

Synthesis, physico-chemical properties and biological activity
of 1-(4-fluorophenyl)-4-[3-(2,3- and 4-
alkyloxyphenylcarbamoyloxy)-2-hydroxypropyl]
piperaziniumchlorides

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Abstract

1-(4-fluorophenyl)-4-[3-(2,3- and 4-alkyloxyphenylcarbamoyloxy)-2-hydroxy-propyl]piperaziniumchlorides, with one to four carbon atoms in the alkoxy group on aromatic ring have been synthesized as the derivatives of substituted phenylcarbamic acid. The structures were confirmed by their spectral data. Potential antiarrhythmic activity was evaluated in guinea-pigs model. Preliminary studies demonstrated that the evaluated compounds, using ouabain arrhythmia model, appear to possess only moderate antiarrhythmic activity. Only compound marked as **4f** appears to be more potent and lead us to focus our attention on structures with more bulky substituent in the *m*-position at aromatic ring in the hydrophilic part of molecule.

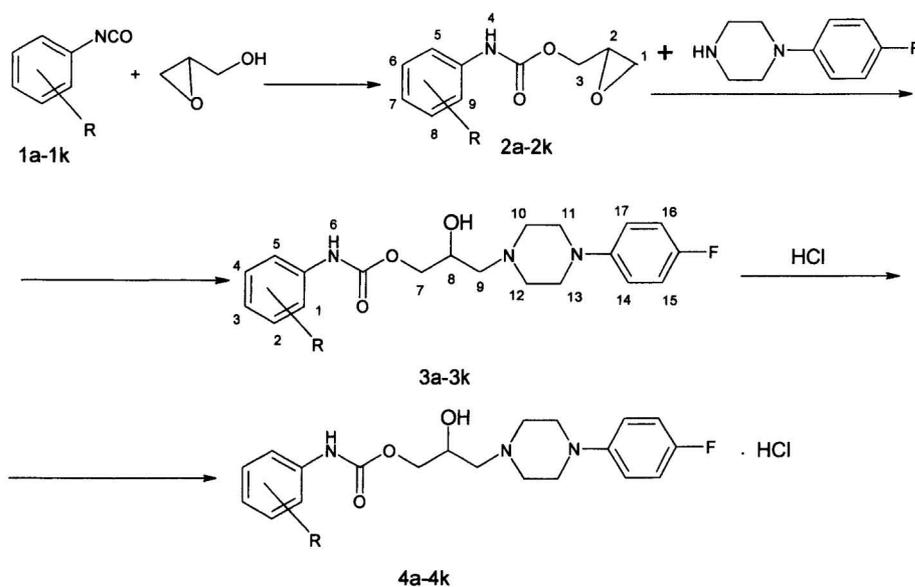
Keywords: phenylcarbamic acid derivatives, antiarrhythmic activity

Introduction

Many esters of alkoxyphenylcarbamic acids exhibit various, e.g. local anaesthetic [1, 2, 3], antiarrhythmic [4], antiulcer [5], antimycobacterial [6] and other activities [7]. In connection with systematic investigations in the group of basic substituted phenylcarbamates the attention was given to the preparation of the basic esters of alkoxy substituted phenylcarbamic acids. Some derivatives from phenylcarbamate compounds are assumed to possess, except of local anaesthetic activity, also antiarrhythmic and antihypertensive effects due to the presence of oxyaminopropanol (i.e. beta-adrenoreceptor blocking) and *N*-phenylpiperazine, or substituted *N*-phenylpiperazine, (i.e. vasodilatative) structural fragments in one molecule. The aim of this paper is synthesis, identification and determination of some physico-chemical properties of eleven novel phenylcarbamate derivatives. Four of them have been then evaluated on their potential antiarrhythmic activity.

Results and Discussion

The aim of our work was mainly synthesis of the new compounds and only some representatives have been chosen to inform us about their potential pharmacological activity. The compounds 1-(4-fluorophenyl)-4-[3-(2-,3- and 4-alkyloxyphenylcarbamoxy)-2-hydroxypropyl]piperaziniumchlorides with one to four carbon atoms in the alkoxy group on aromatic ring have been synthesized by reaction of (4-fluorophenyl)piperazin-1-yl with corresponding 2,3-epoxypropylesters of 2-, 3- or 4-alkoxysubstituted phenylcarbamic acids (**2a-2k**), which were prepared by reaction of 2,3-epoxypropan-1-ol with corresponding 2-, 3- or 4-alkoxysubstituted phenylisocyanates (**1a-1k**), and then isolated as the salts (**4a-4k**) with hydrochloric acid (Scheme) according to similar procedure which was described in the literature [8].

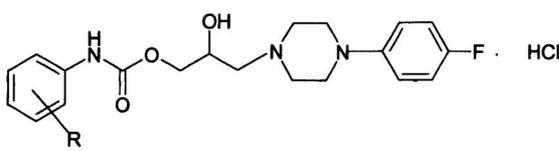


R=2-OCH₃ (1a,2a,3a,4a), 2-OC₂H₅ (1b,2b,3b,4b), 2-OC₃H₇ (1c,2c,3c,4c),
 3-OCH₃ (1d,2d,3d,4d), 3-OC₂H₅ (1e,2e,3e,4e), 3-OC₃H₇ (1f,2f,3f,4f),
 3-OC₄H₉ (1g,2g,3g,4g), 4-OCH₃ (1h,2h,3c,4h), 4-OC₂H₅ (1i,2i,3i,4i),
 4-OC₃H₇ (1j,2j,3j,4j),4-OC₄H₉(1k,2k,3k,4k)

Scheme. Synthetic protocol of the compounds, ring labelling of compounds (2a-2k and 3a-3k) from the point of ¹H-NMR spectra.

The methods of identification included the ultraviolet spectra (UV), infrared spectra (IR), mass spectra, ¹H-NMR spectra and thin-layer chromatography. The prepared phenylcarbamates were solid, colorless compounds and their m.p.'s and R_f's are designed in Tab. 1. The spectrum in ultraviolet area showed three λ maxima at 206 nm, 234 nm and 278 nm in distilled water. IR spectra of prepared compounds showed absorption bands around 3300 cm⁻¹ (N-H stretching, str.), 2600

cm^{-1} (N⁺-H str.), 1730 cm^{-1} (C=O str.), 1600 cm^{-1} (aromatic C-C str.) and 1200 cm^{-1} (NHC deformation). The mass spectra of prepared compounds revealed the presence of $[\text{M}+\text{H}]^+$ ions in all cases, the $^1\text{H-NMR}$ spectra of the synthesized compounds **4a-4k** revealed the presence of signals at 2.40 – 2.86 ppm range (t, 4H, H-10, H-12), 2.80 – 4.04 ppm range (t, 4H, H-11, H-13) and 6.82 – 7.26 ppm range (m, 8H, Ar C-H). All the compounds were checked for purity also by thin-layer chromatography. Lipophilicity of the evaluated compounds depends on the number of carbon atoms in the alkoxy chain as well as on the character of substituent added in the hydrophilic part of molecule. It was found that dependence of R_M values on lipophilicity is linear in all cases. These results contribute to quantitative structure-activity relationship studies in group of phenylcarbamate type compounds.

								
Compd.	R	Mol. formula	Mol. wt.	M.p. (°C)	Yield (%)	Solv. for recrystall.	R_f	R_M
4a	2-OCH ₃	C ₂₁ H ₂₇ O ₄ N ₃ FCl	439,91	163-168	66	propan-2-ol	0,52	-0,46
4b	2-OC ₂ H ₅	C ₂₂ H ₂₉ O ₄ N ₃ FCl	453,94	138-144	76	propan-2-ol	0,68	-0,09
4c	2-OC ₃ H ₇	C ₂₃ H ₃₁ O ₄ N ₃ FCl	467,97	145-152	65	propan-2-ol	0,78	0,24
4d	3-OCH ₃	C ₂₁ H ₂₇ O ₄ N ₃ FCl	439,91	196-199	76	propan-2-ol	0,35	-0,51
4e	3-OC ₂ H ₅	C ₂₂ H ₂₉ O ₄ N ₃ FCl	453,94	201-204	74	propan-2-ol	0,45	-0,19
4f	3-OC ₃ H ₇	C ₂₃ H ₃₁ O ₄ N ₃ FCl	467,97	137-142	79	propan-2-ol	0,64	0,03
4g	3-OC ₄ H ₉	C ₂₄ H ₃₃ O ₄ N ₃ FCl	481,99	154-157	75	propan-2-ol	0,74	0,18
4h	4-OCH ₃	C ₂₁ H ₂₇ O ₄ N ₃ FCl	439,91	195-200	80	propan-2-ol	0,32	-0,78
4i	4-OC ₂ H ₅	C ₂₂ H ₂₉ O ₄ N ₃ FCl	453,94	188-193	61	propan-2-ol	0,38	-0,61
4j	4-OC ₃ H ₇	C ₂₃ H ₃₁ O ₄ N ₃ FCl	467,97	197-201	74	propan-2-ol	0,58	-0,48
4k	4-OC ₄ H ₉	C ₂₄ H ₃₃ O ₄ N ₃ FCl	481,99	199-205	82	propan-2-ol	0,64	-0,15

Tab. 1. 1 - (4 - fluorophenyl) – 4 - [3 - (2 -,3 - and 4-alkyloxyphenylcarbamoxyloxy)-2-hydroxypropyl]piperaziniumchlorides

All evaluated compounds given intravenously at conc. $1 \cdot 10^{-6}$ mol.kg⁻¹ decreased heart rate which was measured during 5 min after application. Significant negative chronotropy was, however, noticed only after standard propaphenon and compound 4f. Similary, all evaluated compounds delayed or prevented the onset of extrasystoles, fibrillation and cardiac arrest. The most effective compound, except of propaphenon, seems to be 4f, which significantly increased consumption of ouabain needed to elicit extrasystoles and heart arrest. Summarized data are shown in Tab. 2.

Compound	Δ HR (% beats.min ⁻¹)	Ouabain consumption (μ g.kg ⁻¹)		
		Extrasystoles	Fibrillation	Heart arrest
Saline	-0,2 \pm 1,2	206,0 \pm 11,4	279,9 \pm 13,2	361,5 \pm 12,4
4b	-4,3 \pm 1,8	226,9 \pm 11,6	272,9 \pm 7,8	361,4 \pm 9,9
4c	-6,3 \pm 2,5	240,3 \pm 16,0	303,5 \pm 16,3	432,6 \pm 29,0
4e	-3,7 \pm 2,1	227,2 \pm 11,6	294,2 \pm 15,4	389,7 \pm 13,5
4f	-4,7 \pm 3,2***	267,0 \pm 10,8**	328,5 \pm 22,5	428,8 \pm 16,4*
Propaphenone	-35,9 \pm 1,4***	290,5 \pm 26,5*	374,4 \pm 19,1**	462,0 \pm 12,0***

Each value represents mean from 5-6 experiments \pm SEM.

* p<0,05; ** p<0,01; *** p<0,001 in comparison to saline

Tab. 2. Relative negative chronotropic effect of evaluated compounds at conc. $1 \cdot 10^{-6}$ mol.kg⁻¹ in guinea-pigs (Δ HR) and their protective effect against ouabain-induced arrhythmogenity.

Our preliminary studies demonstrated that the evaluated compounds, using ouabain arrhythmia model, appear to possess only moderate antiarrhythmic activity. Only compound 4f appears to be more potent and lead us to focus our attention on structures with more bulky substituent in the *m*-position.

Due to with problem with experimental animals the structure-activity relationships will be discussed in our next, more extensive paper. Our next studies would be also concentrated to evaluation of others, mainly antihypertensive activities of the compounds.

Experimental

Equipments: Melting points were determined using a Kofler hotplate apparatus (HMK Franz Kústner, Germany) and they are uncorrected. Spectra in UV region were measured on spectrometer 8452 A Diode Array Spectrophotometer, Vectra 286/12 Desk Jet 500 (Hewlett Packard), IR spectra (KBr disc) were recorded on FTIR spectrophotometer Nicolet Model Impact 410. $^1\text{H-NMR}$ spectra were scanned on spectrophotometer Varian Gemini 2000, 200 MHz, coupling constants are quoted ($^{\beta}\text{J}$) in Hz, chemical shifts (δ -scale) are quoted in parts per million and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. Mass spectra were recorded by mass spectrometer Agilent 1100, LC/MSD VL Trap with linear mechanic pump (KD Scientific, USA).

Due to a structural similarity of the prepared compounds only one type is characterized by spectroscopic data (3-alkyloxysubstituted compounds, metoxy- to butoxy-derivatives). Structure of prepared compounds **2d-2g**, **3d-3g** was checked by IR and $^1\text{H-NMR}$ spectra. MS and UV/VIS spectra of compounds **4d-4g** were measured.

Synthesis of compounds. 1 - (4 - fluorophenyl) – 4 - [3 - (2 -, 3 - and 4-alkyloxyphenyl-carbamoyloxy)-2-hydroxypropyl]piperaziniumchlorides (Tab.1) with number of carbon atoms in the alkyloxy chain ranging from one to four were prepared in a few steps.

2,3-epoxypropylesters of alkyloxy substituted phenylcarbamic acids

2,3-epoxypropylesters of corresponding alkyloxy substituted phenylcarbamic acids as the intermediate products were prepared by addition of 2,3-epoxypropan-1-ol

to corresponding alkoxyphenylisocyanates [8]. Mixture of these compounds was solved in benzene, p.a., then was heated 10h at $t=60^{\circ}\text{C}$. Purity and identity of prepared intermediates was checked by IR spectra and $^1\text{H-NMR}$ spectra. IR spectra (KBr disc): **2d**: 3283 cm^{-1} (N-H str.), 2956 cm^{-1} (C-H str.), 1728 cm^{-1} (C=O str.), 1600 cm^{-1} (aromatic C-C str.), **2e**: 3289 cm^{-1} (N-H str.), 2976 cm^{-1} (C-H str.), 1738 cm^{-1} (C=O str.), 1601 cm^{-1} (aromatic C-C str.), **2f**: 3298 cm^{-1} (N-H str.), 2974 cm^{-1} (C-H str.), 1732 cm^{-1} (C=O str.), 1599 cm^{-1} (aromatic C-C str.), **2g**: 3268 cm^{-1} (N-H str.), 2966 cm^{-1} (C-H str.), 1730 cm^{-1} (C=O str.), 1598 cm^{-1} (aromatic C-C str.), $^1\text{H-NMR}$ (200 MHz, CDCl_3): **2d**: 2,69 (t, 2H, H-1, $^{\beta}\text{J}=2,7\text{ Hz}$); 2,88 (t, 2H, H-3, $^{\beta}\text{J}=2,8\text{ Hz}$); 3,28 (m, 1H, H-2); 3,79 (s, 3H, O-CH₃); 6,6-6,9 (m, 4H, ArH); 7,27 (s, 1H, H-4, NH), **2e**: 1,40 (t, 3H, O-CH₂-CH₃, $^{\beta}\text{J}=7,0\text{ Hz}$); 2,68 (t, 2H, H-1, $^{\beta}\text{J}=2,6\text{ Hz}$); 2,88 (t, 2H H-3, $^{\beta}\text{J}=2,6\text{ Hz}$); 4,07 (q, 2H, O-CH₂-CH₃, $^{\beta}\text{J}=7,0\text{ Hz}$); 6,85-6,89 (m, 4H, ArH); 7,1 (s, 1H, H-4, NH), **2f**: 1,02 (t, 3H, O-CH₂-CH₂-CH₃, $^{\beta}\text{J}=7,2\text{ Hz}$); 1,39 (m, 2H, O-CH₂-CH₂-CH₃); 1,81 (m, 2H, O-CH₂-CH₂-CH₃); 2,69 (t, 2H, H-1, $^{\beta}\text{J}=2,9\text{ Hz}$); 2,87 (t, 2H, H-3, $^{\beta}\text{J}=2,9\text{ Hz}$); 3,20 (m, 1H, H-2); 6,59-7,18 (m, 4H, ArH); 7,14 (s, 1H, H-4, NH) **2g**: 0,99 (t, 3H, O-CH₂-CH₂-CH₂-CH₃); 1,45 (m, 2H, O-CH₂-CH₂-CH₂-CH₃); 1,65 (m, 2H, O-CH₂-CH₂-CH₂-CH₃); 1,80 (t, 2H, H-1); 2,8 (t, 2H, H-3); 3,6 (m, 1H, H-2); 3,80 (t, 2H, O-CH₂-CH₂-CH₂-CH₃); 6,40-7,15 (m, 4H, ArH); 7,05 (s, 1H, H-4, NH). After isolation and checking the identity and purity of 2,3-epoxypropylesters the 1-(4-fluorophenyl)piperazine compound was added. Mixture was heated 8h. The products obtained were purified by crystallization from appropriate solvent (presented in the Tab. 1). $^1\text{H-NMR}$ (200 MHz, CDCl_3): **3d**: 2,56 (t, 4H, H-10, H-12); 2,90 (t, 4H, H-11, H-13); 3,89 (s, 3H, OCH₃); 6,88-7,24 (m, 8H, ArH), **3e**: 1,40 (t, 3H, O-CH₂-CH₃); 2,52 (t, 4H, H-10, H-12); 3,15 (t, 4H, H-11, H-13); 3,72 (q, 2H, O-CH₂-CH₃); 6,88-7,23 (m, 8H, ArH), **3f**: 1,032 (t, 3H, O-CH₂-CH₂-CH₃); 2,55 (m, 2H, O-CH₂-CH₂-CH₃); 2,86 (t, 4H, H-10, H-12); 3,15 (t, 2H, O-CH₂-CH₂-CH₃); 3,14 (t, 4H, H-11, H-13); 4,04 (t, 4H, H-11, H-13); 6,89-6,97 (m, 8H, ArH), **3g**: 0,99 (t, 3H, O-CH₂-CH₂-CH₂-CH₃); 1,87 (m, 2H, O-CH₂-CH₂-CH₂-CH₃); 1,99 (m, 2H, O-CH₂-CH₂-CH₂-CH₃); 2,48 (t, 4H, H-10, H-12); 3,13 (t, 3H, O-CH₂-CH₂-CH₂-CH₃); 3,92 (t, 4H, H-11, H-13); 6,90-7,10 (m, 8H, ArH).

After addition of etheric hydrochloric acid solution the corresponding salts were isolated. Mass spectra (MS): **4d**: $m/e = 404,2$; **4e**: $m/e = 418,2$; **4f**: $m/e = 432,3$; **4g**: 446,2. UV/VIS spectra (distilled water, nm): **4d**: $\lambda_1 = 204$ nm ($\log \varepsilon_1 = 4,73$), $\lambda_2 = 234$ nm ($\log \varepsilon_2 = 4,36$), $\lambda_3 = 278$ nm ($\log \varepsilon_3 = 3,67$); **4e**: 204 (4,64), 234 (4,28), 278 (3,42); **4f**: 204 (4,52), 234 (4,12), 278 (3,31); **4g**: 204 (4,48), 234 (4,25), 278 (3,37).

R_f values from thin-layer chromatography were obtained in phase petrolether : diethylamine (2:1), R_M values were obtained in phase hydrochloric acid : acetone (1:1) after silica gel plates impregnation with 7% silica oil in heptan.

Ouabain-induced arrhythmias. Potential antiarrhythmic activity was evaluated in guinea-pigs, which were anesthetized i.p. with urethane ($1,5 \text{ g.kg}^{-1}$). Ouabain ($36 \mu\text{g.ml}^{-1}$) was infused into v. jugularis at a rate of $0,34 \text{ ml.min}^{-1}$. Five min. before starting ouabain infusion, compound solutions were given intravenously and using ECG (BTL-08MT, Medical technologie, Czech Republic) heart rate and onset of extrasystoles, fibrillation and heart arrest were registered. From the consumption of ouabain, which was needed for the particular heart disturbances, correspondent doses were calculated.

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