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ORIGINAL ARTICLE

Calix[4]arene *N*-chalconeamides: synthesis and influence on Mg^{2+},ATP-dependent Ca^{2+} accumulation in the smooth muscle subcellular structures

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Abstract The *cone*-shaped calixarene *N*-chalconeamides were synthesized by the reaction of calix[4]arene carboxylic acids or acid chlorides with aminochalcones. It was found, that calixarene chalconeamides influence the Mg^{2+}, ATP-dependent Ca^{2+} accumulation in mitochondria and sarcoplasmatic reticulum of the smooth muscle cells.

Keywords Calix[4]arene · Chalcone · Ca^{2+} accumulation · Smooth muscle subcellular structure

Introduction

Calixarenes [1, 2] are perspective scaffolds in the design of the extractants [3, 4], sensors [5, 6], catalysts [7, 8] and bioactive compounds [9, 10] due to their unique capability to bind ions and neutral molecules selectively.

During the last decade calixarenes are in the focus of biochemical studies. As it was remarkably shown, these macrocycles are able to influence biological events. Functionalized calixarenes show antibacterial activity [11], are effective anticoagulants, antitrombotics [12], and antitumor agents [13]. Zinc and copper complexes of calixarenes demonstrate enzymatic activity [14, 15]. Aminophosponic acids derivatives are effective inhibitors of alkaline phosphatases [16, 17]. Calixarenes are effective inhibitors of chlorine channels [18] and effectors of Na^+ or K^+ transfer through biomembranes [19, 20].

It is known that calcium cations are unique secondary messengers, transferring electrical and pharmacological signals in the cells. It is shown in our recent study that calixarenephosphonic acids suppress the activity of Na^+,K^+-ATP-ases of the plasmatic membrane [21]. It was found that calixarenes containing amide and sulfonylamidine moieties at the wide rim are effective inhibitors of the smooth muscles cells calcium pumps [22].

The synthesis of the narrow rim functionalized calix[4]-arene amides, possessing chalcone residues and their influence Ca^{2+} on accumulation in subcellular structures of smooth muscles is the subject of this work. It is known that chalcones have a wide spectrum of biological activity, e.g. antibiotic, bactericidal, bacteriostatic, antimicrobial, and some of them are able to inhibit ferments [23]. Coupling of calixarenes and chaclones opens new potential for the design of membrane-coupled ion-transport systems effectors.

Experimental

Melting points were determined on a Boëtius apparatus and are uncorrected. The synthesis of amides was carried out in anhydrous solvents and in dry atmosphere. TLC was performed on silica gel 60 W Merck plates. Column chromatography was carried out using Acros Organics silica gel (0.35–0.07 mm, pore diameter 6 nm). ^{1}H NMR spectra were recorded on a VXR 300 instrument operating at 300 MHz. The chemical shifts are referenced to TMS as

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internal standard. IR spectra were recorded on a M 80 spectrometer. Monomethyloxycalixarene **1** (this compound was obtained by the method [24] but MeONa was used as a base), acid **8** [25], acid chloride **10** [25], 4′-aminochalcone [26], 3′-aminochalcone [27] and 4′-acetamidochalcone **12** [28] were prepared according to the described procedures.

5,11,17,23-Tetra-*tert*-butyl-25-methoxy-26,28-dihydroxy-27-(ethoxycarbonylmethoxy)calix[4]arene **2**

A suspension of 5,11,17,23-tetra-*tert*-butyl-25-methoxycalix[4]arene **1** (1.44 g, 2.17 mmol), ethyl bromoacetate (0.40 g, 2.39 mmol) and K_2CO_3 (0.17 g, 1.20 mmol) in acetonitrile (150 mL) was stirred at reflux for 6 h. After cooling, the solvent was removed under reduced pressure. The remaining solid was taken up in $CHCl_3$ (15 mL) and washed with 10% HCl (10 mL) and then with water (20 mL). The organic layer was dried over Na_2SO_4, the solvent was evaporated *in vacuo* and the residue was recrystalized from MeOH (30 mL). White solid: yield 64%, Mp 165–166 °C.

^{1}H NMR ($CDCl_3$), δ: 0.90 (s, 9H, Me_3C), 0.98 (s, 9H, Me_3C), 1.28 (s, 18H, Me_3C), 1.34 (t, 3H, CH_3CH_2O), 3.31 (d, 2H, $ArCH_{2eq}$, $^2J_{HH}$ = 13.1 Hz), 3.33 (d, 2H, $ArCH_{2eq}$, $^2J_{HH}$ = 13.1 Hz), 3.96 (s, 3H, CH_3O), 4.26 (d, 2H, $ArCH_{2ax}$, $^2J_{HH}$ = 13.1 Hz), 4.30 (q, 2H, OCH_2CH_3), 4.44 (d, 2H, $ArCH_{2ax}$, $^2J_{HH}$ = 13.1 Hz), 4.67 (s, 2H, $-OCH_2COOEt$), 6.73 (s, 2H, ArH), 6.82 (s, 2H, ArH), 7.05 (m, 4H, ArH), 7.15 (s, 2H, OH). IR (KBr, ν/cm^{-1}): ν_{OH} = 3450 (associated OH···OH···OMe), $\nu_{C=O}$ = 1735. Anal. Found: C 79.57%; H 8.52%. Calc. for $C_{49}H_{64}O_6$: C 78.56%; H 8.61%.

5,11,17,23-Tetra-*tert*-butyl-25-methoxy-26,28-dihydroxy-27-(hydroxycarbonylmethoxy)-calix[4]arene **3**

Compound **2** (1.04 g, 1.38 mmol) was added to a solution of KOH (3.87 g, 6.91 mmol) in EtOH/H_2 O (30/30 mL) and the reaction mixture was refluxed for 11 h. After cooling, the precipitated salt (0.7 g) was filtered off and treated with the 5% solution of HCl in EtOH/H_2O (25/25 mL). The precipitated acid **3** was filtered off and dried *in vacuo*. White solid: yield 56%, Mp 231–232 °C.

^{1}H NMR ($CDCl_3$), δ: 1.08 (s, 9H, Me_3C), 1.10 (s, 9H, Me_3C), 1.25 (s, 18H, Me_3C), 3.43 (d, 2H, $ArCH_{2eq}$, $^2J_{HH}$ = 13.1 Hz), 3.45 (d, 2H, $ArCH_{2eq}$, $^2J_{HH}$ = 13.1 Hz), 4.05 (s, 3H, CH_3O), 4.08 (d, 2H, $ArCH_{2ax}$, $^2J_{HH}$ = 13.1 Hz), 4.20 (d, 2H, $ArCH_{2ax}$, $^2J_{HH}$ = 13.1 Hz), 4.66 (s, 2H, $-OCH_2$-COOH), 6.96 (s, 2H, ArH), 7.00 (s, 2H, ArH), 7.06 (s, 4H, ArH), 7.89 (s, 2H, OH). IR (KBr, ν/cm^{-1}): $\nu_{C=O}$ = 1690 (associated), $\nu_{C=O}$ = 1725 (monomeric), $\nu_{OH(COOH)}$ = 2550 (dimeric), ν_{OH} = 3450 (associated OH···OH···OMe). Anal. Found: C 78.76%; H 8.52%. Calc. for $C_{47}H_{60}O_6$: C 78.28%; H 8.38%.

5,11,17,23-Tetra-*tert*-butyl-25,27-dipropoxy-26,28-bis(hydroxycarbonylmethoxy)calix[4]arene **5**

5,11,17,23-Tetra-*tert*-butyl-25,27-dipropoxy-26,28-bis(methoxycarbonylmethoxy)calix[4]arene (2.54 g, 2.89 mmol) was added to a solution of KOH (14.60 g, 260.7 mmol) in EtOH/H_2O (65/65 mL) and the reaction mixture was refluxed for 4.5 h. The solution was concentrated under reduced pressure to the volume of 40 mL and treated with 10% HCl. The precipitated product was filtered off, washed with water and dried. White solid: yield 86%, Mp 205–206 °C.

^{1}H NMR ($CDCl_3$), δ: 0.84, 1.34 (2s, 18H, 18H, Me_3C), 0.91 (t, 6H, CH_3) 1.88 (m, 4H, CH_2), 3.29 (d, 4H, $ArCH_{2eq}$, $^2J_{HH}$ = 13.1 Hz), 3.84 (t, 4H, $CH_3CH_2CH_2O$), 4.25 (d, 4H, $ArCH_{2ax}$, $^2J_{HH}$ = 13.1 Hz), 4.65 (s, 4H, OCH_2), 6.56 (s, 2H, ArH), 7.18 (s, 2H, ArH). Anal. Found: C 76.34%; H 8.6%. Calc. for $C_{54}H_{72}O_8$: C 76.38; H 8.55.

5,11,17,23-Tetra-*tert*-butyl-25,27-dipropoxy-26,28-bis(chlorocarbonylmethoxy)calix[4]arene **7**

Acid **5** (1.50 g, 1.77 mmol) was dissolved in thionyl chloride (10 mL), then DMF (3–4 drops) was added and the solution was stirred at reflux in anhydrous conditions for 3 h. After removal of the thionyl chloride under reduced pressure hexane (10 mL) was added to the residue. The solvent was evaporated again under reduced pressure. This procedure was repeated twice to remove $SOCl_2$. Product was dried *in vacuo* at 40 °C for 10 h. White solid: yield 86%, Mp 198.5–200 °C.

^{1}H NMR ($CDCl_3$), δ: 0.83, 1.33 (2s, 18H, 18H, Me_3C), 1.06 (t, 6H, CH_3), 1.93 (m, 4H, CH_2), 3.23 (d, 4H, $ArCH_{2eq}$, $^2J_{HH}$ = 13.5 Hz), 3.71 (t, 4H, $CH_3CH_2CH_2O$), 4.57 (d, 4H, $ArCH_{2ax}$, $^2J_{HH}$ = 13.5 Hz), 5.34 (s, 4H, OCH_2), 6.43, 7.11 (2s, 4H, 4H, ArH). Anal. Found: C 72.34%; H 7.74%; Cl 8,24%. Calc. for $C_{54}H_{70}Cl_2O_6$: C 73.20%; H 7.96%; Cl 8.00%.

General procedure for the syntheses of compounds 4, 6 and 9a (method A)

The solutions of acids **3**, **5**, **8** (0.113 mmol) in dry ethyl acetate (25 mL) were added dropwise to a solution of DCC and 4′-aminochalcone (0.119 mmol per each acid group) in the same solvent (10 mL). The reaction mixture was stirred at 60 °C for 24 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography. Product was dried *in vacuo* for 10 h.

5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26-methoxy-28-((4′benzylideneacetophenonyl)amino-carbonylmethoxy)calix[4]arene **4**

Eluent for the column ethyl acetate/hexane 1:2, $R_f = 0.32$. Pale-yellow solid: yield 77%, Mp 226–228 °C.

^{1}H NMR ($CDCl_3$), δ: 1.05 (s, 9H, Me_3C), 1.06 (s, 9H, Me_3C), 1.28 (s, 18H, Me_3C), 3.45 (d, 2H, ArCH_{2eq}, $^2J_{HH}$ = 13.4 Hz), 3.47 (d, 2H, ArCH_{2eq}, $^2J_{HH}$ = 13.4 Hz), 3.96 (s, 3H, CH_3O), 4.19 (d, 2H, ArCH_{2ax}, $^2J_{HH}$ = 13.4 Hz), 4.27 (d, 2H, ArCH_{2ax}, $^2J_{HH}$ = 13.4 Hz), 4.67 (s, 2H, OCH_2), 6.94 (s, 2H, ArH), 6.95 (s, 2H, ArH), 7.10 (s, 4H, ArH), 7.42 (m, 3H, ArH), 7.58 (d, 1H, $^3J_{HH}$ = 15.8 Hz, CH=CH), 7.61 (s, 2H, OH[1]) 7.67 (m, 2H, ArH), 7.84 (d, 1H, $^3J_{HH}$ = 15.8 Hz, CH=CH), 8.01 (d, 2H, $^3J_{HH}$ = 8.9 Hz), 8.10 (d, 2H, $^3J_{HH}$ = 8.9 Hz), 10.67 (br. s, 1H, NH). IR (KBr, ν/cm^{-1}): $\nu_{C=C}$ = 1585, $\nu_{C=O}$ = 1645 (associated), $\nu_{C=O}$ = 1685 (monomeric), ν_{NH} = 3330 (associated), ν_{OH} = 3450 (associated OH···OH···OMe). Anal. Found: C 80.56%; H 7.52%; N 1.70%. Calc. for $C_{62}H_{71}O_6N$: C 80.43%; H 7.68%; N 1.51%.

5,11,17,23-Tetra-*tert*-butyl-25,27-dipropoxy-26,28-bis((4′-benzylideneacetophenonyl)-aminocarbonylmethoxy)calix[4]arene **6**

Eluent for the column ethyl acetate/hexane 1:1, $R_f = 0.3$. Yellow solid: yield 78%, Mp 135–136 °C.

^{1}H NMR ($CDCl_3$), δ: 0.60 (t, 6H, $^3J_{HH}$ = 7.3 Hz, CH_3CH_2CH_2O), 0.90 (s, 18H, Me_3C), 1.33 (s, 18H, Me_3C), 1.55 (m, 4H, $^3J_{HH}$ = 7.8 Hz, CH_3CH_2CH_2O), 3.33 (d, 4H, ArCH_{2eq}, $^2J_{HH}$ = 13 Hz), 3.65 (t, 4H, $^3J_{HH}$ = 7.8 Hz, CH_3CH_2CH_2O), 4.45 (d, 4H, ArCH_{2ax}, $^2J_{HH}$ = 13 Hz), 4.63 (s, 4H, OCH_2), 6.65 (s, 4H, ArH), 7.18 (s, 4H, ArH), 7.40 (m, 6H, ArH), 7.48 (d, 2H, $^3J_{HH}$ = 15.9 Hz, CH=CH), 7.67 (m, 4H, ArH), 7.80 (d, 4H, $^3J_{HH}$ = 7.8 Hz, ArH), 7.82 (d, 2H, $^3J_{HH}$ = 15.9 Hz, CH=CH), 7.98 (d, 4H, $^3J_{HH}$ = 8.9 Hz, ArH), 9.68 (br. s, 2H, NH). IR (KBr, ν/cm^{-1}): $\nu_{C=C}$ = 1585, $\nu_{C=O\ (amide)}$ = 1645 (associated), $\nu_{C=O\ (amide)}$ = 1675 (monomeric), ν_{NH} = 3310 (associated). Anal. Found: C 79.50%; H 7.52%; N 2.50%. Calc. for $C_{84}H_{94}O_8N_2$: C 80.13%; H 7.47%; N 2.22%.

5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetra((4′-benzylideneacetophenonyl)aminocarbonyl-methoxy)calix[4]arene **9a**

Eluent for chromatography $CHCl_3$/MeOH 8:2, $R_f = 0.49$. Pale-yellow solid: yield 73%, Mp 301–302 °C.

[1] This signal disappeared after addition of CF_3COOD.

^{1}H NMR ($CDCl_3$), δ: 1.08 (s, 36H, Me_3C), 3.29 (d, 4H, ArCH_{2eq}, $^2J_{HH}$ = 13.2 Hz), 4.66 (d, 4H, ArCH_{2ax}, $^2J_{HH}$ = 13.2 Hz), 4.80 (s, 8H, –OCH_2), 6.82 (s, 8H, ArH), 7.31 and 7.37 (m + d, 12H + 4H, $^3J_{HH}$ = 15.9 Hz, CH=CH + ArH), 7.49 (m, 8H, ArH), 7.65 (d + m, 4H + 8H, $^3J_{HH}$ = 15.9 Hz, CH=CH + ArH), 7.83 (d, 8H, $^3J_{HH}$ = 8.4 Hz, ArH), 9.40 (br. s, 4H, NH). IR (KBr, ν/cm^{-1}): $\nu_{C=C}$ = 1605, $\nu_{C=O}$ = 1663, $\nu_{C=O\ (amide)}$ = 1697, ν_{NH} = 3320 (associated). Anal. Found: C 78.87%; H 6.41%; N 3.27%. Calc. for $C_{112}H_{108}O_{12}N_4$: C 79.06%; H 6.35%; N 3.29%.

Synthesis of the compounds 6 and 9a,b (method B)

5,11,17,23-Tetra-*tert*-butyl-25,27-dipropoxy-26,28-bis((4′-benzylideneacetophenonyl)amino-carbonylmethoxy)calix[4]arene **6**

A solution of acid chloride **7** (0.100 g, 0.113 mmol), 4′-aminochalcone (0.056 g, 0.250 mmol) and Et_3N (0.073 g, 0.72 mmol) in dry benzene (8 mL) was stirred at reflux for 4 h. The precipitate of Et_3N·HCl was filtered off. Benzene was removed under reduced pressure. The crude product was purified by column chromatography. Eluent used ethyl acetate/hexane 1:1, $R_f = 0.3$. Yellow solid: yield 63%. All physical constants and spectral data are analogous to the compound **6**, obtained according to the method A.

5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetra((4′-benzylideneacetophenonyl)aminocarbonyl-methoxy)calix[4]arene **9a**

A solution of acid chloride **10** (0.50 g, 0.52 mmol) and 4′-aminochalcone (0.50 g, 2.24 mmol) in dry toluene (8 mL) was stirred at reflux for 3 h. The progress of the reaction was followed by TLC (eluent CH_3CN). After cooling of the reaction mixture the precipitated product was filtered off, washed with toluene and dried *in vacuo*. Pale-yellow solid: yield 60%. All physical constants and spectral data are analogous to the compound **9a**, obtained according to the method A.

5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetra((3′-benzylideneacetophenonyl)aminocarbonyl-methoxy)calix[4]arene **9b**

A solution of acid chloride **10** (0.50 g, 0.52 mmol), 3′-aminochalcone (0.50 g, 2.24 mmol) and Et_3N (0.317 g, 3.14 mmol) in dry benzene (12 mL) was stirred at reflux. The precipitate of Et_3N·HCl was filtered off. The filtrate was washed with water and brine, dried over Na_2SO_4 and

evaporated to give a product **9b**. Yellow solid: yield 70%, Mp 142–143 °C.

^{1}H NMR ($CDCl_3$), δ: 1.10 (s, 36H, Me_3C), 3.44 (d, 4H, $ArCH_{2eq}$, $^2J_{HH}$ = 12.9 Hz), 4.71 and 4.76 (s + d, 8H + 4H, OCH_2 + $ArCH_{2ax}$, $^2J_{HH}$ = 12.9 Hz), 6.89 (s, 8H, ArH), 7.32 and 7.39 (m + d, 14H + 4H, ArH + CH=CH, $^3J_{HH}$ = 15.7 Hz), 7.50 (m, 10H, ArH), 7.60 (d, 4H, ArH, $^3J_{HH}$ = 7.1 Hz), 7.70 (d, 4H, $^3J_{HH}$ = 15.7 Hz, CH=CH), 7.91 (d, 4H, $^3J_{HH}$ = 7.1 Hz, ArH), 8.22 (s, 4H, ArH), 9.58 (br. s, 4H, NH). IR (KBr, ν/cm^{-1}): $\nu_{C=C}$ = 1600, $\nu_{C=O}$ = 1668, $\nu_{C=O\ (amide)}$ = 1690, ν_{NH} = 3320 (associated). Anal. Found: C 78.96%; H 6.29%; N 3.35%. Calc. for $C_{112}H_{108}O_{12}N_4$: C 79.06%; H 6.35%; N 3.29%.

5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetra(phenylaminocarbonylmethoxy)calix[4]arene **11**

Aniline (0.08 g, 0.86 mmol) was added to a solution of acid chloride **10** (0.20 g, 0.21 mmol) in dry toluene (10 mL). The reaction mixture was stirred at reflux for 5 h. The solvent was removed under reduced pressure, and the residue was recrystalized from CH_3CN. White solid: yield 86%, Mp 277–278 °C.

^{1}H NMR ($CDCl_3$), δ: 1.08 (s, 36H, Me_3C), 3.28 (d, 4H, $ArCH_{2eq}$, $^2J_{HH}$ = 13.1 Hz), 4.63 (d, 4H, $ArCH_{2ax}$, $^2J_{HH}$ = 13.1 Hz), 4.65 (s, 8H, OCH_2), 6.80 (s, 8H, ArH), 7.03 (m, 4H, ArH), 7.16 (m, 8H, ArH), 7.49 (m, 8H, ArH), 9.04 (br. s, 4H, NH). Anal. Found: C 77.52%; H 6.99%; N 4.65%. Calc. for $C_{76}H_{84}O_8N_4$: C 77.26%; H 7.16%; N 4.74%.

Biochemical methods

Myocyte suspensions from the rat uterus were obtained by the method [29] with some modifications [30]. Total cell number was estimated using a hemocytometer. Mg^{2+},ATP-dependent Ca^{2+} transport in smooth muscle mitochondria was monitored using $^{45}Ca^{2+}$ as radioactive tracer. The composition of the incubation medium (0.5 mL) was as follows (mM): HEPES-NaOH buffer (pH 7.4), 20; $MgCl_2$, 3; ($^{45}CaCl_2$ + $^4CaCl_2$) (0,5 μCi/mL), 0.01; ATP, 3; sodium succinate, 3; KCl, 125; NaCl, 25; thapsigargin, 0.0001; potassium phosphate, 2. Final cell concentration was about 0.2×10^6 in mL. The digitonin concentration in the incubation medium was 0.1 mg/mL. Just this digitonin concentration is known to disturb the integrity of the plasma membrane, but do not affect the intracellular membrane structures [31]. Thapsigargin was used for the selective inhibition of Mg^{2+},ATP-dependent sarcoplasmic reticulum Ca^{2+} pump activity [32]. The incubation medium temperature was 37 °C, and the incubation time was 5 min. The energy-dependent Ca^{2+} accumulation in mitochondria was quantified regarding the difference between the accumulation of the cation in the standard medium and its adsorption from ruthenium red containing medium.

Mg^{2+},ATP-dependent Ca^{2+} transport in smooth muscle sarcoplasmic reticulum was also monitored using $^{45}Ca^{2+}$ as radioactive tracer. The composition of the incubation medium (0.5 mL) was as follows (mM): HEPES-NaOH buffer (pH 7.4), 20; $MgCl_2$, 3; ($^{45}CaCl_2$ + $^4CaCl_2$) (0,5 μCi/mL), 0.01; ATP, 3; KCl, 125; NaCl, 25; ruthenium red, 0.01; potassium oxalate, 10. Final cell concentration was about 0.2×10^6 in mL. The digitonin concentration in the incubation medium was 0.1 mg/mL. Ruthenium red was used for selective inhibition of mitochondria Ca^{2+} uniporter activity [32]. The incubation medium temperature was 37 °C, and the incubation time was 5 min. The energy-dependent Ca^{2+} accumulation in sarcoplasmic reticulum was quantified regarding the difference between the accumulation of the cation in the standard medium and its adsorption from thapsigargin containing medium.

In all the experiments Ca^{2+} accumulation was arrested by rapid vacuum filtration of the incubation mixture through membrane filtres (pore diameter 0.45 μm). The radioactivity of the filters was measured with a Beckman liquid scintillation spectrometer.

Results and discussion

Synthesis

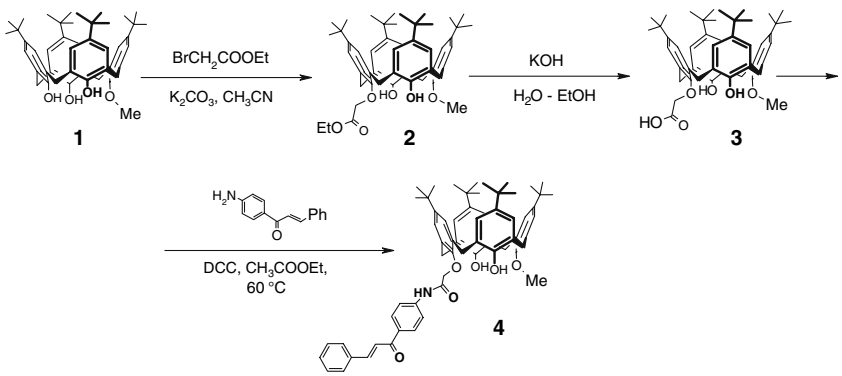
Amides **4**, **6**, **9a**,**b** were synthesized by the reaction of aminochalcones with calixarene carboxylic acids in the presence of DCC (method A) or with the acid chlorides (method B).

Calixarene monamide **4** was synthesized by the method A starting from monomethoxycalixarene **1** (Scheme 1). Calixarene **1** was alkylated with ethyl bromacetate in the presence of K_2CO_3 as the base, yielding 64% of monoester **2**. Further basic hydrolysis of **2** yields in monocarboxylic acid **3**. Coupling of monoacid **3** with 4′-aminochalcone in the presence of DCC in ethyl acetate at 60 °C after 24 h yields 77% of the monoamide **4**.

By the same procedure, the reaction of dicarboxylic acid **5** with 4′-aminochalcone yields 78% of calixarene diamide **6** (Scheme 2). Calixarene diamide **6** was also obtained with 63% yield by refluxing of acid chloride **7** and 4′-aminochalcone in benzene for 4 h in the presence of triethylamine (Scheme 2).

Calixarene tetraamides **9a**,**b** were synthesized by the methods A or B starting from tetraacid **8** or acid chloride **10** correspondingly. The reaction of acid chloride **10** with 4′-aminochalcone has been performed in refluxing toluene without a base. At the same time, the reaction of **10** with 3′-

Scheme 1 Synthesis of calixarene monoamide **4**



aminochalcone was carried out in refluxing benzene in the presence of triethylamine (Scheme 3).

Structure

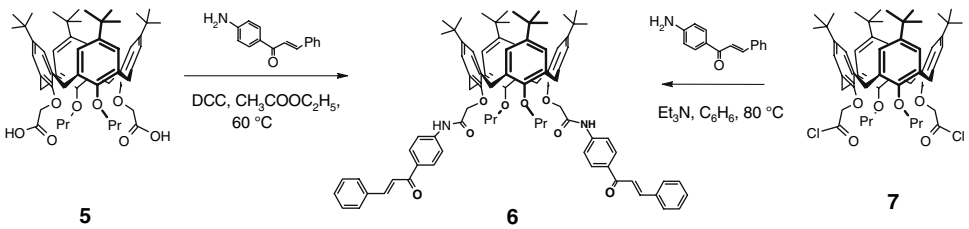
The structure of the amides **4**, **6**, **9a**,**b** was proved by ^{1}H NMR spectroscopy. In the NMR spectra characteristic signals of axial and equatorial protons of methylene bridges appear as doublets with an average coupling constant $^2J_{HH}$ = 13 Hz. In the spectrum of the C_s-symmetrical monoamide **4** the methylene bridges appear as four doublets. In the spectra of the C_{2v}- and C_{4v}-symmetrical diamide **6** and tetraamides **9a**,**b** the bridges are observed as one pair of doublets. The mentioned splitting pattern suggests that all the macrocycles are in the *cone* conformation. The distance between the resonance signals of the axial and equatorial protons of monoamide **4**, $\Delta\delta < 1$ ppm, evidences the *flattened cone* conformation of the macrocyclic skeleton with two opposite phenol rings oriented outwards the cavity. In contrast, for diamide **6** and tetraamides **9a**,**b** this value is more than 1 ppm, suggesting a *regular cone* conformation [33, 34]. Signals of NH protons of calixarenes **4**, **6**, **9a**,**b** appear as broad singlets in the area of 9.4–10.7 ppm, allowing to conclude these groups are hydrogen bonded.

Apparently, in the IR spectra of amides **9a**,**b** wide bands of associated NH (3320 cm^{-1}) and C=O (1663, 1668 cm^{-1}) are observed. These bands are not affected by dilution, this fact evidences that in the amides **9a**,**b** strong intramolecular hydrogen bonds are formed between the amide moieties.

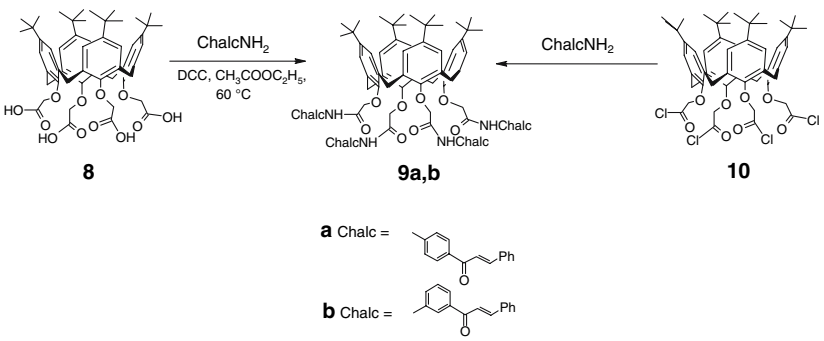
Calixarene effects on Mg^{2+},ATP-dependent Ca^{2+} transport in smooth muscle subcellular structures

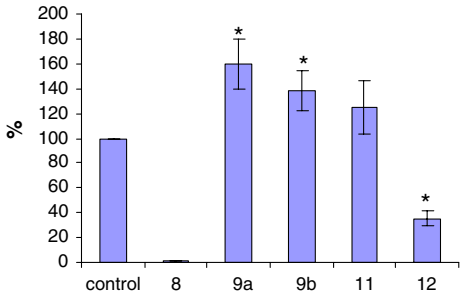
The Mg^{2+},ATP-dependent Ca^{2+} transport in smooth muscle mitochondria and sarcoplasmic reticulum was studied in the presence of the calixarenes **9a** and **9b**. As one can see

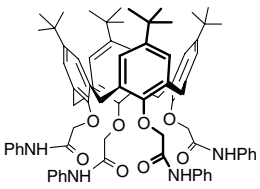
Scheme 2 Synthesis of calixarene diamide **6**



Scheme 3 Synthesis of calixarene tetraamides **9a,b**







11

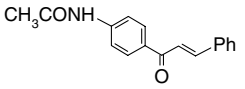


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Fig. 1 Calixarene effects on Mg^{2+},ATP-dependent Ca^{2+} accumulation in rat uterus smooth muscle mitochondria. Data are expressed as mean ± sem, $n = 3–5$. (*)—a value of $p < 0.05$ was considered significant. 100% is a control

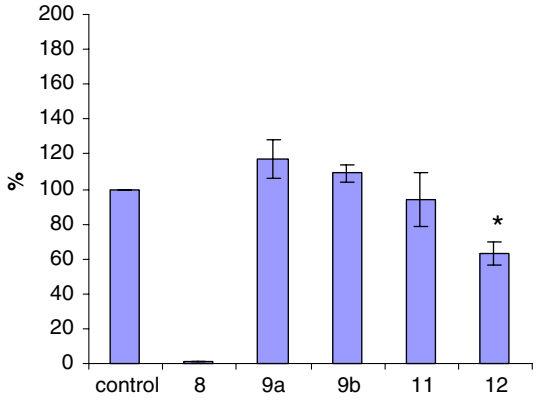

Fig. 2 Calixarene effects on Mg^{2+},ATP-dependent Ca^{2+} accumulation in rat uterus smooth muscle sarcoplasmic reticulum. Data are expressed as mean ± sem, $n = 3–5$. (*)–a value of $p < 0.05$ was considered significant. 100% is a control

on Figs. 1 and 2, calixarenes **9a** and **9b** stimulate Ca^{2+} accumulation in the mitochondria and do not modulate Ca^{2+} accumulation in the sarcoplasmic reticulum.

In order to estimate the effects of chalcone groups, the model compounds—calixarene anilide **11** and 4′-acetamidochalcone **12** have been studied. It was shown, that anilide **11** does not modulate Ca^{2+} accumulation in the mitochondria and sarcoplasmic reticulum. At the same time 4′-acetamidochalcone **12** inhibits cation accumulation in the mitochondria by 65% and in the sarcoplasmic reticulum by 35%. Acid **8** completely suppresses energy-dependent Ca^{2+} accumulation both as in the mitochondria so in sarcoplasmic reticulum.

Conclusion

In this work the synthesis of calixarene amides possessing biorelevant chalcone moieties is described. It was shown that chalconeamide derivatives of calix[4]arenes modulate Mg^{2+},ATP-dependent Ca^{2+} accumulation in the rat uterus smooth muscle subcellular structures. Calixarene chalconeamides could be used as tools for biochemical study of ion-transport mechanisms.

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