

SYNTHESIS AND ENANTIOSEPARATION OF DERIVATIVES  
OF PROPRANOLOL

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Propranolol is one of the first prepared and in therapeutic praxis used beta- adrenolytics. In this paper novel derivatives of propranolol with cyclohexylamino and pyrrolidin-1-yl groups in hydrophilic part were prepared. HPLC-enantioseparation propranolol (as reference compound) and of the prepared derivatives has been achieved using a Chiralpak AD CSP based on the amylose tris (3,5-dimethylphenylcarbamate). (*R*)-enantiomer of the propranolol was prepared by stereoselective synthesis using Jacobsen catalyst.

**Keywords:** propranolol –  $\beta$ -blockers – enantioseparation – HPLC – Chiralpak AD CSP

## INTRODUCTION

Propranolol is the nonselective adrenergic antagonist ( $\beta_1$ ,  $\beta_2$  - blocker) and can be used in the treatment of angina pectoris and hypertension.

This drug can be also used in the treatment or prevention hyperthyroidism, migraine, pheochromocytoma, menopause, and anxiety (Crowter&Smith, 1968, Florenz et al., 1969, Čižmariková, 2002).

Several of derivatives of propranolol with different substitution in hydrophilic part of molecule (piperazine, piperidine, 2-methylpiperidine and morpholine moieties) were studied on the anticonvulsive activity (Fischer, 2002, Lohman, 1990).

Propranolol and its derivatives present an aryloxyaminopropanol structure and have been prepared in two steps from  $\alpha$ -naphthol. Reaction of  $\alpha$ -naphthol with chloromethyloxirane was obtained 1-naphthoxymethyloxirane, which in the next step with amines gives racemic bases (Bruchatá et al., 2006, Eshghi&Porkar, 2003).

Propranolol is chiral molecule and their (*S*)-(-)-enantiomer is 100 more active than (*R*)-(+)-enantiomer [9]. Moreover (*R*)-(-)-propranolol is known to act as a contraceptive (Bevinakatti&Banerji, 1991).

The most widely used technique for separation and quantification of enantiomers is enantioselective HPLC.

Chiral separation by liquid chromatography can be performed either indirectly using achiral derivatization agent forming diastereoisomeric pairs, which can be resolved under a chiral conditions or directly using chiral stationary phases (Bojarski, 2012). There are many of chiral selectors used for separation of  $\beta$ -blockers. Chiral separation of propranolol and analogues can be performed using different chiral stationary phases with proteins (Bojarski, 2012), cyclodextrins (Mathett et al., 1996), and/or cellulose tris(substituted phenylcarbamate) (Aboul-Enein&Bakr, 1998), macrocyclic antibiotics as vancomycin, teicoplanin (Čižmáriková et al., 2005).

(*R*)-propranolol was prepared by stereoselective synthesis using the Jacobsen catalyst. Racemic 1-naphthoxymethyloxirane reacted with catalyst [(*R*, *R*)-N, N'-bis (3,5-di-*tert*-butylsalicylidene)-1,2 diaminato-cyclohexane-(2 -)] cobalt and by kinetic resolution gives the (*S*)-1-naphthoxymethyloxirane and the (*R*)-1-naphthoxypropane-1,2-diol. In the following reaction enantioselective 1-naphthoxymethyloxirane with isopropylamine gives (*R*)-(+)-propranolol.

## EXPERIMENTAL

The melting points were determined using a Kofler Micro Hot Stage and were quoted uncorrected. The purity of the newly prepared compounds was assessed using silicagel plates UV 254 (Merck) and the solvent system of ethylacetate:diethylamine (9:1 v/v) was used. UV spectra were run on spectrophotometer Hewlett-Packard 8452 in methanol. IR spectra were recorded using FTIR IMPACT 400 D (Nicolett) spectrometer in KBr tablets.  $^1\text{H}$  NMR were recorded on the Varian Gemini 2000 Spectrometer operating at 300 MHz for protons.

### Synthesis

Isopropylamino-3-(1-naphthoxy)propan-2-ol, 1-cyclohexylamino-3-(1-naphthoxy)propan-2-ol, 1-pyrrolidin-1-yl-3-(1-naphthoxy)propan-2-ol, were prepared reaction obtained 1-naphthoxyoxirane intermediate with amines (isopropylamine, cyclohexylamine and pyrrolidine) according to [7,8].

Stereoselective (*R*)-enantiomer of propranolol was prepared using Jacobsen catalyst according to [15]. Yield 55 %. M.p. 86-88 °C,  $[\alpha]_{\text{D}}^{20} = -12.5^\circ$  (1%  $\text{CH}_3\text{OH}$ ).

### HPLC-chromatography

#### Instruments

HPLC system AGILENT 1200, consisting of a quaternary pump and diode detector. High-performance liquid chromatography (HPLC) was carried out using the chiral stationary phases (Chiralpac AD) based on the amylose tris (3,5-dimethylphenylcarbamate) (0.46x25). The mobile phase consisted of hexane/ethanol/methanol/diethylamine 78/11/11/0.1, 80/3.75/11.25/0.1, 80/10/10/0.1, 85/2.5/7.5/0.1, 85/3.75/11.25/0.1, 90/2.5/7.5/0.1 v/v/v/v. Samples for analysis were prepared as approximately 1mg .ml<sup>-1</sup> solution in mobile phase. Separation were carried out at flow rate of 0.8 ml /min and

the column temperature was maintained at 25 °C. Chromatograms were scanned at wavelength 267nm ±8nm.

#### Chromatographic characteristics

The separation factor was expressed as  $\alpha = k_2/k_1$ , where  $k_1, k_2$  are retention factors for the first and second eluting enantiomer. The retention factor  $k$  was calculated as follows.  $k_1 = (t_1 - t_0)/t_0$  and  $k_2 = (t_2 - t_0)/t_0$ , where  $t_0, t_1$  and  $t_2$  are the dead elution time and elution time of enantiomers 1 and 2. The stereochemical resolution factor ( $R_s$ ) of the first and second eluting enantiomer was calculated by the ratio of the difference between the retention times  $t_1$  and  $t_2$  to the arithmetic mean of the two peaks widths  $w_1$  and  $w_2$ :

$$R_s = 2(t_2 - t_1) / (w_1 + w_2).$$

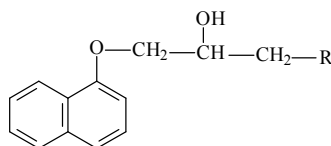
#### Chemicals



All HPLC grade solvents were obtained from Merck (Germany).



## RESULTS AND DISCUSSION

The aim of this study was to prepare new derivatives of propranolol (1-isopropylamine-3-(1-naphthoxy)propan-2-ol) with cyclohexylamino and pyrrolidin-1-yl in basic part of molecule. New compounds were prepared by a two-step synthesis from  $\alpha$ -naphthol. 1-Naphthoxymethyloxirane intermediate prepared by the reaction  $\alpha$ -naphthol with chloromethyloxirane with amine (cyclohexylamine, pyrrolidine) gives final products. These were isolated in the form of free bases or salts with fumaric acid (Table 1).

**Table 1. Physico-chemical parameters of prepared compounds**



Compound	R Form of compound	Summary formula vzo- rec	Mr [g/mol]	M.p. [°C]	Yield [%]
I	NHCH(CH <sub>3</sub> ) <sub>2</sub> base	C <sub>16</sub> H <sub>21</sub> O <sub>2</sub> N	259.35	93-95 <sup>C</sup> A	77 0.37
Ia	NHCH(CH <sub>3</sub> ) <sub>2</sub> fumarate	C <sub>36</sub> H <sub>46</sub> O <sub>8</sub> N <sub>2</sub>	634.77	182-184 B	
II	HN-  base	C <sub>15</sub> H <sub>23</sub> O <sub>2</sub> N	249.35	84-86 A	80 0.46
Ila	HN-  fumarate	C <sub>42</sub> H <sub>56</sub> O <sub>8</sub> N <sub>2</sub>	716.91	175-177 B	

III	 base	C <sub>17</sub> H <sub>21</sub> O <sub>2</sub> N	271.36	71-72 A	73 0.59
IIa	 fumarate	C <sub>38</sub> H <sub>46</sub> O <sub>8</sub> N <sub>2</sub>	658.79	134-136 B	

a- hexane, b-ethylacetate c- m.p.= 95 (Schaus et al., 2002)

The purity of the final products was checked by TLC in mobile phase ethylacetate:diethylamine (9:1 v/v). Structures of prepared compounds were confirmed by IR, UV and <sup>1</sup>H-NMR spectra (Tables 2, 3, 4). The stretching vibration of the characteristic group in the IR spectra were  $\nu_{(\text{OH})}$ ,  $\nu_{(\text{NH})}$  (base),  $\nu_{(\text{C}=\text{C})}$ ,  $\nu_{(\text{C}=\text{Ar})}$  (Table 2).

**Table 2. Values of  $\lambda_{\text{max}}$  and  $\log \epsilon$  in UV spectra,  $\epsilon = \text{m}^2 \text{mol}^{-1}$**

Compounds	$\lambda_{\text{max 1}}$ [nm]	$\log \epsilon_1$	$\lambda_{\text{max 2}}$ [nm]	$\log \epsilon_2$	$\lambda_{\text{max 3}}$ [nm]	$\log \epsilon_3$
I	290	2.74	230	3.48	214	3.62
II	292	2.82	230	3.53	214	3.66
III	288	2.83	230	3.47	214	3.61

The UV spectra of bases display bands corresponding to  $\pi \rightarrow \pi^*$  transition at  $\lambda_{\text{max}} = 214$ -290 nm,  $\log \epsilon = 2.74$ -3.66 (Table 3).

**Table 3. Values of stretching vibration in IR spectra of prepared bases**

Compounds	$\nu (\text{OH})$ [cm <sup>-1</sup> ]	$\nu (\text{NH})$ [cm <sup>-1</sup> ]	$\nu (\text{CH})$ [cm <sup>-1</sup> ]	$\nu (\text{C}=\text{C})$ [cm <sup>-1</sup> ]	$\nu (\text{ArOalk})$ [cm <sup>-1</sup> ]
I	3427	3269	2924-2963	1582, 1596, 1630	1242
II	3417	3278	2927	1582, 1596, 1630	1241
III	3421		2952, 2875, 2818	1578	1239

<sup>1</sup>H-NMR spectra of free bases showed proton signals of the aminopropanol chain (Table 4).

**Table 4. <sup>1</sup>H NMR spectral data of bases δ (ppm)**

Compound	I		II		III	
	σ [ppm]	Number of protons multiplicity	σ [ppm]	Number of protons multiplicity	σ [ppm]	Number of protons multiplicity
Ar-H <sub>2</sub>	6.81	1H, d	6.74	1H, d	6.79	1H, d
Ar-H <sub>5</sub>	7.80	1H, d	7.80	1H, d		1H, d
Ar-H <sub>8</sub>	8.22	1H, d	8.25	1H, d	8.26	1H, d
ArOCH <sub>2</sub> CH(OH)CH <sub>2</sub>	4.21	2H, d	4.21	2H, d	4.19	2H, d
ArOCH <sub>2</sub> CH(OH)CH <sub>2</sub>	3.96	1H, m	3.96	1H, m	3.81	1H, m
ArOCH <sub>2</sub> CH(OH)CH <sub>2</sub>	2.82	2H, d	3.52	2H, d	2.66	2H, d
(CH <sub>3</sub> )CH-NH	1.11	3H, d	-	-	-	-
NHCHCH <sub>2</sub> CH <sub>2</sub> -kruh	-	-	1.25	2H, m	-	-
NHCHCH <sub>2</sub> -kruh	-	-	1.71	2H, m	-	-
NCH <sub>2</sub>	-	-	-	-	2.55	2H, t
NCH <sub>2</sub> CH <sub>2</sub>	-	-	-	-	1.26	2H, m

The prepared compounds (II, III) possess as reference compound propranolol a stereogenic centre and exist in form of two stereoisomers. It thus seems advisable to resolve the racemic mixture and show the express some chromatographic characteristic of each enantiomer.

The HPLC method used in this study employed a derivative amylose as chiral stationary phase and a mixture of hexane:ethanol:methanol:diethylamine at different ratio as mobile phase. The results of an enantioseparation are summarised in Table 5. It is evident that is no significant difference in the separation of the enantiomers on the used chiral stationary phase.

Stereoselective (*R*)-enantiomer of propranolol was prepared using the Jacobsen catalyst. Racemic 1-naphthoxymethyloxirane reacted with catalyst [(*R*, *R*)-N, N'-bis (3,5-di-*tert*-butylsalicylidene) -1,2 diaminato-cyclohexane-(2 -)] cobalt and was kinetic resolved in its (*S*)-1-naphthoxymethyloxirane and (*R*)-3-naphthoxypropane-1,2-diol. (*S*)-1-naphthoxymethyloxirane by reaction with isopropylamine gives (*R*)-(+)-propranolol.

Chromatograms of the prepared derivatives II, III show two peaks as reference compound propranolol I (Table 5, Figures 1, 2, 3).

**Table 5. Chromatographic data for enantioseparation of the prepared compounds on bonded chiral stationary phases**

Compounds	Mobile phase	t <sub>1</sub> [min]	t <sub>2</sub> [min]	k <sub>1</sub>	k <sub>2</sub>	A	R <sub>s</sub>
I	A	6.13	10.15	0.59	1.64	2.70	5.36
I	B	6.77	11.85	0.76	2.97	3.90	8.46
II	C	6.36	10.42	1.22	2.63	1.41	4.39
II	D	5.30	8.10	0.81	1.77	2.18	4.34
III	E	8.78	11.41	1.28	1.96	1.53	2.97
III	F	7.27	10.79	0.93	1.86	2.00	6.40

A - Mobile phase: hexane:ethanol:methanol:diethylamine 80/3,75/11.25/0.1 at compound I

B - Mobile phase: hexane:ethanol:methanol:diethylamine 85/3,75/11.25/0.1 at compound I

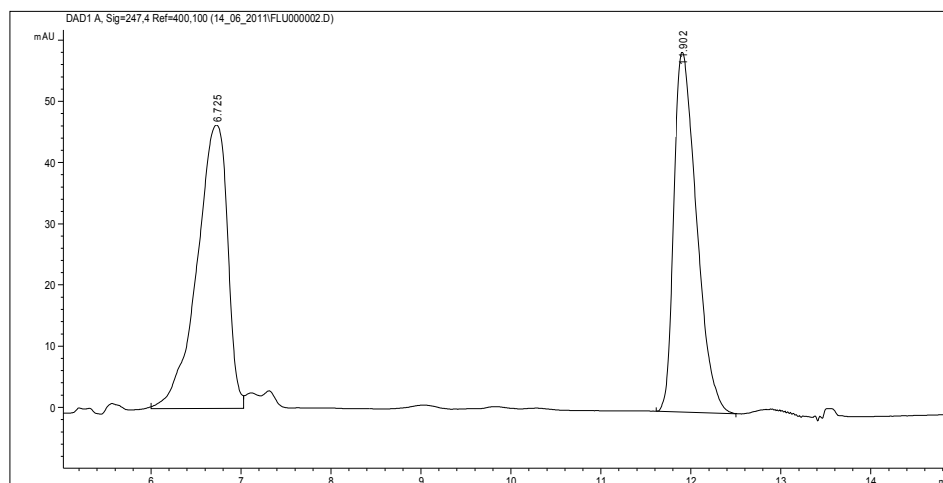
C - Mobile phase: hexane:ethanol:methanol:diethylamine 90/2.5/7.5/0.1 at compound II

D - Mobile phase: hexane:ethanol:methanol:diethylamine 85/2,5/7.5/0.1 at compound II

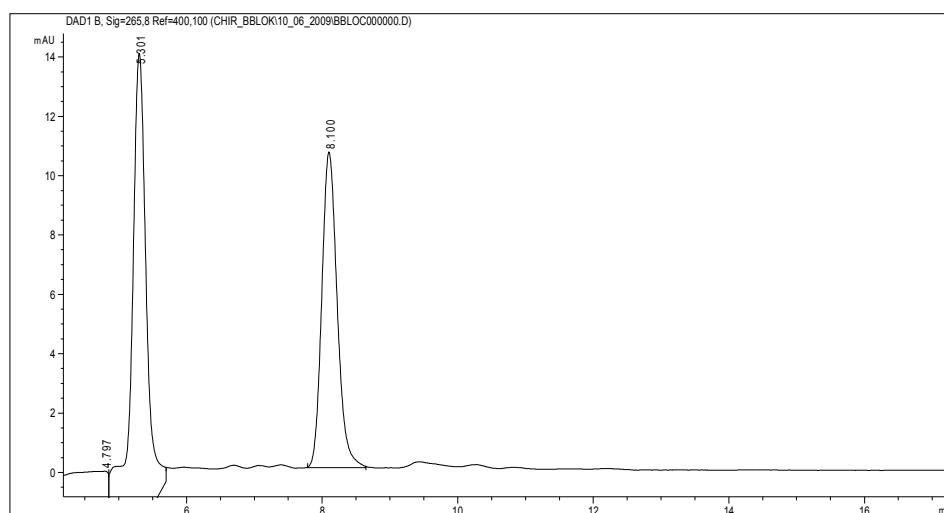
E - Mobile phase: hexane:ethanol:methanol:diethylamine 80/ 10/10/01 at compound III

F - Mobile phase: hexane:ethanol:methanol:diethylamine 78/11/11/01 at compound III

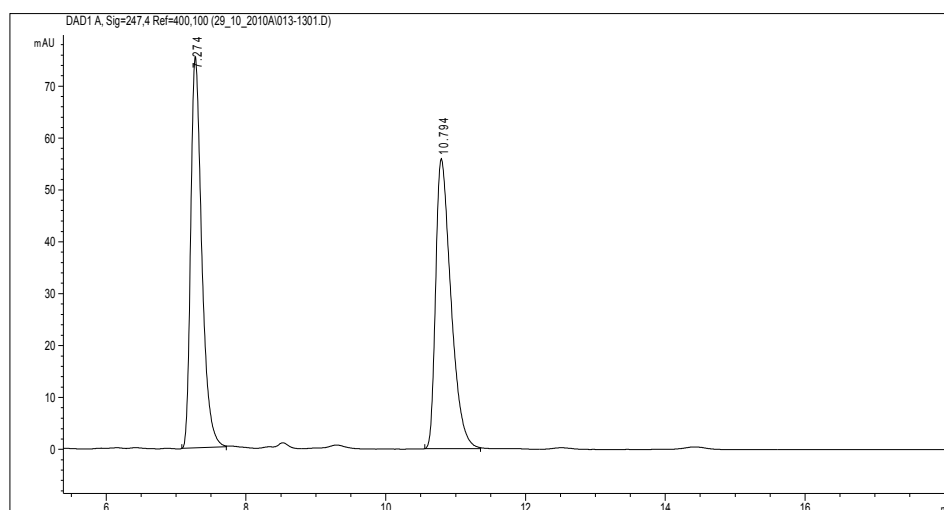
In the used mobile phases was separation of compounds II and III on base line with the separation factors  $\alpha$  in the range 3.90-1.41 and with resolution factor  $R_s$  in the range 6.40-2.97. In the paper (Valentová et al., 2003) racemic propranolol was semipreparative resolved on the CHIRALPAC AD using hexane:ethanol:methanol:diethylamine 85/3.75/11/0.1 v/v/v/v as mobile phase in 99% enantiomeric purity and subsequent using chiroptical characterization was determined absolute configuration the separated enantiomers. The enantiomeric elution order was determined to be (*R*)- before (*S*)-enantiomer induced CD complexes formed in situ with molybdenum tetraacetate.



**Figure 1. Separation of enantiomers of compound I (propranolol)**



**Figure 2 Separation of enantiomers of compound II**



**Figure 3 Separation of enantiomers of compound III**

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### ACKNOWLEDGEMENTS

This publication is the result of the project implementation: Centrum excelentnosti bezpečnostného výskumu kód ITMS: 26240120034 supported by the Research & Development Operational Programme funded by the ERDF.

Autors wish to thank Dr. Karlovska for recording  $^1\text{H}$ -NMR and Ms. Nedorostova for IR and UV spectra and Mr. Lehotsky for recording HPLC.

Registered: March, 1, 2012  
Accepted: July, 18, 2012

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### SYNTÉZA A ENANTIOSEPARÁCIA DERIVÁTOV PROPRANOLOLU

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Propranolol je jedným z prvých pripravených a v klinickej praxi používaných beta-adrenolytík. V tejto práci boli pripravené nové deriváty propranololu s cyklohexylamino a pyrrolidín-1-ylom v hydrofilnej časti molekuly. HPLC-enantioseparácia propranololu ako štandardu a pripravených derivátov bola uskutočnená na kolóne CHIRALPAC AD s chirálnou fázou amylóza tris (3,5-dimetylfenylkarbamát). Stereoselektívnou syntézou bol pripravený pomocou Jacobsenovho katalyzátora (*R*)-enantiomér propranololu.

*Acta Fac. Pharm. Univ. Comen. LIX, 2012, p. 5-13.*