

PHARMACOLOGY

THE ANTICONVULSANT, LOCAL ANESTHETIC AND HEMODYNAMIC PROPERTIES OF SOME CHIRAL AMINOBUTANOL DERIVATIVES OF XANTHONE

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Abstract: In the present study, several pharmacological tests in animals were carried out to assess potential anticonvulsant, local anesthetic and hemodynamic activity of novel 2- and 4-substituted aminobutanol chiral derivatives of xanthone (hydrochlorides of: (R,S)-2-[(7-chloro)-2-xanthonemethyl]-N-methylaminobutan-1-ol (**MH-2(R,S)**), (R,S)-2-(4-xanthonemethyl)-aminobutan-1-ol (**MH-20(R,S)**) and (R,S)-2-[(6-methoxy)-2-xanthonemethyl]-aminobutan-1-ol (**MH-26(R,S)**) and their pure enantiomers R and S). The obtained results provided evidence that the most interesting anticonvulsant (in maximal electroshock-test) activity was shown by compound **MH-2(R)**, which in dose 100 mg/kg *p.o.*, protected the mice against tonic cramp of extensors similarly as phenytoin. Moreover, this compound, in concentrations from 0.25 to 1%, also possessed high local anesthetic activity (in infiltration anesthesia), comparable to the reference compound, mepivacaine. All examined compounds suppressed the spontaneous locomotor activity in mice, especially compound **MH-2(R,S)** and **MH-20(R,S)**, and their enantiomers. The impairment of motor coordination (in chimney test) for applied doses was not observed. Furthermore, compound **MH-20(S)** at dose corresponding to 1/10 LD₅₀ displayed an interesting hemodynamic activity and significantly decreased systolic and diastolic blood pressure in rats. All examined compounds showed chronotropic negative effect in anesthetized rats ECG record. The most reducing heart frequency was observed for enantiomers S of aminobutanol derivatives of xanthone, especially **MH-2(S)**. The LD₅₀ values of the investigated compounds were comparable with LD₅₀ value of the reference compound in local anesthesia tests – mepivacaine. These studies demonstrated different strength of enantiomers and racemic mixture in carried out tests, where the R enantiomers presented rather central and local anesthetic properties, whereas S enantiomers influenced the hemodynamic activity.

Keywords: xanthone, chiral compounds, anticonvulsant and local anesthetic activity

Epilepsy is the most common serious neurological disorder in the world. Worldwide, the prevalence is estimated to be 0.5 – 1%, and there is a lifetime incidence of 1 – 3%. Up to 70% of people suffering epilepsy can be successfully treated with anti-epileptic drugs (AEDs). AEDs aim to prevent seizures but don't cure epilepsy. Drugs that are effective in seizure reduction can block the initiation of the electrical discharge from abnormal electrical discharge to adjacent brain areas. They accomplish this by a variety of mechanisms, including blockade of voltage-gated channels (Na⁺ or Ca²⁺), enhancement of inhibitory GABAergic impulses, or interference with excitatory glutamate receptors (1). Deckers et al. (2) have proposed a classification of AEDs based upon their mechanisms of action, however, a majority of them possessed more than one mechanism of action. First group consists of

antiepileptics which block sustained repetitive firing in individuals neurons, a majority due to the blockade of voltage-dependent sodium or calcium channels (for example: phenytoin, carbamazepine, gabapentin, lamotrigine, oxcarbazepine, valproate, topiramate). This group is effective against generalized tonic-clonic and partial seizures. The second group includes drugs enhancing inhibitory events mediated by γ-aminobutyric acid (GABA) such as benzodiazepines, gabapentin, phenobarbital, tiagabine, valproate and vigabatrin. The third group consists of one drug – ethosuximide, it blocks T-type of calcium channels and is effective in absences. Recently, was suggested also the next group of AEDs which reduce events mediated by excitatory amino acids (glutamate) such as phenobarbital, topiramate and felbamate. However, despite the development of various novel antiepilep-

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tic drugs, about one third of patients with epilepsy are resistant to current pharmacotherapy. Even in patients in whom pharmacotherapy is efficacious, current AEDs do not seem to affect the progression or underlying natural history of epilepsy (1-5). Moreover a large number of new AEDs have been marketed worldwide, but unfortunately most of them possess important side effects, especially in long term therapy (5). Thus, new concepts and original ideas for developing antiepileptic drugs are urgently needed. Current clinically effective AEDs have been found by screening or structural variation of known AEDs and by rational strategies based on knowledge of pathophysiological processes involved in seizures or epilepsy. Also most of AEDs act by a combination of several mechanisms (potentiation of GABA, limitation of glutamatergic excitation, blockade of voltage-dependent sodium channels), so that a "rational" combination of mechanisms in a single drug may be a more successful strategy for creating novel broadly-acting AEDs than development of highly selective compounds (1, 3). The literature data show that the anticonvulsant activity has been demonstrated for compounds without any structural similarity to well-known AEDs, among others: 1-naphthylalkanolamines, O-acyl derivatives of respective aminoalkanols, for which a high anticonvulsant activity has been described (6, 7). Moreover, searching for compounds with potential antiepileptic properties it was noticed that several circulatory drugs with aminoalkanol or aminoalkoxy groups (e.g. beta-blockers) possess also anticonvulsant activity (8). Xanthone derivatives show several beneficial pharmacological properties when tested in biological systems. Depending on the kind and place of substitution in one of xanthone rings, analeptic, hypotensive, antitumor, anitaggregatory, anti-inflammatory and antitubercular properties were described (9-19). Taking into account all these facts, new compounds with potential antiepileptic, local anesthetic, hypotensive and antiarrhythmic activity have been intensively searched in the recent years in xanthones group (20-27). Former pharmacological studies confirmed that new xanthone derivatives, containing chiral 2-amino-1-butanol, can exert central, local anesthetic and/or hemodynamic activity (20-23, 28).

Our previous papers described the synthesis and the preliminary anticonvulsant activity of three new chiral, aminobutanol derivatives of xanthone. From our earlier published findings it follows that the compounds examined in the ADD program (Antiepileptic Drug Development Program) in Bethesda (the maximal electroshock seizure - MES

and subcutaneous pentylenetetrazole seizure threshold - ScMet) possessed a quite good protection index (PI, PI = TD₅₀/ED₅₀, whereby TD₅₀ and ED₅₀ refer to the doses of compounds causing neurotoxicity and anticonvulsant activity in 50% of the animals). The values of PI of new xanthone derivatives were equal 1.9 – 5.84 which correspond to values of PI for standard antiepileptic drug like phenytoin (PI = 6.6), carbamazepine (PI = 4.9) and valproate acid (PI = 1.7), (21, 24, 27). As a continuation of our profound interest in this subject, we present in this paper the anticonvulsant, local anesthetic activity and hemodynamic properties of some racemic and enantiomeric aminobutanol derivatives of xanthone.

EXPERIMENTAL

Animals and experimental conditions

The studies were carried out on male Albino Swiss mice weighing 18 – 24 g, normotensive male Wistar rats weighing 170 – 250 g and guinea pigs of both sexes (300 – 450 g). The animals were kept in plastic cages at a temperature of 20 ± 4°C, under 12/12 h light/dark cycle (light on from 7 a.m. to 7 p.m.). They were fed with granulated feed (standard laboratory pellets; Bacutil, Motycz, Poland) and had free access to water. The control and study groups consisted of 8-10 animals each. Treatment of the used laboratory animals in the present study was in full accordance with the respective Polish and European regulations and was approved by the Local Ethics Committee.

Drugs

2- and 4-substituted aminobutanol derivatives of xanthone: compounds **MH-2(R,S)** (hydrochloride of 2-[(7-chloro)-2-xanthonemethyl]-N-methylaminobutan-1-ol), **MH-20(R,S)** (hydrochloride of 2-(4-xanthonemethyl)-aminobutan-1-ol), **MH-26(R,S)** (hydrochloride of 2-[(6-methoxy)-2-xanthonemethyl]-aminobutan-1-ol) and their pure enantiomers R and S (synthesized at the Department of Chemical Technology and Biotechnology of Drugs CM UJ), mepivacaine (Mepivacain hydrochloride, Rhone – Poulenc Rorer, France), thiopental (Thiopentalum naticum, Sandoz), propranolol hydrochloride (R,S) and its enantiomers [(±), (+) and (-) Propranololum hydrochloricum, Fluka Chemie AG], phenytoin (Phenytoin amp. 250 mg/5mL, Schwarz) were dissolved in 0.9% NaCl. Depending on the experimental method, the tested compounds were given intravenously (*iv*), at doses corresponding to 1/5 – 1/10 of LD₅₀; intradermally or topically, in the form of 0.25, 0.5 and 1% solu-

tions or orally (*po*) in doses 30 and 100 mg/kg bw (in similar doses as in ADD program).

Anticonvulsant activity – MES test

30 min after *po* administration of the investigated compounds in doses 30 and 100 mg/kg in 0.9% NaCl, the mice were tested in MES – test (aural electrodes, alternating current of 50 Hz frequency and 50 mA intensity, duration of individual stimulus 0.2 s). The animals were observed for the following symptoms during the next 2 min: tonic cramp of flexors, tonic cramp of extensors and structural constituent of generalized clonic convulsions. Decreasing of tonic cramp of extensors was assessed as the anticonvulsant activity (30). As the reference compound phenytoin was used (*po*, 15 mg/kg, administrated 1 hour before MES-test) (31, 32).

Spontaneous locomotor activity in mice

The spontaneous locomotor activity of a single mouse was measured in photoresistor actometers (circular cages, 30 cm in diameter, provided with two photocells, and connected to the impulse counter), in 30 min sessions. The investigated compounds were administered *po* 30 min before the test in 0.9% NaCl in doses of 30 and 100 mg/kg.

Chimney test

The motor coordination of the investigated compounds was assessed by means of the chimney test. This test was carried out 30 min after *po* administration of the compounds given to mice in doses: 30 and 100 mg/kg. The animals were previously trained and selected. Then, they were placed in a 25 cm long and 3 cm in diameter horizontally located tube which was reversed in such a way that the mice were able to leave it only climbing backward up as soon as they reached another end. The ability of the mice to leave the tube within 1 min accounted for the lack of motor impairment properties of the investigated compounds (30).

Local anesthetic activity

Local anesthetic activity was evaluated according to Bülbbring and Wajda (33). The obtained results were compared with the duration of corneal or infiltration anesthesia in guinea pigs after mepivacaine administration at the concentrations of 0.25, 0.5 and 1.0% as the reference compound (33). The assessment of EC₅₀ (effective concentration in 50% of animals) was based on the summarized irritation induced by each concentration (in %) of the investigated compounds that guinea pig did not react during the period of observation.

A. Corneal anesthesia

The studied compounds were instilled to the right conjunctival sac as 0.25, 0.5, and 1.0% solutions in a volume of 0.05 mL, and the same volume of 0.9% NaCl was applied to the left eye. The corneal reflex was examined by irritation of right eye conjunctiva (studied eye) and a left eye conjunctiva (control eye) by horse's hair. The strength of local anesthetic activity was determined from the moment of solution instillation to the moment of reflex return. The presence or lack of corneal reflex were considered during an activity assessment. The eye conjunctiva irritation was done 6 times (every 5 s) with the pause of 5 min during the first 30 min.

B. The infiltration anesthesia (intradermal wheal test)

The infiltration anesthesia was tested in guinea pig, by causing the intradermal wheal by injection to the dorsum skin of the studied compounds in a volume of 0.1 mL and at the concentrations of 0.25, 0.5 or 1.0%. The painfull reaction to prick was registered after pricking 3 times the skin at the center of the wheal (every 5 s) with 5 min intervals during the first 30 min of observation and next after 15 min. The experiment was continued until the return of reaction to a prick. The control wheal was done by an intradermal injection of 0.1 mL of 0.9% NaCl.

The effect on ECG

The electrocardiographic recording in rat was done with ASPEL apparatus, with standard lead II, and paper speed of 50 mm/s. The investigated compounds were administered *iv* (to tail vein of thiopental, 75 mg/kg *ip* anaesthetized rat) at doses equal to 1/5 LD₅₀. The ECG was recorded just before administration of the compounds and 1, 5, 10 and 15 min thereafter.

The effect on arterial blood pressure

The normotensive rats were anesthetized with thiopental (75 mg/kg) by *ip* injection. The right carotid artery was cannulated with polyethylene tube filled with heparin in saline to facilitate pressure measurements using a Datamax apparatus (Columbus Instruments). The studied compounds were injected to rats tail vein as solids at 37 – 38°C in doses corresponding to 1/10 LD₅₀ after a 5 min stabilization period, in a volume equivalent to 1 ml/kg.

Acute toxicity

Acute toxicity of compounds in mice was assayed as LD₅₀ (lethal dose in 50% of animals)

according to Litchfield and Wilcoxon (34), calculated from a 24 h mortality rate of mice after *iv* administration of the compounds at gradually increasing doses. The general behavior was observed for 6 h after injection of the tested compounds.

Statistical analysis

The results obtained were presented as the means \pm SEM and evaluated statistically using U-Mann-Whitney test, Student t-test and one way ANOVA-test. Differences were considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

MES test is the most widely used animal model of seizure, because of simple seizure induction and the predictive value for detecting clinically effective antiepileptic drugs is high. The most potent antiepileptic activity in MES test showed compound

MH-2, especially its R enantiomer, which completely (in dose 100 mg/kg *po*) protected mice against tonic stage of cramp of extensors comparable to the reference compound, phenytoin (Table 1). The MES test identifies agents with activity against generalized tonic – clonic seizures, also this test predicts anticonvulsant drug effects against partial seizures (1, 4). The motor coordination (in chimney test) was not disturbed in applied doses (data not shown). Looking for new anticonvulsant compounds also their influence on locomotor activity in animals is very important (3, 30). All tested compounds statistically significantly decreased the spontaneous locomotor activity 60 min after *po* administration of 100 mg/kg dose by about 23 – 60%. The effects of lower doses (30 mg/kg) were statistically significant only in case of **MH-2(R,S)** and **MH-20(S)** compounds (Table 2).

It is well known that anticonvulsants and local anesthetics are similar in many respects, for

Table 1. The influence of investigated compounds on the tonic stage of cramp of extensors in mice in MES-test

Compound	Dose [mg/kg]	The number of mice with tonic stage of cramp of extensors		Mortality [%]	
		X/Y	[%]	Z/Y	[%]
Control	-	6/6	100	0/6	0
Phenytoin	15	0/8	0	0/8	0
MH-2(R, S)	30	7/8	87.5	0/8	0
	100	6/8	75	1/8	12.5
MH-2(S)	30	8/8	100	0/8	0
	100	4/8	50	0/8	0
MH-2(R)	30	8/8	100	1/8	12.5
	100	0/8	0	0/8	0
Control	-	6/6	100	1/6	16.7
MH-20(R, S)	30	5/7	71.4	1/7	14.3
	100	7/8	87.5	1/7	14.3
MH-20(S)	30	8/8	100	1/8	12.5
	100	8/8	100	0/8	12.5
MH-20(R)	30	8/8	100	1/8	12.5
	100	8/8	100	0/8	0
Control	-	6/6	100	0/6	0
MH-26(R, S)	30	8/8	100	0/8	0
	100	1/8	12.5	0/8	0
MH-26(S)	30	8/8	100	0/8	0
	100	2/8	25	0/8	0
MH-26(R)	30	6/8	75	0/8	0
	100	2/8	25	0/8	0

X – the number of mice with tonic stage of cramp of extensors. Z – the number of mice, which were dead during the test. Y – total number of mice used in the test

Table 2. The influence of the investigated compounds on spontaneous locomotor activity in mice 60 min after *po* administration.

Compound	Dose	Locomotor activity (counts during 30 min observation time)
Control	0.9% NaCl	429.33 ± 24.77
MH-2(R,S)	30	191.00 ± 38.86 ^c
	100	222.14 ± 92 ^c
Control	0.9% NaCl	333.40 ± 25.43
MH-2(S)	30	373.17 ± 39.30
	100	134.75 ± 37.89 ^b
Control	0.9% NaCl	383.00 ± 42.10
MH-2(R)	30	405.25 ± 39.16
	100	244.38 ± 31.90 ^a
Control	0.9% NaCl	508.40 ± 49.20
MH-20(R,S)	30	463.50 ± 28.51
	100	338.00 ± 37.96 ^a
Control	0.9% NaCl	508.40 ± 49.20
MH-20(S)	30	213.40 ± 48.33 ^b
	100	200.25 ± 31.45 ^b
Control	0.9% NaCl	508.40 ± 49.20
MH-20(R)	30	409.80 ± 63.98
	100	325.67 ± 18.39 ^a
Control	0.9% NaCl	482.0 ± 44.72
MH-26(R,S)	30	509.0 ± 54.31
	100	368.17 ± 35.87 ^a
Control	0.9% NaCl	480.80 ± 22.91
MH-26(S)	30	502.25 ± 45.50
	100	470.25 ± 38.79
Control	0.9% NaCl	356.87 ± 37.08
MH-26(R)	30	246.0 ± 33.91 ^a
	100	251.0 ± 36.57

Mean number of impulses ± SEM, 60 min after *po* administration of compounds **MH**. The results are the means ± SEM of 8-10 mice per group. U-Mann-Whitney test. ^ap < 0.05, ^bp < 0.02, ^cp < 0.01, ^dp < 0.001

instance: share similarities in their chemical structures, both are known to block sodium channel conduction in nerve cells, and both bind to the same place on the sodium channel (α -units S6 transmembrane segment of domain IV) (35). These known chemically structural similarities and significant anticonvulsant activity of some of the investigated compounds in MES-test led to hypothesis that these derivatives may also possess local anesthetic properties. The obtained results in corneal and infiltration anesthesia tests indicate that the investigated compounds demonstrated potent local anesthetic properties, especially when applied in the infiltration anesthesia method in guinea pig. With this

respect, special attention should be paid to activity of enantiomers R [**MH-20(R)** > **MH-2(R)** >> **MH-26(R)**] of xanthone derivatives which exhibited a local anaesthetic effect stronger than racemic mixtures, enantiomers S and referenced compound in this test – mepivacaine in infiltration and corneal model of anaesthesia (Tables 3-5). These results correspond with data for propranolol, which R enantiomer, without β -adrenoceptor blocking activity, possesses high membrane stabilizing properties, higher than its S enantiomer and racemic mixture (8). Till today, about 60% of local anesthetics have been optical isomers which are mainly used as racemic compounds. For example, the local anes-

Table 3. Local anesthetic activity of the investigated compounds in corneal anesthesia model in guinea pigs.

Compound	Concentration in %	Inhibition of pain reaction in %					
		5 min	30 min	60 min	120 min	240 min	24 h
Mepivacine	0.25%	41.66 ^a	16.67	11.11	2.77	0	0
	0.5%	63.33 ^a	6.66	10.00	6.66	0	0
	1.0%	44.44 ^a	19.44	22.22	8.33	0	0
MH-2 (R, S)	0.25%	0	0	0	0	0	0
	0.5%	0	0	0	0	0	0
	1.0%	0	2.77	0	0	0	0
MH-2(S)	0.25%	0	0	0	0	0	0
	0.5%	0	0	0	0	0	0
	1.0%	0	2.77	0	0	0	0
MH-2(R)	0.25%	2.77	2.77	0	0	0	0
	0.5%	30.55	2.77	8.33	0	0	0
	1.0%	25.00	27.77 ^c	22.22	22.22	2.77	0
MH-20 (R, S)	0.25%	25.00 ^a	0	0	0	0	0
	0.5%	41.67 ^c	0	0	0	0	0
	1.0%	22.22	0	0	0	0	0
MH-20(S)	0.25%	36.11 ^c	0	0	0	0	0
	0.5%	66.11 ^d	11.11 ^d	0	0	0	0
	1.0%	55.78 ^c	5.56	8.33 ^a	5.56	0	0
MH-20(R)	0.25%	2.78	0	0	0	0	0
	0.5%	8.33 ^a	0	0	0	0	0
	1.0%	52.78 ^c	44.44 ^d	27.78 ^a	27.78	8.33	0
MH-26 (R, S)	0.25%	2.78	0	0	0	0	0
	0.5%	22.22	0	0	0	0	0
	1.0%	33.33 ^c	5.56	5.56	0	0	0
MH-26(S)	0.25%	0	0	0	0	0	0
	0.5%	22.22 ^a	0	0	0	0	0
	1.0%	41.67 ^c	8.33	0	0	0	0
MH-26(R)	0.25%	0	0	0	0	0	0
	0.5%	11.11	0	0	0	0	0
	1.0%	61.11 ^c	11.11	8.33	0	0	0

Statistical significance was evaluated using Student t-test : ^ap < 0.05, ^bp < 0.02, ^cp < 0.01, ^dp < 0.001

thetic bupivacaine exists in two stereoisomeric forms, R(+) and S(-)-bupivacaine. Because of its lower cardiac and central nervous system toxicity, attempts were made recently to introduce S(-)-bupivacaine into therapy (36).

All investigated compounds diminished the heart rate from 5 to 30% (Table 6). The most robust changes in rat's ECG record were observed for compound **MH-2 (R,S)** and its enantiomers, specially enantiomer S (data were formerly presented) with: high chronotropic negative effect, prolongation of P-Q and Q-T interval and extension of QRS complex

(28). Remaining compounds did not evoke statistically significant changes in ECG record during experiment (Table 6). The observed changes in rat's ECG record were similar to ECG changes during therapy of the first class of antiarrhythmic drugs according to Vaughan – Williams classification (37). The first class of antiarrhythmic drugs includes substances with membrane stabilizing properties, which block voltage-gated sodium channels in heart, lengthen P-Q and Q-T intervals and extend QRS complex (37, 38). The long-term decrease of blood pressure in anesthetized rat was observed only for compound **MH-**

Table 4. Local anesthetic activity of the investigated compounds in infiltration anesthesia model in guinea pigs.

Compound	Concentration in %	Inhibition of pain reaction in %					
		5 min	30 min	60 min	120 min	240 min	24 h
Mepivacaine	0.25%	55.55 ^d	44.44 ^d	27.77 ^c	33.33	13.33	5.55
	0.5%	100.00 ^d	40.00 ^c	40.00 ^c	46.66	16.67	0
	1.0%	100.00 ^d	94.44 ^d	66.66 ^d	72.77 ^d	50.00 ^c	11.11
MH-2(R, S)	0.25%	33.33 ^a	22.22 ^b	16.67	22.22	16.67	0
	0.5%	55.55 ^d	44.44	38.88 ^d	50.00 ^c	16.67	0
	1.0%	100.00 ^d	94.44 ^d	83.33 ^d	77.77 ^d	77.77 ^d	38.88 ^a
MH-2(S)	0.25%	50.00 ^c	22.22 ^b	22.22 ^b	11.11	11.11	0
	0.5%	44.44 ^c	16.66	38.88 ^d	66.66	11.11	0
	1.0%	100.00 ^d	75.00 ^b	83.33 ^d	94.44 ^d	83.33 ^d	55.55 ^c
MH-2(R)	0.25%	55.55 ^d	44.44 ^c	27.77 ^c	27.77	16.67	0
	0.5%	83.33	61.11	61.11	38.88	16.66	0
	1.0%	100.00 ^d	100.00 ^b	83.33 ^d	83.33 ^d	72.22 ^a	16.66
MH-20 R, S)	0.25%	83.33 ^d	72.22 ^d	72.22 ^c	55.55 ^c	38.89 ^d	5.56
	0.5%	94.44 ^d	83.33 ^c	83.33 ^d	83.33 ^d	83.33 ^d	22.22
	1.0%	83.33 ^d	77.78 ^d	72.22 ^d	66.67 ^c	66.67 ^c	22.22
MH-20(S)	0.25%	77.78 ^d	27.78	22.22	16.67	0	0
	0.5%	94.44 ^d	83.33 ^d	77.78 ^c	61.11	22.22	16.67
	1.0%	83.33 ^c	83.33 ^d	88.89 ^d	88.89 ^d	61.11 ^b	38.89
MH-20(R)	0.25%	83.33 ^d	66.67 ^d	66.67 ^c	44.44	22.22	11.11
	0.5%	88.89 ^d	66.67 ^c	61.11 ^a	55.56 ^d	55.56 ^c	16.67 ^a
	1.0%	100.0 ^d	83.33 ^d	88.89 ^d	83.33 ^d	72.22 ^d	27.78
MH-26 (R, S)	0.25%	83.33 ^c	27.78	38.89	38.89 ^b	38.89	16.67
	0.5%	94.44 ^d	61.11 ^d	72.22 ^c	61.78 ^b	61.78 ^d	33.33 ^c
	1.0%	94.44 ^d	77.78 ^d	83.33 ^d	61.11 ^c	55.55 ^c	22.22 ^b
MH-26(S)	0.25%	100.00 ^c	44.44 ^b	27.78 ^b	33.33 ^b	22.22	11.11
	0.5%	94.44 ^d	83.33 ^d	72.22 ^d	72.22 ^b	38.89	44.44 ^a
	1.0%	100.00 ^d	83.33 ^d	88.89 ^d	88.89 ^c	66.67 ^c	55.56
MH-26(R)	0.25%	61.11 ^c	66.67 ^c	33.33	22.22	5.56	5.56
	0.5%	88.89 ^d	44.44 ^c	33.33	44.44 ^d	33.33 ^a	16.67
	1.0%	100.00 ^d	72.22 ^c	61.11 ^c	61.11 ^d	55.56 ^c	22.22

Statistical significance was evaluated using Student t-test : ^ap < 0.05, ^bp < 0.02, ^cp < 0.01, ^dp < 0.001

20(R,S), and especially its S enantiomer, which decreased systolic and diastolic blood pressure by about 30% (p < 0.001) during 1 h time of observation (Figs. 1, 2). Remaining xanthone racemic mixtures did not evoke statistical significant changes in rat's blood pressure during experiment. The enantiomers R of compounds **MH-2** and **MH-26** practically did not change systolic and diastolic blood pressure during experiment, but their S enantiomers possessed low hypotensive activity (diminishing blood pressure by about 14 to 20% during 1 h time of observation).

The obtained results in acute toxicity test acc. to Litchfield and Wilcoxon demonstrated different strength of enantiomers and racemic mixtures in LD₅₀ value. For compounds **MH-2** and **MH-20** the LD₅₀ values for racemic mixture and enantiomer R were comparable, whereas LD₅₀ value of enantiomer S was significant lower. Quite the reverse results were observed for compound **MH-26**, which enantiomer S possessed the highest LD₅₀ value (Table 7). The LD₅₀ values of the investigated compounds were comparable with LD₅₀ value for reference compound in local anesthesia tests, mepivacaine (32).

Table 5. EC₅₀ values (in %) of the investigated compounds, mepivacaine and propranolol in infiltration anesthesia model in guinea pigs.

Compound	EC ₅₀ (%)	
	60 min	240 min
Mepivacaine	0.60 (0.20 – 1.80)	1.34 (0.42 – 4.20)
MH-2(R,S)	0.53 (0.30 – 0.93)	0.68 (0.35 – 1.29)
MH-2(S)	0.51 (0.26 – 0.97)	0.68 (0.41 – 1.12)
MH-2(R)	0.41 (0.19 – 0.84)	0.74 (0.36 – 1.50)
MH-20(R,S)	0.12 (0.09 – 0.28)	0.34 (0.15 – 0.74)
MH-20(S)	0.40 (0.27 – 0.61)	0.82 (0.62 – 1.07)
MH-20(R)	0.29 (0.13 – 0.67)	0.51 (0.26 – 0.99)
MH-26(R,S)	0.31 (0.15 – 0.64)	0.44 (0.09 – 2.09)
MH-26(S)	0.34 (0.15 – 0.59)	0.63 (0.30 – 1.35)
MH-26(R)	0.68 (0.32 – 1.43)	0.83 (0.50 – 1.40)
Propranolol (±)	0.15 (0.09 – 0.27)	0.33 (0.15 – 0.74)
Propranolol (-)	0.41 (0.24 – 0.69)	0.57 (0.30 – 1.09)
Propranolol (+)	0.20 (0.13 – 0.33)	0.46 (0.23 – 0.94)

Table 6. The influence of chiral xanthone derivatives on rat's ECG. Route: *iv*; anesthesia – thiopental 75 mg/kg *ip*; dose: 1/5 LD₅₀.

Compound	Parameter	Time of observation (min)				
		0	10 s	5 min	10 min	15 min
MH-20 (R,S)	PQ (ms)	33.04 ± 1.08	37.71 ± 1.98 ^b	36.43 ± 0.96	34.09 ± 1.28	36.94 ± 1.31
	QRS (ms)	21.78 ± 1.38	19.90 ± 0.48	19.68 ± 0.54	19.23 ± 0.69	19.99 ± 0.61
	QT (ms)	59.33 ± 2.04	64.03 ± 1.24	62.27 ± 1.85	61.21 ± 1.90	62.07 ± 1.44
	Frequency (beats/min)	373.19 ± 13.96	289.48 ± 15.41 ^d	332.30 ± 8.67 ^b	348.84 ± 10.24	342.24 ± 8.31
MH-20(S)	PQ (ms)	38.05 ± 2.28	41.75 ± 2.65	38.95 ± 1.37	37.6 ± 1.92	38.9 ± 1.53
	QRS (ms)	18.60 ± 0.45	18.85 ± 0.19	18.35 ± 0.19	19.45 ± 0.65	18.85 ± 0.19
	QT (ms)	57.85 ± 2.21	60.25 ± 1.92	60.75 ± 3.07	60.00 ± 3.09	60.30 ± 2.55
	Frequency (beats/min)	343.82 ± 26.35	297.83 ± 25.68	325.45 ± 25.61	324.49 ± 25.78	323.21 ± 25.72
MH-20(R)	PQ (ms)	34.85 ± 1.96	44.80 ± 2.46 ^d	38.22 ± 1.71	38.64 ± 1.13	37.25 ± 1.68
	QRS (ms)	19.39 ± 0.73	21.39 ± 0.47 ^a	19.59 ± 0.64	19.21 ± 0.42	20.14 ± 0.49
	QT (ms)	60.50 ± 1.57	62.78 ± 1.28	61.68 ± 1.46	57.43 ± 1.04	59.60 ± 1.69
	Frequency (beats/min)	354.71 ± 17.07	275.62 ± 17.77 ^c	329.62 ± 24.93	321.11 ± 19.78	323.99 ± 20.30
MH-26 (R,S)	PQ (ms)	38.67 ± 0.59	42.92 ± 1.01 ^d	39.75 ± 0.60	39.42 ± 0.53	39.33 ± 0.26
	QRS (ms)	17.33 ± 0.88	19.5 ± 0.98	18.42 ± 1.16	18.33 ± 1.07	18.42 ± 1.03
	QT (ms)	30.67 ± 1.59	33.00 ± 1.35	31.00 ± 1.50	31.17 ± 1.46	31.67 ± 1.49
	Frequency (beats/min)	372.58 ± 11.09	344.92 ± 12.47	360.17 ± 14.30	361.33 ± 13.81	359.75 ± 12.38
MH-26(S)	PQ (ms)	38.08 ± 1.06	47.25 ± 2.44 ^d	40.00 ± 1.49	39.33 ± 1.13	39.25 ± 1.38
	QRS (ms)	20.27 ± 1.04	21.97 ± 1.44	22.08 ± 1.52	22.97 ± 1.86	21.42 ± 1.40
	QT (ms)	33.09 ± 90	34.36 ± 1.64	36.25 ± 1.66	32.19 ± 1.37	33.19 ± 1.36
	Frequency (beats/min)	385.08 ± 10.09	313.50 ± 7.81 ^d	353.58 ± 9.06	357.33 ± 8.69	360.25 ± 9.08
MH-26(R)	PQ (ms)	38.42 ± 0.58	43.75 ± 1.72 ^c	39.25 ± 1.56	39.00 ± 0.98	39.33 ± 0.86
	QRS (ms)	19.83 ± 1.26	20.33 ± 1.41	19.75 ± 1.12	20.17 ± 1.34	20.17 ± 1.25
	QT (ms)	34.00 ± 1.87	36.25 ± 2.02	34.67 ± 1.69	34.92 ± 1.53	35.25 ± 1.75
	Frequency (beats/min)	376.83 ± 17.07	326.08 ± 16.68 ^a	357.75 ± 16.10	364.08 ± 17.81	360.25 ± 18.24

Values are the means ± SEM of 6-7 preparations in the group. Statistical significance was evaluated using a one-way ANOVA test; ^ap < 0.05, ^bp < 0.02, ^cp < 0.01, ^dp < 0.001

* the data for compound **MH-2(R,S)** and its enantiomers R and S were presented previously (28)

CONCLUSION

Antiepileptic activity in MES test, local anaesthetic activity, the effect on ECG parameters and also

some protective effect in barium chloride-induced arrhythmia may suggest potential membrane stabilizing activity of these investigated compounds (4, 8, 28, 38), which should be examined in some electro-

Table 7. Acute toxicity of the investigated compounds.

Compound	LD ₅₀ [mg/kg]
MH-2(R,S)	47.00 (36.15 – 61.10)
MH-2(S)	36.00 (23.86 – 54.30)
MH-2(R)	47.34 (40.39 – 55.48)
MH-20(R,S)	34.00 (24.83 – 46.56)
MH-20(S)	14.00 (9.82 – 19.96)
MH-20(R)	31.00 (23.2 – 41.42)
MH-26(R,S)	30.60 (22.57 – 41.49)
MH-26(S)	57.60 (51.90 – 63.90)
MH-26(R)	48.00 (41.30 – 55.79)
phenytoin	92 *
mepivacaine	35 **

The experiments were conducted in mice (20 – 25 g) acc. to Litchfield and Wilcoxon (34). The drugs were administered *iv*. The animals were observed for 24 h; n = 6-8.* (33), **(39)

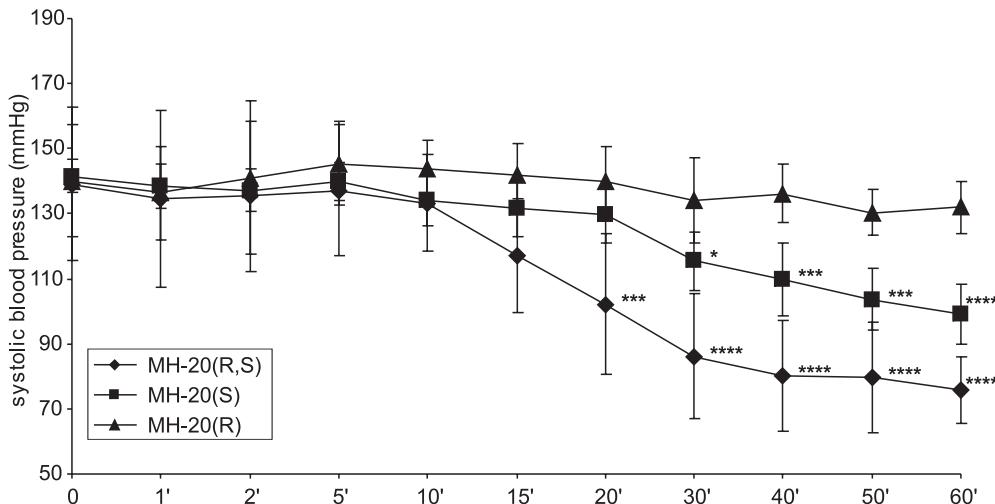


Figure 1. The influence of compound **MH-20(R,S)** and its enantiomers R and S on the systolic blood pressure of anesthetized rat. Route: *iv*; anesthesia – thiopental 75 mg/kg *ip*; dose: 1/10 LD₅₀, * p < 0.05, ** p < 0.02, *** p < 0.01, **** p < 0.001

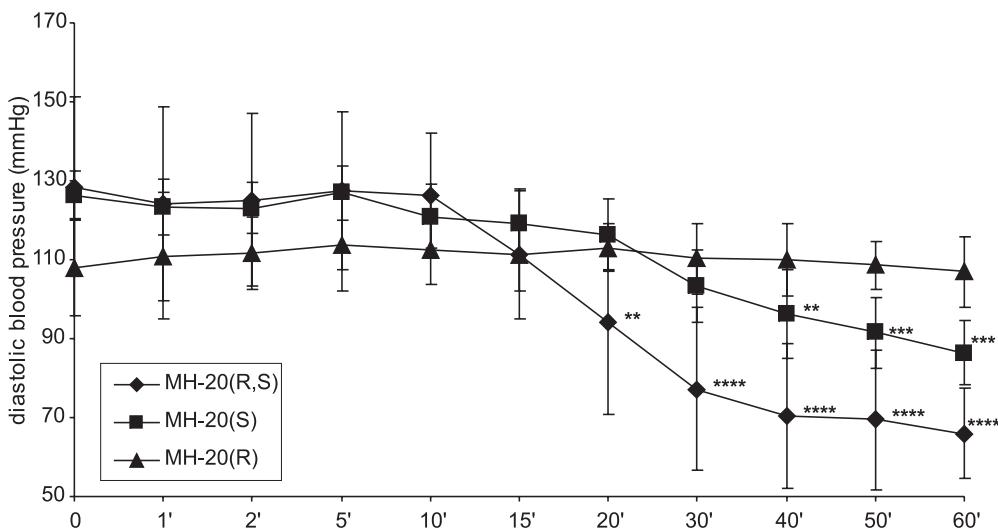


Figure 2. The influence of compound **MH-20(R,S)** and its enantiomers R and S on the diastolic blood pressure of anesthetized rat. Route: *iv*; anesthesia – thiopental 75 mg/kg *ip*; dose: 1/10 LD₅₀, * p < 0.05, ** p < 0.02, *** p < 0.01, **** p < 0.001

physiological tests (for example patch clamp). Special attention should be paid to enantiomers R which possess high anticonvulsant and local anesthetic properties. Enantiomers S of the investigated compounds (especially 4-substituted aminobutanol xanthone derivatives) demonstrated potent hemodynamic properties. The most potent central and local anesthetic pharmacological activity demonstrated 2-substituted compounds with chlorine atom in position 7 of xanthone ring [compound **MH-2(R,S)** and its enantiomers]. Moreover, when aminobutanol chain was moved to 4 position of xanthone ring or chlorine atom was removed [compound **MH-20(R,S)** and its enantiomers] the substances lost their central and local anesthetic properties, but especially for enantiomer S increased its hypotensive activity. The obtained results demonstrated different strength of enantiomers and racemic mixture in affecting CNS functions, local anesthetic and hemodynamic activity, which indicate the importance of pharmacological examination of enantiomers and not only racemic mixtures of newly synthesized compounds.

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