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SYNTHESIS AND PHARMACOLOGICAL STUDY OF 5-PHENOXYMETHYL-1,2,4-OXADIAZOLE DERIVATIVES

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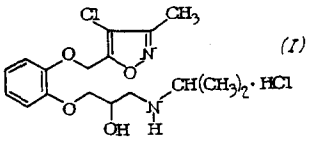
Novel 3-amino-2-hydroxypropoxy derivatives of 5-phenoxymethyl-1,2,4-oxadiazole were synthesized. Compounds were discovered among them that had pronounced β-adrenergic blocking activity combined with moderate α-adrenergic blocking properties.

In current therapy of cardiovascular system diseases, a prominent place is occupied by adrenergic blocking agents. Beta-adrenergic blocking agents (propranolol, atenolol, etc.) are widely used for treating ischemic heart disease, hypertension, heart rhythm disturbances [1 – 5]. In our search for new hybrid (α + β)-adrenergic blocking agents, we synthesized and studied the pharmacolog ofy derivatives of 5-phenoxymethyl-1,2,4-oxadiazole [6].

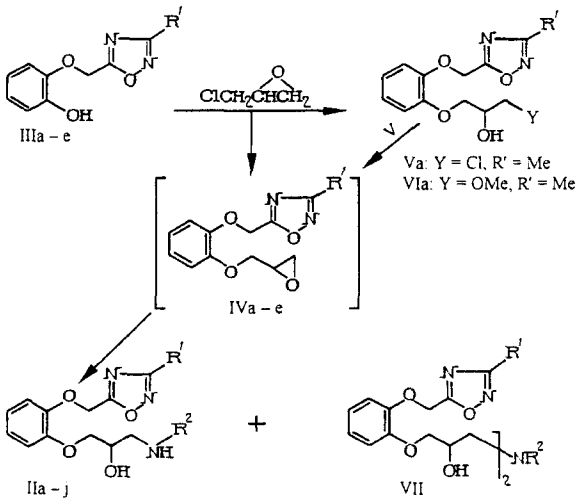
Previously for this purpose, we studied a series of derivatives of 3-amino-2-hydroxypropoxyphenoxymethylisoxazole [7 – 9], and among them we discovered compound (I), exhibiting pronounced β-adrenergic blocking activity combined with a moderate α-adrenergic blocking effect.



(I)

This compound could be of interest for further preclinical and clinical study, but in connection with certain difficulties in synthesis of derivatives of this series, further investigations were undertaken using the more readily available derivatives of phenoxymethyloxadiazole (IIa – j) that are close to it in structure.

The compounds IIa – j were synthesized in a similar way [7, 8] by alkylation of the corresponding 2-hydroxyphenoxymethyl-1,2,4-oxadiazoles (IIIa – e) by epichlorohydrin, with subsequent nucleophilic opening of the formed 2,3-epoxypropoxy derivatives of phenoxymethyl-1,2,4-oxadiazoles (IVa – e) by aliphatic and fatty aromatic amines.



II – IVa – e: R^1 = Me (a), Et (b), $CH(CH_3)_2$ (c), CH_2Ph (d), Ph (e), R^2 = $CH(CH_3)_2$; IIf – j: R^1 = Me; R^2 = $C(CH_3)_3$ (f), $CH(CH_3)CH_2CH_3$ (g), $CH_2CH_2NHCOCH(CH_3)_2$ (h), CH_2CHPh (i), $CH_2CH_2PhMe_2$-3,4 (j).

We described the synthesis of the initial 2-hydroxyphenoxymethyl-1,2,4-oxadiazole IIIa – e separately [3, 10]. As regards the epoxy derivatives IVa – e, we did not succeed in finding satisfactory conditions for their purification, apparently because of the lability of the glycidyl ethers. For this reason, we used IVa – e in the synthesis of compounds II without additional purification. At the same time, the alkylation of

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hydroxy compounds by epichlorohydrin proceeds in a far from unambiguous manner [11, 12]. This produces a large number of impurities in the reaction mixture, where in addition to the initial chlorohydrin, it also contains chlorohydrin of type V, a "bis-ether" (a product of the further reaction of the obtained epoxide IV with the starting phenol III), and a number of other impurities. In this connection, reaction conditions that would ensure a minimum of byproducts becomes quite important.

Indeed, in the alkylation of compounds IIIa-e with a two-fold excess of epichlorohydrin in aqueous dioxane with treatment by a molar amount of sodium hydroxide, in all cases we discovered a complicated mixture of products whose content of epoxy derivatives did not exceed 80%, while the yield of compounds IIa-j from such a reaction mixture averaged 30% (see Table 1).

We showed for the example of compound IIIa that the reaction in a nonaqueous medium (namely in an at least five-fold excess of epichlorohydrin) proceeds more selectively when treated with sodium methoxide. The reason is that the reaction mixture contains practically no "bis-ether" or a number of other impurities that apparently form under the effect of the aqueous alkali. On the other hand, when using an excess of sodium methoxide (about 20%), we also succeed in minimizing the content of chlorohydrin V because the latter is known to actually be an intermediate in this reaction and readily transforms into IV with cleavage of hydrogen chloride [11, 12]. Under the determined conditions, the content of IVa in the product is 93 – 95%. The content of chlorohydrin V and the methoxy derivative VI did not exceed 2 and 3%, respectively (a semiquantitative estimate of the composition was obtained by thin-layer chromatography on Silufol plates with the use of specially obtained chlorohydrin V and methoxy derivative VI as references). It should be noted that the presence of impurities within the indicated limits does not affect the quality of compound IIf, because they are readily separated when compound IIf is transformed into the hydrochloride.

The choice of the optimal conditions for obtaining specifically epoxy compounds is also important because the opening of epoxides, especially by primary amines, is accompanied by the formation of tertiary amines of type (VII) [13], and the use of an excess of amine does not completely suppress the process. Indeed, if epoxide IVa is opened by sterically hindered *tert*-butylamine with the use of even a five-fold (up to a ten-fold) excess in the reaction mixture, the formation of up to 1% of the tertiary amine VIIf is observed (the semiquantitative estimate was obtained by thin-layer chromatography on Silufol).

It should be noted that we did not observe the hypothetical alternative opening of epoxide with the formation of 2-amino-3-hydroxypropoxy derivatives, which is quite consistent with published results [11, 12].

Consequently, the synthesis of hydroxypropanolamine IIf under the above conditions doubled its yield from 35 to 70%, which makes it possible to consider the determined conditions acceptable for industrial synthesis of compounds II.

Compound IIk (differing from IIa – j in the *para* arrangement of the substituents on the benzene ring) was synthesized especially to compare their pharmacological properties. It was synthesized from 3-methyl-5-(4-hydroxyphenoxymethyl)-1,2,4-oxadiazole [6], similarly to the other compounds II.

The constants and physicochemical data of the obtained phenoxypropanolamines are presented in Table 1 and the experimental part.

TABLE 1. Hydrochlorides of 5-[(3-Amino-2-Hydroxypropoxy)phenoxymethyl]-1,2,4-Oxadiazoles IIa-k (Method A)

Compound	M. p., °C	Yield, %	UV spectrum λ_{max} : (log ε)
IIa	133 – 134	50	203 (4.28) : 274 (3.30)
IIb	102 – 103	44	203 (4.23) : 274 (3.23)
IIc	74 – 74.5	47	203 (4.26) : 273 (3.25)
IId	99 – 100	27	226 (4.25) : 274 (3.17)
IIe	152 – 153	40	207 (4.29) : 225 (4.23) : 274 (3.47)
IIf	140 – 141	35(70)*	203 (4.34) : 273 (3.30)
IIg	107 – 108	33	204 (4.27) : 274 (3.27)
IIh	112 – 113	21	203 (4.29) : 273 (3.24)
IIi	95 – 96	10	–
IIj	132 – 133	15	–
IIk**	145 – 146	43	–

* The yields are given for methods A and B.

** The 3-amino-2-hydroxypropanol group is in the *para*-position; R^1 = Me and $R^2 = CH(CH_3)_2$.

CHEMICAL EXPERIMENTAL PART

The IR spectra were obtained on a Perkin – Elmer-457 spectrometer (Sweden) in vaseline oil, and the UV spectra were obtained on a Specord-M-40 instrument (Germany) in ethanol. The PMR spectra were obtained on an XL-200 (Varian) spectrometer with an operating frequency of 200 MHz and with tetramethylsilane as the internal standard. The ^{13}C NMR spectrum was obtained on an XL-100A (Varian) spectrometer with an operating frequency of 25.2 MHz and with tetramethylsilane as the standard. The chemical ionization and electron impact mass spectra were obtained on an SSQ-710 mass spectrometer (Finnigan, USA) with direct injection of the sample into the ion source. The reactant gas was isobutane. Thin-layer chromatography was performed on Silufol in the system chloroform – acetone (40 : 1) and benzene – ethanol – ammonia (2.5%) (45 : 15 : 1) with visualization in ultraviolet light.

3-Methyl-5-[2-(3-*tert*-butylamino-2-hydroxypropoxy)phenoxymethyl]-1,2,4-oxadiazole hydrochloride (IIf). *Method A.* To a solution of 1 g (0.025 mole) of sodium hydroxide in 10 ml of water we added 5.2 g (0.025 mole) of 3-methyl-5-(2-hydroxyphenoxymethyl)-1,2,4-oxadiazole IIIa in 15 ml of dioxane and stirred the mixture at room temperature for 30 min. We added 4.6 g (0.05 mole) of epichlorohydrin and

heated the reaction mixture on a boiling water bath for 3 h. We extracted with ether, dried with Na_2SO_4, and evaporated the ether. We added 10 ml (7.3 g, 0.1 mole) of *tert*-butylamine in 15 ml of methanol to the residue and boiled the mixture for 1 h. We evaporated the mixture under vacuum, recrystallized the residue from petroleum ether (m. p. 40 – 70°C), filtered off the precipitate, and dissolved it in absolute ethanol. To the solution we added ether saturated with HCl until an acidic reaction, filtered off the precipitate, and washed it with absolute ether. The yield was 3.4 g (35%) of IIf, m. p. 140 – 141°C. In a similar way, we prepared compounds IIa – k whose properties are presented in Table 1.

Method B. Into a flask provided with a stirrer, dropping funnel, and a Wurtz attachment with a descending condenser, we put 5.4 g (0.026 mole) of 3-methyl-5-(2-hydroxyphenoxymethyl)-1,2,4-oxadiazole IIIa and 20.0 ml of epichlorohydrin and heated the mixture. At 95 – 100°C, we began to add 30 ml of a 6% solution of MeONa at a rate such that the rate of its addition and that of distillation of the methanol would be the same. After addition was complete (2 – 3 h), we cooled the reaction mixture to 20°C and filtered off the NaCl. We distilled off the epichlorohydrin under vacuum until separation of the distillate completely stopped. We used the remaining yellow oil (technical-grade IVa containing 95.3% of the primary substance, 2% of chlorohydrin Va and 2.5% of the methoxy derivative VIIa as well as two or three impurities in trace amounts) without additional purification in further synthesis. To 6.4 g (0.023 mole) of the obtained technical epoxide IVa we added 13 ml (3 g, 0.12 mole) of *tert*-butylamine in 13 ml of isopropanol and boiled it for 2 h. We distilled off the isopropanol and excess *tert*-butylamine. For complete distillation of the *tert*-butylamine from the reaction mixture, we added pure isopropanol to the flask and again distilled the mixture until it was dry. We dissolved the residue in 13 ml of isopropanol and added isopropanol saturated with HCl up to pH 2 – 3. We held the mixture (with cooling with ice water) for 1 h and filtered off the precipitate containing 0.95% of an admixture of VIIf (according to thin layer chromatography), and recrystallized it from isopropanol. We obtained 6.8 g (70%) of IIf. UV spectrum, λ_{max} (log ε): 203 nm (4.34), 273 nm (3.30). IR spectrum: 3300 cm^{-1} (ν_{OH}). PMR spectrum in $CDCl_3$, δ, ppm: 1.49 (s, 9H, $(CH_3)_3$), 2.41 (s, 3H, 3-CH_3), 3.17 (q, 1H, CH_2NH), 3.38 (q, 1H, CH_2NH), 4.0 – 4.2 (m, 2H, OCH_2), 4.62 (m, 1H, CHOH), 5.29 (s, 2H, 5-CH_2O), 5 – 6 (very broad signal, 1H, CHOH), 6.9 – 7.05 (m, 4H aromatic protons), 9 – 11 (very broad signal, 2H, NH_2^+). ^{13}C NMR spectrum in $CDCl_3$: 11.5 (q, 3-CH_3), 25.8 (q, $(CH_3)_3$), 45.7 (t, CH_2NH), 57.6 (s, $C(CH_3)$), 62.9 (t, 5-CH_2O), 65.6 (d, CHOH), 71.1 (t, OCH_2), 114.0 (d, C^2-arom.), 116.0 (d, C^5-arom.), 121.8 (d, C^3-arom.), 123.6 (d, C^4-arom.), 147.3 (s, C^1-arom.), 149.0 (s, C^6-arom.), 167.0 (s, C^3 of oxadiazole), 174.5 (s, C^5 of oxadiazole). Chemical ionization mass spectrum: 336 (MH^+), 320 $[M–CH_3]^+$, 291 $[M–C_3H_8]^+$, 280 $[M–C_2H_3N_2]^+$, 86 (CH_2=NHC_4H_9). The most intense peak in the spectrum belongs to the quasimolecular ion 336.

3-Methyl-5-[4-(3-isopropylamino-2-hydroxypropoxy)-phenoxymethyl]-1,2,4-oxadiazole hydrochloride (IIk). Using method A, from 5.2 g (0.025 mole) of 3-methyl-5-(4-hydroxyphenoxymethyl)-1,2,4-oxadiazole [3], 4.6 g (0.05 mole) of epichlorohydrin, and 8 ml (5.9 g, 0.1 mole) of isopropylamine, we obtained 3.9 g (43%) of IIk, m. p. 145 – 146°C.

5-[2-(2-Hydroxy-3-chloropropoxy)phenoxymethyl]-3-methyl-1,2,4-oxadiazole (Va). To 3 g (0.011 mole) of specially purified epoxide IVa we added 20 ml of alcohol saturated with HCl, held the mixture for 3 h at room temperature, and then boiled it for 2 h. We evaporated the alcohol under vacuum, dissolved the residue in ether, and washed it with a solution of soda and water. We dried with Na_2SO_4, evaporated the ether, and obtained 3 g (90%) of Va in the form of a light yellow oil. The Beilstein test was positive. Electron impact mass spectrum: 298 (M^+) (the value for the ion containing the ^{35}Cl isotope is given), 249 [$M–CH_2Cl]^+$, 220 $[M–O=CHCH_2Cl]^+$. The most intense peaks belong to the ions 98, 109, and 121.

3-Methyl-5-[2-(3-methoxy-2-hydroxypropoxy)phenoxymethyl]-1,2,4-oxadiazole (IVa). We boiled a mixture of 2 g (0.007 mole) of epoxide IVa in 20 ml of methanol and 5 ml of a 20% solution of MeONa for 2 h. We evaporated the methanol under vacuum, diluted the residue with 20 ml of water, and extracted it with ether. We dried it with calcined potassium carbonate, evaporated the ether, and recrystallized the residue from hexane. We obtained 1.7 g (57%) of VIIa, $C_{14}H_{18}N_2O_5$, m. p. 56 – 57°C.

N-*tert*-Butyl-N,N-bis{2-hydroxy-3[2-(3-methyl-1,2,4-oxadiazole-5-ylmethoxy)phenoxy]propyl}amine (VIIf). We boiled a mixture of 3 g (0.011 mole) of epoxide IVa and 1 ml of *tert*-butylamine in 20 ml of methanol for 2 h. We evaporated the methanol, dissolved the residue in 20 ml of 10% HCl, and extracted with ether. We made the aqueous layer alkaline and extracted it with ether and benzene. We dried with Na_2SO_4 and evaporated until dry. The remaining light yellow oil crystallized while standing. We obtained 2.8 g (85%) of VIIf, m. p. 30 – 32°C. The electron impact mass spectrum was 598 (M^+) (I < 1%), whose decomposition is due to the breaking of α-bonds relative to the tertiary nitrogen atom with the formation of ions 582 $[M–CH_3]^+$ and a more intense fragment 348 $[M–C_{12}H_{13}N_2O_4]^+$. The most intense peaks in the spectrum are 292 $[348 – CH_2 = NC(CH_3)_3]^+$, 57 $[C(CH_3)_3]^+$.

PHARMACOLOGICAL EXPERIMENTAL PART

The existence of β-adrenergic blocking activity was studied on male rats weighing 250 – 300 g narcotized with ethaminal sodium (pentabarbital) (40 – 50 mg/kg intraperitoneally). We determined the influence of the intravenous injection of compounds on the positive chronotropic and depressor effects of isadrine (isoprenaline) (1 μg/kg intravenously). The α-adrenergic blocking activity was studied under similar conditions with respect to the influence on the pressor effect of mezaton (phenylephrine) (0.1 mg/kg intravenously).

The most active compounds (IIa, f) were investigated additionally with respect to some parameters. The β-adrenergic blocking effect was studied upon intravenous injection of the agents to narcotized cats weighing 3 – 4 kg and upon peroral administration to rats weighing 300 – 350 g. In these experiments, we also determined the duration of the β-andrenoceptor blocking effect. On isolated, spontaneously contracting autrium dextrum of guinea pigs, we determined the β-adrenergic blocking activity according to the value of pA_2 with respect to the positive chronotropic effect of isadrine (10^{-7} and 2×10^{-7} M).

On male mice weighing 15 – 17 g, we studied the acute toxicity of the compounds upon intravenous injection. The value of LD_{50} was calculated by the method of Litchfield and Wilcockson [4].

We compared the activity and toxicity of the studied compounds with the toxicity of propanolol, labetolol, and oxprenolol (having a partial structural similarity with the studied compounds).

The results of pharmacological investigation are given in Table 2.

We found that most of the studied compounds exhibit β– and α-adrenergic blocking activity. The most active compounds as regards the β-adrenergic blocking activity are those containing a substituted ring in the *ortho*-position of the aromatic ring (compounds IIa – j). Of these, the highest β-adrenergic blocking activity was found in compounds IIas and IIf containing an isopropyl or *tert*-butyl substituent at the nitrogen of the side chain and a methyl group in position 3 of the oxadiazole ring. Starting with 0.0025 mg/kg, they inhibit the depressor effect depending on the dose, whereas starting with a dose of 0.0025 mg/kg both substances cause a reduction in the frequency of the systoles. The compound IIf lowers the systemic arterial pressure beginning with a dose of 0.01 mg/kg. With respect to the β_1- and β_2-adrenergic blocking activity, the compounds IIa and IIf are close to each other, are superior to propranolol and oxprenolol by an order of magnitude, and are superior to labetolol by two orders of magnitude. The compounds IIg – j, containing a sec-butyl, isobutyric ethylamide, homoveratryl, or phenethyl substituent instead of the isopropyl and *tert*-butyl residue in the side chain, are considerably less active. Similar results were obtained when replacing the methyl group in position 3 of the oxadiazole ring with an ethyl, isopropyl, phenyl, or benzyl radical (compounds IIb – e). The activity practically vanishes when the oxadiazole ring is transferred from the *ortho*- to the *para*-position of the aromatic ring (compound IIk).

TABLE 2. β- and α-Adrenoceptor Blocking Activity and Acute Toxicity of Hydrochlorides of 5-(3-Amino-2-Hydroxypropoxy)phenoxymethyl-1,2,4-Oxadiazoles IIa – k

Compound	β-Adrenoceptor blocking activity (rats)		α-Adrenoceptor blocking activity (rats); dose reducing pressor effect of mezaton by 50% (ED_{50}), mg/kg, intravenously	LD_{50} (mice), mg/kg, intravenously
	dose reducing isadrin tachycardia by 50% (ED_{50}), mg/kg, intravenously	dose diminishing depressive effect of isadrine by 50% (ED_{50}), mg/kg, intravenously		
IIa	0.03	0.003	8.5	87.5
IIb	0.35	0.275	15.0	41.0
IIc	1.75	0.50	15.0	32.0
IId	0.1	0.1	18.0	45.0
IIe	0.65	0.38	17.0	23.0
IIf	0.03	0.008	7.5	72.5
IIg	0.25	1.050	10.5	37.5
IIh	0.30	0.375	15.0	35.0
IIi	2.0	1.7	–	35.0
IIj	0.09	0.23	12.0	65.0
IIk	0.8	>2.0	22.0	298.0
Propranolol	0.42	0.092	–	28.0
Oxprenolol	0.26	0.055	37.5	20.0
Labetalol	0.70	3.30	12.0	97.5

Investigations showed that most compounds have not only β-, but also α-adrenoblocking activity. With respect to the strength of the α-adrenergic blocking effect, they are comparable with one another and do not differ from labetalol.

We additionally studied the compounds IIa, f, which are close in their β- and α-adrenergic blocking activity upon intravenous injection into rats and in their acute toxicity for mice. It was found that the compound IIf with respect to its β_1-adrenergic blocking activity upon peroral administration in rats is superior to compound IIa (ED_{50} with respect to the influence on the chronotropic effect of isadrine is 7.0 and 40 mg/kg, respectively). Similar results were obtained in narcotized cats upon intravenous injection of the compounds (ED_{50} is 0.002 and 0.025 mg/kg, respectively), and also for isolated atriums of guinea pigs (pA_2 is 8.75 and 8.2, respectively). Compound IIf is somewhat superior to IIa with respect to the duration of the β_1-adrenergic blocking effect upon peroral administration in rats (a blockade of the chronotropic effect of isadrine in 4.8 and 12 h after the administration of compound IIf is 92, 40 and 12%, and after the administration of compound IIa it is 84 and 12%; in 12 h, the effect vanishes).

The compounds IIf and IIa, the most active in their β-adrenergic blocking effect, are less toxic than propranolol and oxprenolol, and are comparable in LD_{50} with labetalol.

Consequently, as a result of our studies, we discovered the presence in the series of derivatives of 5-phenoxymethyl-1,2,4-oxadiazole of compounds having a pronounced β-adrenergic blocking activity combined with α-adrenergic blocking properties. We established some correlations between the chemical structure and the adrenergic blocking activity in this series of compounds.

Among the studied compounds, IIf is most active. This compound was named Proxodolol and was subjected to detailed preclinical and clinical investigation [1].

Considering the field of application of modern β-adrenergic blocking agents, we conducted the clinical examination of Proxodolol in two directions, namely, in cardiology as an an-

tihypertensive and antianginal means, and in ophthalmology as an antiglaucomatous means.

The results of the examination confirmed that Proxodolol can to be introduced in medical practice as a new drug.

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