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Pharmacological properties of some aminoalkanolic derivatives of xanthone

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A series of appropriate aminoisopropanoloxy derivatives of 2-, 3- or 6-xanthone was synthesized and evaluated for anticonvulsant activity in the maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole seizure threshold (ScMet) assays and for neurotoxicity (TOX). The most interesting result was the anticonvulsant activity of (±)-3-(2-propylamino)-1-[(2-methyl)-6-xanthonoxy]-2-propanol hydrochloride (**10**), which displayed anti-MES activity with a protective index (TD_{50}/ED_{50}) of 0.80. Some of the obtained compounds were also tested for their effect on the circulatory system (influence on the non-working heart perfusion, protection against adrenaline induced-arrhythmia) and acute toxicity.

1. Introduction

In the course of our investigations of biologically active compounds we have directed our attention to the xanthone derivatives, which show several beneficial properties when tested in biological systems. In dependence on the substitution pattern and on the degree of substitution, antiallergic, antiinflammatory, antituberculotic, antitumor, antiplatelet, β-adrenergic blocking, anticonvulsant and antiacetylcholinesterase properties were described [1–9].

In a previous study we reported the anticonvulsant properties of some aminoisopropanoloxy derivatives of 2-xanthone [7]. The obtained compounds were evaluated for anticonvulsant activity in the maximal electroshock (MES)- and subcutaneous pentylenetetrazole (ScMet)-induced seizure tests and for neurotoxicity (TOX) in the rotorod test in mice and rats. The most interesting results was the anticonvulsant activity of the 3-(*tert*-butyl)-amino- and 3-[*N*-methyl-(*tert*-butyl)-amino]-2-hydroxy-1-(2-xanthonoxy)-propane, which displayed anti-MES activity with a protective index (TD_{50}/ED_{50}) of 1.9 and > 4.5, respectively, corresponding with that for valproate, phenytoin and carbamazepine.

We herein report on the preparation and anticonvulsant activity (for **6**–**12**) of a few appropriate aminoisopropanoloxy derivatives of 2-, 3- or 6-xanthone (**4**–**12**), containing amines like morpholine, 2-amino-2-methyl-1-propanol, 2-aminopropane, 1-*N*-(2-hydroxyethyl)-piperazine, 2,6-(dimethyl)-piperazine. These compounds have some structural moieties (aminoisopropanoloxy group) of known antiarrhythmic agents, such as propranolol, alprenolol, metoprolol etc. This was the reason why some of them (**4**–**7** and **9**) were tested for their effects on the circulatory system (influence on the non-working heart perfusion, antiarrhythmic activity in experimentally adrenaline induced-arrhythmia). Previously, compounds **4** and **5** had been tested for their anticonvulsant effects [7].

2. Investigations, results and discussion

2.1. Chemistry

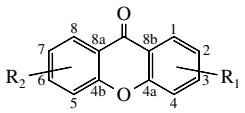
The synthesis and properties of compounds **4** and **5** were described earlier [7]. Compound **6** was synthesized by amination of (±)-3-(2,3-epoxy)-propoxy)-xanthone [5] in n-propanol. The same method was used for the formation of **7**, **9**, **10** and **12** from **2**, which was prepared from 2-methyl-6-hydroxyxanthone (**1**) using propylene epichlorohydrin in the presence of NaOH in water (yield 59%). During the synthesis of **2** the formation of 1,3-bis-(2-methyl-6-xanthonoxy)-2-propanol (**3**) was observed (yield ca. 15%). Compound **1** was readily formylated by demethylation of 2-methyl-6-methoxyxanthone [9] according to a published procedure [10], using xylene with anhydrous aluminium chloride. Compounds **7** and **10** were subjected to N-methylation. The exchange of the secondary amino group of compounds **7** and **10** for a tertiary one (**8**, **11**) was generated by reductive N-methylation. All the obtained compounds with exception of **10** were examined as salts (hydrochlorides). The physical and spectral data for the obtained compounds **1**–**12** are given in the Experimental Part.

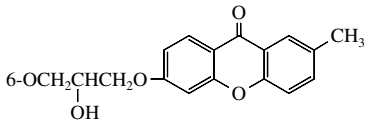
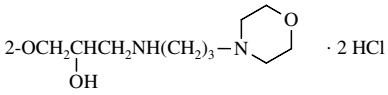
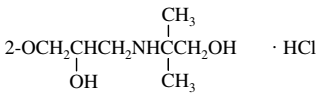
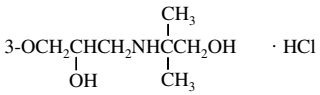
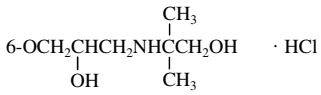
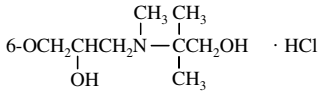
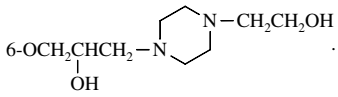
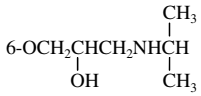
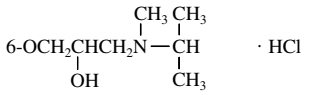
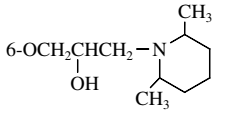
2.2. Pharmacology

2.2.1. Anticonvulsant assays

Preliminary pharmacological and neurotoxicological tests of the synthesized compounds **6**–**12** have been provided by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institutes of Neurological and Communicative Disorders and Stroke (NINCDS), Bethesda, MD, USA, by testing procedures which have been described earlier [11]. Phase I studies involved three tests: MES, ScMet and TOX. The TOX was measured by the rotorod test. The MES assay has predictive value for agents of potential therapeutic value in the management of generalized tonic-clonic (grand mal) epilepsy, whereas these ScMet test is predictive for those likely to be effective against generalized absence (petit mal) seizure [12, 13]. The result from anticonvulsant assays is summarized in Tables 1 and 2.

Table 1 presents results of the phase I according to the ADD Program. Table 2 shows data for compound **10** and compares the effects of **10** to those of some prototype antiepileptic drugs (AEDs). A protective activity in the MES test in mice after i.p. administration was found for compounds **6**, **7** and **10**–**12**. In the ScMet test in mice a protective activity was seen only for **8** in a dose of 100 and 300 mg/kg, 30 min and 4 h after administration, respectively. Neurotoxicity was not observed for compound **9** in doses up to 300 mg/kg, for **10** in doses up to 100 mg/kg and for **6**–**8** and **12** in doses up to 30 mg/kg. The majority of the xanthone derivatives studied demonstrates certain protective activity in a wide range of doses but this effect is accompanied by neurotoxicity in the same range of doses.



Compound	R_1	R_2
3	2-CH_3	6-OH
2	2-CH_3	6-$OCH_2CH(O)CH_2$
3	2-CH_3	
4	 · 2 HCl	H
5	 · HCl	H
6	 · HCl	H
7	2-CH_3	 · HCl
8	2-CH_3	 · HCl
9	2-CH_3	 · 2 HCl
10	2-CH_3	
11	2-CH_3	 · HCl
12	2-CH_3	 · HCl

Modifications in the chemical structures of **7** and **10**, by introduction of a tertiary amine instead of a secondary one did not result in the expected effects in mice in the MES assay. However, compound **8** in the ScMet test in mice was active in comparison to **7** but increased neurotoxicity was observed (Table 1). Table 2 compares the effects of **10** in the quantitative anticonvulsant and neurotoxicity assay in mice dosed i.p. with those of some prototype AEDs. The protective index (PI) in the MES test in mice is lower than that for phenytoin, carbamazepine and valproate.

2.2.2. Non-working heart perfusion

Compounds **4**, **6**, **7** significantly decreased the heart rate by 26–40%, (Fig. 1), prolonged P-Q (by 36–63%), Q-T (by 14–29%) intervals and QRS complex (by 37–60%), (Fig. 2). Compound **5** significantly reduced the heart rate (by 11–20%), (Fig. 1), and prolonged P-Q (by 37–63%) and Q-T (by 11–18%) intervals (Fig. 2). Compound **9** significantly supressed cardiac action (24–29%), produced prolongation of the Q-T interval (20–33%), and not sig-

Table 1: Anticonvulsant screening project (ASP), phase I: Test results in mice after intraperitoneal injection

Compd.	Dose (mg/kg)	Activity										ASP class.[d]
		MES[a]				ScMet[b]		Tox[c]				
		0.25 h	0.5 h	1 h	4h	0.5 h	4 h	0.25 h	0.5 h	1 h	4 h	
6	30		0/1		0/1	0/1	0/1		0/4		0/2	
	100	0/3	0/3	0/3	1/3	0/1	0/1	0/3	2/8	0/3	0/4	1
	300		1/1		1/1	0/1	0/1		4/4		2/2	
7	30		0/1		0/1	0/1	0/1		0/4		0/2	
	100		0/3		1/3	0/1	0/1		1/8		0/4	1
	300		0/1			0/1			3/4		2/2	
8	30		0/1		0/1	0/1	0/1		0/4		0/2	
	100		0/3		0/3	1/5	0/1		5/8		0/4	1
	300		0/1		0/1	0/1	1/5		4/4		1/2	
9	30		0/1		0/1	0/1	0/1		0/4		0/2	
	100		0/3		0/3	0/1	0/1		0/8		0/4	3
	300		0/1		0/1	0/1	0/1		0/4		0/2	
10	3				0/4						0/4	
	10				0/4						0/4	
	30		0/1		1/1	0/1	0/1		0/4		0/2	1
	100	2/3	0/3	0/3	1/3	0/1	0/1	0/3	0/8	0/3	0/4	
	300		1/1		1/1	0/1	0/1		4/4		2/2	
11	30		0/1		0/1	0/1	0/1		1/4		1/2	
	100		1/3		0/3	0/1	0/1		4/8		0/4	4
	300		1/1		1/1	0/1	0/1		4/4		2/2	
12	30		0/1		0/1	0/1	0/1		0/4		0/2	
	100		2/3		2/3	0/1	0/1		4/8		0/4	1
	300		1/1		1/1	0/1	0/1		4/4		2/2	

[a] number of animals protected/number of animals tested in the MES test; [b] number of animals protected/number of animals tested in the ScMet test; [c] number of animals exhibiting toxicity/number of animals tested in the rotorod test; [d] the classification are as follows; 1: anticonvulsant activity at 100 mg/kg or less; 2: anticonvulsant activity at doses greater than 100 mg/kg; 3: compound inactive at 300 mg/kg; 4: toxicity at doses 30 mg/kg

nificantly prolonged P-Q and QRS complex. Some of the compounds of this series slightly reduced the coronary flow (by 2–44%). Compounds **6** and **7** given in a concentration of 10^{-6} M produced arrhythmia and cardiac arrest.

Table 2: Quantitative anticonvulsant activity and neurotoxicity in mice dosed intraperitoneally of 10, and some prototype AED

Compd.	TPE[a] (h)	TD_{50}[b]	ED_{50} MES[c]	ED_{50} ScMet[d]
10	2/1	138.93 (117.24–165.71) [8.51]	173.45 (149.88–207.04) [7.24] PI 0.80	> 125.00 PI<1.11
Phenytoin[e]	1/2, 2	42.8 (36.4–47.5) [10.2]	6.48 (5.65–7.24) [12.4] PI 6.6	> 50 PI<0.9
Carbamazepine[e]	1/4, 1/4	47.8 (39.2–59.2) [7.89]	9.85 (8.77–10.7) [20.8] PI 4.9	> 50 PI < 1.0
Valproate[e]	1/4, 1/4	483 (412–571) [12.3]	287 (237–359) [7.31] PI 1.7	209 (176–249) [8.51] PI 2.3

[a] Time to peak effect. The first value is for the rotorod test; the second is for the anticonvulsant test. In the neurotoxicity assay, dose of **10** (150 mg/kg) was tested at 1/4 h throughout 6 h. [b] Doses (mg/kg) eliciting evidence of minimal neurological toxicity in 50% of animals: 95% confidence interval is shown in parentheses; the slope regression line is shown in brackets. PI: neurotoxic dose/median effective dose (TD_{50}/ED_{50}) for anticonvulsant test. [c] Dose (mg/kg) eliciting the MES protection in 50% animals. [d] Dose (mg/kg) eliciting the ScMET protection in 50% animals. [e] Data from reference [14]

The electrocardiographic changes observed after administration of compounds **4**, **6**, **7** are similar to those seen after administration of quinidine [15].

2.2.3. Adrenaline – induced arrhythmia

The tested compounds, administered intravenously (1/10–1/5 LD_{50}) did not prevent the adrenaline-induced disorders, and did not protect the animals against the lethal effects of full atrioventricular block. The reference compounds- propranolol and quinidine-administered 15 min before adrenaline, showed preventive activity. Their ED_{50} values were 1.05 (0.64–1.73) and 8.7 (8.0–9.4) mg/kg for propranolol and quinidine, respectively.

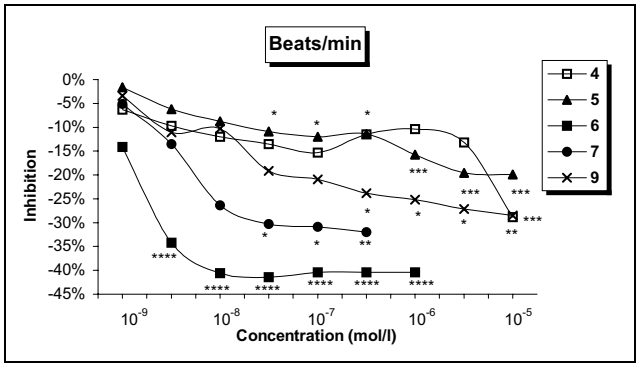


Fig. 1: Influence of tested compounds on heart rate
Statistical analyses were performed using a one-way ANOVA test: *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001

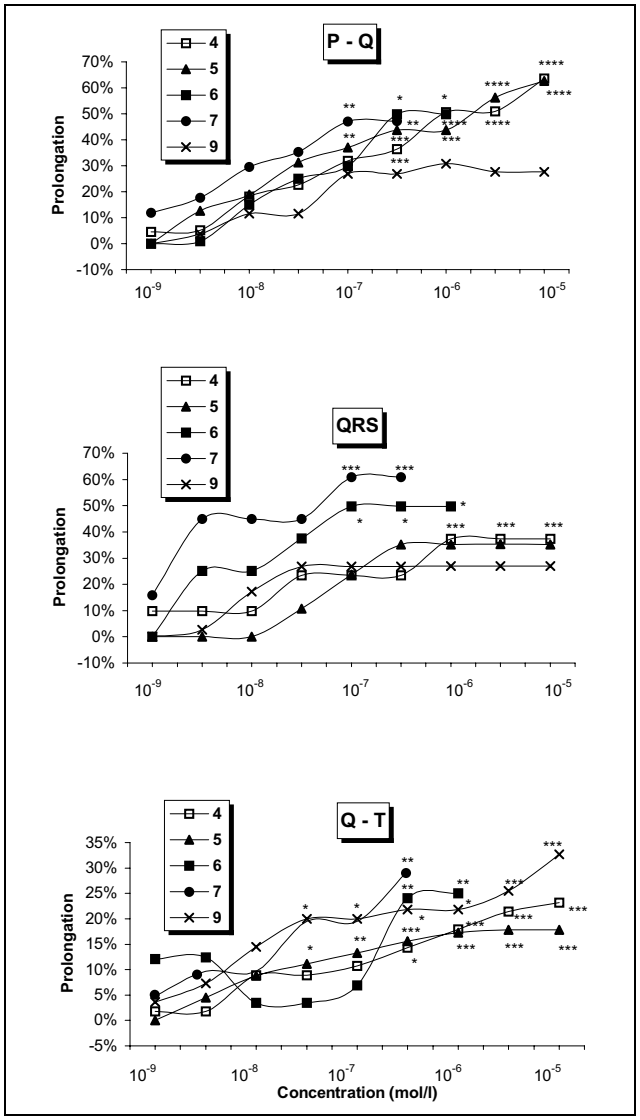


Fig. 2: Influence of tested compounds on ECG parameters
Statistical analyses were performed using a one-way ANOVA test: *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001

2.2.4. Acute toxicity

The LD_{50} values for xanthone derivatives (**4**–**7** and **9**), determined in mice after intravenous administration, are presented in Table 3. The toxicity of the investigated compounds was lower than that of quinidine and propranolol.

3. Experimental

3.1. Chemistry

Melting points (Büchi SMP-20 apparatus) are uncorrected. Elemental analysis was performed at the Department of Pharmaceutical Chemistry, Jagiellonian University Medical College, Kraków. All the results were in an acceptable range. Log P (combined) and log D values prediction were taken with the Pallas program. IR spectra were recorded on a Perkin-Elmer spectrometer using KBr discs. The UV spectra were obtained on a Specord UV-VIS apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer with 200 or 500.13 MHz and 125.17 MHz respectively, using DSS in DMSO-d_6 and TMS in $CDCl_3$ as internal standards and solvents. MS were recorded using an AMD-604 mass spectrometer (70 ev). TLC was performed on silica gel G F_{254} precoated plates (5 × 10 cm, 0.25 mm, Merck), with an appropriate developing system and spots were visualized with UV light. Reagents: 3-(2,3-epoksypropoxy)-xanthone was prepared from 3-hydroxyxanthone [16], according to [5]. Ring opening of the appropriate epoxide derivatives of xanthone with respective amines in n-propanol afforded xanthonoxypropanolamine-related compounds. Bases were converted into hydrochloride salts in propanol/acetone (4/1) with an access of C_2H_5OH saturated with HCl. Reductive N-methylation of **7** and **10** was generated according to well known procedures. Compound **1** was obtained by demethylation of 2-methyl-6-methoxyxanthone [8], according to procedures for 2-hydroxyxanthone [10]. The physical properties of **4** and **5** were described earlier [7].

Table 3: Acute toxicity according to Litchfield and Wilcoxon in mice

Compd.	LD_{50} (mg/kg) i.v.
4	210 (198.1–222.6)
5	140 (160.0–184.8)
6	121 (116.3–125.5)
7	340 (270.2–428.0)
9	380 (342.3–421.8)
Quinidine	90
Propranolol	22

3.1.1. 2-Methyl-6-hydroxyxanthone (**1**)

Yield: 63%, m.p. 278–280 °C (80% CH_3COOH). IR (cm^{-1}): 3252, 2920, 2860, 1648, 1609, 1577, 1479, 1303, 1246, 1215, 1108. ^{1}H NMR (200 MHz) (δ, ppm, DMSO-d_6): 10.91 (1 H, s, OH), 8.0 (1 H, d, J = 6.4 Hz, H-8), 7.89 (1 H, d, J = 1.0 Hz, H-1), 7.57 (1 H, dd, J = 2.1 Hz, J = 8.6 Hz, H-arom.), 7.43 (1 H, d, J = 8.5 Hz, H-arom), 6.87 (1 H, dd, J = 2.2 Hz, J = 8.9 Hz, H-arom.), 6.82 (1 H, d, J = 2.1 Hz, H-arom.), 2.39 (3 H, s, CH_3).
$C_{14}H_{10}O_3$ (226.2)

3.1.2. 2-Methyl-6-(2,3-epoxypropoxy)-xanthone (**2**)

Yield: 59%, m.p. 133–135 °C (n-propanol). R_f = 0.79 (ethylacetate/benzene, 1/1). IR (cm^{-1}): 3062, 2924, 1655, 1620, 1483, 1450, 1307, 1273, 1261, 1233. ^{1}H NMR (500.13 MHz) (δ, ppm, DMSO-d_6): 8.08 (1 H, d, J = 8.9 Hz, H-8), 7.93–7.86 (1 H, m, H-1), 7.67–7.63 (1 H, m, H-3), 7.51 (1 H, d, J = 8.6 Hz, H-4), 7.16 (1 H, d, J = 2.3 Hz, H-5), 7.07 (1 H, dd, J = 2.4 Hz, J = 8.9 Hz, H-7), 4.55 (1 H, dd, J = 2.5 Hz, J = 11.5 Hz, HCHOAr), 4.02 (1 H, dd, J = 6.8 Hz, J = 11.5 Hz, HCHOAr), 3.45–3.37 (1 H, m, CHO), 2.90 (1 H, q, J = 4.3 Hz, J = 5.0 Hz, HCHO), 2.76 (1 H, dd, J = 2.7 Hz, J = 5.0 Hz, HCHO), 2.43 (3 H, s, CH_3). ^{13}C NMR (δ, ppm): 174.77 (C=O), 163.64 (6), 157.32 (4b), 153.76 (4a), 135.94 (3), 133.63 (2), 117.61 (4), 115.09 (8a), 113.62 (7), 101.20 (5), 69.72 (C-1′), 49.31 (C-2′), 43.65 (C-3′), 20.23 (CH_3).
$C_{17}H_{14}O_4$ (282.2)

3.1.3. 1,3-Bis-(2-methyl-6-xanthonoxy)-2-propanol (**3**)

Yield: 15%, m.p. 267–269 °C (DMF). IR (cm^{-1}): 3434, 2929, 1655, 1618, 1591, 1446, 1266, 1230, 1165. ^{1}H NMR (500.13 MHz) (δ, ppm, DMSO-d_6/pyridine-d_5): 8.15 (2 H, d, J = 8.9 Hz, H-8), 7.97 (2 H, d, J = 1.1 Hz, H-1), 7.55 (2 H, dd, J = 2.2 Hz, J = 8.9 Hz, H-3), 7.44 (2 H, d, J = 8.5 Hz, H-4), 7.15 (2 H, d, J = 2.3 Hz, H-5), 7.09 (2 H, dd, J = 2.3 Hz, J = 8.9 Hz, H-7), 4.41–4.31 (5 H, m, $(CH_2)_2$, CH), 6.08 (1 H, bs, OH), 2.34 (6 H, s, CH_3Ar). ^{13}C NMR (δ, ppm): 174.8 (C=O), 164.0 (6), 157.4 (4b), 154.1 (4a), 135.9 (3), 133.6 (2), 127.5 (8), 125.1 (1), 120.8 (8b), 117.6 (4), 115.0 (8a), 113.8 (7), 101.2 (5), 70.1 (CH_2O), 67.3 (CHOH), 20.3 (CH_3).
$C_{31}H_{27}O_7$ (496.5)

3.1.4. (±)-3-[2-N-(2,2-Dimethyl-1-hydroxy)-ethylamino]-1-(3-xanthonoxy)-2-propanol hydrochloride (**6**)

Yield: 66%, m.p. 212–214 °C (n-propanol). R_f = 0.27 ($CHCl_3$/CH_3OH, 1/2). IR (cm^{-1}): 3375, 2996, 2810, 2387, 1654, 1622, 1587, 1466, 1329, 1277, 1259, 1235, 1105. ^{1}H NMR (200 MHz) (δ, ppm, DMSO-d_6): 8.96 (1 H, t, J = 8.3 Hz, NH^+), 8.43 (1 H, t, J = 9.2 Hz, NH), 8.14 (1 H, dd, J = 1.5 Hz, J = 7.9 Hz, H-arom.), 8.07 (1 H, d, J = 8.8 Hz, H-arom.), 7.88–7.81 (1 H, m, H-arom.), 7.49–7.40 (1 H, m, H-arom.), 7.14 (1 H, d, J = 2.2 Hz, H-arom.), 7.06 (1 H, dd, J = 2.3 Hz, J = 8.9 Hz, H-arom.), 6.00 (1 H, d, J = 4.6 Hz, CHOH), 5.63 (1 H, t, J = 4.9 Hz, CH_2OH), 4.43–4.20 (1 H, m, CH), 4.30–4.12 (2 H, m, CH_2OAr), 3.50 (2 H, d, J = 4.6 Hz, CH_2OH), 3.33–3.15 (1 H, m, HCHN), 3.15–2.85 (1 H, m, HCHN), 1.28 (6 H, s, 2 × CH_3). MS (m/z): 358 $(M+1\text{-}(HCl))^+$, 326 $(M\text{-}CH_2OH)^+$ (100%), 251, 213, 163, 114, 102, 70, 58. Log P: 2.43; Log D (pH): −1.24 (1.00), −0.94 (5.00), 0.71 (7.00), 1.09 (7.40).
$C_{20}H_{23}NO_5 \cdot HCl$ (393.8)

3.1.5. (±)-3-[2-N-(2,2-Dimethyl-1-hydroxy)-ethyloamino]-1-[(2-methyl)-6-xanthonoxy]-2-propanol hydrochloride (**7**)

Yield: 58%, m.p. 216–218 °C (n-propanol). R_f = 0.24 ($CHCl_3$/CH_3OH, 1/2). IR (cm^{-1}): 3326, 2975, 2349, 1657, 1619, 1592, 1483, 1445, 1304, 1259, 1231, 1123. UV (ethanol); λ_{max} (log ε): 205 nm (5.41), 249 (5.78),

268 (5.21), 306 (5.36). ^{1}H NMR (500.13 MHz) (δ, ppm, DMSO-d_6): 8.82 (1 H, t, J = 9.2 Hz, NH^+), 8.35 (1 H, t, J = 9.2 Hz, NH), 8.11 (1 H, d, J = 8.9 Hz, H-8), 7.98–7.94 (1 H, m, H-1), 7.66 (1 H, dd, J = 1.9 Hz, J = 9.0 Hz, H-3), 7.53 (1 H, d, J = 8.5 Hz, H-4), 7.17 (1 H, d, J = 2.3 Hz, H-5), 7.08 (1 H, dd, J = 2.4 Hz, J = 8.9 Hz, H-7), 5.92 (1 H, d, J = 5.0 Hz, CHOH), 5.61 (1 H, t, J = 5.0 Hz, CH_2OH), 4.33–4.26 (1 H, m, CH), 4.27–4.17 (2 H, m, CH_2OH), 3.51–3.47 (2 H, m, CH_2OAr), 3.25–3.17 (1 H, m, HCHN), 3.05–2.98 (1 H, m, HCHN), 2.44 (3 H, s, $ArCH_3$), 1.28 (3 H, s, CH_3), 1.27 (3 H, s, CH_3). ^{13}C NMR (δ, ppm): 174.79 (C=O), 163.80 (6), 157.36 (4b), 153.79 (4a), 135.98 (3), 133.68 (2), 127.55 (8), 125.1 (1), 120.81 (8b), 117.64 (4), 115.06 (8a), 113.78 (7), 101.17 (5), 70.50 (CH_2OH), 65.64 (CH_2OAr), 64.5 (CHOH), 59.67 ($C(CH_3)_2$), 44.15 ($N-CH_2$), 20.39, 20.25, 20.19 (3 × CH_3). Log P: 3.01; log D (pH): −0.69 (1.00), −0.40 (5.00), 1.29 (7.00), 1.67 (7.40).
$C_{21}H_{25}NO_5 \cdot HCl$ (407.9)

3.1.6. (±)-*3-[2-N-(2,2-Dimethyl-1-hydroxy)-ethyl-2-N-methylamino]-1-[(2-methyl)-6-xanthonoxy]-2-propanol hydrochloride* (**8**)

Yield: 62%, m.p. 226–228 °C (n-propanol), (m.p. (base) 110–112 °C (toluene/heptane, 1/2). $R_f = 0.35$ ($CHCl_3/CH_3OH$, 1/2). IR (cm^{-1}): 3231, 3108, 2984, 2875, 1658, 1619, 1592, 1444, 1302, 1258, 1231, 1174. ^{1}H NMR (base) (500.13 MHz) (δ, ppm, DMSO-d_6): 8.08 (1 H, d, J = 8.9 Hz, H-8), 7.95 (1 H, dd, J = 1.3 Hz, J = 2.3 Hz, H-1), 7.65 (1 H, ddd, J = 0.7 Hz, J = 2.3 Hz, J = 8.5 Hz, H-3), 7.51 (1 H, d, J = 8.5 Hz, H-4), 7.13 (1 H, d, J = 2.4 Hz, H-5), 7.04 (1 H, dd, J = 2.4 Hz, J = 8.9 Hz, H-7), 4.99 (1 H, s, CHOH), 4.34 (1 H, bs, CH_2OH), 4.20 (1 H, dd, J = 3.4 Hz, J = 10.1 Hz, HCHOAr), 4.06 (1 H, dd, J = 6.0 Hz, J = 10.1 Hz, HCHOAr), 3.92–3.86 (1 H, m, CHOH), 3.61 (1 H, dd, J = 2.2 Hz, J = 11.1 Hz, HCHOH), 3.23 (1 H, dd, J = 3.8 Hz, J = 11.1 Hz, HCHOH), 2.55 (1 H, dd, J = 6.7 Hz, J = 13.25 Hz, HCHN), 2.46 (1 H, dd, J = 6.7 Hz, J = 13.25 Hz, HCHN), 2.44 (3 H, dd, J = 0.7 Hz, J = 1.3 Hz, CH_3Ar), 2.24 (3 H, s, C′H_3–N), 0.94 (3 H, s, C′H_3–C), 0.93 (3 H, s, CH_3–C). Log P: 3.30; log D (pH): 1.09 (1.00 and 2.00), 1.29 (5.00), 2.74 (7.00), 2.99 (7.40).
$C_{22}H_{27}NO_5 \cdot HCl$ (422.1)

3.1.7. (±)-*3-[4-(Hydroxyethyl)-1-piperazinylo]-1-[(2-methyl)-6-xanthonoxy]-2-propanol dihydrochloride* (**9**)

Yield: 60%, m.p. 228–230 °C (n-propanol), (m.p. (base) 104–106 °C (ethanol)). $R_f = 0.48$ ($CHCl_3/CH_3OH$, 1/1). IR (cm^{-1}): 3183, 2936, 2826, 1661, 1620, 1590, 1485, 1446, 1307, 1257, 1233, 1170, 1099. UV (ethanol; λ_{max} (log ε): 238 nm (5.68), 265 (5.13), 305 (5.26). ^{1}H NMR (500.13 MHz) (δ, ppm, DMSO-d_6): 12.8 (2 H, bb, NH^+), 8.11 (1 H, d, J = 8.9 Hz, H-8), 7.97–7.95 (1 H, m, H-1), 7.67 (1 H, ddd, J = 0.5 Hz, J = 2.3 Hz, J = 8.5 Hz, H-3), 7.54 (1 H, d, J = 8.5 Hz, H-4), 7.20 (1 H, d, J = 2.3 Hz, H-5), 7.08 (1 H, dd, J = 2.4 Hz, J = 8.9 Hz, H-7), 6.2 (1 H, bb, OH), 4.57–4.40 (1 H, m, CHOH), 4.48 (1 H, bb, CH_2OH), 4.24–4.14 (2 H, m, CH_2OH), 4.10–2.95 (14 H, m, $N(CH_2)_6$, OCH_2), 2.45 (3 H, s, CH_3). ^{13}C NMR (δ, ppm): 174.80 (C=O), 163.78 (6), 157.38 (4b), 153.79 (4a), 136.00 (3), 133.69 (2), 127.56 (8), 125.11 (1), 120.81 (8b), 117.64 (4), 115.09 (8a), 113.80 (7), 101.20 (5), 70.76 (CH_2OAr), 63.95 (CH), 58.48 ($N-CH_2-CH$), 57.8 (CH_2-CH_2N), 55.17 (CH_2OH), 48.20–48.60 ($(CH_2)_4$ (piperazine)), 20.25 (CH_3). Log P: 1.78; Log D (pH): −1.69 (1.00), −0.25 (5.00), 1.16 (7.00), 1.42 (7.40).
$C_{23}H_{28}N_2O_5 \cdot 2HCl$ (485.4)

3.1.8. (±)-*3-(2-Propylamino)-1-[(2-methyl)-6-xanthonoxy]-2-propanol* (**10**)

Yield: 64%, m.p. 122–124 °C (toluene/heptane, 2/1), (m.p. (hydrochloride) 205–207 °C (n-propanol)). $R_f = 0.18$ ($CHCl_3/CH_3OH$, 1/2). IR (cm^{-1}): 3365, 3072, 2978, 2927, 2802, 2763, 2724, 2488, 1655, 1621, 1592, 1483, 1449, 1308, 1278, 1255, 1234, 1175, 1031. UV (base) (ethanol); λ_{max} (log ε): 205 nm (5.30), 238 (5.63), 268 (5.18), 305 (5.20). ^{1}H NMR (base) (200 MHz) (δ, ppm, DMSO-d_6): 8.06 (1 H, d, J = 8.8 Hz, H-8), 7.93 (1 H, d, J = 1.1 Hz, H-1), 7.64 (1 H, dd, J = 2.2 Hz, J = 8.8 Hz, H-3), 7.50 (1H, d, J = 8.5 Hz, H-4), 7.12 (1 H, d, J = 2.2 Hz, H-5), 7.02 (1 H, dd, J = 2.2 Hz, J = 8.8 Hz, H-7), 5.30–4.80 (1 H, bb, CHOH), 4.22–4.00 (2 H, m, $O-CH_2$), 3.98–3.82 (1 H, m, CHOH), 2.78–2.62 (2 H, m, $N-CH_2$), 2.62–2.51 (1 H, m, CH), 2.48 (3 H, dd, J = 1.1 Hz, 2.2 Hz, CH_3Ar), 2.10–1.80 (1 H, bb, NH), 0.998 (6 H, d, J = 6.1 Hz, $C(CH_3)_2$). Log P: 4.02; log D (pH): 0.64 (1.00, 2.00), 0.65 (5.00), 1.11 (7.00), 1.41 (7.40).
$C_{20}H_{23}NO_4$ (341.4)

3.1.9. (±)-*3-(2-Propyl-2-N-methylamino)-1-[(2-methyl)-6-xanthonoxy]-2-propanol hydrochloride* (**11**)

Yield: 58%, m.p. 192–194 °C (n-propanol), (m.p. (base) 100–102 °C (heptane)). $R_f = 0.29$ ($CHCl_3/CH_3OH$, 1/2). IR (cm^{-1}): 3299, 3214, 3060, 2972, 2934, 2634, 2481, 1646, 1615, 1589, 1482, 1445, 1310, 1276, 1255, 1231, 1172. ^{1}H NMR (base) (500.13 MHz) (δ, ppm, DMSO-d_6): 8.09 (1 H, d, J = 8.8 Hz, H-8), 7.96 (1 H, dd, J = 1.3 Hz, J = 2.3 Hz, H-1), 7.66 (1 H, ddd, J = 0.7 Hz, J = 2.3 Hz, J = 8.5 Hz, H-3), 7.52 (1 H, d, J = 8.5 Hz, H-4), 7.14 (1 H, d, J = 2.3 Hz, H-5), 7.05 (1 H, dd, J = 2.3 Hz, J = 8.8 Hz, H-7), 4.90 (1 H, s, CHOH), 4.19 (1 H, dd, J = 3.1 Hz, J = 10.1 Hz, HCH–OAr), 4.07 (1 H, dd, J = 4.2 Hz, J = 10.1 Hz, HCH–OAr), 3.95–3.88 (1 H, m, CHOH), 2.78 (1 H, t, J = 6.6 Hz, $CH(CH_3)_2$), 2.51 (1 H, dd, J = 7.5 Hz, J = 12.8 Hz, HCHN), 2.44 (3 H, dd, J = 0.7 Hz, J = 1.3 Hz, $Ar-CH_3$), 2.36 (1 H, dd, J = 6.0 Hz, J = 12.8 Hz, HCHN), 2.21 (3 H, s, $N-CH_3$), 0.95 (3 H, d, J = 6.6 Hz, $C-CH_3$), 0.94 (3 H, d, J = 6.6 Hz, $C-CH_3$). Log P: 4.31; Log D (pH): 0.48 (1.00), 0.53 (5.00), 0.75 (7.00), 0.96 (7.40).
$C_{21}H_{25}NO_4 \cdot HCl$ (391.9)

3.1.10. (±)-*3-[(2,6-Dimethyl)-1-piperidine)-1-[(2-methyl)-6-xanthonoxy)-2-propanol hydrochloride* (**12**)

Yield: 64%, m.p. 238–240 °C (n-propanol). $R_f = 0.31$ ($CHCl_3/CH_3OH$, 1/2). IR (cm^{-1}): 3262, 2936, 2869, 2646, 2583, 1655, 1623, 1593, 1444, 1305, 1272, 1231, 1170. UV (ethanol): λ_{max} (log ε): 240 (5.60), 270 (5.07), 305 (5.17), 330 (4.90). ^{1}H NMR (500.13 MHz) (δ, ppm, DMSO-d_6): 10.26 (1 H, bb, NH^+), 8.11 (1 H, d, J = 8.8 Hz, H-8), 7.98–7.94 (1 H, m, H-1), 7.67 (1 H, dd, J = 2.2 Hz, J = 8.9 Hz, H-3), 7.54 (1 H, d, J = 8.5 Hz, H-4), 7.18 (1 H, d, J = 2.3 Hz, H-5), 7.13–7.04 (1 H, m, H-7), 6.05–5.90 (1 H, m, OH), 4.42–4.35 (1 H, m, CHOH), 4.30–4.13 (2 H, m, $Ar-OCH_2$), 3.60–3.26 (3 H, m, $N(CH)_3$), 3.08–2.98 (1 H, m, $N-CH_2$), 2.44 (3 H, s, $ArCH_3$), 1.88–1.45 (4 H, m, $(CH_2)_2$ (piperidine)), 1.44 (2 H, d, J = 6.1 Hz, CH_2 (piperidine), 1.38 (d, J = 6.1 Hz), 1.31 (d, J = 6.2 Hz), 1.24 (d, J = 6.5 Hz) (6 H, 2 × CH_3). Log P: 5.40; log D (pH): 1.71 (1.00), 2.17 (5.00), 3.94 (7.00), 4.32 (7.40).
$C_{24}H_{329}NO_4 \cdot HCl$ (431.9)

3.2. Pharmacology

3.2.1. Materials

Compounds: Adrenaline hydrochloride (Polfa), thiopental sodium (Biochemie Gmbh, Vienna), sodium heparin (Polfa).
Animals: The experiments were carried out on male albino Swiss or albino CF-1 mice (18–25 g), male albino Wistar (180–250 g) or Sprague-Dawley (110–150 g) rats. The animals were housed in wire mesh cages in room at 20 ± 2 °C with natural light-dark cycles. The animals had free access to standard pellet diet and water, and used after a minimum of 3 days acclimation to the housing conditions. Control and experimental group consisted of 6–8 animals each.
Statistical analysis: The data are expressed as means. The results were statistically analysed by the one-way ANOVA test. Differences were considered significant when $p < 0.05$.

3.2.2. Anticonvulsant assays

The data in Tables 1 and 2 were generated by the National Institute of Neurological Disorders and Stroke, NIH Bethesda, USA [11]. The compounds were suspended in 0.5% methylcellulose/H_2O mixture and were administered either intraperitoneally to mice (CF-1) or orally to rats (Sprague-Dawley) at dose levels of 3.0–300.0 mg/kg. The MES were elicited by 60 Hz alternating current of 50 mA (mice) or 150 mA (rats) delivered for 0.2 s via corneal electrodes. A drop of 0.9% NaCl solution was instilled in each eye prior to application of the electrodes. Abolition of the hindlimb tonic extensions component of the seizure was defined as protection in the MES test. The ScMet test was conducted by administering 85 mg/kg of pentylenetetrazole dissolved in 0.9% NaCl solution in the posterior midline of mice. A minimal time 30 min subsequent to s.c. administration of pentylenetetrazole was used for seizure detection. A failure to observe even a threshold seizure (a single episode of clonic spasm of at least 5 s duration) was regarded as protection. Neurological deficit was measured in mice by the rotorod test. The mouse was placed on 1 inch diameter knurled plastic rod rotating at 6 rpm. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of three trials. In rats, neurological deficit was indicated by ataxia and loss of placing response and muscle tone. Anticonvulsant quantification, i.e., the doses of drug required to produce the biological responses in 50% of animals (ED_{50}), and the respective 95% confidence intervals, were determined on selected compounds displaying sufficient antiepileptic activity and low neurotoxicity from the above primary evaluations by means of a computer program using probit analysis.

3.2.3. Non-working heart perfusion

Hearts from thiopental-anaesthetised (45–60 mg/kg, i.p.) rats were perfused according to the Langendorff technique [17] at a constant pressure of 70 cm H_2O (6.87 kPa) with Chenoweth-Koelle solution continuously gassed with 95% O_2 plus 5% CO_2 of the following composition (mmol/l): NaCl (120.0), KCl (5.6), $MgCl_2$ (2.2), $NaHCO_3$ (19.0), $CaCl_2$ (2.4), and glucose (10.0). The effect of tested compounds, in concentration of 10^{-9} to 10^{-4} M, coronary flow (cardiac effluent), and electrocardiogram (obtained by two stainless steel electrodes, one inserted into the muscle of the ventricular wall and another attached to the metal aortic cannula) was assessed after 15–20 min of initial stabilisation.

3.2.4. Adrenaline-induced arrhythmia according to Szekeres [18]

The arrhythmia was evoked in rats anaesthetised with thiopental (75 mg/kg i.p.) by i.v. injection of adrenaline (20 μg/kg). The tested compounds were administered intravenously, 15 min before adrenaline. The criterion of antiarrhythmic activity was the lack of premature beats and inhibition of cardiac arrhythmia in comparison with the control group.

3.2.5. Acute toxicity according to Litchfield and Wilcoxon [19]

The compounds, dissolved in 0.9% saline, were injected into the caudal vein (1 ml/kg). Each dose was given to 6 animals. The LD_{50} were calculated acc. to the method of Litchfield and Wilcoxon after a 24 h observation period.

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