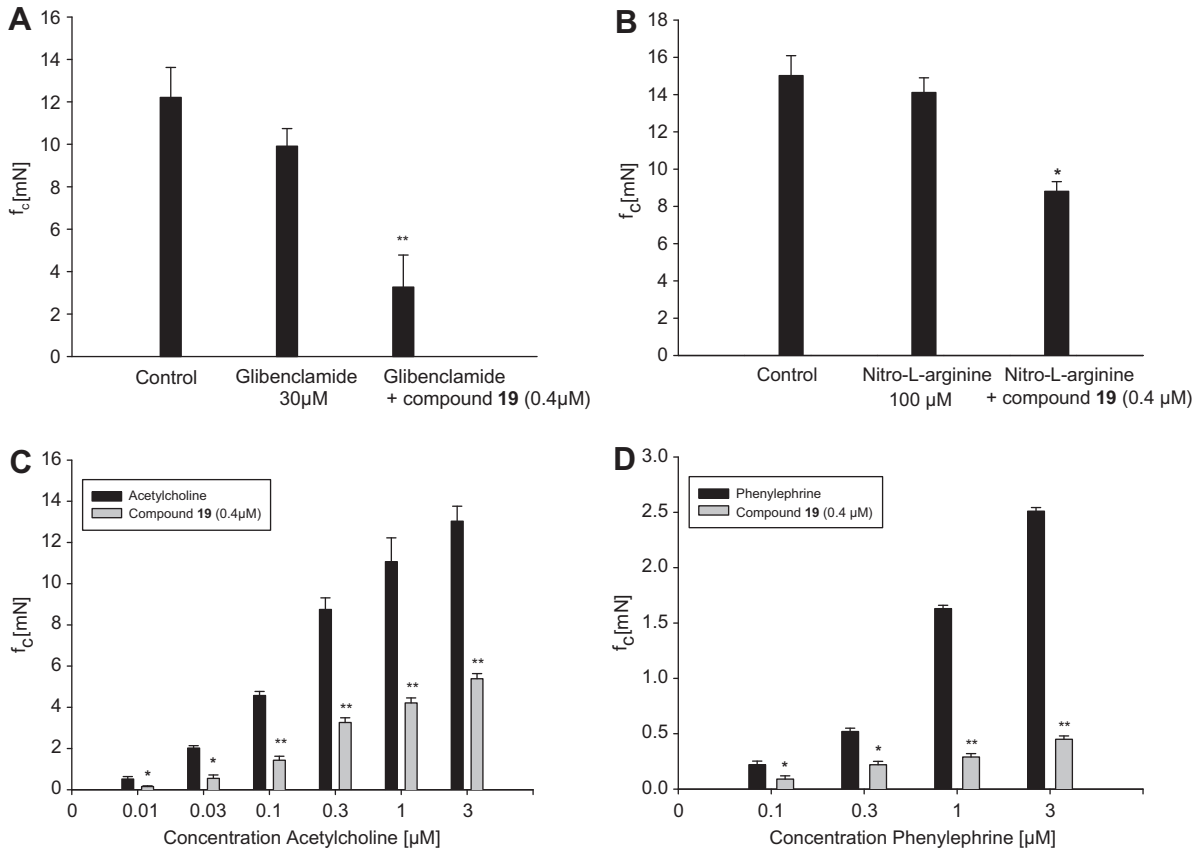


Figure 3. Mode of action of compound **19** on terminal ileum preparations in presence of glibenclamide (panel A), nitro-L-arginine (panel B), acetylcholine (panel C) and phenylephrine (panel D). The decrease or increase of contraction force (f_c) in mN is plotted on the ordinate. The bars represent the arithmetic means ± SEM from four experiments. Significance level: $^{*}P$ <0.05; $^{**}P$ <0.01.

standard benchmark in the evaluation of drug-like properties. The following physicochemical properties were calculated using MOE 2008.10; the guidelines proposed by Lipinski to prevent poor absorption and permeation are given in brackets: molecular weight 456.43 (<500), two H-bond donors (<5), two H-bond acceptors (<10), and log *P* 5.4 (<5). Three of four rules are fulfilled by compound **19** and only the log *P* value is higher than the postulated value. Hence, these data suggest a good likelihood of absorption for this compound.

Different mechanisms of action can be responsible for this effect. To identify a possible involvement of the endothelial nitric oxide synthase (eNOS), cholinergic and α-adrenergic mechanisms we studied the effect of the dithiobenzanilides in presence of nitro-L-arginine, an inhibitor of eNOS, as well as the effect of the compounds on contractions induced by the neurotransmitter acetylcholine and the sympathomimetic phenylephrine. Since it has been demonstrated that ATP-dependent potassium channels (K_{ATP}-channels) play an important role in smooth muscle relaxation we investigated a possible involvement of these channels using glibenclamide as K_{ATP}-channel blocker.[12,13] Investigations concerning the mechanism of action were done for all of the herein presented compounds but only the data of compound **19**, the most potent compound, are shown exemplarily. The concentration studied was the IC_{50} value of **19** (0.4 μM). However, as can be seen in Figure 3A and B, inhibition of the K_{ATP}-channels and eNOS did not show significant changes of the spasmolytic activity of the substances. In contrast to simple benzanilide derivatives, which are described to possess selective spasmolytic activity and which had no influence on contractions caused by acetylcholine, dithiobenzanilide compounds inhibited significantly spasms evoked by this spasmogen.[1] Moreover, according to the results, the compounds appeared to act via the adrenergic route. There was also a significant antagonization of the phenylephrine-evoked effect in presence of the test compounds detectable. Bolton stated that contractions induced by high potassium ion concentration (>30 mM) depended on the influx of calcium ions into the cells through voltage-dependent calcium channels.[14] Hence, compounds, which are able to inhibit high potassium ion induced contractions, can be considered as calcium channel blockers.[15,16] Thus, this phenomenon is probably also partly responsible for the observed inhibition of force of contraction by the compounds presented herein. Taken together, we were able to show that antispasmodic dithiobenzanilides mediated their potency through at least three different modes of action. The observed antagonism against the spasmogens acetylcholine and phenylephrine and the supposed calcium channel blockade indicate polypharmacological characteristics of these compound class. In a recent article, Mestres and Gregori-Puigjané defined polypharmacology, in short, as 'the binding of a ligand to multiple protein targets' stating that this principle is a current trend in drug design now.[17]

4. Conclusion

The current study demonstrates the structure–activity guided synthesis of spasmolytic compounds. We further developed the general scaffold of benzanilides with respect of their antispasmodic potential. The resulting dithiobenzanilides showed an up to eightfold higher concentration dependent spasmolytic activity than simple benzanilide derivatives. The IC_{50} value of the most

potent compound (**19**) was 0.4 μM. This activity was dependent on the substitution of the second amide linker at the connective ring and increased in the order para < meta ≪ ortho. Moreover, the data demonstrated that 3,4,5-trifluoro substituents at both terminal aromatic rings were most favorable for activity. On the other hand, methoxy substituents—especially 3,4,5-trimethoxy—showed no or weak spasmolytic activity. Investigations concerning the mode of action of the compounds revealed that neither eNOS inhibition nor activation of K_{ATP}-channels contributed to the activity. However, the results showed that the compounds are able to antagonize significantly effects evoked by acetylcholine and phenylephrine. We also suppose the involvement of a calcium channel blocked caused by the test substances. Based on these polypharmacological properties the herein presented compounds could be used in the treatment of multifactorial diseases and gastrointestinal disorders, like for example the irritable bowl syndrome and gut spasms.

5. Experimental section

5.1. Chemistry

5.1.1. General experimental methods

All chemicals obtained from commercial suppliers were used as received and were of analytical grade. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPx200 (200 and 50 MHz). Chemical shifts are reported in δ units (ppm) relative to Me_4Si line as internal standard and J values are reported in Hertz. Mass spectra were obtained by a Hewlett Packard (GC: 5890; MS: 5970) spectrometer. Solutions in organic solvents were dried over anhydrous sodium sulfate.

5.1.2. General procedure for the synthesis of the benzanilide derivatives of set I

To a solution of 10 mmol appropriate *o*-nitroaniline in 50 ml pyridine was quickly added the appropriate benzoyl chloride. The reaction was stirred for 5–20 h at room temperature. Afterwards the reaction mixture was purged into ice water and the formed solid was isolated and recrystallized.

To a solution of 6 mmol 2-nitrobenzanilide derivative in ethanol 10% Pd/C-catalyst relating to the initial weight were added carefully under argon atmosphere and the reaction was stirred under hydrogen atmosphere until the usage of hydrogen has stopped. The reaction mixture was purged into ice water, the solid product was filtered off and recrystallized.

To a solution of 2 mmol *o*-aminobenzanilide derivative in THF the appropriate benzoyl chloride was added. The reaction was stirred for 30 min at room temperature and purged into ice water. The crude product was recrystallized in ethanol.

5.1.2.1. 2-{2-[(4-Methoxybenzoyl)amino]anilino}-2-oxoethyl acetate (1). Yield: 1.81 g (91.2%), mp 165 °C; ^{1}H NMR ($CDCl_3$): δ 9.90 (s, 1H), 9.58 (s, 1H), 7.98 (AB-system, J_{AB} = 8.6 Hz, 2H), 7.75–7.44 (m, 2H), 7.35–7.17 (m, 2H), 7.08 Hz (AB-system, J_{AB} = 8.6 Hz, 2H), 4.67 (s, 6H), 3.85 (s, 3H), 2.04 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.8, 166.0, 165.1, 162.1, 130.5, 129.7, 126.0, 125.8, 125.5, 125.3, 124.7, 113.7, 62.6, 55.5, 20.3; MS *m/z* 342 [M^+, 2%], 135 [100%]. Anal. Calcd for $C_{18}H_{18}N_2O_5$: C, 63.15; H, 5.30; N, 8.18. Found: C, 63.13; H, 3.35; N, 8.10.

5.1.2.2. 2-Oxo-2-{2-[(3,4,5-trimethoxybenzoyl)amino]anilino}-ethyl acetate (2). Yield: 0.57 g (70.9%), mp 185 °C; ^{1}H NMR ($CDCl_3$): δ 9.11 (s, 1H), 9.08 (s, 1H), 7.49–7.32 (m, 2H), 7.23 (s, 2H), 7.16–6.99 (m, 2H), 4.66 (s, 2H), 3.96 (s, 3H), 3.94 (s, 3H), 3.93 (s, 3H), 2.19 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 170.0, 167.3, 166.2, 153.6, 149.2, 141.9, 130.9, 129.8, 128.9, 127.0, 126.6, 126.0, 125.9, 105.3, 63.0, 61.5, 56.7, 21.0; MS *m/z* 402 [M^+, 6%], 195 [100%]. Anal. Calcd for $C_{20}H_{22}N_2O_7$x0.2H_2O: C, 59.13; H, 5.56; N, 6.90. Found: C, 59.12; H, 5.42; N, 6.55.

5.1.2.3. 2-{2-[(4-Methoxybenzoyl)amino]-4,5-dimethylanilino}-2-oxoethyl acetate (3). Yield: 0.45 g (33.0%), mp 172 °C; ^{1}H NMR ($CDCl_3$): δ 9.17 (s, 1H), 8.94 (s, 1H), 7.93 (d, J_{AB} = 8.8 Hz, 2H), 7.11 (s, 1H), 7.06 (s, 1H), 6.99 (d, J_{AB} = 8.8 Hz, 2H), 4.60 (s, 2H), 3.89 (s, 3H), 2.19 (s, 3H), 1.99 (s, 3H), 1,98 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 170.2, 167.1, 166.2, 163.1, 135.5, 135.2, 129.9, 128.4, 127.5, 126.8, 126.6, 126.1, 114.3, 62.9, 55.9, 21.1, 19.5; MS *m/z* 370 [M^+, 4 %], 135 [100%]. Anal. Calcd for $C_{20}H_{22}N_2O_5$: C, 64.85; H, 5.99; N, 7.56. Found: C, 64.70; H, 6.11; N, 7.44.

5.1.2.4. 2-{4,5-Dimethyl-2-[(3,4,5-trimethoxybenzoyl)amino]-anilino}-2-oxoethyl acetate (4). Yield: 0.49 g (38.0%), mp 219 °C; ^{1}H NMR ($CDCl_3$): δ 9.02 (s, 1H), 9.01 (s, 1H), 7.26 (s, 2H), 7.13 (s, 1H), 7.09 (s, 1H), 4.64 (s, 2H), 3.98 (s, 6H), 3.93 (s, 3H), 2.22 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 170.0, 167.1, 165.9, 153.7, 141.8, 135.7, 135.4, 129.1, 128.3, 127.3, 126.7, 126.6, 105.3, 63.0, 61.4, 56.8, 21.1, 19.6, 19.5; MS *m/z* 430 [M^+, 7%], 195 [100%]. Anal. Calcd for $C_{22}H_{26}N_2O_7$·0.3H_2O: C, 60.63; H, 6.15; N, 6.43. Found: C, 60.47; H, 6.10; N, 6.26.

5.1.2.5. 2-{4-Methoxy-2-[(4-methoxybenzoyl)amino]anilino}-2-oxoethyl acetate (5). Yield: 0.74 g (90.4%), mp 143 °C; ^{1}H NMR ($CDCl_3$): δ 9.81 (s, br, 1H), 9.51 (s, br, 1H), 8.00 (AB-system, J_{AB} = 8.8 Hz, 2H), 7.51–7.26 (m, 2H), 7.09 (AB-system, J_{AB} = 8.8 Hz, 2H), 6.84 (dd, J = 8.8 Hz and 2.9 Hz, 1H), 4.68 (s, 2H), 3.86 (s, 3H), 3.78 (s, 3H), 2.05 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 169.7, 165.9, 165.2, 162.1, 156.9, 132.2, 129.7, 127.2, 126.0, 122.8, 113.6, 110.6, 109.2, 62.6, 55.4, 55.3, 20.3; MS *m/z* 372 [M^+, 5%], 135 [100%]. Anal. Calcd for $C_{19}H_{20}N_2O_6$: C, 61.28; H, 5.41; N, 7.52. Found: C, 61.36; H, 5.36; N, 7.49.

5.1.2.6. 2-{4-Fluoro-2-[(4-methoxybenzoyl)amino]anilino}-2-oxoethyl acetate (6). Yield: 0.44 g (87.3%), mp 121 °C; ^{1}H NMR ($CDCl_3$): δ 9.91 (s, 1H), 9.57 (s, 1H), 8.00 (AB-system, J_{AB} = 8.7 Hz, 2H), 7.77–7.36 (m, 2H), 7.29–6.95 (m, 3H), 4.68 (s, 2H), 3.85 (s, 3H), 2.03 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 170.1, 166.5, 165.8, 159.6 (d, J_{CF} = 240 Hz), 133.1 (d, J_{CF} = 11 Hz), 130.2, 128.4 (d, J_{CF} = 9.6 Hz), 126.3 (d, J_{CF} = 8.1 Hz), 114.0, 111.9 (d, J_{CF} = 23 Hz), 110.7 (d, J_{CF} = 23 Hz), 62.9, 55.8, 20.7; MS *m/z* 360 [M^+, 2%], 135 [100%]. Anal. Calcd for $C_{18}H_{17}FN_2O_5$: C, 60.00; H, 4.76; N, 7.77. Found: C, 59.64; H, 4.59; N, 7.50.

5.1.2.7. *N*-{2-[(2,2-Dimethylpropanoyl)amino]-phenyl}-3,4,5-trimethoxybenzamide (7). Yield: 0.69 g (89.0%), mp 158–160 °C; ^{1}H NMR ($CDCl_3$): δ 9.39 (s, 1H), 8.54 (s, 1H), 7.53 (dd, J = 7.4 and 2.0 Hz, 1H), 7.37 (s, 2H), 7.32 (d, J = 7.4 Hz, 1H), 7.21–7.00 Hz (m, 2H), 4.07 (s, 6H), 4.04 (s, 3H), 1.38 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 179.1, 165.9, 153.6, 141.7, 131.6, 131.1, 129.3, 126.5, 126.3, 126.0, 105.4, 61.4, 56.8, 39.9, 28.0; MS *m/z* 386 [M^+, 10%], 195 [100%]. Anal. Calcd for $C_{21}H_{26}N_2O_5$: C, 65.27; H, 6.78; N, 7.25. Found: C, 64.86; H, 6.66; N, 7.69.

5.1.2.8. *N*1-{2-[(2,2-Dimethylpropanoyl)amino]-4-methoxyphenyl}-3,4,5-trimethoxybenzamide (8). Yield: 0.70 g (80.0%), mp 170 °C; ^{1}H NMR ($CDCl_3$): δ 9.00 (s, 1H), 8.53 (s, 1H), 7.27–7.16 (m, 3H), 6.82 (d, J = 2.8 Hz, 1H), 6.53 (dd, J = 8.9 Hz and 2.8 Hz, 1H), 3.94 (s, 6H), 3.90 (s, 3H), 3.55 (s, 3H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$): δ 179.0, 166.0, 158.0, 153.6, 141.5, 132.7, 129.4, 127.0, 124.1, 112.0, 111.2, 105.2, 61.4, 56.7, 55.7, 39.9, 27.9; MS *m/z* 416 [M^+, 11%], 195 [100%]. Anal. Calcd for

$C_{22}H_{28}N_2O_6$: C, 63.45; H, 6.78; N, 6.73. Found: C, 63.38; H, 6.78; N, 6.66.

5.1.3. General procedure for the synthesis of the dibenzamide derivative 9 and dithiodibenzamide derivatives of set II, III, and IV

To a solution of 15 mmol appropriate phenylenediamine in 50 ml THF 35 mmol of the adequate benzoyl chloride were added quickly and stirred at room temperature for 2–3 h. Afterwards, the reaction mixture was purged into ice water. The formed solid was filtered off and recrystallized.

To a solution of 10 mmol dibenzamide derivative in THF 20 mmol Lawesson's reagent (LR) were added and the reaction mixture was refluxed for 2–5 h. Then, the solvent was removed in vacuo and the crude product was purified by column chromatography and recrystallisation.

5.1.3.1. *N,N′*-(1,4-Phenylene)bis(3,4,5-trifluorobenzamide) (9). Yield: 4.01 g (63.0%), mp >350 °C; ^{1}H NMR (DMSO-d_6): δ 10.49 (s, 2H), 8.13–7.40 (m, 8H); ^{13}C NMR (DMSO-d_6): δ 166.7, 157.4 (dd, J_{CF} = 8.3 and 10.6 Hz), 153.3–152.9 (m, 1C), 152.5–152.3 (m, 1C), 148.7–147.9 (m, 1C), 143.8 (dd, J_{CF} = 9 and 16 Hz), 134.9, 121.9 (d, J_{CF} = 13 Hz), 120.4, 118.3 (dd, J_{CF} = 4 and 21 Hz), 107.1 (q, J_{CF} = 29 and 8 Hz); MS *m/z* 424 [M$^+$, 9%], 159 [100%]. Anal. Calcd for $C_{20}H_{10}F_6N_2O_2$: C, 56.62; H, 2.38; N, 6.60. Found: C, 56.32; H, 2.18; N, 6.63.

5.1.3.2. *N,N′*-(1,4-Phenylene)bis(4-fluorobenzothioamide) (10). Yield: 2.41 g (63.0%), mp 279–289 °C; ^{1}H NMR (DMSO-d_6): δ 11.83 (s, 2H), 8.16–7.64 (m, 8H), 7.32 (t, J = 8.7 Hz, 4H); ^{13}C NMR (DMSO-d_6): δ 196.1, 163.9 (J_{CF} = 247.5 Hz), 139.1 (d, J_{CF} = 2.9 Hz), 137.9, 130.2 (d, J_{CF} = 8.9 Hz), 124.4, 115.1 (d, J_{CF} = 21.9 Hz); MS *m/z* 384 [M$^+$, 7%], 139 [100%]. Anal. Calcd for $C_{20}H_{14}F_2N_2S_2$: C, 62.48; H, 3.67; N, 7.29. Found: C, 62.78; H, 3.56; N, 7.23.

5.1.3.3. *N,N′*-(1,4-Phenylene)bis(3,4,5-trifluorobenzothioamide) (11). Yield: 1.47 g (68.0%), mp 200–203 °C; ^{1}H NMR (DMSO-d_6): δ 12.27 (s, 2H), 8.01 (s, 4H), 7.94–7.52 (m, 4H); ^{13}C NMR (DMSO-d_6): δ 188.7, 154.9–154,3 (m, 1C), 152.5–152.1 (m, 1C), 150.0–149.7 (m, 1C), 148.5–148.2 (m, 1C), 147.7–147.4 (m, 1C), 143.7–143.4 (m, 1C), 137.3, 129.2–128.9 (m, 1C), 123.3, 118.3 (dd, J_{CF} = 4 and 24 Hz), 106.7 (q, J_{CF} = 7 and 50 Hz); MS *m/z* 456 [M$^+$, 4%], 175 [100%]. Anal. Calcd for $C_{20}H_{10}F_6N_2S_2$: C, 52.63; H, 2.21; N, 6.14. Found: C, 52.22; H, 2.10; N, 5.92.

5.1.3.4. *N,N′*-(1,4-Phenylene)bis(3,4,5-trimethoxybenzothioamide) (12). Yield: 2.05 g (77.7%), mp 265–270 °C; ^{1}H NMR (DMSO-d_6): δ 11.65 (s, 2H), 7.86 (s, 4H), 7.23 (s, 4H), 3.86 (s, 12H), 3.74 (s, 6H); ^{13}C NMR (DMSO-d_6): δ 196.8, 152.3, 140.1, 138.0, 137.7, 124.6, 105.5, 60.3, 56.2; MS *m/z* 528 [M$^+$, 2%], 211 [100%]. Anal. Calcd for $C_{26}H_{28}N_2O_6S_2$: C, 59.07; H, 5.34; N, 5.30. Found: C, 58.79; H, 5.65; N, 5.08.

5.1.3.5. *N,N′*-(1,3-Phenylene)bis(4-fluorobenzothioamide) (13). Yield: 1.61 g (42.0%), mp 238–239 °C; ^{1}H NMR (DMSO-d_6): δ 11.87 (s, 2H), 8.37 (s, 1H), 8.10–7.82 (m, 4H), 7.79–7.61 (m, 2H), 7.57–7.43 (m, 1H), 7.41–7.19 (m, 4H); ^{13}C NMR (DMSO-d_6): δ 196.4, 164.1 (J_{CF} = 249 Hz), 140.3, 139.1 (d, J_{CF} = 3 Hz), 130.3 (d, J_{CF} = 3 Hz), 128.7, 122.6, 120.0, 115.3 (d, J_{CF} = 22 Hz); MS *m/z* 384 [M$^+$, 31%], 139 [100%]. Anal. Calcd for $C_{20}H_{14}F_2N_2S_2$: C, 62.48; H, 3.67; N, 7.29. Found: C, 62.17; H, 3.45; N, 7.25.

5.1.3.6. *N,N′*-(1,3-Phenylene)bis(3,4,5-trifluorobenzothioamide) (14). Yield: 1.14 g (25.0%), mp 211–216 °C; ^{1}H NMR (DMSO-d_6): δ 12.02 (s, 2H), 8.41 (s, 1H), 7.94–7.76 (m, 4H), 7.75–7.60 (m, 2H), 7.58–7.48 (m, 1H); ^{13}C NMR (DMSO-d_6): δ 193.2, 152.1 (dd, J_{CF} = 4 and 10 Hz), 147.2 (dd, J_{CF} = 4 and 10 Hz), 143.1, 140.0, 138.7–138.1 (m), 129.0, 122.7, 119.7, 112.9 (q, J_{CF} = 23 and 5 Hz); MS *m/z* 456 [M$^+$, 3%], 175 [100%]. Anal. Calcd for $C_{20}H_{10}F_6N_2S_2$: C, 52.63; H, 2.21; N, 6.14. Found: C, 52.27; H, 2.35; N, 6.03.

5.1.3.7. *N,N′*-(1,3-Phenylene)bis(4-methoxybenzothioamide) (15). Yield: 3.83 g (94.0%), mp 233–238 °C; ^{1}H NMR (DMSO-d_6): δ 11.63 (s, 2H), 8.27 (s, 1H), 7.90 (d, J_{AB} = 8.5 Hz, 4H), 7.78–7.59 (m, 2H), 7.55–7.39 (m, 1H), 7.02 (d, J_{AB} = 8.5 Hz, 4H), 3.83 (s, 6H); ^{13}C NMR (DMSO-d_6): δ 196.8, 161.9, 140.5, 134.7, 129.8, 128.5, 122.4, 120.5, 113.4, 55.7; MS *m/z* 408 [M$^+$, 35%], 151 [100%]. Anal. Calcd for $C_{22}H_{20}N_2O_2S_2$: C, 64.68; H, 4.93; N, 6.86. Found: C, 64.73; H, 4.94; N, 6.79.

5.1.3.8. *N,N′*-(1,3-Phenylene)bis(3,4,5-trimethoxybenzothioamide) (16). Yield: 1.10 g (34.7%), mp 258–260 °C; ^{1}H NMR (DMSO-d_6): δ 11.67 (s, 2H), 8.27 (s, 1H), 7.77–7.57 (m, 1H), 7.22 (s, 4H), 3.86 (s, 12H), 3.73 (s, 6H); ^{13}C NMR (DMSO-d_6): δ 197.0, 152.3, 140.4, 140.1, 137.6, 128.7, 122.8, 120.6, 105.5, 60.3, 56.2; MS *m/z* 528 [M$^+$, 2%], 211 [100%]. Anal. Calcd for $C_{26}H_{28}N_2O_6S_2$: C, 59.07; H, 5.34; N, 5.30. Found: C, 58.80; H, 5.64; N, 5.09.

5.1.3.9. *N,N′*-(1,2-Phenylene)bis(4-fluorobenzothioamide) (17). Yield: 2.07 g (59.0%), mp 170–171 °C; ^{1}H NMR ($CDCl_3$): δ 9.53 (s, 2H), 8.05–7.72 (m, 4H), 7.68–7.35 (m, 4H), 7.17–6.89 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 198.2, 165.0 (J_{CF} = 254 Hz), 136.9 (d, J_{CF} = 3 Hz), 134.6, 129.5 (d, J_{CF} = 9 Hz), 129.1, 115.6 (d, J_{CF} = 22 Hz); MS *m/z* 384 [M$^+$, 2%], 139 [100%]. Anal. Calcd for $C_{20}H_{14}F_2N_2S_2$: C, 62.48; H, 3.67; N, 7.29. Found: C, 62.17; H, 3.71; N, 7.01.

5.1.3.10. *N,N′*-(1,2-Phenylene)bis(4-trifluoromethoxybenzothioamide) (18). Yield: 0.57 g (27.6%), mp 203–204 °C; ^{1}H NMR ($CDCl_3$): δ 9.48 (s, 2H), 7.86 (AB-system, J_{AB} = 8.7 Hz, 4H), 7.67–7.32 (m, 4H), 7.19 (AB-system, J_{AB} = 8.7 Hz, 4H); ^{13}C NMR ($CDCl_3$): δ 197.8, 151.7 (d, J_{CF} = 2 Hz), 138.9, 134.4, 129.0 (d, J_{CF} = 7 Hz), 127.7, 120.5, 120.2 (d, J_{CF} = 257 Hz); MS *m/z* 516 [M$^+$, 1%], 205 [100%]. Anal. Calcd for $C_{22}H_{14}F_6N_2O_2S_2$: C, 51.16; H, 2.73; N, 5.42. Found: C, 51.32; H, 2.75; N, 5.32.

5.1.3.11. *N,N′*-(1,2-Phenylene)bis(3,4,5-trifluorobenzothioamide) (19). Yield: 2.32 g (55.0%), mp 184–187 °C; ^{1}H NMR ($CDCl_3$): δ 9.34 (s, 2H), 7.75–7.36 (m, 8H); ^{13}C NMR ($CDCl_3$): δ 195.4, 153.3 (dd, J_{CF} = 4 and 10 Hz), 148.3 (dd, J_{CF} = 4 and 10 Hz), 144.8, 139.7, 134.2, 129.6, 127.7, 111.9 (q, J_{CF} = 23 and 5 Hz); MS *m/z* 456 [M$^+$, 1%], 175 [100%]. Anal. Calcd for $C_{20}H_{10}F_6N_2S_2$: C, 52.63; H, 2.21; N, 6.14. Found: C, 52.69; H, 2.28; N, 6.10.

5.1.3.12. *N,N′*-(1,2-Phenylene)bis(4-methoxybenzothioamide) (21). Yield: 0.87 g (44.4%), mp 200–201 °C; ^{1}H NMR (DMSO-d_6): δ 11.20 (s, 2H), 7.85 (AB-system, J_{AB} = 8.5 Hz, 4H), 7.46 (s, 4H), 6.93 (AB-system, J_{AB} = 8.5 Hz, 4H), 3.78 (s, 4H); ^{13}C NMR (DMSO-d_6): δ 197.0, 161.9, 136.0, 133.7, 129.9, 127.9, 127.8, 113.4, 55.7; MS *m/z* 408 [M$^+$, 0.2%], 151 [100%]. Anal. Calcd for $C_{22}H_{20}N_2O_2S_2$: C, 64.68; H, 4.93; N, 6.86. Found: C, 64.45; H, 4.77; N, 6.94.

5.1.3.13. *N,N′*-(1,2-Phenylene)bis(3,4,5-trimethoxybenzothioamide) (22). Yield: 0.79 g (37.5%), mp 195–199 °C; ^{1}H NMR (DMSO-d_6): δ 11.36 (s, 2H), 7.48 (s, 4H), 7.18 (s, 4H), 3.68 (s, 6H), 3.67 (s, 12H); ^{13}C NMR (DMSO-d_6): δ 197.5, 152.1, 140.0, 136.7, 136.1, 128.1, 127.7, 106.0, 60.3, 56.0; MS *m/z* 528 [M$^+$, 0.1%], 211 [100%]. Anal. Calcd for $C_{26}H_{28}N_2O_6S_2$: C, 59.07; H, 5.34; N, 5.30. Found: C, 58.79; H, 5.65; N, 5.08.

5.1.4. General procedure for the synthesis of the dimethylated dithiodibenzamide derivative 20

To a solution of 5 mmol *N*,*N*′-(1,2-phenylene)bis(3,4,5-trifluorobenzamide) in 100 ml THF 10 mmol sodium hydride were carefully added under argon atmosphere. After stirring for 30 min, 12 mmol methyl iodide were added. After four days stirring at room temperature the reaction was stopped. The excessively sodium hydride was neutralized with ethanol/water and then, the reaction mixture was purged into ice water. The solid product was separated and recrystallized in ethanol.

To a solution of 1.4 mmol *N*,*N*′-(1,2-phenylene)bis(3,4,5-trifluoro-*N*-methylbenzothioamide in THF 4 mmol Lawesson's reagent (LR) were added and the reaction mixture was refluxed for 2 h. Then, the solvent was removed in vacuo and the crude product was recrystallized in ethanol.

5.1.4.1. *N*,*N*′-(1,2-Phenylene)bis(3,4,5-trifluoro-N-methylbenzothioamide) (20). Yield: 0.43 g (63.4%), mp 224–229 °C; ^{1}H NMR ($CDCl_3$): δ 7.67–7.26 (m, 8H), 7.20–6.91 (m, 8H), 3.92 (N-CH_3, 3H), 3.56 (N-CH_3, 3H), 3.12 (N-CH_3, 3H), 2.96 (N-CH_3, 3H); ^{13}C NMR ($CDCl_3$): mixture of rotational isomers; MS *m/z* 484 [M^+, 1%], 280 [100%]. Anal. Calcd for $C_{22}H_{14}F_6N_2S_2$: C, 54.54; H, 2.91; N, 5.78. Found: C, 54.43; H, 2.77; N, 5.76.

5.2. Pharmacological studies

To evaluate the spasmolytic activity of the compounds isometric contraction measurements were used according to the procedure described elsewhere.[1,18]

Due to insolubility of the test compounds in aqueous nutrient solution, stock solutions of the compounds were dissolved in dimethylsulfoxide (DMSO) every day and were further diluted with modified Krebs-Henseleit solution to the required concentrations. To exclude the DMSO effect, a series of experiments with DMSO only were performed at the same experimental conditions. The DMSO effect was subtracted from the results of the compounds.

The antagonist of K_{ATP}-channels, glibenclamide, and the inhibitor of the eNOS, nitro-L-arginine, were used to elucidate a possible mode of action of the compounds. After precontraction of the terminal ileum preparations by 60 mM KCl, glibenclamide (30 μM) or nitro-L-arginine (100 μM) were added to the bathing solution for 45 min. After this period of time the test compounds were administered in their IC_{50} concentration for another 45 min.

To determine a possible α-adrenoceptor blocking activity the terminal ileum preparations were contracted with the agonist phenylephrine in concentrations of 0.1, 0.3, 1, and 3 μM. The contractions caused by phenylephrine were recorded for 40 s. A wash-out period with bathing solution for 10 min was performed between each concentration. After addition of the highest concentration of phenylephrine followed by a 10 min wash-out period, the test compound was added in the IC_{50} concentration for 45 min. Then phenylephrine was used again in concentrations of 0.1, 0.3, 1, and 3 μM. After each concentration a wash-out period for 10 min with compound-containing bathing solution was carried out. For the investigation of a possible anticholinergic effect acetylcholine in concentrations of 0.01, 0.03, 0.1, 0.3, 1, and 3 μM was used. These experiments were performed in the same way as described for phenylephrine.

References and notes

1. Brunhofer, G.; Handler, N.; Leisser, K.; Studenik, C. R.; Erker, T. *Bioorg. Med. Chem.* **2008**, *16*, 5974.
2. Howard, R. M. *Gastroenterol. Clin. North Am.* **2003**, *32*, xi.
3. Howard, R. M. *Gastroenterol. Clin. North Am.* **2003**, *32*, 463.
4. Navarrete-Vázquez, G.; Moreno-Diaz, H.; Aguirre-Crespo, F.; León-Rivera, I.; Villalobos-Molina, R.; Muñoz-Muñiz, O.; Estrada-Soto, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4169.
5. Frank, R.; Jakob, M.; Thunecke, F.; Fischer, G.; Schutkowski, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 1120.
6. Gannon, M. K.; Holt, J. J.; Bennett, S. M.; Wetzel, B. R.; Loo, T. W.; Bartlett, M. C.; Clarke, D. M.; Sawada, G. A.; Higgins, J. W.; Tombline, G.; Raub, T. J.; Detty, M. R. *J. Med. Chem.* **2009**, *52*, 3328.
7. Lincke, T.; Behnken, S.; Ishida, K.; Roth, M.; Hertweck, C. *Angew. Chem., Int. Ed.* **2010**, *49*, 2011.
8. Reiner, A.; Wildemann, D.; Fischer, G.; Kiefhaber, T. *J. Am. Chem. Soc.* **2008**, *130*, 8079.
9. Tran, T. T.; Zeng, J.; Treutlein, H.; Burgess, A. W. *J. Am. Chem. Soc.* **2002**, *124*, 5222.
10. Wang, F.; Langley, R.; Gulten, G.; Dover, L. G.; Besra, G. S.; Jacobs, W. R.; Sacchettini, J. C. *J. Exp. Med.* **2007**, *204*, 73.
11. Kaneda, T.; Shimizu, K.; Nakajyo, S.; Urakawa, N. *Eur. J. Pharmacol.* **1998**, *355*, 149.
12. Ceron, P. I. B.; Cremonez, D. C.; Bendhack, L. M.; Tedesco, A. C. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 686.
13. Li, L.; Whiteman, M.; Guan, Y. Y.; Neo, K. L.; Cheng, Y.; Lee, S. W.; Zhao, Y.; Baskar, R.; Tan, C.-H.; Moore, P. K. *Circulation* **2008**, *117*, 2351.
14. Bolton, T. B. *Physiol. Rev.* **1979**, *59*, 606.
15. Godfraind, T.; Miller, R.; Wibo, M. *Pharmacol. Rev.* **1986**, *38*, 321.
16. Shah, A. J.; Gowani, S. A.; Zuberi, A. J.; Ghayur, M. N.; Gilani, A. H. *Phytother. Res.* **2010**, *24*, 28.
17. Mestres, J.; Gregori-Puigjané, E. *Trends Pharmacol. Sci.* **2009**, *30*, 470.
18. Galanski, M. E.; Erker, T.; Handler, N.; Lemmens-Gruber, R.; Kamyar, M.; Studenik, C. R. *Bioorg. Med. Chem.* **2006**, *14*, 826.