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European Journal of Pharmaceutical Sciences 24 (2005) 421–431
EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES
www.elsevier.com/locate/ejps

Synthesis and pharmacological profile of non-peptide vasopressin antagonists

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Received 28 July 2004; received in revised form 24 November 2004; accepted 16 December 2004

Abstract

Recently we presented a series of 6-ethyl and 6-benzylthieno[2,3-b][1,4]thiazine derivatives with relaxing effects on vascular smooth muscle and terminal ileum. In this report the synthesis of further thieno[2,3-b][1,4]thiazine derivatives and related compounds with a thieno[2,3-b][1,4]thiazepine or thieno[3,2-b][1,4]thiazine ring system is described. The pharmacological effect of the agents was tested in isolated smooth (terminal ileum, pulmonary artery, aortic rings, myometrial strips) and heart (papillary muscle, spontaneously beating right atrium) muscle preparations of the guinea pig. Contractions were measured isometrically, and smooth muscle preparations were either precontracted with high K^+ (60 or 90 mM KCl containing nutrient solution) or with agonists, while papillary muscles were electrically stimulated (1 Hz). The vasopressin antagonistic activity of the test compounds was tested in isolated papillary muscles in which the V_{1A}-receptor subtype is located. The biphasic response to vasopressin was antagonized, dependent on the chemical structure of the test compound. Thieno[3,2-b][1,4]thiazines were more potent than thieno[2,3-b][1,4]thiazine and thieno[2,3-b][1,4]thiazepine compounds. In addition, substitution of a methyl substituted terminal benzyl ring instead of a phenyl- or dichlorobenzoyl moiety attenuated the vasopressin antagonistic effect.

Keywords: Thienothiazines; Thienothiazepines; Vasopressin antagonism; Smooth muscle; Papillary muscle; Guinea pig

1. Introduction

The anterior pituitary hormone vasopressin plays an important role in various regulatory effects such as fluid and electrolyte homeostasis and blood pressure, depending on the tissue and the vasopressin receptor subtype to which it binds. So far four vasopressin receptor subtypes V_{1A}, V_{1B}, V_2 and V_3 have been identified (Birnbaumer et al., 1992; Lolait et al., 1992; Morel et al., 1992; De Keyzer et al., 1994; Sugimoto et al., 1994; Thibonnier et al., 1994). The V_{1A} subtype is located e.g. in vascular smooth muscle cells, cardiomyocytes, hepatocytes and platelets as has been shown by radioligand binding experiments (Thibonnier and Roberts, 1985; Phillips et al., 1990; Howl et al., 1991; Serradeil-Le Gal et al., 1995). Via binding to V_{1A} vasopressin causes potent vasoconstriction. The V_{1B} subtype was found in the anterior pituitary, pancreatic β-cells and adrenal medulla (Jard et al., 1986; Lee et al., 1995; Grazzini et al., 1996) and there it stimulates release of hormones and mediators. The V_2 subtype is present in the kidney (Guillon et al., 1982; Jans et al., 1989), being responsible for water retention, but there are also extrarenal V_2 or V_2-like receptors that are involved in vascular and clotting factor responses (Serradeil-Le Gal, 2001). Consequently the effects of vasopressin may also contribute to pathophysiological states like congestive heart failure, liver cirrhosis, renal disease and many others (Mah and Hofbauer, 1987; Laszlo et al., 1991; Naitoh et al., 1994; Thibonnier et al., 2002). There is ample evidence that vasopressin is a component of the neurohormonal response to congestive heart

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doi:10.1016/j.ejps.2004.12.008

failure, and that it might play a role in the development, progression and worsening of this disease. It could be shown that non-peptide vasopressin antagonists are able to improve the fluid status, osmotic balance and hemodynamics of patients with congestive heart failure (Thibonnier, 2003). This new therapeutic approach led researchers to develop non-peptide vasopressin receptor antagonists, which have the advantage of oral application.

Examples for synthetized non-peptide V_{1A} antagonists are OPC-21268 (Yamamura et al., 1991) and SR 49059 (Serradeil-Gal et al., 1993), whereas conivaptan (Tahara et al., 1997; Burnier et al., 1999; Yatsu et al., 1999; Matsuhisha et al., 2000) and YM471 (Tsukada et al., 2002) are potent non-selective V_{1A}/V_2 vasopressin receptor antagonists.

Preliminary data of aromatically substituted 6-ethyl- and 6-benzyl-2,3-dihydro-1H-thieno[2,3-b][1,4]thiazines suggest vasopressin receptor antagonistic activity of the compounds (personal communication, master theses). The 6-ethyl derivatives showed more potent relaxing effects on smooth muscle preparations than the 6-benzyl analogues. In this paper we present some 6-ethyl- and 6-benzyl-2,3-dihydro-1H-thieno[2,3-b][1,4]thiazines, showing that the ethyl group in position 6 on the thienothiazine ring is responsible for the potent relaxing effects. Furthermore these results prompted us to modify the ring system and synthesize some new 2-benzyl-4,5,6,7-tetrahydrothieno[2,3-b][1,4]thiazepines and 3,4-dihydro-2H-thieno[3,2-b][1,4] thiazines and to investigate their effects on smooth and heart muscle preparations.

2. Materials and methods

2.1. Chemistry

2.1.1. General methods

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 Varian UnityPlus-300 (300 MHz). Chemical shifts are reported in δ values (ppm) 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) spektrometer. The obtained elemental analysis results were within ±0.4% of the theoretical values for the formulas given. Column chromatography was performed using silica gel 60, 70–230 mesh ASTM (Merck). Solutions in organic solvents were dried over anhydrous sodium sulfate.

*2.1.1.1. (2-Benzyl-4,5,6,7-tetrahydrothieno[2,3-b][1,4]thiazepin-4-yl)(4-nitrophenyl)methanone (**8**).* A solution of **7** in anhydrous methylene chloride (40 ml) was treated with triethylamine (1.04 g, 10 mmol) followed by 4-nitro benzoic acid chloride (1.9 g, 10 mmol) under argon atmosphere. After stirring at room temperature for 3 h the reaction mixture was washed with water, 5% sodium hydrogen carbonate solution and brine. The dried and evaporated organic layer was recrystallized from ethanol to yield 3.3 g (80%) of an olive solid, mp 153 °C. ^{1}H NMR ($CDCl_3$): δ = 2.27–2.43 (m, 2H, $SCH_2C\mathbf{H}_2$), 2.74–2.88 (m, 2H, SCH_2), 3.75 (s, 2H, phenyl CH_2), 3.33–4.77 (m, 2H, NCH_2), 6.00 (s, 1H, thiophene H), 6.74–6.86 (m, 2H, aromat. H), 7.09–7.21 (m, 3H, aromat. H), 7.42 (B-part of an AB-system, J = 8.8 Hz, 2H), 8.02 (A-part of an AB-system, J = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ = 32.6, 33.0, 36.1, 45.8, 123.0, 125.0, 126.8, 127.9, 128.3, 128.4, 138.8, 142.3, 143.5, 145.3, 148.0, 167.2 (1 C could not be detected). MS: *m/z* 91 (100%), 150 (27%), 260 (25%), 410 (59%). Anal. $C_{21}H_{18}N_2O_3S_2$.

*2.1.1.2. (4-Aminophenyl)(2-benzyl-4,5,6,7-tetrahydrothieno[2,3-b][1,4]thiazepin-4-yl)methanone (**9**).* A solution of **8** in a mixture of glacial acetic acid (100 ml), methanol (7 ml) and water (7 ml) was warmed up to 70 °C. Then iron powder (3.92 g, 70 mmol) was added in portions. After stirring the reaction mixture at 70 °C for 1 h it was poured onto ice water. The crude product was recrystallized from ethyl acetate to yield 3.41 g (90%) of a solid, mp 163 °C. ^{1}H NMR ($CDCl_3$): δ = 2.18–2.38 (m, 2H, $SCH_2C\mathbf{H}_2$), 2.65–2.82 (m, 2H, SCH_2), 3.81 (s, 2H, CH_2 phenyl), 3.88 (s, 2H, NH_2), 3.31–4.30 (m, 2H, NCH_2), 6.11 (s, 1H, thiophene H), 6.84–6.98 (m, 2H, aromat. H), 6.46 (B-part of an AB-system, J = 8.5 Hz, 2H), 7.15 (A-part of an AB-system, J = 8.5 Hz, 2H), 7.08–7.32 (m, 3H, aromat. H). ^{13}C NMR ($CDCl_3$): δ = 32.6, 33.2, 36.2, 45.7, 113.5, 124.3, 125.5, 125.8, 126.5, 128.2, 128.4, 130.1, 139.1, 141.9, 147.3, 148.3, 169.3. MS: *m/z* 120 (100%), 261 (14%), 380 (9%). Anal. $C_{21}H_{20}N_2OS_2$.

*2.1.1.3. 3,4-Dihydro-2H-thieno[3,2-b][1,4]thiazine (**13**).* To a solution of **12** (0.684 g, 4 mmol) in absolute tetrahydrofuran (20 ml) a 1.0 M solution of lithium aluminium hydride (4 ml, 4 mmol) in tetrahydrofuran was added dropwise under argon atmosphere. After stirring at room temperature for 4 h ethyl acetate (2–3 ml) was added carefully and the mixture stirred for further 20 min. By-products were removed by filtration of the reaction mixture through aluminium oxide 90 and eluting with tetrahydofuran. The filtrate was concentrated at room temperature and immediately transformed to **14**. MS: *m/z* 102 (52%), 115 (25%), 129 (23%), 142 (56%), 157 (100%).

*2.1.1.4. 3,4-Dihydro-2H-thieno[3,2-b][1,4]thiazin-4-yl(4-nitrophenyl)methanone (**14**).* The concentrated solution of **13** was treated with triethylamine (0.56 ml, 4 mmol) followed by 4-nitro benzoic acid chloride (0.74 g, 4 mmol), diluted in 4 ml tetrahydrofuran. After stirring at room temperature for 6 h the reaction mixture was evaporated and the residue diluted in ethyl acetate. The organic layer was washed with 5% sodium hydrogen carbonate solution and water, dried and evaporated. The product was purified by crystallisation from dimethylformamide/water (7 + 3) to yield 1.0 g (82%) of a solid, mp 197–198 °C. ^{1}H NMR (d_6-DMSO/$CDCl_3$): δ = 3.17–3.29 (m, 2H, NCH_2), 3.93–4.06 (m, 2H, SCH_2),

6.78 (B-part of the thiophen AB-system, $J = 5.8$ Hz, 1H), 7.17 (A-part of the thiophen AB-system, $J = 5.8$ Hz, 1H), 7.84 (B-part of the phenyl AB-system, $J = 8.7$ Hz, 2H), 8.32 (A-part of the phenyl AB-system, $J = 8.7$ Hz, 2H). ^{13}C NMR (d_6-DMSO/$CDCl_3$): $\delta = 23.1$, 46.8, 113.0, 117.7, 121.3, 121.8, 126.9, 138.8, 146.3, 163.8 (1 C could not be detected). MS: *m/z* 104 (36%), 129 (12%), 150 (58%), 156 (100%), 306 (47%). Anal. $C_{13}H_{10}N_2O_3S_2$.

*2.1.1.5. 4-Aminophenyl-(3,4-dihydro-2H-thieno[3,2-b][1,4]thiazin-4-yl)methanone (**15**).* To a solution of **14** in a mixture of glacial acetic acid (20 ml), methanol (1.4 ml) and water (1.4 ml) iron powder was added in portions. After stirring the reaction mixture at 50 °C for 4 h it was poured onto ice water. The crude product was filtered, diluted in methylene chloride and washed with 5% sodium hydrogen carbonate and water. The organic layer was dried and evaporated in vacuo to yield 0.37 g (67%) of a solid. The product was purified by column chromatography (toluene/ethyl acetate 7 + 3), mp 160–161 °C. ^{1}H NMR ($CDCl_3$): $\delta = 3.11$–3.23 (m, 2H, NCH_2), 3.98 (s, 2H, NH_2), 4.14–4.26 (m, 2H, SCH_2), 6.57–6.73 (m, 2H, aromat. H), 6.93 (d, $J = 5.8$ Hz, 1H, thiophene H), 7.09–7.37 (m, 3H, aromat. H, thiophene H). ^{13}C NMR ($CDCl_3$): $\delta = 26.3$, 48.3, 114.1, 119.1, 123.1, 123.3, 129.9, 149.0, 168.6 (1 C could not be detected). MS: *m/z* 92 (22%), 120 (100%), 276 (5%). Anal. $C_{13}H_{12}N_2OS_2$.

*2.1.1.6. General procedure for the preparation of compounds **3**–**6**, **10**, **11** and **16**–**19**.* To a solution of **1** respectively **2** respectively **9** respectively **15** in dry tetrahydrofuran triethylamine and 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.

*N-[4-(6-Ethyl-2,3-dihydro-1H-thieno[2,3-b][1,4]thiazine-1-ylcarbonyl)phenyl]-2,4-dimethylbenzamide (**3**):* Reagents: **1** (0.61 g, 2 mmol) in dry tetrahydrofuran (10 ml), 2,4-dimethylbenzenecarbonyl chloride (0.336 g, 2 mmol), triethylamine (0.28 ml, 2 mmol). Reaction time: 2 h. Eluent: toluene/ethyl acetate 7 + 3. Yield: 0.625 g (72%) of a beige solid, mp 80 °C. ^{1}H NMR ($CDCl_3$): $\delta = 1.11$ (t, $J = 7.5$ Hz, 3H, $CH_2C\mathbf{H}_3$), 2.34 (s, 3H, CH_3 phenyl), 2.43 (s, 3H, CH_3 phenyl), 2.58 (q, $J = 7.5$ Hz, 2H, $CH_2C\mathbf{H}_3$), 3.10–3.24 (m, 2H, SCH_2), 4.02–4.15 (m, 2H, NCH_2), 6.22 (broad s, 1H, thiophene H), 6.99 (d, $J = 7.7$ Hz, 1H, aromat. H), 7.04 (s, 1H, aromat. H), 7.33 (d, $J = 7.7$ Hz, 1H, aromat. H), 7.41 (B-part of an AB-system, $J = 8.7$ Hz, 2H), 7.61 (A-part of an AB-system, $J = 8.7$ Hz, 2H), 8.22 (broad s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 15.5$, 19.8, 21.2, 23.4, 28.5, 43.8, 115.4, 119.0, 121.0, 126.3, 126.8, 129.5, 130.6, 132.0, 132.8, 132.9, 136.6, 140.5, 140.6, 141.6, 168.2, 168.3. MS: *m/z* 105 (30%), 133 (100%), 185 (20%), 252 (40%), 436 (10%). Anal. $C_{24}H_{24}N_2O_2S_2$.

*N-[4-(6-Benzyl-2,3-dihydro-1H-thieno[2,3-b][1,4]thiazine-1-ylcarbonyl)phenyl]-2,4-dimethylbenzamide (**4**):* Reagents: **2** (0.732 g, 2 mmol) in dry tetrahydrofuran (10 ml), 2,4-dimethylbenzenecarbonyl chloride (0.336 g, 2 mmol), triethylamine (0.28 ml, 2 mmol). Reaction time: 2 h. Eluent: toluene/ethyl acetate 7 + 3. Yield: 0.971 g (97%) of a solid, mp 170–178 °C. ^{1}H NMR ($CDCl_3$): $\delta = 2.26$ (s, 3H, CH_3 phenyl), 2.37 (s, 3H, CH_3 phenyl), 3.00–3.17 (m, 2H, SCH_2), 3.76 (s, 2H, CH_2 phenyl), 3.91–4.09 (m, 2H, NCH_2), 6.06 (broad s, 1H, thiophene H), 6.86–7.04 (m, 4H, aromat. H), 7.05–7.23 (m, 3H, aromat. H), 7.23–7.27 (m, 1H, aromat. H), 7.32 (B-part of an AB-system, $J = 8.4$ Hz, 2H), 7.53 (A-part of an AB-system, $J = 8.4$ Hz, 2H), 8.01 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 19.8$, 21.2, 28.6, 36.1, 43.3, 117.0, 118.9, 119.7, 122.9, 126.4, 126.5, 126.8, 128.3, 128.4, 129.5, 130.6, 132.1, 133.0, 136.7, 138.3, 139.2, 140.5, 140.7, 168.2 (1 C could not be detected). MS: *m/z* 105 (31%), 133 (100%), 252 (50%), 498 (19%). Anal. $C_{29}H_{26}N_2O_2S_2$.

*N-[4-(6-Ethyl-2,3-dihydro-1H-thieno[2,3-b][1,4]thiazine-1-ylcarbonyl)phenyl]-2,6-dichlorobenzamide (**5**):* Reagents: **1** (0.61 g, 2 mmol) in dry tetrahydrofuran (10 ml), 2,6-dichlorobenzenecarbonyl chloride (0.391 g, 2 mmol), triethylamine (0.28 ml, 2 mmol). Reaction time: 20 h. Eluent: toluene/ethyl acetate 6 + 4. Yield: 0.744 g (78%) of a solid, mp 116 °C. ^{1}H NMR ($CDCl_3$): $\delta = 1.12$ (t, $J = 7.5$ Hz, 3H, $CH_2C\mathbf{H}_3$), 2.60 (q, $J = 7.5$ Hz, 2H, $C\mathbf{H}_2CH_3$), 3.12–3.26 (m, 2H, SCH_2), 3.99–4.13 (m, 2H, NCH_2), 6.26 (broad s, 1H, thiophene H), 7.18–7.32 (m, 3H, aromat. H), 7.40 (B-part of an AB-system, $J = 8.7$ Hz, 2H), 7.62 (A-part of an AB-system, $J = 8.7$ Hz, 2H), 8.58 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): $\delta = 15.4$, 23.4, 28.4, 44.1, 115.6, 119.6, 121.0, 128.0, 129.3, 130.8, 131.2, 132.2, 132.6, 135.6, 139.7, 141.6, 162.8, 168.3. MS: *m/z* 173/175/177 (100%/64%/11%), 185 (46%), 292/294/296 (54%/34%/6%), 476/478/480 (14%/10%/2%). Anal. $C_{22}H_{18}Cl_2N_2O_2S_2$. HRMS: calcd. 476.0187, found 476.0165.

*N-[4-(6-Benzyl-2,3-dihydro-1H-thieno[2,3-b][1,4]thiazine-1-ylcarbonyl)phenyl]-2,6-dichlorobenzamide (**6**):* Reagents: **2** (0.732 g, 2 mmol) in dry tetrahydrofuran (10 ml), 2,6-dichlorobenzenecarbonyl chloride (0.391 g, 2 mmol), triethylamine (0.28 ml, 2 mmol). Reaction time: 24 h. Eluent: toluene/ethyl acetate 6.5 + 3.5. Yield: 0.709 g (66%) of a light orange oil. ^{1}H NMR ($CDCl_3$/d_6-DMSO): $\delta = 3.22$–3.43 (m, 2H, SCH_2), 3.95 (s, 2H, CH_2 phenyl) 3.87–4.13 (m, 2H, NCH_2), 6.53 (broad s, 1H, thiophene H), 7.10 (B-part of an AB-system, $J = 7.0$ Hz, 2H), 7.21 (A-part of an AB-system, $J = 7.0$ Hz, 2H), 7.15–7.40 (m, 1H, aromat. H), 7.44–7.69 (m, 3H, aromat. H), 7.51 (B-part of an AB-system, $J = 8.5$ Hz, 2H), 7.80 (A-part of an AB-system, $J = 8.5$ Hz, 2H), 11.05 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$/d_6-DMSO): $\delta = 27.6$, 35.1, 115.6, 118.8,

123.2, 126.3, 128.2, 128.3, 128.4, 129.1, 130.9, 131.2, 131.6, 132.7, 136.1, 137.5, 139.6, 140.4, 162.3, 167.7 (1 C could not be detected). MS: *m/z* 91 (59%), 145/147/149 (24%/17%/3%), 173/175/177 (100%/64%/10%), 247 (39%), 292/294/296 (49%/32%/5%), 538/540/542 (17%/14%/3%). Anal. $C_{27}H_{20}Cl_2N_2O_2S_2$.

*N-[4-(2-Benzyl-4,5,6,7-tetrahydrothieno[2,3-b][1,4]thiazepine-4-ylcarbonyl) phenyl]-2-methylbenzamide (***10***):* Reagents: **9** (0.38 g, 1 mmol) in dry tetrahydrofuran (10 ml), 2-methylbenzoyl chloride (0.155 g, 1 mmol), triethylamine (0.14 ml, 1 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate 7 + 3. Yield: 0.449 g (90%) of a yellow oil. ^{1}H NMR ($CDCl_3$): δ = 2.12–2.30 (m, 2H, $SCH_2C\mathbf{H}_2$), 2.44 (s, 3H, CH_3 phenyl), 2.60–2.79 (m, 2H, SCH_2), 3.77 (s, 2H, CH_2 phenyl), 3.19–4.22 (m, 2H, NCH_2), 6.04 (s, 1H, thiophene H), 6.77–6.92 (m, 2H, aromat. H), 7.07–7.53 (m, 11H, aromat. H), 7.92 (s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): δ = 19.8, 32.8, 32.9, 36.2, 45.9, 118.5, 125.3, 125.6, 125.9, 126.5, 126.6, 128.2, 128.4, 129.1, 130.3, 131.3, 131.7, 136.1, 136.4, 139.0, 139.7, 142.5, 146.6, 168.1, 168.8. MS: *m/z* 119 (100%), 133 (27%), 238 (49%), 261 (38%), 498 (27%). Anal. $C_{29}H_{26}N_2O_2S_2$.

*N-[4-(2-Benzyl-4,5,6,7-tetrahydrothieno[2,3-b][1,4]thiazepine-4-ylcarbonyl) phenyl]-4-methoxybenzamide (***11***):* Reagents: **9** (0.38 g, 1 mmol) in dry tetrahydrofuran (10 ml), 3-methoxybenzenecarbonyl chloride (0.171 g, 1 mmol), triethylamine (0.14 ml, 1 mmol). Reaction time: 1 h. Eluent: toluene/ethyl acetate 7 + 3. Yield: 0.432 g (84%) of a yellow oil. ^{1}H NMR ($CDCl_3$): δ = 2.09–2.33 (m, 2H, $SCH_2C\mathbf{H}_2$), 2.60–2.80 (m, 2H, SCH_2), 3.74 (s, 2H, CH_2 phenyl), 3.80 (s, 3H, OCH_3), 3.27–4.65 (m, 2H, NCH_2), 6.03 (broad s, 1H, thiophene H), 6.78–6.96 (m, 2H, aromat. H), 6.99–7.48 (m, 3H, aromat. H), 7.24 (B-part of an AB-system, J = 8.4 Hz, 2H), 7.49 (A-part of an AB-system, J = 8.4 Hz, 2H), 6.89 (B-part of an AB-system, J = 8.8 Hz, 2H), 7.80 (A-part of an AB-system, J = 8.8 Hz, 2H), 8.21 (broad s, 1H, CO–NH). ^{13}C NMR ($CDCl_3$): δ = 32.8, 33.0, 36.2, 45.9, 55.4, 113.9, 118.8, 125.2, 125.5, 126.5, 126.9, 128.3, 128.4, 128.9, 129.0, 131.4, 139.0, 139.9, 142.7, 146.6, 162.5, 165.2, 169.0. MS: *m/z* 91 (100%), 135 (73%), 254 (28%), 261 (23%), 514 (16%). Anal. $C_{29}H_{26}N_2O_3S_2$.

*N-[4-(3,4-Dihydro-2H-thieno[3,2-b][1,4]thiazine-4-ylcarbonyl)phenyl]-2,6-dichlorobenzamide (***16***):* Reagents: **15** (0.552 g, 2 mmol) in dry tetrahydrofuran (10 ml), 2,6-dichlorobenzenecarbonyl chloride (0.418 g, 2 mmol), triethylamine (0.28 ml, 2 mmol). Reaction time: 20 h. Eluent: toluene/ethyl acetate 8 + 2. Yield: 0.223 g (24.8%) of a solid, mp 241–243 °C. ^{1}H NMR (d_6-DMSO): δ = 3.20–3.35 (m, 2H, NCH_2), 4.00–4.20 (m, 2H, SCH_2), 6.83 (d, J = 8.6 Hz, 1H, thiophene H), 7.21 (d, J = 8.6 Hz, 1H, thiophene H), 7.47–7.72 (m, 5H, phenyl H), 7.85 (d, J = 12.8 Hz, 2H, phenyl H), 11.06 (s, 1H, CO–NH). ^{13}C NMR (d_6-DMSO): δ = 25.1, 48.6, 114.2, 119.0, 119.3, 123.2, 128.2, 128.6, 129.3, 129.6, 131.1, 131.5, 136.0, 140.2, 162.3, 167.2. MS: *m/z* 173 (100%), 292 (78%), 448/450/452 (8%/9%/1%). Anal. $C_{20}H_{14}Cl_2N_2O_2S_2$.

*N-[4-(3,4-Dihydro-2H-thieno[3,2-b][1,4]thiazine-4-ylcarbonyl)phenyl]-4-fluoro benzamide (***17***):* Reagents: **15** (0.552 g, 2 mmol) in dry tetrahydrofuran (10 ml), 4-fluorobenzenecarbonyl chloride (0.316 g, 2 mmol), triethylamine (0.28 ml, 2 mmol). Reaction time: 22 h. Eluent: toluene/ethyl acetate 8 + 2. Yield: 0.218 g (27.3%) of a solid, mp 248–249 °C. ^{1}H NMR (d_6-DMSO): δ = 3.23–3.33 (m, 2H, NCH_2), 4.04–4.18 (m, 2H, SCH_2), 6.83 (d, J = 8.6 Hz, 1H, thiophene H), 7.21 (d, J = 8.6 Hz, 1H, thiophene H), 7.34–7.49 (m, 2H, phenyl H), 7.59 (d, J = 13.2 Hz, 2H, phenyl H), 7.92 (d, J = 13.2 Hz, 2H, phenyl H), 8.01–8.14 (m, 2H, phenyl H), 10.52 (s, 1H, CO–NH). ^{13}C NMR (d_6-DMSO): δ = 30.3, 53.8, 119.3, 120.6 (d, $^2J_{C,F}$ = 21.9 Hz), 124.5, 124.9, 128.4, 133.6, 134.3, 134.6, 135.7 (d, $^3J_{C,F}$ = 9.2 Hz), 136.3 (d, $^4J_{C,F}$ = 2.9 Hz), 146.2, 169.4 (d, $^1J_{C,F}$ = 249.2 Hz), 170.0, 172.5. MS: *m/z* 123 (100%), 242 (55%), 398 (4%). Anal. $C_{20}H_{15}FN_2O_2S_2$.

*N-[4-(3,4-Dihydro-2H-thieno[3,2-b][1,4]thiazine-4-ylcarbonyl)phenyl]-2-methylbenzamide (***18***):* Reagents: **15** (0.552 g, 2 mmol) in dry tetrahydrofuran (10 ml), 2-methylbenzenecarbonyl chloride (0.308 g, 2 mmol), triethylamine (0.28 ml, 2 mmol). Reaction time: 24 h. Eluent: toluene/ethyl acetate 7 + 3. Yield: 0.229 g (26.8%) of a solid, mp 208–209 °C. ^{1}H NMR (d_6-DMSO): δ = 2.54 (s, 1H, CH_3), 3.36–3.46 (m, 2H, NCH_2), 4.16–4.29 (m, 2H, SCH_2), 6.95 (d, J = 8.6 Hz, 1H, thiophene H), 7.33 (d, J = 8.6 Hz, 1H, thiophene H), 7.41–7.76 (m, 6H, phenyl H), 8.02 (d, J = 12.8 Hz, 2H, phenyl H), 10.71 (s, 1H, CO-NH). ^{13}C NMR (d_6-DMSO): δ = 19.2, 25.1, 48.6, 114.1, 119.0, 119.3, 123.2, 125.6, 127.7, 128.4, 128.9, 129.4, 129.8, 130.5, 135.2, 136.9, 141.1, 167.3, 168.1. MS: *m/z* 91 (67%), 119 (100%), 238 (52%), 394 (4%). Anal. $C_{21}H_{18}N_2O_2S_2$.

*N-4-(3,4-Dihydro-2H-thieno[3,2-b][1,4]thiazine-4-ylcarbonyl)phenylacetamide (***19***):* Reagents: **15** (0.552 g, 2 mmol) in dry tetrahydrofuran (10 ml), 2-phenylacetylchloride (0.308 g, 2 mmol), triethylamine (0.28 ml, 2 mmol). Reaction time: 24 h. Eluent: toluene/ethyl acetate 8 + 2. Yield: 0.204 g (25.9%) of a solid, mp 210–211 °C. ^{1}H NMR (d_6-DMSO): δ = 3.20–3.31 (m, 2H, NCH_2), 3.69 (s, 2H, CH_2 phenyl), 3.99–4.11 (m, 2H, SCH_2), 6.80 (d, J = 8.6 Hz, 1H, thiophene H), 7.18 (d, J = 8.6 Hz, 1H, thiophene H), 7.22–7.39 (m, 5H, phenyl H), 7.52 (B-part of an AB-system, J = 13.0 Hz, 2H), 7.73 (A-part of an AB-system, J = 13.0 Hz, 2H), 10.45 (s, 1H, CO-NH). ^{13}C NMR (d_6-DMSO): δ = 25.1, 43.3, 48.5, 114.1, 118.5, 119.3, 123.2, 126.5, 128.3, 128.5, 128.6, 129.1, 129.4, 135.7, 141.0, 167.3, 169.5. MS: *m/z* 91 (45%), 120 (100%), 238 (59%), 394 (4%). Anal. $C_{21}H_{18}FN_2O_2S_2$.

2.2. Pharmacology

2.2.1. Isolated preparations

The effect of the compounds was studied in isolated preparations of the guinea pig (supplied by the Institute for Laboratory Animal Science and Genetics, A-2325 Himberg). From one guinea pig up to 10 different preparations (isolated right

atrium, 1–2 papillary muscles, 2–3 pieces of terminal ileum, 2 rings of thoracic aorta and 1–2 rings of pulmonary artery) can be dissected. The animals (either sex, 320–430 g) were killed by a blow on the neck. After quick removal of the preparations they were placed in oxygenated nutrient solution. A small silver hook was attached to 2–3 cm long pieces of the terminal ileum, to papillary muscles and spontaneously beating right atria to allow mounting in the experimental set-up. Thoracic aorta and pulmonary artery were cut into small rings of about 5 mm and were fixed in the apparatus.

2.2.2. Nutrient solution

The preparations were isolated and stored at room temperature in Krebs–Henseleit Solution with the following composition (in mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, NaH_2CO_3 24.0, NaH_2PO_4 0.43, glucose 11.0. During the experiments the preparations were superfused with oxygenated (95% O_2–5% CO_2) Krebs–Henseleit Solution at a temperature of $37 \pm 1\,^{\circ}C$ to guarantee sufficient oxygen supply and appropriate pH of 7.2–7.4.

2.2.3. Test compounds

The compounds **3**, **4**, **6**, **10**, **14**, **16**, **18**, **19** were tested in various isolated preparations and in presence of vasopressin.

Vasopressin was used as [Arg^8]-vasopressin acetate (Sigma-Aldrich Co.) (MW: 1084.20). In order to validate the applied assay the selective V_{1A} antagonist [$Phenylacetyl^1$, O-Me-D-Tyr^2, $Arg^{6,8}$, Lys^9]-vasopressin amide (Sigma-Aldrich Co., MW: 1239; Howl et al., 1993) was used as the reference agent.

Phenylephrine, prostaglandine F2α and angiotensin were purchased by Sigma-Aldrich Co.

Because of insolubility of the test compounds in aqueous nutrient solution, an appropriate amount of DMSO had to be used. Therefore, a series of experiments was performed at the same experimental conditions, but using the solvent containing bathing solution without any test compound present.

2.2.4. Experimental set-up

Isometric measurement of contraction force and spontaneous rate of activity was performed with a force transducer (Type Fort 10 with a Transbridge™ 4-Channel Transducer Amplifier, World Precision Instruments, Sarasota, FL, USA). Papillary muscles were electrically stimulated with rectangular pulses of 3 ms duration delivered from a A310 Accupulser™ (World Precision Instruments, Inc., FL, USA) at a constant driving rate of 1 Hz. Intensity was adjusted to 10% above threshold. Signals were recorded continuously with a chart recorder (BD 112 Dual Channel, Kipp&Zonen, NL).

2.2.5. Experimental protocol

A resting tension of 3.9 mN (papillary muscle), 4.9 mN (terminal ileum), 9.81 mN (pulmonary artery), 10.4 mN (right atrium) or 19.62 mN (aorta) was applied in order to obtain maximum contractility of the respective preparation, and was kept constant throughout the experiments.

2.2.5.1. Concentration–response curves. Following an equilibrium period of half an hour the terminal ileum was precontracted with 60 mM KCl containing Krebs–Henseleit Solution, aorta and pulmonary artery were precontracted with 90 mM KCl containing nutrient solution. After a constant level of contraction force of precontracted preparations and electrically driven papillary muscles as well as constant rate of spontaneous activity of right atria was reached, a control period of 15 min followed. Then each test compound was added cumulatively to the bathing solution every 30 min after a steady-state effect had been reached. The IC_{30}-values were estimated graphically. In addition control experiments with the appropriate amount of solvent were performed.

For measurement of contractions evoked by agonists, rings of thoracic aorta and pulmonary artery were contracted with phenylephrine (0.1, 0.3, 1.0 and 3.0 μM), prostaglandin F2α (0.1, 0.3, 1.0 and 3.0 μM) or angiotensin (0.1 μM). After maximum contraction was observed at a distinct concentration of the injected agonist the preparation was washed three times before the agonist was applied at the next higher concentration. The same procedure was then performed in presence of the test compound.

2.2.5.2. Vasopressin antagonism. In order to avoid tachyphylaxis, each preparation was exposed to vasopressin only once. For control experiments vasopressin was added to electrically stimulated papillary muscles (1 Hz) at concentrations of 1, 3 and 10 μM, and registration of signals was done continuously over a period of 5 min. The effect of the agents was tested at concentrations of 1, 10 and 100 μM. For this purpose 45 min after addition of one of these test compounds 3 μM vasopressin was added to the bathing solution as described for control experiments.

2.2.6. Statistics

For statistical analysis the arithmetic means and standard error of the mean (S.E.M.) of *n* experiments were calculated. Statistical significance of the results was evaluated by the Student's *t*-test for paired observations, except for experiments with vasopressin. In this series of experiments different preparations for control and test compound experiments had to be taken to avoid desensitization. For these experiments the Student's *t*-test for unpaired observations was used.

3. Results

3.1. Chemistry

The additionally synthesized thienothiazine compounds **3**–**6** were prepared by derivatisation of the previously de-

scribed amines **1** and **2** (Galanski et al., submitted) with 2,4-dimethylbenzoyl chloride and 2,6-dichlorobenzoyl chloride (Fig. 1).

The synthetic route to the target compounds **10** and **11** is outlined in Fig. 2. Treatment of **7** (Erker, 1996) with 4-nitrobenzoyl chloride gave **8**, which was reacted with iron powder in glacial acetic acid, water and methanol to reduce the nitro group to amine **9** in good yield. Reaction with the appropriate acid chlorides was carried out under standard conditions to obtain the products **10** and **11**.

The preparation of the additional 3,4-dihydro-2H-thieno[3,2-b][1,4]thiazine analogs **16**–**19** is summarized in Fig. 3. Treatment of intermediate **12** (Paulmier and Outurquin, 1983) with lithium aluminium hydride under argon atmosphere provided the desired amine **13**, which turned out to be very instable. After filtration through aluminium oxide with tetrahydrofuran and concentration without further purification **13** was immediately reacted with 4-nitrobenzoyl chloride to **14**. Amine **13** was characterized by GC–MS. Reduction of compound **14** by the standard procedure with glacial acetic acid and iron powder and derivatisation of the resulting product **15** with 2,6-dichlorobenzoyl chloride, 4-fluorobenzoyl chloride, 2-methylbenzoyl chloride and phenylacetyl chloride gave the target compounds **16**–**19** in moderate yields.

3.2. Effects on smooth muscle contractility

The effect of the test compounds on the contractility of isolated smooth muscle preparations largely depended on the type of tissue. Only compounds **3** and **4** reduced the force of contraction (f_c) in each of the three tested smooth muscle preparations. However, the course of the concentration–response curves was rather flat, even for test compounds for which an IC_{30} could be estimated, so that IC_{50}-values were not reached within the tested concentration range up to 100 μM.

In terminal ilea f_c was concentration dependently decreased by every test substance. The most effective compound was **19** ($n = 4$) with an IC_{30} of 3 ± 1 μM ($n = 4$). The weakest effect on the terminal ileum was found with **6** ($n = 5$), which did not reach an IC_{30}-value (Table 1).

In pulmonary arteries the effect of the test compounds on f_c was generally less pronounced. The most effective compounds were **3** ($n = 5$), **4** ($n = 4$), **14** ($n = 6$) and **19** ($n = 4$) for which IC_{30}-values could be estimated (Table 1). All the other compounds (**6**, $n = 5$; **10**, $n = 5$; **16**, $n = 3$; **18**, $n = 5$) did not decrease f_c significantly ($P > 0.05$).

The effect on the contractility of aortic rings was inconsistent. Whereas **6** ($n = 5$), **10** ($n = 5$), **14** ($n = 5$), **16** ($n = 4$) and **18** ($n = 6$) did not significantly reduce f_c, for **3** ($n = 5$) and **4** ($n = 5$) an IC_{30} could be estimated (Table 1). In contrast, **19** increased force of contraction by $26.9 \pm 7.5\%$ at a concentration of 100 μM ($n = 3$, $P < 0.05$).

3.3. Vasoinhibitory effect

None of the test compounds ($n = 3$ for each agent, each preparation and concentration of 10, 30 and 100 μM) significantly affected phenylephrine- (0.1, 0.3, 1 and 3 μM) or prostaglandine F2α (0.1, 0.3 1 and 3 μM) induced contractions in pulmonary arteries and aortic strips. Angiotensin (0.1 and 0.3 μM) provoked contractions were concentration dependently (1, 10 and 100 μM test compound) and significantly (10 and 100 μM, $P < 0.05$) decreased by all compounds. A representative original recording is represented for **16** in Fig. 4.

3.4. Effect on spontaneous rate of activity of right atria

The spontaneous rate of activity of isolated right atria was slightly decreased, but none of the test compounds (**4**, $n = 3$; **6**, $n = 3$; **10**, $n = 3$; **14**, $n = 3$; **16**, $n = 3$; **18**, $n = 4$; **19**, $n = 3$) except **3** ($n = 3$) reached the IC_{30} level (Table 1). Although

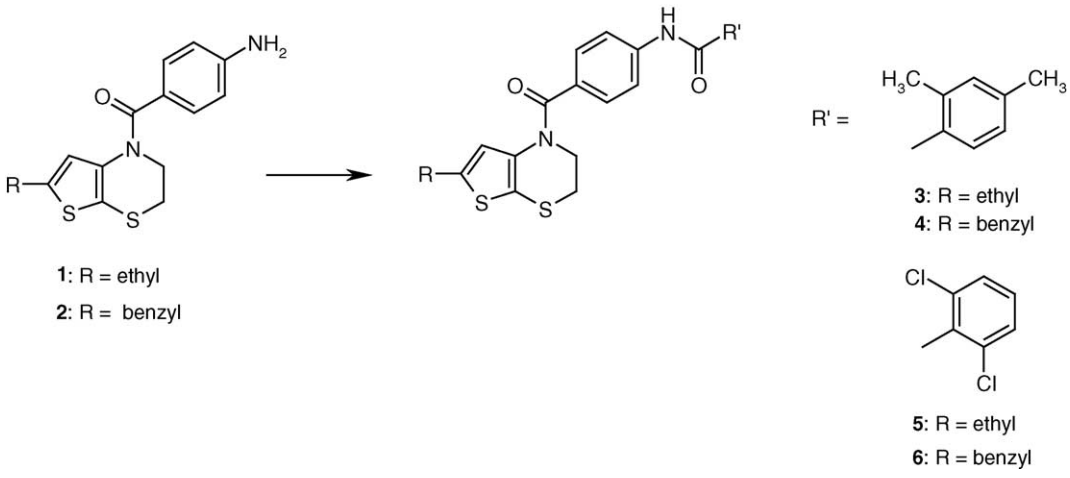

Fig. 1. Synthesis of the compounds **3**–**6**.

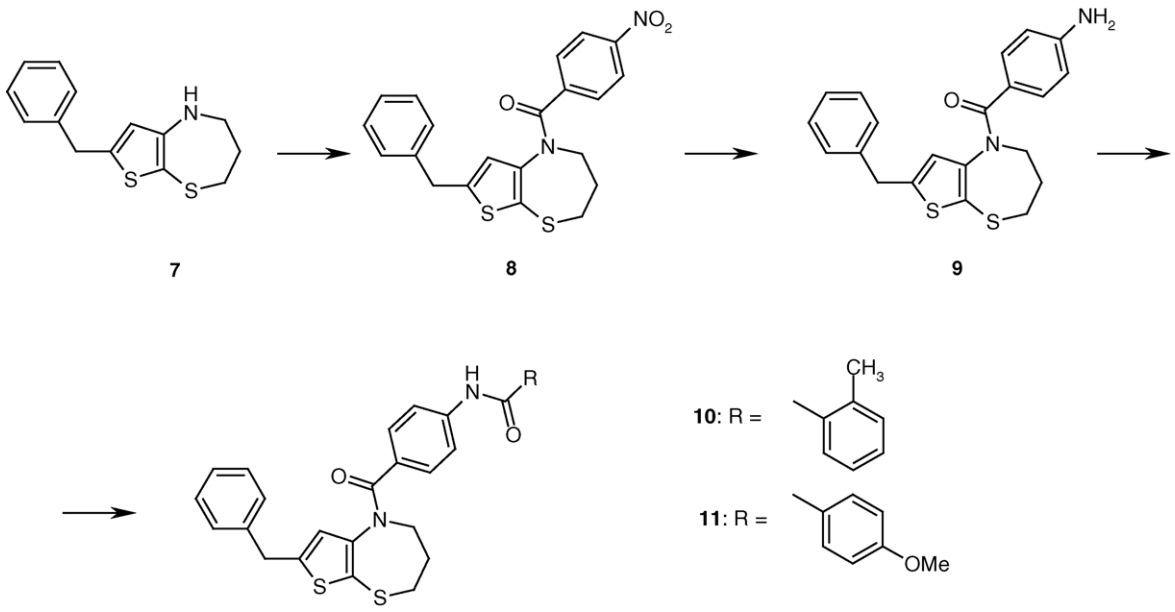

Fig. 2. Synthesis of the compounds **10** and **11**.

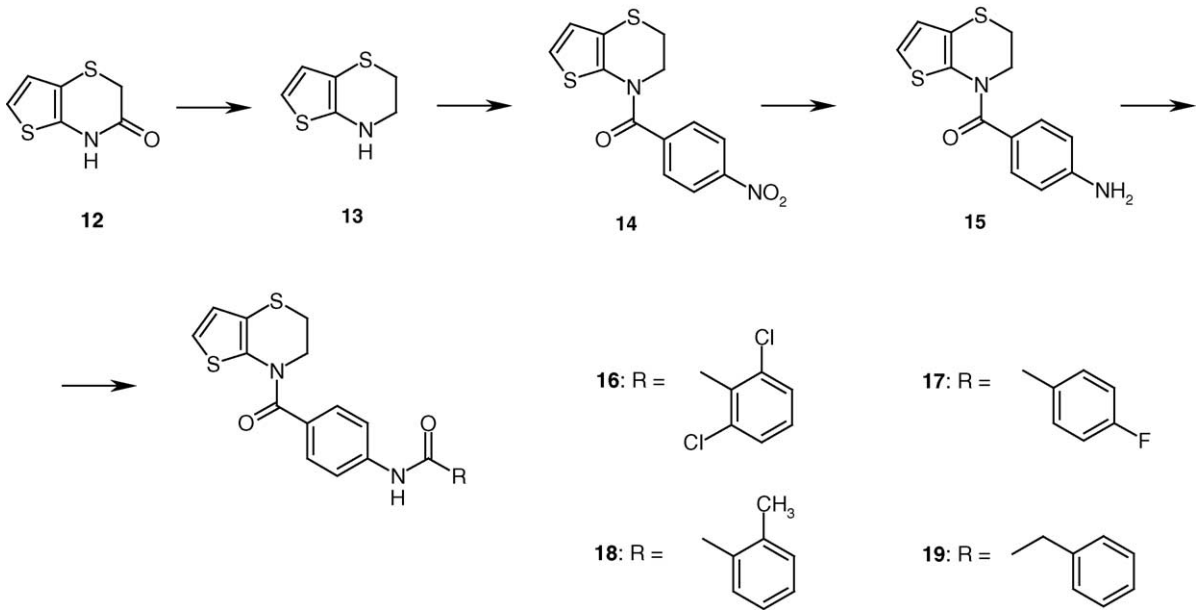

Fig. 3. Synthesis of the compounds **14** and **16**–**19**.

Table 1
Effect of the test compounds on f_c (terminal ileum, pulmonary artery, aortic rings, papillary muscle) and spontaneous rate of frequency (right atrium)

Test agent	Terminal ileum	Pulmonary artery	Aortic rings	Right atrium	Papillary muscle
3	8 ± 2 (5)	10 ± 3 (5)	26 ± 6 (5)	95 ± 6 (3)	10 ± 3 (5)
4	7 ± 2 (5)	30 ± 5 (4)	22 ± 5 (5)	– (3)	31 ± 5 (5)
6	– (5)	– (5)	– (5)	– (3)	– (5)
10	15 ± 4 (3)	– (5)	– (5)	– (3)	12 ± 4 (5)
14	15 ± 3 (5)	20 ± 5 (6)	– (5)	– (3)	– (4)
16	11 ± 4 (4)	– (3)	– (4)	– (3)	– (4)
18	5 ± 2 (7)	– (5)	– (6)	– (4)	– (6)
19	3 ± 1 (4)	96 ± 8 (4)	– (3)	– (3)	– (4)

The effect of the test compounds is given as IC_{30} (mean value ± S.E.M. in μM) for each preparation. No significant ($P < 0.05$) effect on f_c or spontaneous rate of activity is indicated with a dash. The number of experiments is given in parentheses.

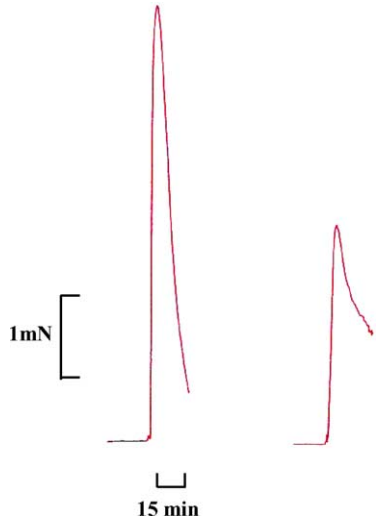

Fig. 4. Original recordings of an angiotensin (0.1 μM)-induced contraction on an aortic ring without (recording on the left) and in presence of 10 μM **16** (recording on the right).

there was a tendency of **14** for positive chronotropic activity up to a concentration of 3 μM (+8.6 ± 2.8%, $n = 3$), which turned into a weak negative chronotropic effect at 100 μM (−10.8 ± 6.7%, $n = 3$).

3.5. Effect on papillary muscle

16 ($n = 4$), **18** ($n = 6$) and **19** ($n = 4$) did not significantly affect the contractility of papillary muscles, while **3** ($n = 5$), **4** ($n = 5$) and **10** ($n = 5$) exerted a concentration dependent negative inotropic effect (Table 1).

In contrast, **6** and **14** even showed a weak positive inotropic effect, which was most pronounced at a **14**-concentration of 1 μM with an increase from 2.4 ± 1.4 to 3.1 ± 1.6 mN (+26.9 ± 11.8%, $n = 4$); but this increase was attenuated upon reduction or enhancement of **14** concentration. A weak positive inotropic effect (+10 ± 4%, $n = 5$) was also seen with **6** at concentrations higher than 3 μM.

3.6. Vasopressin antagonism

Vasopressin (1 μM: $n = 4$, 3 μM: $n = 14$ and 10 μM: $n = 4$) affected the contractility of papillary muscles in a biphasic way. 30 s after addition of vasopressin a maximal negative inotropic response was seen (at 3 μM: −7.04 ± 2.2%, $n = 14$, $P < 0.05$), which attenuated gradually, and after 1.5 min the control value was reached again. This transient negative inotropic effect was followed by a positive inotropic response which reached its maximum after 2.5–3 min (at 3 μM: +21.4 ± 3.4%, $n = 14$, $P < 0.01$; Table 2) and which lasted about 5 min. This latter effect was accompanied by a transient increase of the resting tension (+0.5 ± 0.2 mN, $n = 14$) (Fig. 5). These responses were concentration dependent, but were not significantly more pronounced at 10 μM, so that a concentration of 3 μM was chosen for further experiments.

When 3 μM vasopressin was added in presence of one of the test compounds, the vasopressin-induced negative inotropic response remained unaffected by **3** (1, 10 and 100 μM, each $n = 3$), **4** (1 μM, $n = 5$; 10 and 100 μM, each $n = 3$), **6** (1, 10, 100 μM, each $n = 3$) and **10** (1 and 10, each $n = 3$; 100 μM, $n = 4$), but was diminished by **14** (100 μM, $n = 5$), **16** (100 μM, $n = 4$), **18** (100 μM, $n = 3$) and **19** (100 μM, $n = 4$) (Fig. 5, Table 2).

The positive inotropic activity of 3 μM vasopressin was concentration dependently and significantly antagonized by **6** (at 100 μM, $n = 3$), **14** (1 μM, 10 μM, 100 μM, each $n = 5$), **16** (10 and 100 μM, $n = 4$ each) and **19** (10 and 100 μM, $n = 4$ each) (Table 2). A weak, non-significant reduction of the positive inotropic vasopressin response was seen with **4** (at 10 and 100 μM, $n = 3$ each) and **10** (at 100 μM, $n = 4$). The two compounds **3** (1, 10 and 100 μM, $n = 3$ each) and **18** (1, 10 and 100 μM, $n = 3$ each) did not antagonize the vasopressin-induced contraction at either concentration (Fig. 5).

Table 2
The effect of the test compounds on the transient negative inotropic and maximum positive inotropic effect of vasopressin

Control [−7 ± 2/+21 ± 3 ($n = 14$)]

Test agent	Vasopressin + 1 μM	Vasopressin + 10 μM	Vasopressin + 100 μM
Reference	–	$-2 \pm 1^{**} / +4 \pm 2^{**}$ (3)	–
3	−6 ± 2/ + 16 ± 3 (3)	−8 ± 2/ + 14 ± 4 (3)	−6 ± 3/ + 19 ± 2 (3)
4	−8 ± 2/ + 19 ± 4 (5)	−7 ± 3/ + 15 ± 3 (3)	−7 ± 2/ + 14 ± 2 (3)
6	−8 ± 2/ + 16 ± 4 (3)	−5 ± 3/ + 15 ± 3 (3)	$-9 \pm 1 / + 11 \pm 2^{*}$ (3)
10	−7 ± 1/ + 20 ± 1 (3)	−8 ± 2/ + 16 ± 3 (3)	−8 ± 2/ + 9 ± 3 (4)
14	−7 ± 1/ + 8 ± 4 (5)	$-7 \pm 1 / + 7 \pm 2^{**}$ (5)	$-4 \pm 1^{*} / +5 \pm 1^{**}$ (5)
16	−6 ± 2/ + 23 ± 2 (3)	$-5 \pm 1 / + 8 \pm 5^{*}$ (4)	$-3 \pm 2^{*} / + 9 \pm 1^{*}$ (4)
18	−6 ± 2/ + 16 ± 5 (3)	−4 ± 2/ + 18 ± 3 (3)	−5 ± 1/ + 24 ± 2 (3)
19	−6 ± 2/ + 14 ± 2 (3)	$-6 \pm 2 / + 8 \pm 3^{*}$ (4)	$-1 \pm 1^{**} / + 6 \pm 1^{**}$ (4)

In the upper most row control values (in %) for the transient, maximal negative inotropic and the maximum positive inotropic effect of 3 μM vasopressin in isolated papillary muscles are given. Further the influence on the negative and positive inotropic effect of 3 μM vasopressin is presented for the test compounds at concentrations of 1, 10 and 100 μM. The first values always indicate the decrease of f_c in percent, 30 s after addition of vasopressin (without a test compound = control, and in presence of a test compound, respectively). The second values give the increase of f_c in percent, 3 min after vasopressin addition (without a test compound = control, and in presence of the test compound, respectively). Mean values ± S.E.M. in % of n experiments are given. Number of experiments is written in parentheses. Significant changes compared to control are indicated with asterisks ($^{*}P < 0.05$, $^{**}P < 0.01$).

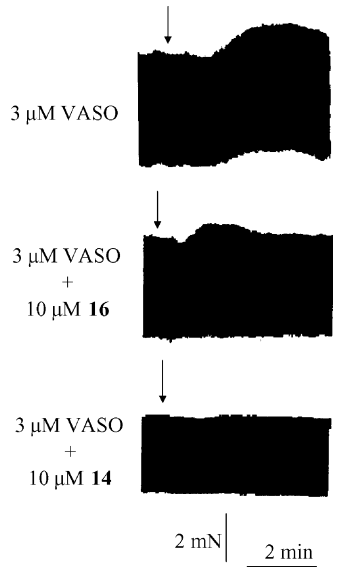

Fig. 5. Original recordings from papillary muscles illustrating the vasopressin antagonistic activity. In the upper panel the effect of 3 μM vasopressin (VASO) on f_c is shown. The addition of vasopressin is indicated with arrows. In the middle row the weak vasopressin antagonistic effect of 10 μM **16** is presented, and in the lower panel the marked antagonistic activity of 10 μM **14** is illustrated.

The transient increase in resting tension was attenuated by the test compounds (Fig. 5) except by **3** and **18**.

The effect of the most effective vasopressin antagonistic acting compound in the papillary muscle was also studied on strips of the uterus ($n = 3$). **14** (30 and 100 μM) concentration dependently reduced the response to vasopressin. The contraction induced by 0.1 μM vasopressin was reduced from 37.6 ± 3.5 to 27.4 ± 4.3 mN ($-27.1 \pm 6.1\%$, $n = 3$) at 30 μM **14** and to 23.9 ± 3.1 mN ($-36.4 \pm 6.3\%$, $n = 3$) at 100 μM **14**.

4. Discussion

In the cardiovascular system vasopressin produces vasoconstriction through V_1-receptors on vascular smooth muscles, but also the modulation of myocardial contractility is mediated by the V_1-receptors. In terms of vasopressin's direct effects on the myocardial contractility, diverse results have been reported. Positive inotropy was observed in rat (Walker et al., 1988), cat (Schoemaker et al., 1990) and guinea pig myocardium (Yamaguchi et al., 1995), while negative inotropy was observed in dog (Furukawa et al., 1992) and rabbit myocardium (Endoh et al., 1992). In neonatal rat cardiomyocytes and guinea pig ventricular myocytes V_1-receptor stimulation caused an increase of cytosolic free calcium (Xu and Gopalakrishnan, 1991) and L-type calcium current (Kurata et al., 1999) resulting in an increase of resting and developing tension. Yamaguchi et al. (1995) studied the effect of the selective V_1-receptor antagonist OPC-21268 (Yamamura et al., 1991) and the V_2 -receptor antagonist OPC-31260 (Yamamura et al., 1992) on isolated papillary muscles of the guinea-pig heart. They showed that OPC-21268 almost completely abolished the vasopressin induced increase in the developed tension of guinea pig papillary muscles, but resting tension was only affected at higher concentrations. The V_2-antagonist OPC-31260 failed to antagonize the vasopressin caused positive inotropic effect, thus indicating that V_1-receptors are responsible for the positive inotropy in guinea pig papillary muscles. Except **18** and **3** our test compounds concentration dependently antagonized the positive inotropic effect of vasopressin with the ranking potency **14** = **19** > **16** = **10** > **6** > **4**. These data show that the substitution of a phenyl- or dichlorobenzyl moiety does not significantly influence the vasopressin antagonistic activity, but that the effect was lost by substitution of a methylbenzyl group. Further, the results show that the thieno[3,2-b][1,4]thiazine compounds are more effective in suppressing the positive inotropic effect of vasopressin than the thieno[2,3-b][1,4]thiazine compounds, and that a thieno[2,3-b][1,4]thiazepine structure instead of the latter heterocycle does not markedly influence the effect. In addition the increase of the resting tension was antagonized by these test compounds.

In contrast to the findings of Yamamura et al. (1992) Fujisawa and Iijima (1999) observed a transient negative inotropic effect shortly after vasopressin application in addition, which was accompanied by a transient decrease of I_{Ca} that turned to a slowly developing increase of I_{Ca} and force of contraction in isolated ventricular myocytes and papillary muscles of the guinea pig. Also this transient negative inotropic response was antagonized by the V_1-antagonist OPC-21268, but not by OPC-31260 (Fujisawa and Iijima, 1999). Our data coincide with these observations of negative inotropy. The transient decrease in force of contraction by vasopressin was attenuated by the thieno[3,2-b][1,4]thiazine compounds **14** and **16** or even abolished by **18** and **19** at higher concentrations, but was not significantly affected by the tested thieno[2,3-b][1,4]thiazine compounds **3**, **4** and **6**, and the thieno[2,3-b][1,4]thiazepine **10**. These findings imply an influence of the heterocyclic structure on the vasopressin antagonistic activity.

The suggestion that the test compounds can be classified as V_1-receptor antagonists is further strengthened by the fact that **14** was able to antagonize the vasopressin-induced uterine contraction which is mediated via V_{1a}-receptors, similar to results reported for the V_1-antagonists SR 49059 (Kawamata et al., 2003; Steinwall et al., 2004) and OPC-21268 (Atke et al., 1995), keeping in mind that the receptors mediating the effects of vasopressin on the myometrium are species-dependent (Kawamata et al., 2003). For that reason our findings will be verified by receptor binding studies.

In vascular smooth muscle, the mechanism of contraction induced by membrane depolarisation is different from that induced by receptor agonists (Karaki et al., 1997). Receptor agonists not only open the L-type calcium channel, but also the non-selective cation channel (Kuriyama et al.,

1995). In addition they increase Ca^{2+} sensitivity of contractile elements (Kitazawa et al., 1991) and may release Ca^{2+} from intracellular storage sites (Karaki et al., 1997). Thus, our data suggest that the weak vasodilatory effect of some of the test compounds is only partly due to a Ca^{2+} antagonistic effect. Although the mechanism of the effect on the angiotensin-induced contractions has to be further elucidated, these assumptions could explain the difference in the reduction of contraction force in various tissues.

In conclusion, the test compounds if at all, only slightly reduced force of contraction. The thieno[3,2-b][1,4]thiazine compounds exerted a more effective vasopressin antagonistic activity than thieno[2,3-b][1,4]thiazine and thieno[2,3-b][1,4]thiazepine compounds, and in addition substitution of a methylbenzyl group instead of a phenyl- or dichlorobenzyl moiety attenuated the vasopressin antagonistic potency.

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