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Studies on the chemistry of thienoanellated *O,N*- and *S,N*-containing heterocycles. Part 30: Synthesis and pharmacological properties of thieno[2,3-*b*][1,4]thiazines with potential vasopressin receptor antagonistic activity

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Abstract—A series of new nonpeptide vasopressin antagonists with a 6-ethyl-thieno[2,3-*b*][1,4]thiazine or 6-benzyl-thieno[2,3-*b*][1,4]thiazine skeleton and structural modifications of the aryl side chain were synthesized in this study. The effects on guinea pig heart and smooth muscle preparations were investigated. In the presence of AVP the compounds showed an antagonistic effect. The compounds did not change spontaneous rate in right atria and exerted a slight but not significant negative inotropic effect in papillary muscles. The relaxing effect on vascular smooth muscle and terminal ileum was far more pronounced. Generally the relaxing effect on terminal ilea was more potent maybe due to difference in V_{1a} receptor density. Our results demonstrate that compounds with an ethyl group in position six on the thienothiazine ring (**14**, **16**, **18** and **22**) exerted the most potent relaxing activity in terminal ilea, whereas compounds with a phenyl ring in position six reduced this effect.

1. Introduction

The peptide hormone arginine vasopressin (AVP) is well known for its multiple biological effects in mammals. It exerts its vasoconstrictive activities through the vascular V_{1a} receptors and its antidiuretic action through renal epithelial V_2 receptors. AVP antagonists are useful therapeutic agents for the treatment of congestive heart failure, some types of hypertension or peripheral vascular diseases as well as for diseases caused by excessive renal reabsorption of water. First AVP receptor antagonists were peptide analogues[1,2] of AVP with poor oral bioavailability followed by a number of various nonpeptide compounds, which show V_{1a} and/or V_2 antagonistic activities and are orally effective.

The selective V_{1a} antagonist **1**[3] (OPC-21268), a tetrahydroquinoline derivative and the benzazepine derivative **2**[4] (OPC-31260), a selective V_2 antagonist, are the leading compounds among the first nonpeptide AVP antagonists (Fig. 1). In the past few years several other selective, orally active AVP antagonists with diverse basic ring skeletons, including benzodiazepines,[5,6] benzothiazepines[7] and thienoazepines[8] have been investigated. Results of clinical trials with a number of AVP receptor antagonists, among them compound **2**,[9] conivaptan[10] (a V_{1a} and V_2 antagonist), tolvaptan[11] (a selective V_2 antagonist) and relcovaptan[12] (a selective V_{1a} antagonist), are promising.

The therapeutic potential of selective V_{1a} or V_2 AVP antagonists, the presumed benefits of dual V_{1a}/V_2 antagonists in therapy of congestive heart failure[13] and the observations that AVP could be involved in coronary vasoconstriction[14,15] cause continuing interest in a novel series of compounds.

These aspects prompted us to design new compounds with a modified ring structure. In continuation to our previous work[16] by following the concept of

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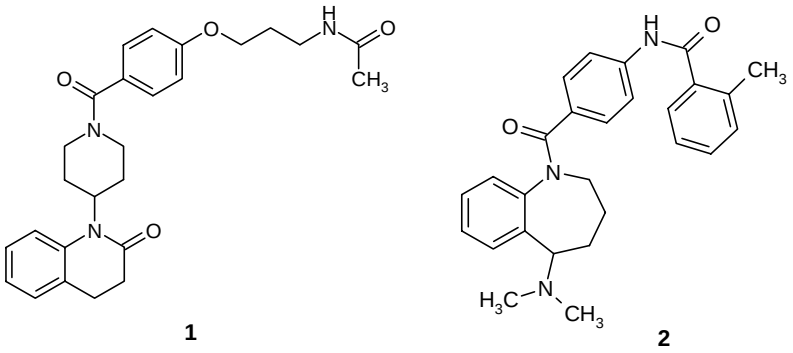
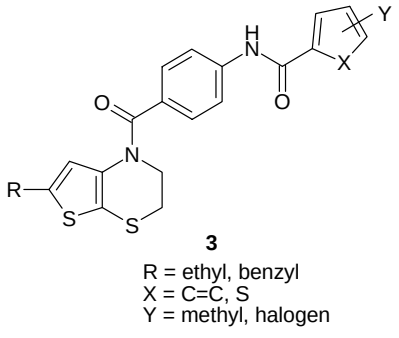


Figure 1. Structures of the vasopressin receptor antagonists **1** (OPC-21268) and **2** (OPC-31260).

bioisosterism, where a chemical group in a biologically active compound can be replaced with another showing similar pharmacological properties, we chose replacement of the phenyl ring by a thiophene ring. Thiophene ring and phenyl ring show about the same size and lipophilicity. Thus, thiophene analogues of biologically active benzene derivatives may exhibit similar activities.[17] Except for some quinoline derivatives[5,18] almost all published bicyclic AVP antagonists have a benzene ring fused with a seven-membered ring as skeleton. Therefore, we considered to construct a new skeleton containing thiophene and a six-membered ring. Additionally, we chose a thioether isosteric linker[19] instead of a methylene moiety. Thus we synthesized a number of compounds with the new ring structure 2,3-dihydrothieno[2,3-*b*][1,4]thiazine substituted with various 4-(benzoylamino)benzoyl and 4-(thiophenecarbonylamino)benzoyl moieties. The general formula **3** is shown in Figure 2. Generally it is known that an alkyl group next to the sulfur atom in thiophene ring containing drugs can increase their biological activity.[20] Thus, the preparation of both ethyl and benzyl substituted ring systems was chosen to investigate whether the target compounds show antagonistic activity on AVP receptors. To increase the lipophilicity of the terminal aromatic ring, which can enhance the AVP receptor affinity,[21] we considered to introduce methyl and fluoro substituted benzoyl moieties, and additionally methyl and chloro thiophenecarbonyl moieties. In this study, the syntheses and the results of the chronotropic as well as the inotropic action on guinea pig smooth and heart muscle preparations of these new compounds are presented. We only investigated the possible effect of the compounds on V_{1a} receptors in the presence or absence of AVP. These receptors are available in the preparations used.



3

R = ethyl, benzyl
X = C=C, S
Y = methyl, halogen

Figure 2. General structure of the thienothiazine-based compounds described here.

2. Chemistry

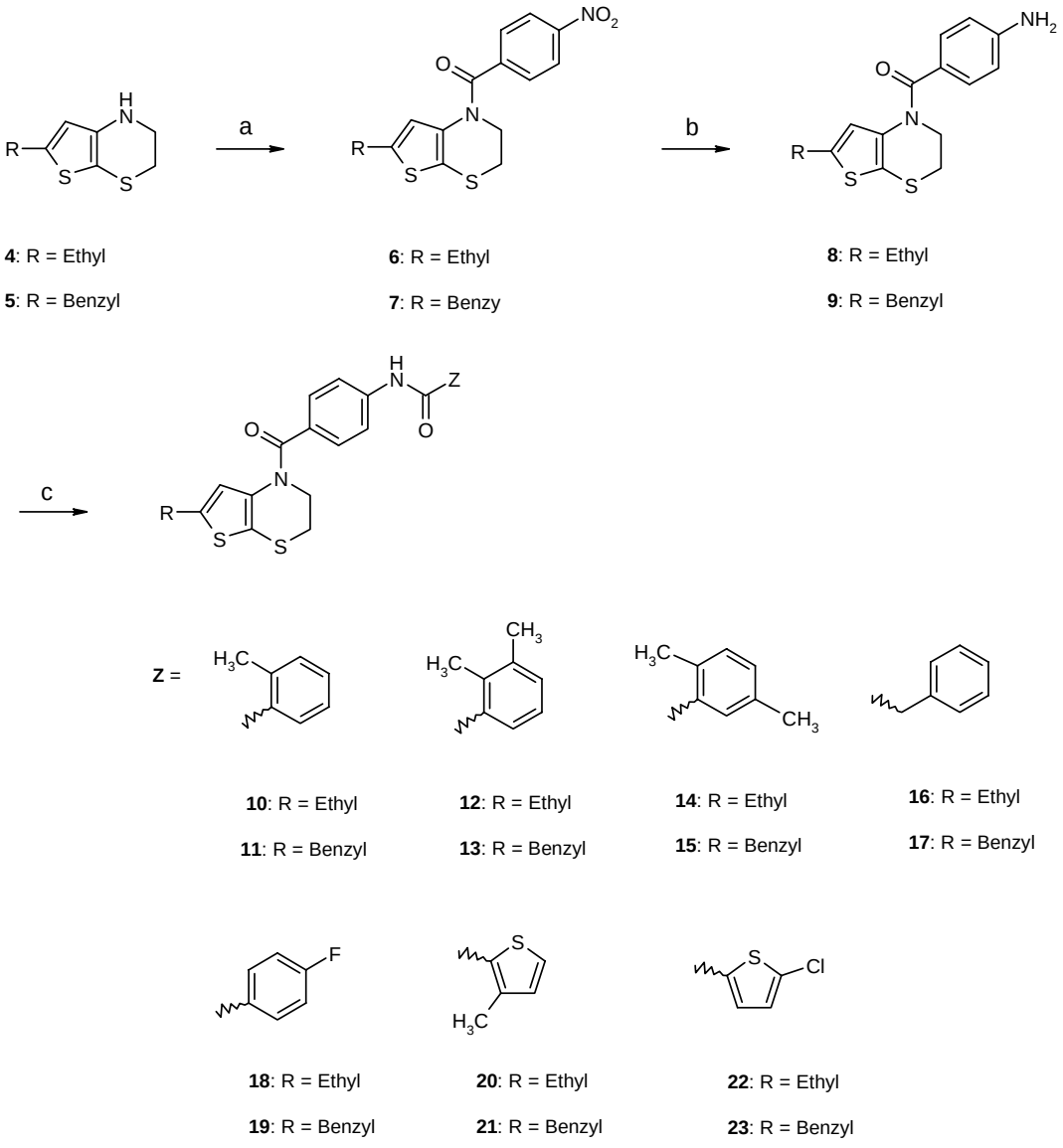
The synthetic pathway for the preparation of the target compounds **10**–**23** is shown in Scheme 1. The starting materials **4**[22] and **5**[23] were treated with 4-nitrobenzoyl chloride in methylene chloride to obtain compounds **6** and **7** in good yields. Reduction of the nitro groups in **6** and **7** was accomplished with iron powder in glacial acetic acid with small amounts of water and methanol to give the amines **8** and **9**. Finally, derivatization of **8** and **9** with 2-methylbenzoyl chloride, 2,3-dimethylbenzoyl chloride, 2,5-dimethylbenzoyl chloride, phenylacetyl chloride, 4-fluorobenzoyl chloride, 3-methythiophene-2-carbonyl chloride and 5-chlorothiophene-2-carbonyl chloride at room temperature afforded the desired compounds **10**–**23** in good yields.

3. Pharmacological results and discussion

The electromechanical effect of the compounds was studied in papillary muscles in the presence and absence of AVP, terminal ilea, aortic and arteria pulmonalis rings.

3.1. Effects on papillary muscles in the presence of AVP

AVP (3 μmol/l, $n = 3$) showed a time-dependent effect on papillary muscles. It had a negative inotropic effect during 1.5 min that was reversed after 2 min into a positive inotropic effect becoming less positive after 5 min. The compounds **15** (1 μmol/l, $n = 3$; 10 μmol/l, $n = 3$), **16** (1 μmol/l, $n = 3$; 10 μmol/l, $n = 3$), **18** (1 μmol/l, $n = 3$; 10 μmol/l, $n = 3$), **19** (1 μmol/l, $n = 3$; 10 μmol/l, $n = 3$), **21** (1 μmol/l, $n = 3$; 10 μmol/l, $n = 3$) and **22** (1 μmol/l, $n = 3$; 10 μmol/l, $n = 3$) antagonized the time-dependent AVP effect. Compounds **14** (1 μmol/l, $n = 3$; 10 μmol/l, $n = 3$) and **23** (1 μmol/l, $n = 3$; 10 μmol/l, $n = 3$) also exerted an antagonistic effect that was less pronounced. These data are shown in Figure 3.



Scheme 1. Reagents and conditions: (a) 4-nitro benzoic acid chloride, TEA, CH_2Cl_2, Ar, rt; (b) Fe^0, glacial acetic acid, MeOH, H_2O, 50 °C, respectively, 70 °C; (c) ZCOCl, TEA, THF, rt.

3.2. Effects on papillary muscles and right atria

The inotropic effect of the compounds was studied in isolated papillary muscles at a constant stimulation rate of 1 Hz in a concentration range between 0.03 and 100 μmol/l ($n = 3$–6). All compounds slightly but not significantly decreased force of contraction (f_c). No effects were observed in right atria testing the chronotropic activity. Spontaneous rate was not changed by all compounds in a concentration range between 0.03 and 100 μmol/l ($n = 3$–5) compared to control values.

3.3. Effects on aortic rings

Aortic rings were stimulated with 90 mmol/l KCl. The relaxing effect of the compounds on contraction was studied in concentrations between 0.03 and 100 μmol/l. Compound **14** reduced f_c from 14.68 ± 0.85 to 0.98 ± 0.6 mN ($n = 4$, $P < 0.001$), **15** from 8.14 ± 1.49 to 7.17 ± 1.4 mN ($n = 5$), **16** from 10.01 ± 1.97 to 4.22 ± 1.3 mN ($n = 5$, $P < 0.001$), **18** from 8.91 ± 1.92 to 5.96 ± 1.17 mN ($n = 5$, $P < 0.01$), **19** from 9.0 ± 1.68 to 4.28 ± 1.02 mN ($n = 5$, $P < 0.01$), **21** from 9.92 ± 3.02 to 5.1 ± 1.33 mN ($n = 4$, $P < 0.01$), **22** from 12.51 ± 3.02 to 7.96 ± 1.83 mN ($n = 3$, $P < 0.05$) and **23** from 20.19 ± 4.06 to 1.42 ± 0.76 mN ($n = 4$, $P < 0.001$). The IC_{50} values were 84 μmol/l (**14**), 22.9 μmol/l (**16**), 86.75 μmol/l (**19**) and 92 μmol/l (**23**). The decrease in percent is shown in Figure 4.

3.4. Effects on arteria pulmonalis rings

Similar experiments as on aortic rings were carried out using arteria pulmonalis rings. The preparations were stimulated with 90 mmol/l KCl. Again all compounds concentration-dependently relaxed the KCl-induced contraction in concentrations between 0.3 and 100 μmol/l. Compound **14** reduced f_c from 18.35 ± 1.46

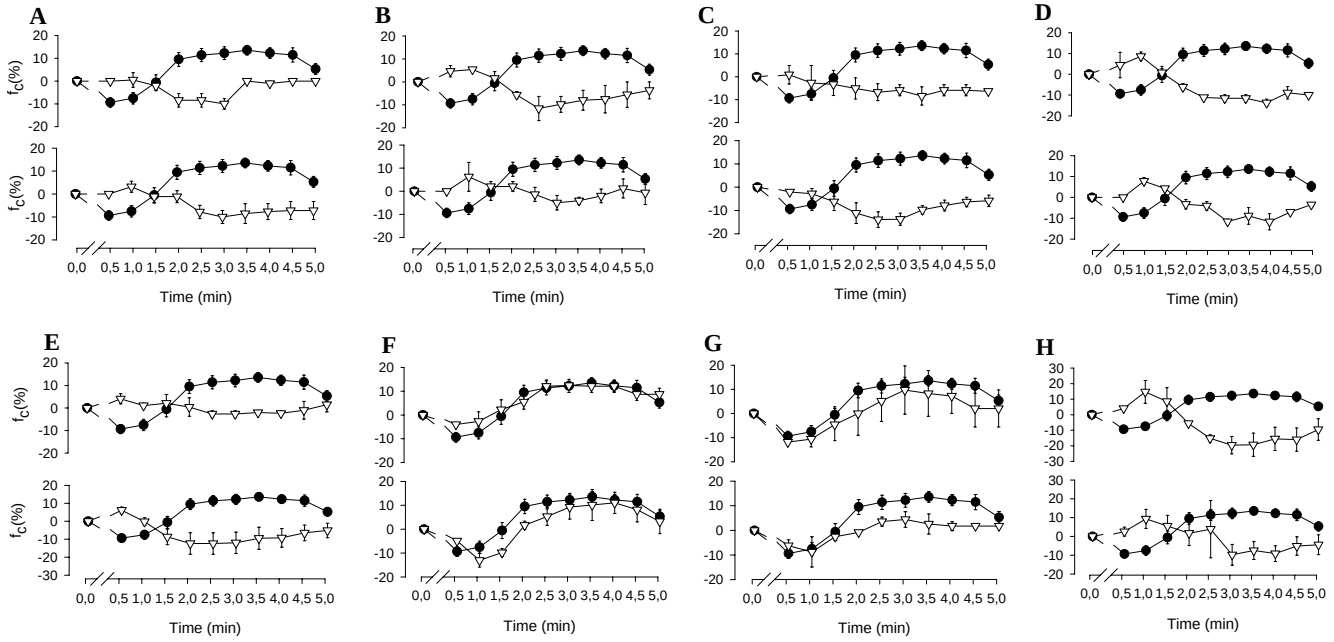


Figure 3. Panel A shows the effect of AVP (3 μmol/l) (•), the effect of 1 μmol/l **16** (▽) in the presence of 3 μmol/l AVP on top and the effect of 10 μmol/l **16** (▽) in the presence of 3 μmol/l AVP on the bottom. Panel B shows the effect of AVP (3 μmol/l) (•), the effect of 1 μmol/l **18** (▽) in the presence of 3 μmol/l AVP on top and the effect of 10 μmol/l **18** (▽) in the presence of 3 μmol/l AVP on the bottom. Panel C shows the effect of AVP (3 μmol/l) (•), the effect of 1 μmol/l **19** (▽) in the presence of 3 μmol/l AVP on top and the effect of 10 μmol/l **19** (▽) in the presence of 3 μmol/l AVP on the bottom. Panel D shows the effect of AVP (3 μmol/l) (•), the effect of 1 μmol/l **21** (▽) in the presence of 3 μmol/l AVP on top and the effect of 10 μmol/l **21** (▽) in the presence of 3 μmol/l AVP on the bottom. Panel E shows the effect of AVP (3 μmol/l) (•), the effect of 1 μmol/l **22** (▽) in the presence of 3 μmol/l AVP on top and the effect of 10 μmol/l **22** (▽) in the presence of 3 μmol/l AVP on the bottom. Panel F shows the effect of AVP (3 μmol/l) (•), the effect of 1 μmol/l **23** (▽) in the presence of 3 μmol/l AVP on top and the effect of 10 μmol/l **23** (▽) in the presence of 3 μmol/l AVP on the bottom. Panel G shows the effect of AVP (3 μmol/l) (•), the effect of 1 μmol/l **14** (▽) in the presence of 3 μmol/l AVP on top and the effect of 10 μmol/l **14** (▽) in the presence of 3 μmol/l AVP on the bottom. Panel H shows the effect of AVP (3 μmol/l) (•), the effect of 1 μmol/l **15** (▽) in the presence of 3 μmol/l AVP on top and the effect of 10 μmol/l **15** (▽) in the presence of 3 μmol/l AVP on the bottom. The percentage of contraction force is plotted on the ordinate against the time in minutes on the abscissa. Symbols represent the arithmetic mean ± SEM of three experiments.

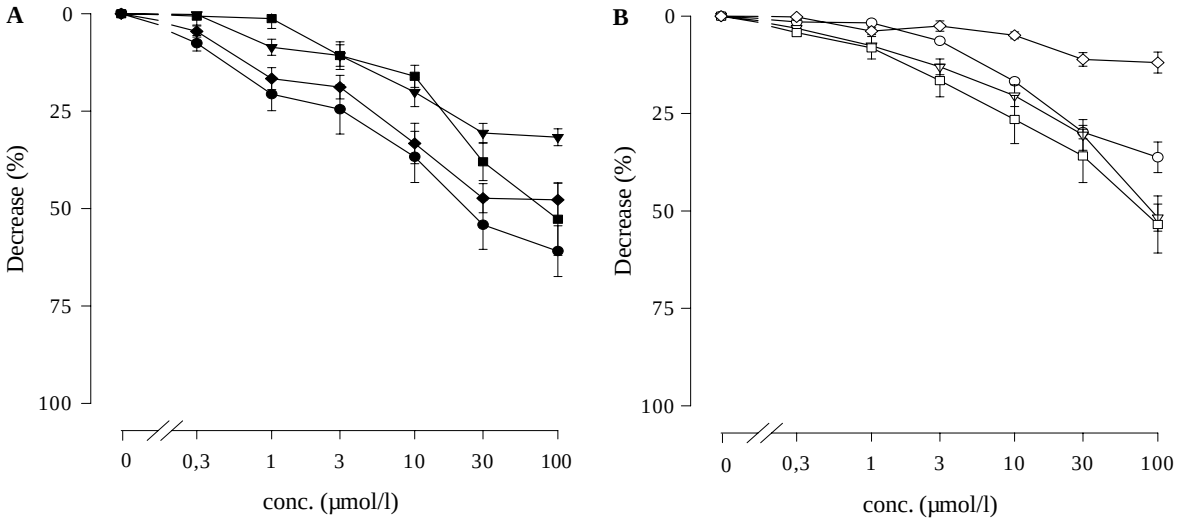


Figure 4. Panel A shows the effect of **16** (•), **18** (▼), **19** (■) and **21** (♦) and panel B the effect of **22** (○), **23** (▽), **14** (□) and**15** (◊) on aortic rings contracted by 90 mmol/l KCl. The decrease in percent of contraction force is semilogarithmically plotted on the ordinate against the concentration of the compounds on the abscissa. Symbols represent the arithmetic mean ± SEM from three to five experiments.

to 0.91 ± 0.15 mN ($n = 4$, $P < 0.001$), **15** from 18.1 ± 2.73 to 12.55 ± 2.08 mN ($n = 4$), **16** from 10.04 ± 0.71 to 1.93 ± 0.41 mN ($n = 5$, $P < 0.001$), **18** from 13.43 ± 2.34 to 1.22 ± 0.27 mN ($n = 5$, $P < 0.01$), **19** from 21.2 ± 2.53 to 13.81 ± 2.03 mN ($n = 4$, $P < 0.05$), **21** from 18.69 ± 1.92 to 11.66 ± 0.93 mN ($n = 4$, $P < 0.05$), **22** from 17.49 ± 6.17 to 9.37 ± 4.34 mN ($n = 3$, $P < 0.05$) and **23** from 18.3 ± 2.9 to 7.65 ± 1.07 mN ($n = 4$, $P < 0.001$). The IC_{50} values were 30 μmol/l (**14**), 14.4 μmol/l (**16**),

12.7 μmol/l (**18**), 100 μmol/l (**22**) and 100 μmol/l (**23**). The decrease in percent is shown in Figure 5.

3.5. Effects on terminal ilea

The relaxing effect of the compounds was also studied in terminal ilea. The preparations were stimulated with 60 mmol/l KCl. Again all compounds concentration-dependently relaxed the KCl-induced contraction. The relaxing effect of the compounds on contraction was studied in concentrations between 0.03 and 100 μmol/l. Compound **14** reduced f_c from 11.01 ± 1.79 to 0.07 ± 0.07 mN ($n = 6$, $P < 0.001$), **15** from 22.48 ± 2.77 to 20.34 ± 3.05 mN ($n = 5$), **16** from 13.24 ± 2.35 to 2.33 ± 0.49 mN ($n = 5$, $P < 0.01$), **18** from 12.34 ± 2.69 to 4.0 ± 1.33 mN ($n = 5$, $P < 0.01$), **19** from 10.18 ± 0.57 to 0 mN ($n = 7$, $P < 0.001$), **21** from 26.26 ± 2.54 to 20.08 ± 2.04 mN ($n = 4$), **22** from 12.86 ± 1.76 to 0 mN ($n = 8$, $P < 0.001$) and **23** from 22.03 ± 2.26 to 0.85 ± 0.39 mN ($n = 5$, $P < 0.001$). The IC_{50} values were 7.7 μmol/l (**14**), 1.5 μmol/l (**16**), 4.8 μmol/l (**18**), 15.15 μmol/l (**19**), 1.82 μmol/l (**22**) and 23.3 μmol/l (**23**). The decrease in percent is shown in Figure 6.

3.6. Receptor binding assay

Compound **21** was chosen as an example to study the receptor binding activity and the possible antagonistic effect. In each experiment, the respective reference compound was tested concurrently with **21** in order to assess the assay suitability. It was tested at several concentrations for IC_{50} value determination. The assay was rendered valid if the suitability criteria were met. The IC_{50} value for reference compound [$d(CH_2)_5^1$, $Tyr(Me)_2$]-AVP was 1.8 ± 0.15 nM, the inhibitory constant (K_i) was 1.1 ± 0.11 nM and the Hill coefficient was

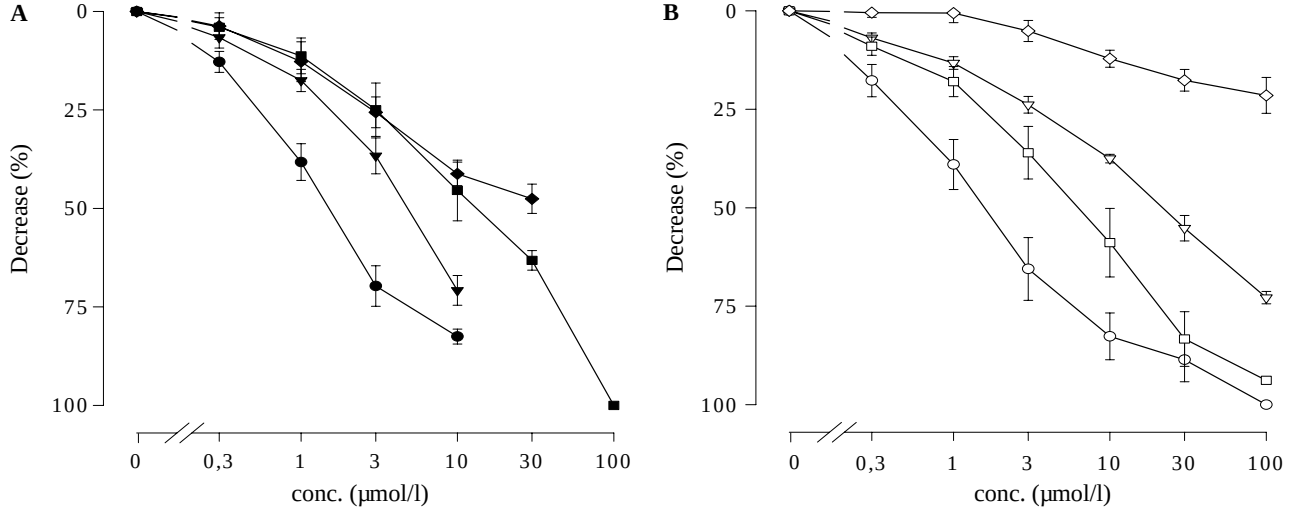


Figure 5. Panel A shows the effect of **16** (●), **18** (▼), **19** (■) and **21** (♦) and panel B the effect of **22** (○), **23** (▽), **14** (□) and **15** (◊) on arteria pulmonalis rings contracted by 90 mmol/l KCl. The decrease in percent of contraction force is semilogarithmically plotted on the ordinate against the concentration of the compounds on the abscissa. Symbols represent the arithmetic mean ± SEM from three to five experiments.

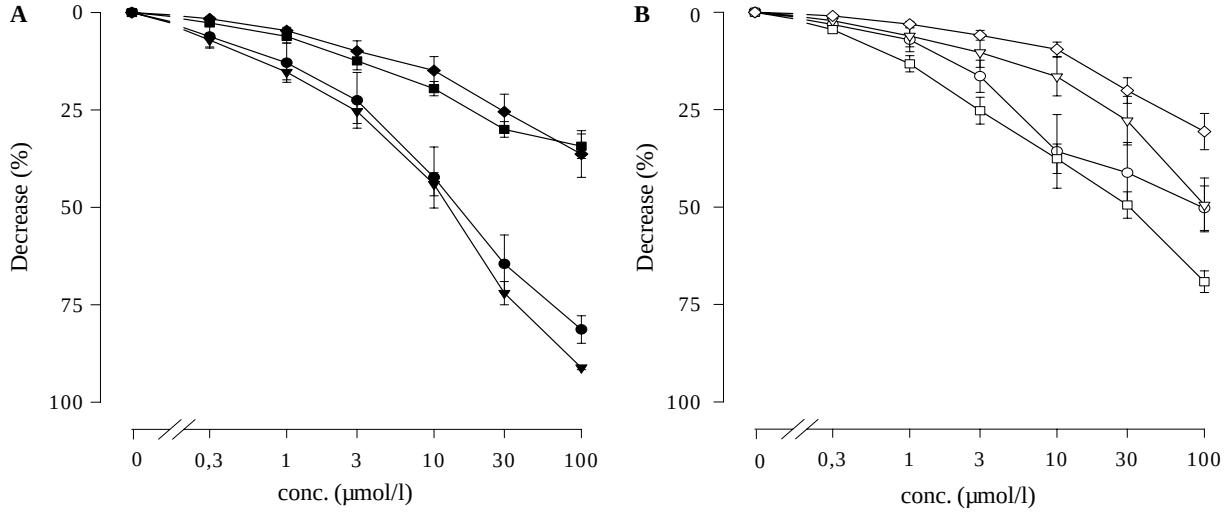


Figure 6. Panel A shows the effect of **16** (●), **18** (▼), **19** (■) and **21** (♦) and panel B the effect of **22** (○), **23** (▽), **14** (□) and **15** (◊) on terminal ilea contracted by 60 mmol/l KCl. The decrease in percent of contraction force is semilogarithmically plotted on the ordinate against the concentration of the compounds on the abscissa. Symbols represent the arithmetic mean ± SEM from five to eight experiments.

1.1 ± 0.06 (n = 3). The IC_{50} value for reference compound AVP was 0.82 ± 0.17 nM, the inhibitory constant (K_i) was 0.59 ± 0.08 nM and the Hill coefficient was 1.0 ± 0.07 (n = 3). Compound **21** (10 μmol/l, n = 3) showed a moderate effect on V_{1a} (h) (K_i > 10 μmol/l, 72.6%) and on V_2 (h) (K_i > 20 μmol/l, 79.7%).

Our results demonstrate that the relaxing effect on smooth muscle preparations is far more pronounced than the negative inotropic effect on heart muscle preparations. AVP in low concentrations exerts a negative inotropic effect in guinea pig hearts,[24] whereas higher concentrations (>0.1 μmol/l) increase contractility.[25] This is consistent with the findings of Fujisawa and Iijima.[26] They initially found a negative inotropic effect followed by a positive inotropic effect using AVP in concentrations between 0.3 and 3 μmol/l. In our experiments, a concentration of 3 μmol/l AVP caused a negative inotropic effect followed by a positive effect. When the compounds in a concentration of 1 and 10 μmol/l were added in the presence of 3 μmol/l AVP this effect was reversed. Compounds **14** and **23** only showed a weak antagonism. There also seems to be a species specificity.[27] OPC-21268, a nonpeptide V_{1a} receptor antagonist, could reverse the cardiac effects of AVP, whereas OPC-31260, a V_2 receptor antagonist did not have any effect.[26] In other studies, OPC-31260 was found to bind to V_{1a} receptors. Therefore, this compound is considered to be a V_2/V_{1a} ligand.[28]

Vasopressin also stimulates V_{1a} receptors in smooth muscles causing a vasoconstriction.[29] The effect of the new compounds on aortic, arteria pulmonalis rings and terminal ilea was more pronounced than on heart muscle preparations. Among the smooth muscle preparations the effect of the compounds on terminal ilea was more potent. This might be due to a difference in receptor density in vascular smooth muscles and terminal ilea. Compounds **14**, **16**, **18** and **22** had the most potent effect on terminal ilea, **19** and **23** were less effective and **15** and **21** cause a weak relaxing effect. The AVP antagonism of the compounds was either slightly increased by concentrations of 10 μmol/l or concentrations of 3 and 10 μmol/l showed similar effects. Compound **14** for example had a strong relaxing effect but the AVP antagonism was weak, whereas **15** and **21** showed a weak relaxing effect but their AVP antagonism was more pronounced. Previously we studied another series of potential vasopressin antagonists and compared them with the selective V_{1a} antagonist [phenylacetyl[1],*O*-Me-D-Tyr[2],Arg[6,8],Lys[9]]-vasopressin amide.[30] This compound antagonized the AVP effects similar to compounds **15**, **16**, **18**, **19** and **21** and (for values see Galanski et al. 2005[30] and Fig. 3).

The antagonistic vasopressin effect of **21** was studied in in vitro receptor binding assays using human V_{1a} and V_2 receptors. A moderate inhibitory effect on V_{1a} and V_2 was found and there was only a slight difference in the inhibition of V_{1a} and V_2 receptors. Therefore, some of the compounds can be considered to be moderate V_{1a}/V_2 ligands. Similar results were found for OPC-21268.[31] Thus the vasopressin antagonistic effect of the compounds cannot be solely responsible for the vasodilation. From earlier studies we know that the thienothiazine moiety causes a more or less pronounced calcium antagonistic effect depending on the different substitution pattern.[32] We conclude that the vasodilatory effect of the compounds is not only due to V_{1a} antagonism but also due to a calcium antagonistic effect. Concerning structure activity relationships we demonstrated that an ethyl group in position six on the thienothiazine moiety exert potent relaxing effects (**14**, **16**, **18** and **22**). A substitution of the ethyl group by a benzyl unit reduces this effect (**15**, **19**, **21** and **23**).

4. Conclusion

The aim of our investigation was to synthesize new nonpeptide AVP receptor antagonists and to study their vasoactive and cardiac effects in comparison to those of AVP. Yet, this study has a limitation as there are species differences that influence vasopressin responses. We did our experiments in guinea pig preparations that seem to be more sensitive to our compounds than human vasopressin receptors. Nevertheless, we discovered some nonpeptide compounds with a moderate AVP antagonism similar to the V_{1a} antagonist OPC21268.[31]

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 with a Kofler hot-stage apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Varian UnityPlus-300 (300 MHz). Chemical shifts are reported in δ values (parts per million) relative to Me_4Si line as internal standard and J values are reported in Hertz. Mass spectra were obtained by a Shimadzu GC/MS QP 1000 EX or Hewlett Packard (GC: 5890; MS: 5970) spectrometer. Column chromatography was performed using silica gel 60, 70–230 mesh ASTM (Merck). Solutions in organic solvents were dried over anhydrous sodium sulfate.

5.1.2. General procedure for the preparation of 6 and 7. A solution of **4**, respectively, **5** in anhydrous methylene chloride (50 ml) was treated with triethylamine (2.02 g, 20 mmol) followed by 4-nitro benzoic acid chloride (3.71 g, 20 mmol) under argon atmosphere. After stirring at room temperature the reaction mixture was washed with water, 5% sodium hydrogen carbonate solution and brine. The dried and evaporated organic layer was purified.

5.1.2.1. (6-Ethyl-2,3-dihydro-1*H*-thieno[2,3-*b*][1,4]thiazin-1-yl)(4-nitrophenyl) methanone (6). Reagent: compound **4** (3.70 g, 20 mmol). Reaction time: 3 h. Purification: crystallization with ethanol. Yield: 4.36 g (62%) of a solid, mp 155–158 °C. ^1H NMR ($CDCl_3$): δ = 0.86–1.20 (m, 3H, CH_3), 2.32–2.77 (m, 2H, CH_2), 3.13–3.45 (m, 2H, SCH_2), 3.98–4.37 (m, 2H, NCH_2),

5.68 (br s, 1H, thiophene H), 7.65 (B-part of an AB-system, $J = 8.4$ Hz, 2H), 8.22 (A-part of an AB-system, $J = 8.4$ Hz, 2H). ^{13}C NMR ($CDCl_3$): $\delta = 15.4$, 23.3, 28.6, 120.6, 123.4, 129.3, 141.5, 142.4, 148.6, 166.2 (3C could not be detected). MS: *m*/*z* 184 (11%), 334 (100%). Anal. Calcd for $C_{15}H_{14}N_2O_3S_2$: C, 53.88; H, 4.22; N, 8.38. Found: C, 53.69; H, 4.27; N, 8.10.

5.1.2.2. (6-Benzyl-2,3-dihydro-1*H*-thieno[2,3-*b*][1,4]thiazin-1-yl)(4-nitrophenyl) methanone (7). Reagent: compound **5** (4.94 g, 20 mmol). Reaction time: 4 h. Purification: flash chromatography with methylene chloride. Yield: 7.66 g (97%) of a solid, mp 168–171 °C. ^{1}H NMR ($CDCl_3$): $\delta = 3.14$–3.36 (m, 2H, SCH_2), 3.62–3.89 (m, 2H, phenyl CH_2), 4.04–4.32 (m, 2H, NCH_2), 5.47 (br s, 1H, thiophene H), 6.69–7.05 (m, 2H, aromat. H), 7.07–7.28 (m, 3H, aromat. H), 7.51 (B-part of an AB-system, $J = 8.1$ Hz, 2H), 8.11 (A-part of an AB-system, $J = 8.1$ Hz, 2H). ^{13}C NMR ($CDCl_3$): $\delta = 28.7$, 35.9, 122.4, 123.4, 126.7, 128.2, 128.4, 129.1, 138.8, 139.1, 141.4, 148.5, 166.3 (3C could not be detected). MS: *m*/*z* 246 (31%), 396 (100%). Anal. Calcd for $C_{20}H_{16}N_2O_3S_2 \cdot 0.2CH_2Cl_2$: C, 58.68; H, 4.00; N, 6.78. Found: C, 58.72; H, 3.92; N, 6.84.

5.1.3. General procedure for the preparation of 8 and 9. Reagent **6**, respectively, **7** was dissolved in a warmed mixture of glacial acetic acid (100 ml), methanol (7 ml) and water (7 ml). Then, iron powder (3.92 g, 70 mmol) was added in portions. The reaction mixture was heated and poured onto ice water. The crude product was diluted in ethyl acetate and washed with sodium hydrogen carbonate solution. The organic layer was dried and evaporated.

5.1.3.1. (4-Aminophenyl)(6-ethyl-2,3-dihydro-1*H*-thieno[2,3-*b*][1,4]thiazin-1-yl) methanone (8). Reagent: compound **6** (3.34 g, 10 mmol). Reaction temperature: 50 °C. Reaction time: 1 h. Yield: 2.84 g (93%) of an oil. ^{1}H NMR ($CDCl_3$): $\delta = 1.12$ (t, $J = 7.5$ Hz, 3H, CH_3), 2.58 (q, $J = 7.5$ Hz, 2H, CH_2), 3.15–3.30 (m, 2H, SCH_2), 3.99 (s, 2H, NH_2), 4.09–4.23 (m, 2H, NCH_2), 6.22 (s, 1H, thiophene H), 6.56 (B-part of an AB-system, $J = 8.5$ Hz, 2H), 7.32 (A-part of an AB-system, $J = 8.5$ Hz, 2H). ^{13}C NMR ($CDCl_3$): $\delta = 15.5$, 23.4, 28.6, 43.7, 113.7, 114.3, 121.3, 124.5, 130.7, 133.6, 141.2, 149.1, 169.0. MS: *m*/*z* 120 (69%), 185 (9%), 304 (100%). Anal. Calcd for $C_{15}H_{16}N_2OS_2$: C, 59.18; H, 5.30; N, 9.20. Found: C, 59.30; H, 5.54; N, 9.00.

5.1.3.2. (4-Aminophenyl)(6-benzyl-2,3-dihydro-1*H*-thieno[2,3-*b*][1,4]thiazin-1-yl) methanone (9). Reagent: compound **7** (3.96 g, 10 mmol). Reaction temperature: 70 °C. Reaction time: 4 h. Yield: 3.50 g (96%) of a solid, mp 130–132 °C. ^{1}H NMR ($CDCl_3$): $\delta = 3.10$–3.24 (m, 2H, SCH_2), 3.85 (s, 2H, phenyl CH_2), 3.96 (br s, 2H, NH_2), 4.07–4.19 (m, 2H, NCH_2), 6.19 (br s, 1H, thiophene H), 7.01–7.12 (m, 2H, aromat. H), 7.14–7.36 (m, 3H, aromat. H), 6.55 (B-part of an AB-system, $J = 8.4$ Hz, 2H), 7.29 (A-part of an AB-system, $J = 8.4$ Hz, 2H). ^{13}C NMR ($CDCl_3$): $\delta = 28.7$, 36.1, 43.3, 113.8, 115.9, 123.1, 124.5, 126.5, 128.3, 128.4, 130.7, 133.7, 137.9, 139.4, 149.0, 168.9. MS: *m*/*z* 69 (100%), 91 (46%), 170 (24%), 185 (24%), 366 (0.3%). Anal. Calcd for $C_{20}H_{18}N_2OS_2$: C, 65.55; H, 4.95; N, 7.64. Found: C, 65.66; H, 4.77; N, 7.39.

5.1.4. General procedure for the preparation of compounds 10–23. To a solution of **8** (0.609 g, 2 mmol), respectively, **9** (0.732 g, 2 mmol) in dry tetrahydrofuran (10 ml) triethylamine (0.28 ml, 2 mmol) and 2 mmol of the corresponding acid chloride, dissolved in dry tetrahydrofuran (1 ml), were added dropwise. After stirring the reaction mixture at room temperature, the solvent was removed under vacuo. The residue was diluted with ethyl acetate and washed with 5% aqueous hydrogen carbonate solution and water. The organic layer was dried and evaporated. The residue was purified by column chromatography. The following compounds were prepared according to this method:

5.1.4.1. N1-{4-[(6-Ethyl-2,3-dihydro-1*H*-thieno[2,3-*b*]-[1,4]thiazin-1-yl)carbonyl]phenyl}-2-methylbenzamide (10). Reagents: compound **8**, 2-methylbenzoyl chloride (0.309 g, 2 mmol). Reaction time: 2.5 h. Eluent: toluene/ethyl acetate, 7:3. Yield: 0.665 g (79%) of a white solid, mp 102–105 °C. ^{1}H NMR ($CDCl_3$): $\delta = 1.12$ (t, $J = 7.5$ Hz, 3H, CH_3), 2.47 (s, 3H, CH_3 phenyl), 2.59 (q, $J = 7.5$ Hz, 2H, CH_2), 3.10–3.28 (m, 2H, SCH_2), 4.02–4.20 (m, 2H, NCH_2), 6.22 (br s, 1H, thiophene H), 7.15–7.30 (m, 2H, aromat. H), 7.31–7.50 (m, 2H, aromat. H), 7.44 (B-part of an AB-system, $J = 8.4$ Hz, 2H), 7.62 (A-part of an AB-system, $J = 8.4$ Hz, 2H), 8.02 (br s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 15.5$, 19.8, 23.4, 28.6, 115.5, 119.1, 121.1, 125.8, 126.6, 129.6, 130.5, 130.9, 131.3, 132.9, 135.9, 136.5, 140.3, 141.7, 168.2, 168.3 (1C could not be detected). MS: *m*/*z* 119 (100%), 185 (17%), 238 (30%), 422 (6%). Anal. Calcd for $C_{23}H_{22}N_2O_2S_2$: C, 65.38; H, 5.25; N, 6.63. Found: C, 65.17; H, 5.01; N, 6.45.

5.1.4.2. N1-{4-[(6-Benzyl-2,3-dihydro-1*H*-thieno[2,3-*b*]-[1,4]thiazin-1-yl)carbonyl]phenyl}-2-methylbenzamide (11). Reagents: compound **9**, 2-methylbenzoyl chloride (0.309 g, 2 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate, 7:3. Yield: 0.585 g (60%) of a yellow oil. ^{1}H NMR ($CDCl_3$): $\delta = 2.46$ (s, 3H, phenyl CH_3), 3.11–3.23 (m, 2H, SCH_2), 3.84 (s, 2H, phenyl CH_2), 4.02–4.15 (m, 2H, NCH_2), 6.11 (br s, 1H, thiophene H), 6.99–7.47 (m, 9H, aromat. H), 7.38 (B-part of an AB-system, $J = 8.4$ Hz, 2H), 7.61 (A-part of an AB-system, $J = 8.4$ Hz, 2H), 8.25 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 19.7$, 28.5, 36.0, 117.0, 118.9, 122.8, 125.8, 126.5, 126.6, 128.3, 128.4, 129.5, 130.4, 130.6, 131.2, 132.9, 135.9, 136.4, 138.3, 139.2, 140.4, 168.2 (2C could not be detected). MS: *m*/*z* 119 (100%), 238 (33%), 247 (14%), 484 (9%). Anal. Calcd for $C_{28}H_{24}N_2O_2S_2$: C, 69.39; H, 4.99; N, 5.78. Found: C, 67.51; H, 4.86; N, 5.34. HRMS: Calcd: 484.1279. Found: 484.1267.

5.1.4.3. N1-{4-[(6-Ethyl-2,3-dihydro-1*H*-thieno[2,3-*b*]-[1,4]thiazin-1-yl)carbonyl]phenyl}-2,3-dimethylbenzamide (12). Reagents: compound **8**, 2,3-dimethylbenzoyl chloride (0.336 g, 2 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate, 7:3. Yield: 0.812 g (93%) of a yellow oil. ^{1}H NMR ($CDCl_3$): $\delta = 1.12$ (t, $J = 7.5$ Hz, 3H, CH_3), 2.30 (s, 3H, CH_3 phenyl), 2.33 (s, 3H, CH_3 phenyl), 2.59 (q, $J = 7.5$ Hz, 2H, CH_2), 3.11–3.26 (m, 2H, SCH_2),

4.01–4.17 (m, 2H, NCH_2), 6.22 (br s, 1H, thiophene H), 7.04–7.31 (m, 3H, aromat. H), 7.43 (B-part of an AB-system, $J = 8.5$ Hz, 2H), 7.61 (A-part of an AB-system, $J = 8.5$ Hz, 2H), 8.05 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 15.5$, 16.3, 20.2, 23.4, 28.5, 43.9, 115.5, 119.0, 121.1, 124.2, 125.5, 129.6, 130.8, 131.7, 132.8, 134.4, 136.8, 138.1, 140.4, 141.6, 168.3, 169.0. MS: *m/z* 105 (39%), 133 (100%), 185 (19%), 252 (46%), 436 (14%). Anal. Calcd for $C_{24}H_{24}N_2O_2S_2$: C, 66.03; H, 5.54; N, 6.42. Found: C, 66.25; H, 5.40; N, 6.20.

5.1.4.4. N1-{4-[(6-Benzyl-2,3-dihydro-1*H*-thieno[2,3-*b*]-[1,4]thiazin-1-yl)carbonyl]phenyl}-2,3-dimethylbenzamide (13). Reagents: compound **9**, 2,3-dimethylbenzoyl chloride (0.336 g, 2 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate, 7:3. Yield: 0.916 g (92%) of an orange oil. ^1H NMR ($CDCl_3$): $\delta = 2.29$ (s, 3H, phenyl CH_3), 2.33 (s, 3H, phenyl CH_3), 3.11–3.23 (m, 2H, SCH_2), 3.84 (s, 2H, phenyl CH_2), 4.01–4.13 (m, 2H, NCH_2), 6.11 (br s, 1H, thiophene H), 7.00–7.29 (m, 8H, aromat. H), 7.36 (B-part of an AB-system, $J = 8.4$ Hz, 2H), 7.61 (A-part of an AB-system, $J = 8.4$ Hz, 2H), 8.13 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 16.3$, 20.2, 28.6, 36.1, 47.8, 117.0, 118.8, 122.9, 124.2, 125.6, 126.5, 128.3, 128.4, 129.6, 130.7, 131.6, 133.0, 134.4, 136.8, 138.1, 138.3, 139.2, 140.4, 168.2, 168.9. MS: *m/z* 91 (51%), 105 (43%), 133 (100%), 247 (19%), 252 (31%), 498 (10%). Anal. Calcd for $C_{29}H_{26}N_2O_2S_2$: C, 69.85; H, 5.26; N, 5.62. Found: 69.73; H, 5.28; N, 5.57.

5.1.4.5. 1-{4-[(6-Ethyl-2,3-dihydro-1*H*-thieno[2,3-*b*][1,4]-thiazin-1-yl)carbonyl]phenyl}-2,5-dimethylbenzamide (14). Reagents: compound **8**, 2,5-dimethylbenzenecarbonyl chloride (0.336 g, 2 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate, 7:3. Yield: 0.667 g (76%) of a beige solid, mp 182–184 °C. ^1H NMR ($CDCl_3$): $\delta = 1.11$ (t, $J = 7.5$ Hz, 3H, CH_2CH_3), 2.30 (s, 3H, CH_3 phenyl), 2.39 (s, 3H, CH_3 phenyl), 2.58 (q, $J = 7.5$ Hz, 2H, CH_2CH_3), 3.09–3.26 (m, 2H, SCH_2), 3.99–4.15 (m, 2H, NCH_2), 6.21 (br s, 1H, thiophene H), 7.04–7.19 (m, 2H, aromat. H), 7.24 (s, 1H, aromat. H), 7.40 (B-part of an AB-system, $J = 8.4$ Hz, 2H), 7.61 (A-part of an AB-system, $J = 8.4$ Hz, 2H), 8.23 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 15.5$, 19.2, 20.8, 23.4, 28.5, 43.8, 115.4, 119.0, 121.0, 127.3, 129.5, 130.6, 131.0, 131.1, 132.8, 133.1, 135.3, 135.8, 140.4, 141.6, 168.3, 168.4. MS: *m/z* 91 (100%), 105 (32%), 133 (73%), 185 (16%), 252 (38%), 436 (10%). Anal. Calcd for $C_{24}H_{24}N_2O_2S_2$: C, 66.03; H, 5.54; N, 6.42. Found: 66.30; H, 5.39; N, 6.21.

5.1.4.6. N1-{4-[(6-Benzyl-2,3-dihydro-1*H*-thieno[2,3-*b*]-[1,4]thiazin-1-yl)carbonyl]phenyl}-2,5-dimethylbenzamide (15). Reagents: compound **9**, 2,5-dimethylbenzenecarbonyl chloride (0.336 g, 2 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate, 7:3. Yield: 0.721 g (72%) of a solid, mp 80 °C. ^1H NMR ($CDCl_3$): $\delta = 2.32$ (s, 3H, CH_3 phenyl), 2.43 (s, 3H, CH_3 phenyl), 3.03–3.30 (m, 2H, SCH_2), 3.84 (s, 2H, CH_2 phenyl) 3.96–4.20 (m, 2H, NCH_2), 6.12 (br s, 1H, thiophene H), 6.94–7.34 (m, 8H, aromat. H), 7.40 (B-part of an AB-system, $J = 8.0$ Hz, 2H), 7.61 (A-part of an AB-system, $J = 8.0$ Hz, 2H), 8.08 (br s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 19.3$, 20.8, 28.6, 36.1, 117.0, 118.9, 122.9, 126.5, 127.2, 128.3, 128.4, 129.6, 130.7, 131.1, 131.2, 133.0, 133.2, 135.4, 135.8, 138.3, 139.2, 140.4, 168.2, 168.3 (1C could not be detected). MS: *m/z* 105 (51%), 133 (100%), 252 (47%), 498 (22%). Anal. Calcd for $C_{29}H_{26}N_2O_2S_2$: C, 69.85; H, 5.26; N, 5.62. Found: C, 69.83; H, 5.28; N, 5.39.

5.1.4.7. N1-{4-[(6-Ethyl-2,3-dihydro-1*H*-thieno[2,3-*b*]-[1,4]thiazin-1-yl)carbonyl]phenyl}-2-phenylacetamide (16). Reagents: compound **8**, phenylacetyl chloride (0.309 g, 2 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate, 4:6. Yield: 0.582 g (69%) of a yellow oil. ^1H NMR ($CDCl_3$): $\delta = 1.08$ (t, $J = 7.5$ Hz, 3H, CH_3), 2.55 (q, $J = 7.5$ Hz, 2H, CH_2), 3.05–3.27 (m, 2H, SCH_2), 3.67 (s, 2H, phenyl CH_2), 3.98–4.19 (m, 2H, NCH_2), 6.22 (br s, 1H, thiophene H), 7.23–7.54 (m, 9H, aromat. H), 8.05 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 15.4$, 23.4, 28.5, 44.6, 115.6, 119.0, 121.0, 127.5, 129.0, 129.3, 130.7, 132.7, 134.2, 140.1, 141.7, 168.4, 169.4 (2C could not be detected). MS: *m/z* 120 (100%), 185 (19%), 238 (49%), 422 (11%). Anal. Calcd for $C_{23}H_{22}N_2O_2S_2$: C, 65.38; H, 5.25; N, 6.63. Found: C, 65.09; H, 5.19; N, 6.38.

5.1.4.8. N1-{4-[(6-Benzyl-2,3-dihydro-1*H*-thieno[2,3-*b*][1,4]thiazin-1-yl)carbonyl]phenyl}-2-phenylacetamide (17). Reagents: compound **9**, phenylacetyl chloride (0.309 g, 2 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate, 6:4. Yield: 0.623 g (64%) of a solid, mp 138–140 °C. ^1H NMR ($CDCl_3$): $\delta = 3.20$–3.36 (m, 2H, SCH_2), 3.80 (s, 2H, CO–CH_2), 3.90 (s, 2H, phenyl CH_2), 4.13–4.30 (m, 2H, NCH_2), 6.20 (br s, 1H, thiophene H), 7.00–7.15 (m, 2H, aromat. H), 7.18–7.33 (m, 3H, aromat. H), 7.37–7.59 (m, 9H, aromat. H), 7.99 (br s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 28.5$, 36.0, 44.6, 60.3, 117.1, 118.9, 122.8, 126.5, 127.5, 128.3, 128.4, 129.1, 129.3, 129.4, 130.6, 132.9, 134.3, 138.3, 139.2, 140.1, 168.3, 169.4. MS: *m/z* 91 (61%), 120 (100%), 238 (36%), 247 (15%), 484 (9%). Anal. Calcd for $C_{28}H_{24}N_2O_2S_2$: C, 69.39; H, 4.99; N, 5.78. Found: C, 69.11; H, 5.06; N, 5.67.

5.1.4.9. N1-{4-[(6-Ethyl-2,3-dihydro-1*H*-thieno[2,3-*b*]-[1,4]thiazin-1-yl)carbonyl]phenyl}-4-fluorobenzamide (18). Reagents: compound **8**, 4-fluorobenzoyl chloride (0.317 g, 2 mmol). Reaction time: 1.5 h. Eluent: toluene/ethyl acetate, 7:3. Yield: 0.677 g (79%) of an orange solid, mp 184–186 °C. ^1H NMR ($CDCl_3$): $\delta = 1.10$ (t, $J = 7.5$ Hz, 3H, CH_3), 2.63 (q, $J = 7.5$ Hz, 2H, CH_2), 3.19–3.40 (m, 2H, SCH_2), 3.92–4.14 (m, 2H, NCH_2), 6.61 (br s, 1H, thiophene H), 7.28–7.46 (m, 2H, aromat. H), 7.52 (B-part of an AB-system, $J = 8.6$ Hz, 2H), 7.87 (A-part of an AB-system, $J = 8.6$ Hz, 2H), 7.99–8.18 (m, 2H, aromat. H), 10.49 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 15.5$, 22.8, 27.4, 44.6, 114.0, 115.4 (d, $^2J_{C,F} = 21.4$ Hz), 119.5, 121.4, 128.8, 130.3, 130.5 (d, $^3J_{C,F} = 9.2$ Hz), 131.1 (d, $^4J_{C,F} = 2.9$ Hz), 132.5, 140.0, 141.1, 164.2 (d, $^1J_{C,F} = 249.5$ Hz), 164.7, 168.0. MS: *m/z* 95 (42%), 123 (100%), 185 (44%), 242 (47%), 426 (9%). Anal. Calcd for $C_{22}H_{19}FN_2O_2S_2$: C, 61.95; H, 4.49; N, 6.57. Found: C, 61.69; H, 4.52; N, 6.30.

5.1.4.10. N1-{4-[(6-Benzyl-2,3-dihydro-1*H*-thieno[2,3-*b*][1,4]thiazin-1-yl)carbonyl]phenyl}-4-fluorobenzamide (19). Reagents: compound **9**, 4-fluorobenzoyl chloride (0.317 g, 2 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate, 6:4. Yield: 0.837 g (86%) of a solid, mp 194–196 °C. ^1H

NMR ($CDCl_3$/DMSO-d_6): δ = 3.21–3.41 (m, 2H, SCH_2), 3.94 (s, 2H, phenyl CH_2), 3.97–4.11 (m, 2H, NCH_2), 6.53 (br s, 1H, thiophene H), 7.03–7.57 (aromat. H, B-part of an AB-system, J = 8.4 Hz, 9H), 7.87 (A-part of an AB-system, J = 8.5 Hz, 2H), 8.00–8.17 (m, 2H, aromat. H), 10.52 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$/DMSO-d_6): δ = 28.4, 36.0, 44.9, 116.2, 116.3, 116.4, 120.3, 124.0, 127.2, 129.3, 129.7, 131.2, 131.3, 132.0, 133.6, 138.3, 140.6, 142.0, 163.8, 165.6, 166.3. MS: *m*/*z* 123 (82%), 242 (98%), 247 (100%), 488 (29%). Anal. Calcd for $C_{27}H_{21}FN_2O_2S_2$: C, 66.37; H, 4.33; N, 5.73. Found: C, 66.16; H, 4.34; N, 5.68.

5.1.4.11. N1-{4-[(6-Ethyl-2,3-dihydro-1*H*-thieno[2,3-*b*][1,4]thiazin-1-yl)carbonyl]phenyl}-3-methyl-2-thiophenecarboxamide (20). Reagents: compound **8**, 3-methylthiophene-2-carbonyl chloride (0.320 g, 2 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate, 7:3. Yield: 0.720 g (84%) of a yellow solid, mp 108–114 °C. ^{1}H NMR ($CDCl_3$): δ = 1.11 (t, J = 7.5 Hz, 3H, CH_3), 2.56 (s, 3H, thiophene CH_3), 2.47–2.65 (m, 2H, CH_2), 3.08–3.26 (m, 2H, SCH_2), 4.03–4.18 (m, 2H, NCH_2), 6.24 (br s, 1H, thiophene H), 6.93 (d, J = 5.0 Hz, 1H, thiophene H), 7.33 (d, J = 5.0 Hz, 1H, thiophene H), 7.45 (B-part of an AB-system, J = 8.6 Hz, 2H), 7.60 (A-part of an AB-system, J = 8.6 Hz, 2H), 7.93 (br s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): δ = 15.5, 15.8, 23.4, 28.5, 43.9, 115.4, 119.3, 121.0, 127.1, 129.5, 130.3, 130.8, 132.2, 132.8, 140.1, 141.6, 142.8, 161.2, 168.2. MS: *m*/*z* 125 (100%), 185 (28%), 244 (49%), 428 (12%). Anal. Calcd for $C_{21}H_{20}N_2O_2S_3$: C, 58.85; H, 4.70; N, 6.54. Found: C, 58.69; H, 4.62; N, 6.38.

5.1.4.12. N1-{4-[(6-Benzyl-2,3-dihydro-1*H*-thieno[2,3-*b*][1,4]thiazin-1-yl)carbonyl]phenyl}-3-methyl-2-thiophenecarboxamide (21). Reagents: compound **9**, 3-methylthiophene-2-carbonyl chloride (0.320 g, 2 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate, 4:6. Yield: 0.550 g (56%) of a light brown oily solid, mp 150–151 °C. ^{1}H NMR ($CDCl_3$): δ = 2.59 (s, 3H, CH_3), 3.13–3.28 (m, 2H, SCH_2), 3.84 (s, 2H, phenyl CH_2), 4.07–4.20 (m, 2H, NCH_2), 6.12 (br s, 1H, thiophene H), 6.95 (d, J = 5.0 Hz, 1H, thiophene H), 6.99–7.11 (m, 2H, aromat. H), 7.11–7.30 (m, 3H, aromat. H), 7.35 (d, J = 5.0 Hz, 1H, thiophene H), 7.43 (B-part of an AB-system, J = 8.6 Hz, 2H), 7.58 (A-part of an AB-system, J = 8.6 Hz, 2H), 7.86 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): δ = 15.8, 28.6, 36.1, 116.8, 119.2, 122.9, 126.5, 127.1, 128.3, 128.4, 129.6, 130.2, 130.8, 132.3, 133.1, 138.3, 139.3, 140.1, 142.9, 161.1, 168.2 (1C could not be detected). MS: *m*/*z* 91 (25%), 125 (100%), 244 (34%), 490 (10%). Anal. Calcd for $C_{26}H_{22}N_2O_2S_3$: C, 63.65; H, 4.52; N, 5.71. Found: 63.89; H, 4.48; N, 5.52.

5.1.4.13. N1-{4-[(6-Ethyl-2,3-dihydro-1*H*-thieno[2,3-*b*]-[1,4]thiazin-1-yl)carbonyl]phenyl}-5-chloro-2-thiophenecarboxamide (22). Reagents: compound **8**, 5-chlorothiophene-2-carbonyl chloride (0.360 g, 2 mmol). Reaction time: 2.5 h. Eluent: toluene/ethyl acetate, 6:4. Yield: 0.217 g (24%) of a beige solid, mp 105–112 °C. ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ = 1.07 (t, J = 11.4 Hz, 3H, CH_3), 2.59 (q, J = 11.4 Hz, 2H, CH_2), 3.19–3.39 (m, 2H, SCH_2), 3.90–4.06 (m, 2H, NCH_2), 6.54 (br s, 1H, thiophene H), 7.28 (d, J = 6.2 Hz, 1H, thiophene H), 7.44 (B-part of an AB-system, J = 12.8 Hz, 2H), 7.76 (A-part of an AB-system, J = 12.8 Hz, 2H), 7.93 (d, J = 6.2 Hz, 1H, thiophene H), 10.47 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$/DMSO-d_6): δ = 13.8, 21.4, 26.2, 58.1, 112.8, 117.8, 119.5, 125.9, 127.2, 127.4, 128.7, 130.9, 133.3, 137.2, 138.7, 139.0, 157.5, 166.3. MS: *m*/*z* 120 (100%), 145/147 (74%/30%), 185 (65%), 264/266 (45%/17%), 304 (14%), 448/450 (26%/13%). Anal. Calcd for $C_{20}H_{17}ClN_2O_2S_3 \cdot 0.1$toluene: C, 54.26; H, 3.92; N, 6.11. Found: 54.29; H, 4.20; N, 5.94.

5.1.4.14. N1-{4-[(6-Benzyl-2,3-dihydro-1*H*-thieno[2,3-*b*][1,4]thiazin-1-yl)carbonyl]phenyl}-5-chloro-2-thiophenecarboxamide (23). Reagents: compound **9**, 5-chlorothiophene-2-carbonyl chloride (0.360 g, 2 mmol). Reaction time: 2.5 h. Crystallization from toluene. Yield: 0.345 g (34%) of a beige solid, mp 203-205 °C. ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ = 3.19-3.35 (m, 2H, SCH_2), 3.92 (s, 2H, phenyl CH_2), 3.96–4.11 (m, 2H, NCH_2), 6.50 (br s, 1H, thiophene H), 7.02–7.35 (m, 5H, aromat. H), 7.32 (d, J = 4.1 Hz, 1H, thiophene H), 7.49 (B-part of an AB-system, J = 8.5 Hz, 2H), 7.80 (A-part of an AB-system, J = 8.5 Hz, 2H), 7.99 (d, J = 4.1 Hz, 1H, thiophene H), 10.53 (s, 1H, CO–NH). ^{13}C NMR (DMSO-d_6): δ = 27.5, 35.1, 115.4, 119.4, 123.1, 125.3, 126.4, 128.3, 128.4, 128.9, 129.5, 130.6, 132.6, 134.4, 137.4, 138.8, 139.7, 140.4, 159.0, 167.8 (1C could not be detected). MS: *m*/*z* 91 (100%), 145/147 (33%/14%), 247 (22%), 264/266 (14%/5%), 510/512 (5%/3%). Anal. Calcd for $C_{25}H_{19}ClN_2O_2S_3$: C, 58.75; H, 3.75; N, 5.48. Found: C, 59.05; H, 3.93; N, 5.25.

5.2. Electromechanical studies on heart and smooth muscle preparations

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 stored at room temperature in gassed (95% O_2 and 5% CO_2), modified Krebs–Henseleit solution with the following composition (in millimoles per litre): NaCl 118.0, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4$ 5.8, KH_2PO_4 1.4, $NaHCO_3$ 11.9 and glucose 10. The aorta was cleaned of loosely adhering fat and connective tissue and cut into rings of 2 mm width. Aortic rings were precontracted with 90 mmol/l KCl.

The terminal portion of the ileum was removed and the 10 cm nearest to the caecum was discarded. The intestine was placed in a nutrient solution. The intestine was cleaned by flushing with nutrient solution and cut into pieces of 2–3 cm length.

The arteria pulmonalis 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 fibres were carefully removed to prevent spontaneous activity. Only muscles with a diameter of less than 0.87 mm were used in order to have a sufficient oxygen supply.[33] Chronotropic activity was tested on guinea pig isolated right atria. After the isolation, the preparations (terminal ilea, arteria pulmonalis rings, papillary muscles and right

atria) were stored at room temperature in gassed (95% O_2 and 5% CO_2) Krebs–Henseleit solution with the following composition (in millimoles per litre): 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. Isometric contraction force of electrically stimulated papillary muscles (1 Hz), terminal ilea precontracted by 60 mmol/l KCl, aortic and arteria pulmonalis rings precontracted by 90 mmol/l KCl as well as spontaneous activity of right atria was measured by the method described by Reiter.[34] The preparations were placed in a continuously oxygenated (95% O_2 and 5% CO_2) bath of 35 ml nutrient solution to guarantee sufficient oxygen supply and appropriate pH as well as circulation of nutrient solution with and without test compound. The experiments were performed at a temperature of 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). Papillary muscles were electrically driven with an Anapulse Stimulator Model 301-T and an Isolation Unit Model 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), 10.37 mN (right atria) or 19.6 mN (aortic and arteria pulmonalis rings) was kept constant throughout the experiments.

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 *n* experiments were calculated. Statistical significance of the results was evaluated by Student's *t*-test for paired observations.

Stock solutions of the compounds **14**–**16**, **18**, **19** and **21**–**23** were dissolved in dimethylsulfoxide (DMSO) and distilled water every day and were further diluted with modified Krebs–Henseleit solution to the required concentrations. To exclude the DMSO effect experiments with DMSO 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.

5.3. Receptor binding assays

The effects of **21** were investigated in in vitro human V_{1a} and V_2 receptor binding assays. [$d(CH_2)_5^1$, Tyr(Me)$_2$]-AVP and AVP were used as reference compounds. For competition studies [^{3}H]AVP (0.3 nM) was added to each membrane preparation that was then incubated with a concentration of 10 μmol/l of compound **21**. After the incubation period (V_{1a} (*h*), 60 min/22 °C and V_2 (*h*), 90 min/22 °C), the reaction was terminated, followed by rapid filtration. The filters were rinsed and the retaining radioactivity was counted with a scintillation counter (for details, see Tahara et al. 1998[31]).

The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabelled ligand. The results are expressed as a percent of control specific binding and as a percent inhibition of control specific binding obtained in the presence of **21**. Individual and mean values are presented in the Results section 3.6. The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (n_H) were determined by nonlinear regression analysis of the competition curves using Hill equation curve fitting. The inhibition constants (K_i) were calculated from the Cheng Prusoff equation ($K_i = IC_{50}/(1 + (L/K_D))$, where L = concentration of radioligand in the assay and K_D = affinity of the radioligand for the receptor).

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