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ACTA FACULTATIS PHARMACEUTICAE UNIVERSITATIS COMENIANAE

STUDY OF PHYSICO-CHEMICAL PROPERTIES OF POTENTIAL BETA-ADRENOLYTICS

ŠTÚDIUM FYZIKÁLNO-CHEMICKÝCH VLASTNOSTÍ POTENCIÁLNYCH BETA-ADRENOLYTÍK

Original research article

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Abstract The aim of this paper is the study of physico-chemical properties of the chosen compounds, derivatives of 2-hydroxy-3-[2-(4-methoxyphenyl)ethylamino]propyl-4-[(alkoxycarbonyl)amino]benzoates and 2-hydroxy-3-[2-(2-methoxyphenyl)ethylamino]propyl-4-[(alkoxycarbonyl)amino]benzoates with potential ultra-short beta-adrenolytic activity. The studied compounds are different in the position of the substituent on the benzene ring in the side chain as well as in the aromatic ring in position 4 with alkyl- (methyl- to butyl-) carbamate. The physico-chemical characteristics, for example, lipophilicity, surface activity, adsorbability, acidobasic properties etc., are very important for the explanation of the relationship between structure and biological activity of the drug. These parameters serve as the base of quantitative structure-activity study. The goal of this work is to establish the spectral characteristics of studied compounds in UV-area, pK_a values, the parameters of lipophilicity (the values of R_f and R_M from thin layer chromatography, retention time t'_R and capacity factor k´ from liquid chromatography and experimental partition coefficients log P´ values), surface tension, critical micelle concentrations, the adsorbability of compounds expressed by percent of adsorbed compound on active charcoal β% as well as by Freundlich adsorption isotherms. The obtained values are correlated with the parameters characterising the size of molecule, for example, the number of carbon atoms on carbamate functional group.

Slovak abstract Zámerom práce je štúdium fyzikálno-chemických vlastností vybraných látok, derivátov 2-hydroxy-3-[2-(4-metoxyfenyl)-etylamino]-propyl-4-[(alkoxykarbonyl)-amino]-benzoátov a 2-hydroxy-3-[2-(2-metoxyfenyl) etylamino]-propyl-4-[(alkoxykarbonyl)amino]-benzoátov s potenciálnym ultra-krátkym beta-adrenolytickým účinkom. Študované látky sa líšia v polohe substituenta na benzénovom kruhu, v bočnom reťazci, ako aj na aromatickom kruhu v polohe 4- s alkyl (metyl- až butyl-) karbamátom. Fyzikálno-chemické charakteristiky, ako lipofilita, povrchová aktivita, schopnosť adsorpcie, acidobázické vlastnosti, atď. sú veľmi významné pre objasnenie vzťahu medzi štruktúrou a biologickou aktivitou liečiva. Tieto parametre slúžia ako základ pre štúdium QSAR. Cieľom práce je stanovenie spektrálnych charakteristík v UF oblasti, hodnôt pK_a, parametrov lipofility (hodnoty R_f a R_M, z chromatografie na tenkej vrstve, retenčný čas t'_R a kapacitný factor k´ z kvapalinovej chromatografie a experimentálne rozdeľovacie koeficienty), povrchového napätie, kritickej micelovej koncentrácie c.m.c., schopnosť adsorpcie látok na aktívne uhlie, vyjadrené percentom adsorbovaného množstva β % ako aj Freundlichovou adsorpčnou izotermou. Získané hodnoty sú korelované s parametrami charakterizujúcimi veľkosť molekuly, napr. počet atómov uhlíka v karbamátovej funkčnej skupine.

Keywords *ultra-short-acting beta-blockers, lipophilicity, adsorbability, critical micelle concentration*

Kľúčové slová: *ultra krátko účinné beta-blokátory, lipofilita, adsorbabilita, kritická micelová koncentrácia*

INTRODUCTION

The studied compounds are derivatives of [(arylcarbonyl)oxy] aminopropanol, with carbamate substitution on benzene ring. The traditional isopropyl or tertiary butyl residues on basic nitrogen atom are replaced with bulky 2- or 4-(methoxyphenyl) ethylamine group. Such structural change enhances the lipophilic character of the molecule. The introduction of a bulky substituent on the N-atom can lead to compounds with significant β^1-adrenoreceptor selectivity (Graham, 2009 & Griffith, 2003). The compounds were prepared at the Department of Chemical drugs of the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic (Mokrý *et al*., 2011). These drugs are potential ultra-short-acting beta-adrenergic

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receptor blocking agents. The short duration of action is realised by incorporation of a metabolically unstable ester group on the benzene ring (Jackman *et al*. 2002). The list of studied compound is in Table 1. The stability studies of the compounds were examined in acidic and alkaline media, in buffers and due oxidation at room and at elevated temperature chromatographically and R_f values of incipient products and degradation products were detected. Kinetics of acid and base hydrolysis in various solutions at temperature 80°C and 100°C was examined through ultraviolet-area spectrophotometry. Kinetic parameters such as rate constant *k*, half-life period $t_{1/2}$ and usable life t_{90} were determined (Stankovičová *et al*., 2012). For clarification of relationships between structure and biological activity of potential drugs, it is necessary to state their physicochemical parameters that are related to biological effect such lipophilicity, surface activity, capability of binding on phase boundary, and acidobasic properties. These parameters serve as the base of quantitative structure-activity relationship study, which leads to the projection of new drugs.

MATERIALS AND METHODS

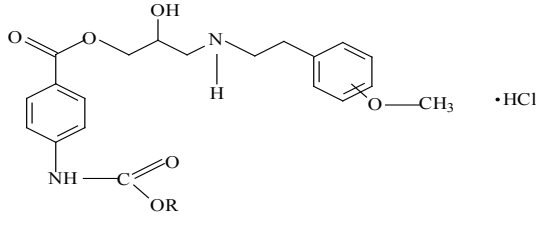
Studied compounds

The studied compounds were synthesised as hydrochloride by authors (Mokrý *et al*., 2011) and they are listed in Table 1. All other chemicals and solvents were of analytical reagent grade.

Apparatus

The Thermostat Memmert WB 10 (Germany), UV-VIS spectrophotometer Shimadzu UV-1800 (Japan), HPLC system consisting of high pressure pump DeltaChrom SDS 030 Watrex (Slovakia), injector loop with volume 20 µl Watrex (Slovakia), column Sepharon SDX C18 with size 250 × 4 mm Watrex (Slovakia), size of grain 7 µm, flow UV detector Delta Chrom UVD 200 Watrex (Slovakia), Camag Universal UV Lampe TL-900N (Switzerland), InoLab pH 720 WTW Series pH meter (Germany), WTW pH-Electrode SenTix 61 (Germany).

Table 1. List of the studied compounds



Compound	R	Position in ring	Mr
1	CH3	4-OCH3	438.91
2	C2H5	4-OCH3	452.94
3	C3H7	4-OCH3	466.96
4	C4H9	4-OCH3	480.99
5	CH3	2-OCH3	438.91
6	C2H5	2-OCH3	452.94
7	C3H7	2-OCH3	466.96
8	C4H9	2-OCH3	480.99

Spectral characteristics of compounds

Ultraviolet spectra of water solutions of compounds were measured in the region from wavelength 200 to 450 nm. The solutions of compounds ($c = 1 \times 10^{-5}$ mol/l) were prepared by diluting of storage solution of compound in methanol c = 0.1% to the volume 100.0 ml by distilled water. The value of absorbance at each maximum was recorded, the values ε ($m^2 \cdot mol^{-1}$) and $A^{1\%}_{1cm}$ were calculated.

Thin-layer chromatography

The chromatographic separation of compounds was carried out on silica gel layer Silufol® UV 254 15 × 15 cm. 5 µl of examined solution of 0.1% solution of compounds in methanol was applied on the plate. Development distance was 10 cm. A UV light lamp Camag was used for detection at wavelength λ = 254 nm. Chromatographic system S1: petroleum ether/diethyl-amine (6.0/3.0 v/v), saturation of chromatographic chamber 20 min, chromatographic system S2: hexane/diethyl-amine (6.0/4.0 v/v) under the same conditions.

Liquid chromatography

The mobile phase was 90% methanol; pH value adjusted to 6.0 by addition of sodium acetate. The separation was carried out under pressure 7.9 MPa at flow rate 0.6 ml/min and the column temperature was maintained at 25°C. The chromatograms were scanned at wavelength 272 nm. The injection volume was 20 µl. The analytes were dissolved in methanol ($c = 5 \times 10^{-4}$ mol/l) and were applied three times.

Experimental partition coefficient (P´)

The experimental partition coefficient of studied compounds was measured by the shake-flask method in octan-1-ol/phosphate buffer medium at pH = 7.0 using 0.2 ml organic solvent and 10.0 ml of phosphate buffer solution of analysed compound ($c = 2.3 \times 10^{-5}$ mol/l). The concentration of compounds in the aqueous phase after shaking (1 h) and equilibration (1h) was determined spectrophotometrically at wavelength 270 nm.

Relative surface activity

The relative surface activity of compounds 1 – 4 was determined at temperature 22°C by Traube stalagmometric method. Activity of the water solutions of the compounds $c = 1 \times 10^{-3}$ mol/l was measured. The reference solution was distilled water.

Critical micelle concentration

The critical micelle concentration (c.m.c.) of compounds 5 – 8 was determined spectrophotometrically at wavelength 270 nm. The storage solution of compound in methanol c = 0.1% was diluted to volume 25.0 ml by distilled water. The concentration of prepared solutions was from $c = 1.1 \times 10^{-5}$ mol/l to $c = 1.95 \times 10^{-4}$ mol/l. All the measurements were performed at temperature 22°C. The calculation of c.m.c. value was realised from the point of intersection of the two extrapolated linear parts of dependence of absorbance vs concentration.

Dissociation constant (pKa)

The pKa values of compounds were determined by the ultraviolet spectrophotometric method. Absorbance values of the solutions of compounds were measured in NaOH (c = 0.1 mol/l), HCl (c = 0.1 mol/l), and six trometamol buffer solutions with pH values 7.2, 7.4, 7.6, 8.0, 8.4 and 9.0 at wavelengths that were responsible for the maximum difference between absorbance values in acidic and alkaline medium. Values of pKa were calculated according to Henderson and Hasselbach equation. The concentration of measured solutions was c = 2.0 × 10^{-5} mol/l. These were prepared by dilution of storage solutions of compounds in methanol c = 0.1% to volume 25.0 ml by buffer solutions or NaOH and HCl solutions, respectively.

Adsorption study

The activated carbon was first washed three-times (for 15 min by shaking) with hot distilled water and dried at 110°C for 2 days and then kept in a desiccator containing silica gel. The adsorption of studied compounds 1, 3 and 4 was estimated at pH 7.0 (phosphate buffer). The flasks with 50.0 ml of studied compound solution and with 2 mg of activated carbon were shaken at temperature 20°C for 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0 and 2.5 h. The time duration of another adsorption study was 90 min. The original concentration of studied compounds in buffer solution varied from 4.0 × 10^{-5} mol/l for time dependence study, and from 1.8 × 10^{-5} mol/l to 5.1 × 10^{-5} mol/l. for concentration dependence study. After equilibration, the sample was filtered through thick filter paper. The compound content in the filtrate solution was determined spectrophotometrically in the UV region at wavelength 272 nm. The measured solutions of compounds were prepared by dilution of the storage solution of compound in methanol c = 0.1%.

CALCULATIONS

Retardation factor Rf and RM value

$$R_f = \frac{b}{a} \tag{1}$$

$$R_M = \log\left(\frac{1}{R_f} - 1\right) \tag{2}$$

a = migration distance of the solvent front,
b = migration distance of the analyte

Capacity factor k´

$$k' = \frac{t_R - t_M}{t_M} \tag{3}$$

t_R = retention time, t_M = hold-up time

Specific absorbance $A^{1\%}_{1cm}$

$$A^{1\%}_{1cm} = \frac{10\varepsilon}{M_r} \tag{4}$$

ε = molar absorptivity, M_r = relative molecular mass

Experimental partition coefficient P´

$$P' = \frac{(1000 \times m) - (a \times c_{H2O} \times M_r)}{b \times c_{H2O} \times M_r} \tag{5}$$

m = weight of compound in solution (g), a = volume of aqueous phase (ml), b = volume of organic phase (ml) Mr = relative molecular weight, c_{H2O} = concentration of compound in aqueous phase after shaking

Critical micelle concentration

straight line $y_1 = a\,x_1 + b$ and straight line $y_2 = c\,x_2 + d$

$$c.m.c. = \left|\frac{b-d}{c-a}\right| \tag{6}$$

pK_a value

$$pK_a = pH + \frac{A-B}{B-C} \tag{7}$$

pH = pH value of measured solution, A = absorbance in NaOH 0.1 mol/l, B = absorbance in buffer solutions, C = absorbance in HCl 0.1 mol/l

Percent of adsorbed compound β

$$\beta = \frac{100 \times C_v}{c_v + c_{eq}}\% \tag{8}$$

c_v = concentration of adsorbed compound (mol/l),
c_{eq} = concentration of unbounded compound (mol/l)

Freundlich adsorption constants k, N

$$\log m = \log k + 1/N \times \log c \tag{9}$$

c = equilibrium solute concentration in the bulk (mg/l), m = amount of solute adsorbed (mg/g), k, N = *Freundlich constants*

The linear regression equation

$$y = a_0 + a_1 x \tag{10}$$

was computed by the least squares techniques, where a_0 is intercept, a_1 is slope.

RESULTS AND DISCUSSION

The studied compounds are derivatives of 2-hydroxy-3-[2-(4-methoxyphenyl)ethylamino]propyl-4-[(alkoxycarbonyl)amino]-benzoates and 2-hydroxy-3-[2-(2-methoxyphenyl)ethylamino] propyl-4- [(alkoxycarbonyl)amino]benzoates where the aromatic ring is substituted in para position with alkyl (methyl to butyl) carbamate group. The substances were prepared by

five-step synthesis. The final bases were converted to their hydrochloride salts. The structure of prepared compounds was confirmed by ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy as well as Fourier transformation infrared spectrophotometry and mass spectroscopy. The purity of prepared compounds was examined by means of thin layer chromatography (Mokrý *et al*., 2011).

In this paper, the substances were characterized by ultraviolet spectra. The values log ε were determined as well as the specific absorbance $A^{1\%}_{1cm}$. Ultraviolet absorption spectrum of aqueous methanol solutions of studied compounds between 200 and 450 nm have two absorbance maxima. 2-methoxyderivatives have absorbance maximum λ_{max1} around 270 nm and λ_{max2} around 212 nm, 4-methoxyderivatives – λ_{max1} around 272 nm and λ_{max2} around 218 nm. The values of molar absorption coefficients ε and specific absorbance $A^{1\%}_{1cm}$ are in Table 2. The absorption bands of the 2-methoxyderivatives are stronger than that of 4-methoxyderivatives. The values of absorption coefficients are used for identification of compounds and values of specific absorbance are used in quantitation of compounds.

Chromatographic parameters, the R_f and R_M values from TLC for two mobile phases are in Table 2. In both the systems, the stationary phase is more polar than the mobile phase, the R_f values rise with the number of carbon atoms, the R_M values decrease, which is in accordance with the lipophilicity of compounds. The R_f values of 2-methoxyderivatives are higher than R_f values of 4-methoxyderivatives in both systems.

Lipophilicity of studied compounds was evaluated by HPLC method with reverse-phase system. Chemically modified silica gel with bounded octadecyl groups was used as nonpolar stationary phase. The most favourable mobile phase was methanol 90% with adjusted pH value by sodium acetate at pH = 6.0. The t'_R as well as k´ values of compounds rise with the height of relative molar weight Mr. The 2-methoxyderivatives in comparison with 4-methoxyderivatives show these higher values. The parameters obtained represent the efficiency of interaction of compound with hydrophobic surface of stationary phase as well as their lipophilicity, which rise with increase of alkyl chain of molecule.

The transport of a drug in biological system depends upon its partition coefficient. The experimental partition coefficient P´ is influenced by the ratio of total concentrations of drug in lipid and aqueous phases; however, the drug is present in several forms. The values of experimental partition coefficients log P´of studied compounds are between 1.48 and 2.47 (Table 3). The dependence of log P´ vs number of carbon atoms is linear for 2-methoxyderivatives; these values are in accordance with R_M and log k´ values. The theoretical values of log P for basic compounds were obtained by several computation techniques, the values MLOGP were the nearest to the experimental. Results of regression analysis are in Table 4.

The pK_a value of a compound is, formally, the negative logarithm of its acid dissociation constant K_a. The pKa value is a convenient numerical way to compare the relative acidity or basicity of weakly ionising compounds in aqueous or miscible solvent-aqueous solutions (Newton & Kluza, 1978). The pKa values of studied compounds (Table 3) determined spectrophotometrically are between 8.29 and 9.59, which is in accordance with that of β-adrenolytics such as pindolol (8.8), practolol (9.5) and propranolol (9.45) (Newton &Kluza, 1978).

The values of relative surface activity γ [Nm^{-1}] of studied 4-methoxyderivatives determined by Traube stalagmometric method are in Table 3. Because this method was not suitable for determination of concentration dependence of surface activity to evaluate c.m.c. we have chosen ultraviolet spectrophotometry for this. The values of c.m.c. of 2-methoxyderivatives are in Table 3. The dependence of surface activity vs number of carbon atoms is not linear in both groups of compounds.

Table 2. UV spectral characteristics and chromatographic parameters of the studied compounds

Compound	UV spectral characteristics			Chromatographic parameters			
	λ_{max} nm	ε m^2 mol^{-1}	$A^{1\%}_{1cm}$	R_M		t'_R min	log k´
				S_1	S_2		
1	271	2379	542	0.31	0.43	0.71	-0.149
	218	1514	345				
2	271	2148	474	0.14	0.23	0.79	-0.102
	218	1332	294				
3	272	2339	501	-0.02	0.09	0.91	-0.041
	218	1482	317				
4	272	2265	471	-0.27	-0.05	1.05	0.021
	219	1518	316				
5	270	2962	675	0.21	0.37	0.75	-0.125
	212	2461	561				
6	271	3116	688	0.02	0.21	0.83	-0.081
	212	2324	513				
7	271	2526	541	-0.12	0.10	0.95	-0.022
	215	1521	326				
8	271	2470	513	-0.31	0.03	1.10	0.041
	214	1540	320				

Table 3. The results of the physico-chemical parameters of studied compound

Parameters of lipophilicity, dissociation constants, surface tension and critical micelle concentration						Results of adsorption study		
Compound	**log P´**	**MLOGP**	**pKa**	**Surface tension γ [N m^{-1}]**	**Critical micelle conc. [mol/l×10^4]**	**β %**	**k**	**N**
1	1.48		8.78	0.0733		60.5	32.6	1.150
2	1.92		8.55	0.0741				
3	2.47		8.95	0.0718		63.5	31.93	1.188
4	2.11		9.34	0.0704		75.2	36.34	1.204
5	1.77	1.74	9.59		1.237			
6	1.95	1.96	8.61		1.174			
7	2.09	2.18	8.29		1.185			
8	2.26	2.39	8.43		1.167			

Table 4. Results of the regression analysis

Function	**R_M = f (C) a)**	**R_M = f (C) b)**	**log k´= f (C) a)**	**log k´= f (C) b)**	**log P′ = f (C) a)**	**log P′= f (C) b)**
n	4	4	4	4	4	4
r	0.984	0.996	0.997	0.999	0.999	0.948
F	61.004	231.06	322.12	831.83	960.04	4.400
s	0.0319	0.232	0.0069	0.0057	0.0116	0.2281
a_0	0.460	0.570	-0.186	-0.210	1.615	0.385
a_1	-0.113	-0.158	0.0557	0.0571	0.161	1.244
a_2						-0.200

a) 2-methoxyderivatives; b) 4-methoxyderivatives,
n is the number of points, r is the correlation coefficient, s is the standard deviation, F is the F-test.

Active charcoal is one of the important adsorbents capable of binding on their surface other substances in relatively large amounts. This property is often used in pharmacy as well as in the study of structures-biological activity relationships, where activated carbon serves as a model substance for the study of hydrophobic interactions (Abe *et al*., 1990). The activated carbon adsorption property (expressed by the partition coefficient of drugs at infinite dilution) was successfully correlated with drug potencies of local anaesthetics as a parameter for the QSAR (Abe *et al*., 1990 & Abe *et al*., 1988). Carbon surface adsorption often follows the Freundlich adsorption isotherms (Abe *et al*., 1988 & Tanada *et al*., 1997) in which the logarithm of the adsorbed amount bears a linear relationship with the logarithm of the free drug concentrations. The aim of this work is investigation of the adsorption property of the 4-methoxyderivatives compared to active charcoal according to Freundlich model and according to the amount of the bound substance β percents dependending on time. The results are in Table 3. The adsorption of these compounds shows the rise of adsorbed amount depending on time with maximum in time between 80 to 120 min. The time of duration of another adsorption studies was 90 min. The adsorption of compounds rises with number of carbon atoms as well as with relative molecular weight (the size of molecule). The Freundlich model of adsorption was employed to evaluate the course of adsorption in dependence on the concentration of substances.

CONCLUSION

The obtained results of physico-chemical evaluation of ultra-short-acting beta-adrenolytics may serve as the base for investigation of new drugs in this group of compounds. A study like this was not performed on such compounds. The studied and determined physico-chemical parameters of lipophilicity, surface activity, capability of binding on phase boundary, provide a measure for interfacial hydrophobic – hydrophilic interactions that play, together with the acidobasic properties, an important role in various biological processes and may be used as a convenient tool in the QSAR study, investigating drug actions. The work by such experiences also expands the knowledge about derivatives of phenylcarbamic acid.

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