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Synthesis and pharmacological properties of new GABA- and TRP allosteric modulators

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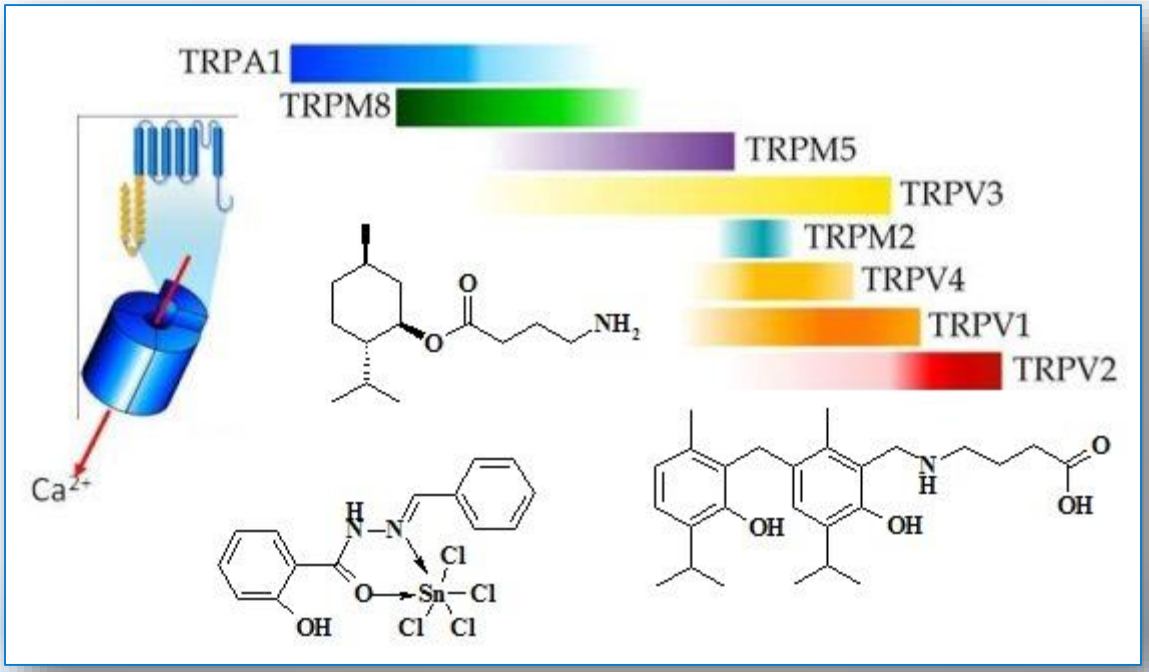


Abstract:

Research and development of drugs with combined pharmacological effect – those that affect the different receptor types – is an urgent problem of modern pharmacology. In this work we are presenting the synthesis and pharmacological investigation of some new menthol, thymol and salicylic acid derivatives designed as GABA- and TRP allosteric modulators.

Our findings identified menthyl ester of GABA (2-isopropyl-5-methylcyclohexyl 4-aminobutyrate) as a compound with anticonvulsant activity over a wide range of doses: 87-1350 mg/kg whereas menthyl ester of glycine (2-isopropyl-5-methylcyclohexyl 2-aminoacetate) shows significant sedative effect over 6 hours after oral administration at 175 mg/kg.

Complex compounds obtained by $SnCl_4$ interaction with salicyloylhydrazones have demonstrated anti-inflammatory, anxiolytic, antidepressant and analgesic activity in different models *in vivo*. All aforementioned compounds can be considered as those that exhibit a combined pharmacological activity due to their simultaneous binding to different receptor types.

Keywords: menthol esters, GABA, TRP, salicyloylhydrazones, pharmacological activity.

Introduction

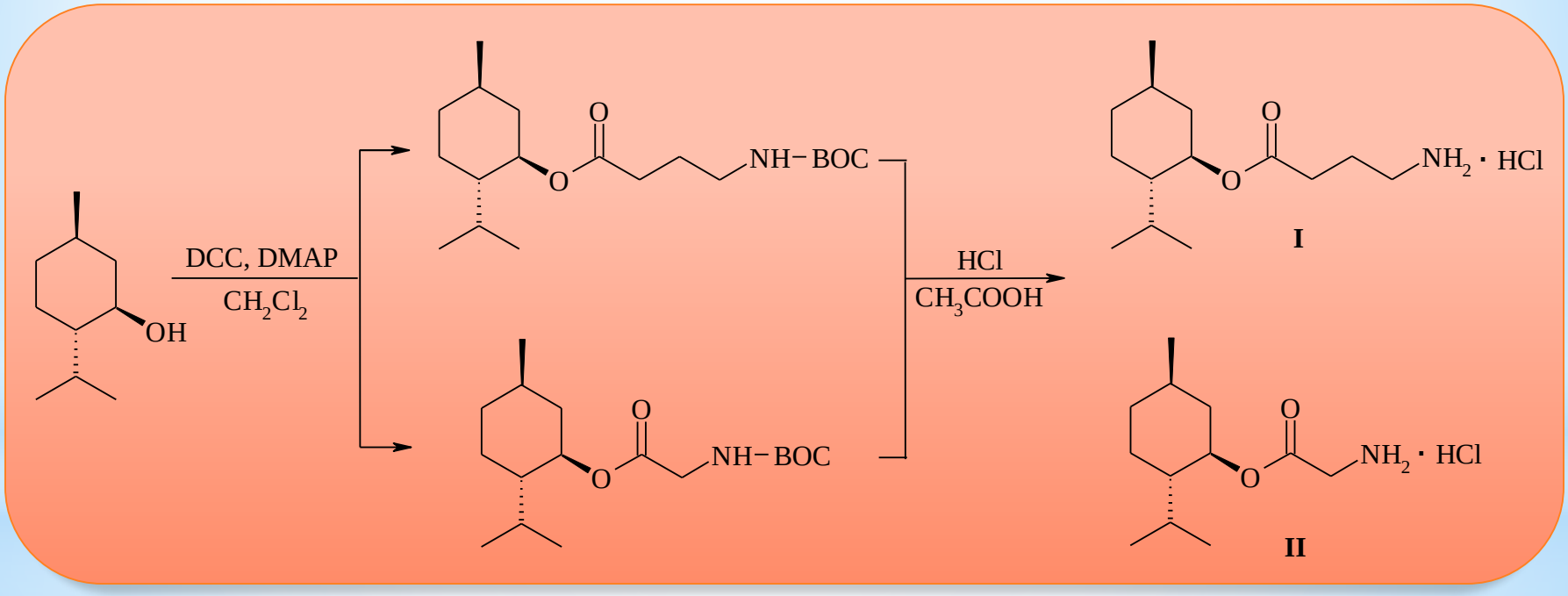
GABAergic and glycinergic systems are the targets of a wide range of drugs active on the CNS, including anxiolytics, sedative-hypnotics, general anesthetics and anticonvulsants (Macdonald and Olsen, 1994). Recent studies have reported that cyclic monoterpenes menthol and thymol also have actions within the CNS (Zhang et al., 2008) and act as a potent positive allosteric modulator of $GABA_A$ receptors (Hall et al., 2004).

It has been reported that salicylic acid derivatives activate heterologously expressed TRPA1 (Bandell et al., 2004), a member of the TRP channel family expressed by nociceptors and its potential role as a sensor of noxious cold (Story et al., 2003; Bandell et al., 2004).

In the present study, menthyl esters of inhibitory neurotransmitters (GABA and glycine), thymol derivatives and $SnCl_4$ complexes with salicyloylhydrazones were investigated for anticonvulsant, sedative and anti-inflammatory activities.

Results and discussion

Menthyl ester of GABA (2-isopropyl-5-methylcyclohexyl 4-aminobutyrate) and glycine (2-isopropyl-5-methylcyclohexyl 2-aminoacetate) were synthesized using DCC/DMAP coupling method followed by deprotection of the amino groups in the HCl/CH_3COOH medium.



Pharmacological properties of menthyl GABA ester

Acute toxicity

The acute oral LD_{50} of menthyl ester to adult white mice was measured and estimated at 2700 mg/kg; noteworthy is the fact that experimental animals show signs of convulsions and respiratory rhythm inhibition before dying.

Anticonvulsant activity (PTZ test)

Table 1. Dose-response relationship for GABA menthyl ester in 6 h after oral administration (mean ± SEM)

Dose, mg/kg		87	175	350	700	1350	Control
MED of pentylenetetrazole, % of control	DCTC	147±4.7	170±4.0	195±4.3	200±5.7	200±6.2	100±5.3
	DTE	144±5.3	158±2.8	186±6.0	183±4.0	217±4.2	100±5.1

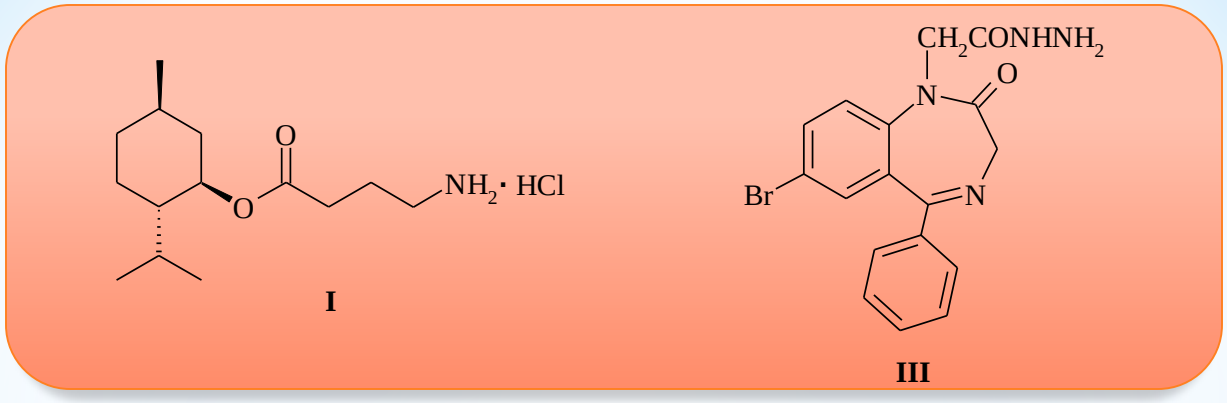
[a]MED – minimum effective dose. [b]DCTC – dose of pentylenetetrazole for inducing clonic-tonic convulsions in experimental animals. [c]DTE – dose of pentylenetetrazole for inducing tonic extension in experimental animals.

Table 2. Time-response relationship for GABA menthyl ester after oral administration at dose 175 mg/kg (mean ± SEM)

Time, h	MED of pentylenetetrazole, % of control	
	DCTC	DTE
0.5	109 ± 3.3	114 ± 4.1
1	152 ± 2.8	167 ± 4.0
3	167 ± 3.0	167 ± 3.7
6	164 ± 4.2	169 ± 5.3
18	191 ± 2.9	196 ± 3.3
24	186 ± 4.5	189 ± 3.7
48	155 ± 2.7	161 ± 4.0
72	139 ± 3.1	142 ± 2.9
96	133 ± 4.0	142 ± 4.2
Control	100 ± 5.0	100 ± 4.2

[a]MED – minimum effective dose. [b]DCTC – dose of pentylenetetrazole for inducing clonic-tonic convulsions in experimental animals. [c]DTE – dose of pentylenetetrazole for inducing tonic extension in experimental animals.

Synergistic anticonvulsant effect after oral co-administration of gidazepam and GABA menthyl ester



As seen, both gidazepam and GABA menthyl ester reveal anticonvulsant effect in 3 h after oral administration with DCTC and DTE values: 250 and 238% for compound **I**; 215 and 196% for compound **III**. Co-administration of compound **I** and **III** was shown to increase anticonvulsant activity compared with each compound alone with DCTC and DTE values: 370 and 358%. These data demonstrate that orally co-administered gidazepam and GABA menthyl ester produce synergistic effect in seizures prevention.

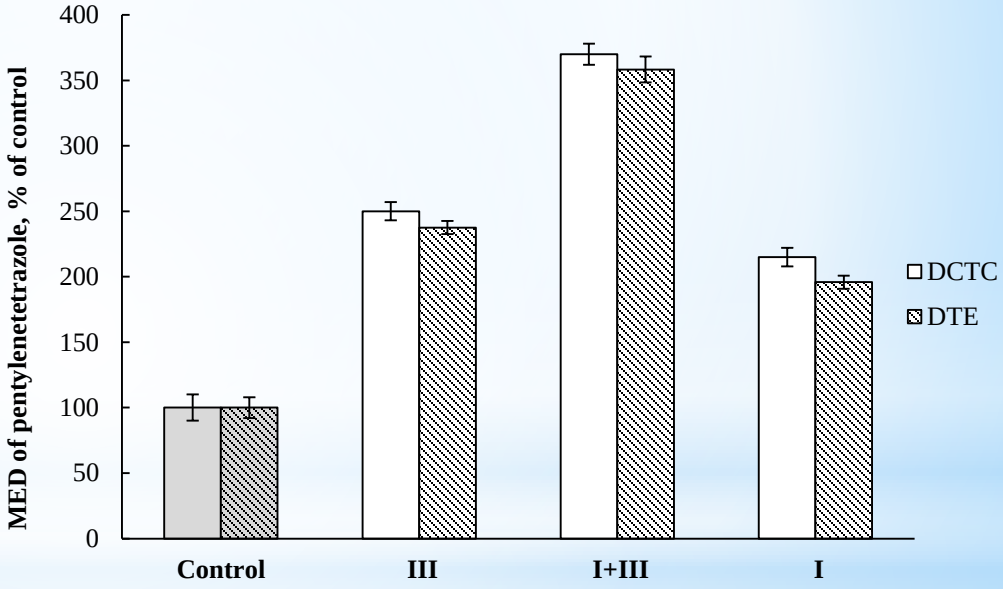


Fig. 5. Anticonvulsant activity of compounds I, III and their mixture.

Pharmacological properties of menthyl glycine ester

Acute toxicity

Acute toxicity study of glycine menthyl ester revealed the following LD_{50} values: 1350 mg/kg by oral route administration and 50 mg/kg intravenously.

Anticonvulsant activity (PTZ test)

Glycine menthyl ester was not shown to demonstrate significant anticonvulsant activity at the doses of 87-350 mg/kg via oral route at 3 hour after oral administration. However, at the dose of 700 mg/kg it produces anticonvulsant effect as indicated by increasing of DCTC and DTE values that amounts to 203 % and 172%, respectively.

Anticonvulsant activity (Strychnine test)

Strychnine has been demonstrated to have a well-defined mechanism of convulsant action reported to be by directly antagonizing the inhibitory spinal cord and brainstem reflexes of glycine and thus increasing spinal reflexes. Taking into account this fact, we have evaluated ester anticonvulsant effect in strychnine induced seizure model. Menthyl ester at the dose of 700 mg/kg was found to modify strychnine action slightly with DCTC and DTE values 148 % and 141%, accordingly; this might be assigned to menthol predominant contribution into anticonvulsant activity of synthesized ester.

Sedative activity (OFT test)

Our results demonstrate that the oral administration of menthyl ester at doses of 350-700 mg/kg causes a marked reduction both in lateral (Fig. 1) and vertical (Fig. 2) activities, but does not affect the research activity.

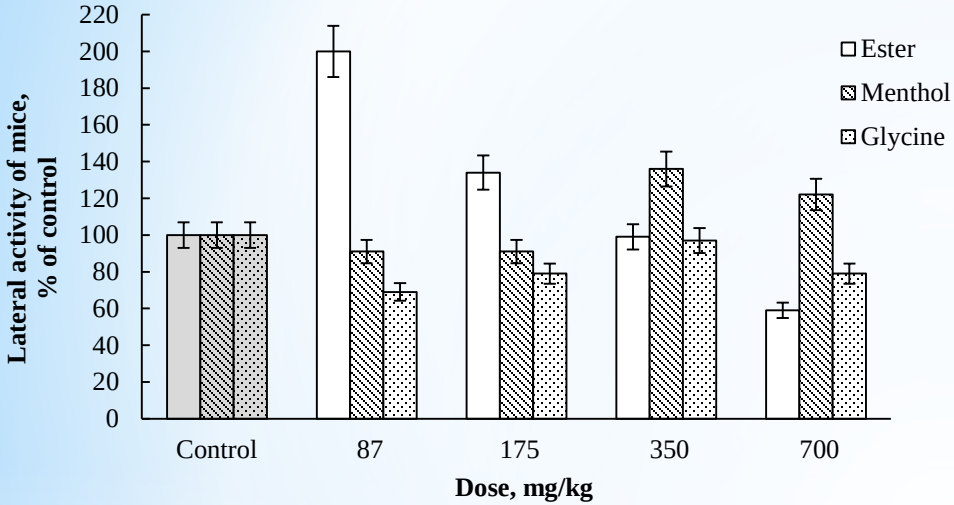


Fig. 1. Comparable lateral activity of mice in 3 h after oral administration of glycine menthyl ester, menthol and glycine (dose-response relationship).

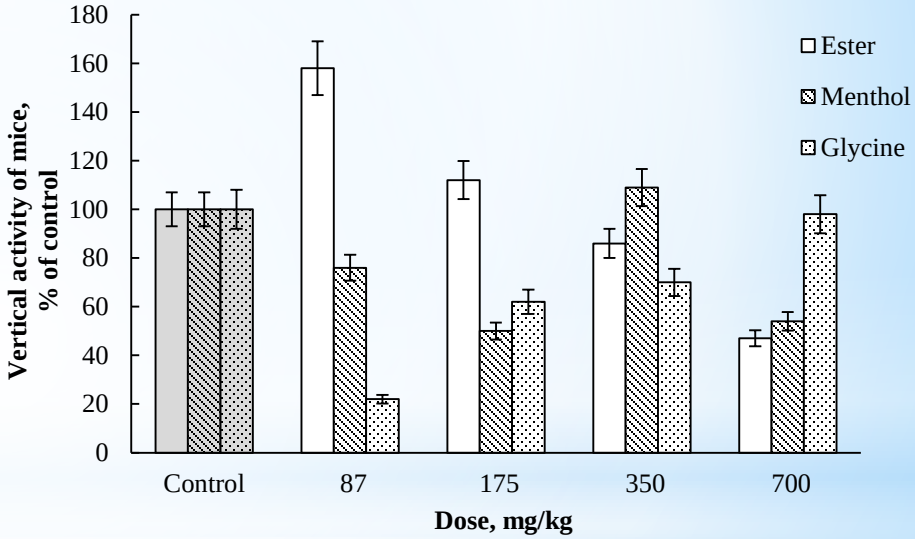


Fig. 2. Comparable vertical activity of mice in 3 h after oral administration of glycine menthyl ester, menthol and glycine (dose-response relationship).

Considering the possible prolonged action of obtained ester, sedative effect was estimated over the time range: 1-24 hours. This enables the pharmacokinetics of synthesized compound to be expressed as a function of time after oral administration. Our data reveal that menthyl ester of glycine at 175 mg/kg dose causes a time-dependent reduction of locomotor (Table 3) and research activity (Fig. 4). Maximum suppressive effect was found within the time of 3-6 hours and continued up to 24 hours after oral administration, indicating prolonged sedative action.

Table 3. Locomotor activity of mice after oral administration of glycine menthyl ester at dose 175 mg/kg (time-response relationship)

Time after oral administration, h	Locomotor activity	
	lateral	vertical
Control	100,0 ± 8,1	100,0 ± 7,7
1	130,3 ± 7,4	80,9 ± 7,2
3	64,7 ± 5,2	19,2 ± 5,4
6	48,1 ± 6,0	31,3 ± 6,6
24	78,7 ± 4,9	83,6 ± 9,1

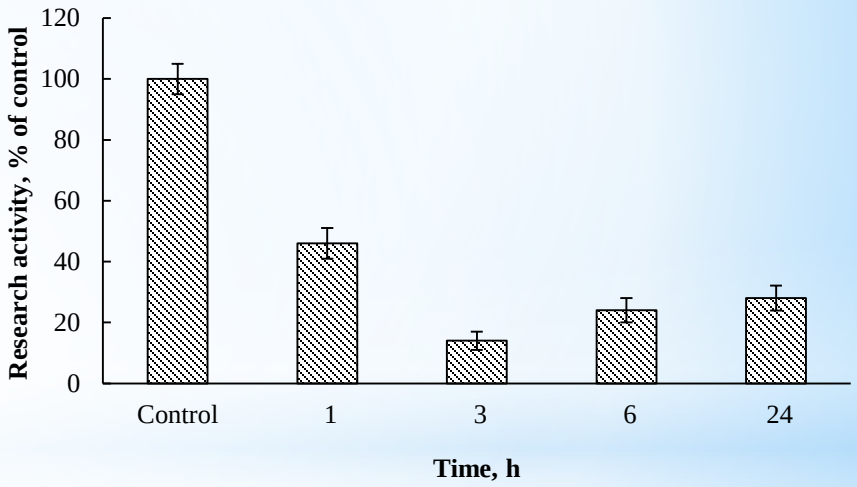


Fig. 4. Research activity of mice after oral administration of glycine menthyl ester at dose 175 mg/kg (time-response relationship).

Pharmacological properties of thymol GABA ester

Thymol ester of GABA were synthesized using DCC/DMAP coupling method followed by deprotection of the amino groups in the HCl/CH_3COOH medium.

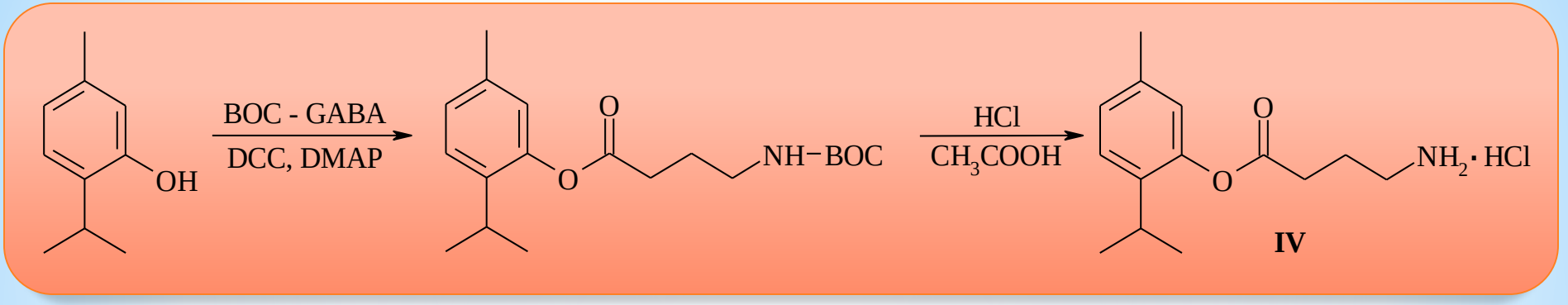


Table 4. Dose-response relationship for GABA thymol ester in 6 h after oral administration (mean ± SEM)

Dose, mg/kg		20	40	60	80	100	Control
MED of pentylenetetrazole, % of control	DCTC	211±5.7	200±4.2	211±5.7	206±4.8	200±5.9	100±3.3
	DTE	188±5.0	167±3.1	178±5.0	171±4.0	158±3.5	100±5.5

Pharmacological properties of thymol-GABAderivative

Thymol derivative **V** were synthesized by reaction between thymol and GABA in the presence of formalin solution at 60-80 °C.

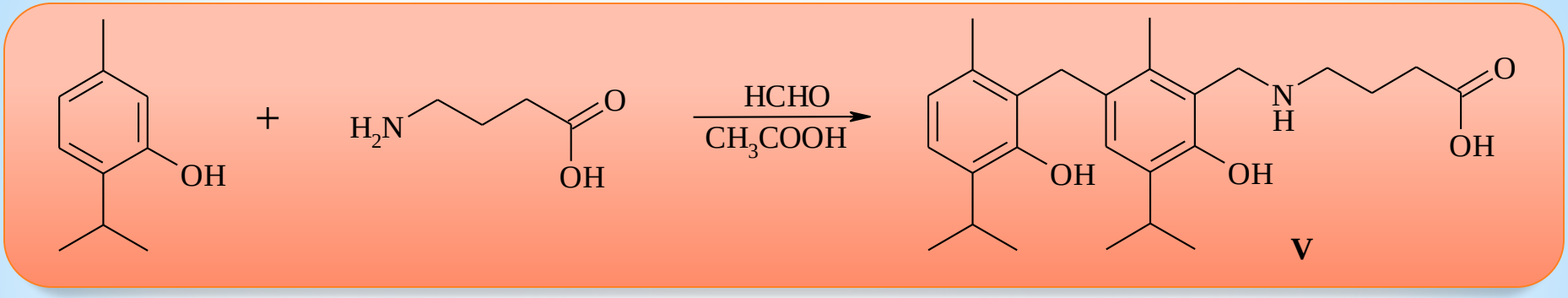
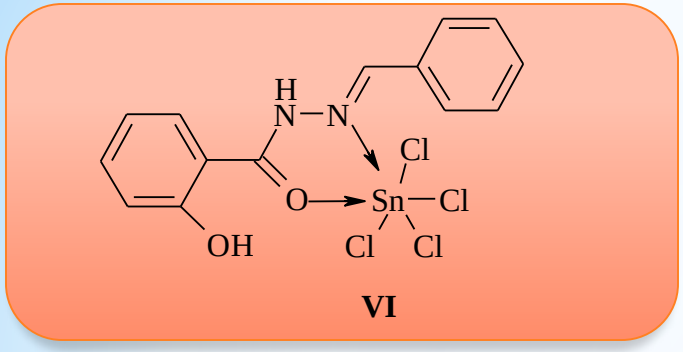


Table 5. Dose-response relationship for thymol-GABA derivative in 6 h after oral administration (mean ± SEM)

Dose, mg/kg		25	50	100	Control
MED of pentylenetetrazole, % of control	DCTC	227±4.9	175±3.8	200±3.9	100±3.2
	DTE	221±5.0	177±4.2	205±4.2	100±4.5

Pharmacological properties of $SnCl_4$ complexes with salicyloylhydrazones

Anti-inflammatory activity (Carrageenan-Induced Model)



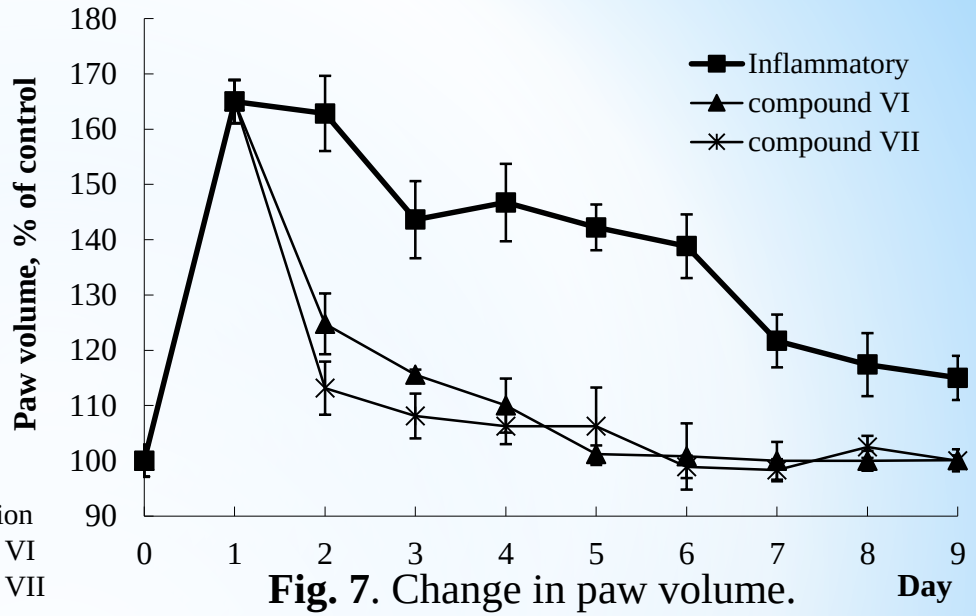
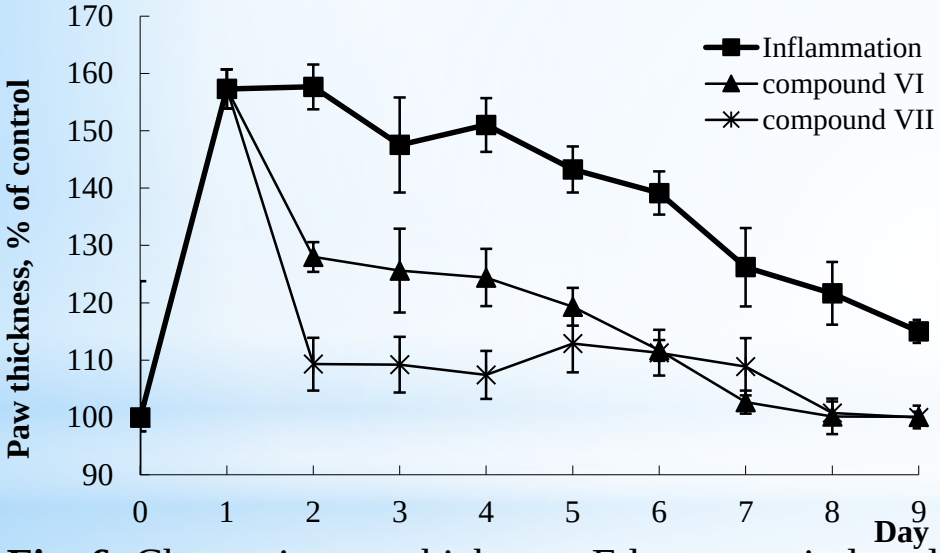


Fig. 7. Change in paw volume.



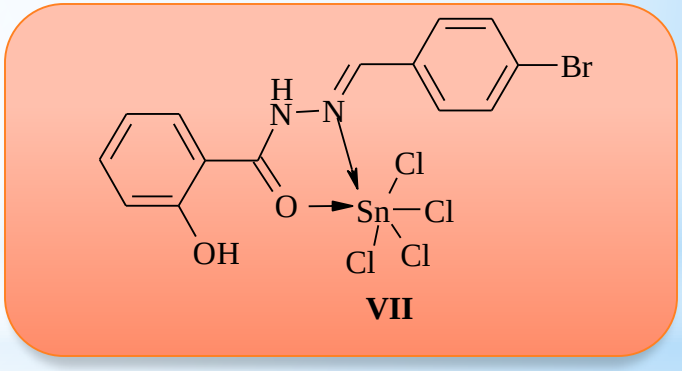
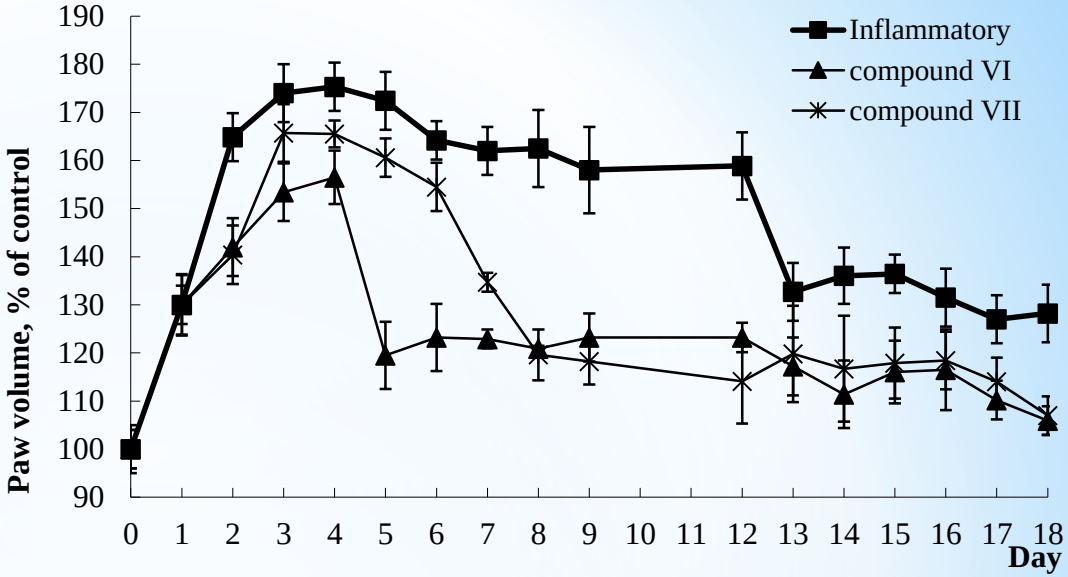


Fig. 6. Change in paw thickness. Edema was induced by injecting 0.1 mL of 1% solution of carrageenan into the sub plantar surface of right-hind paw.

Anti-inflammatory activity (Formalin-Induced Model)

Fig 8. Change in paw volume. Data are expressed as mean ± standard error.



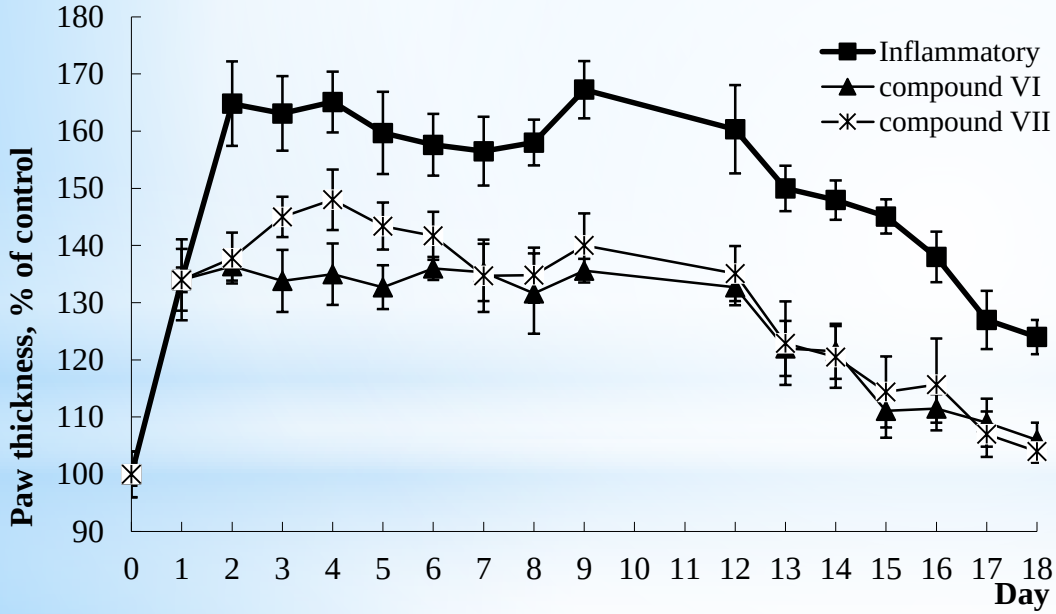
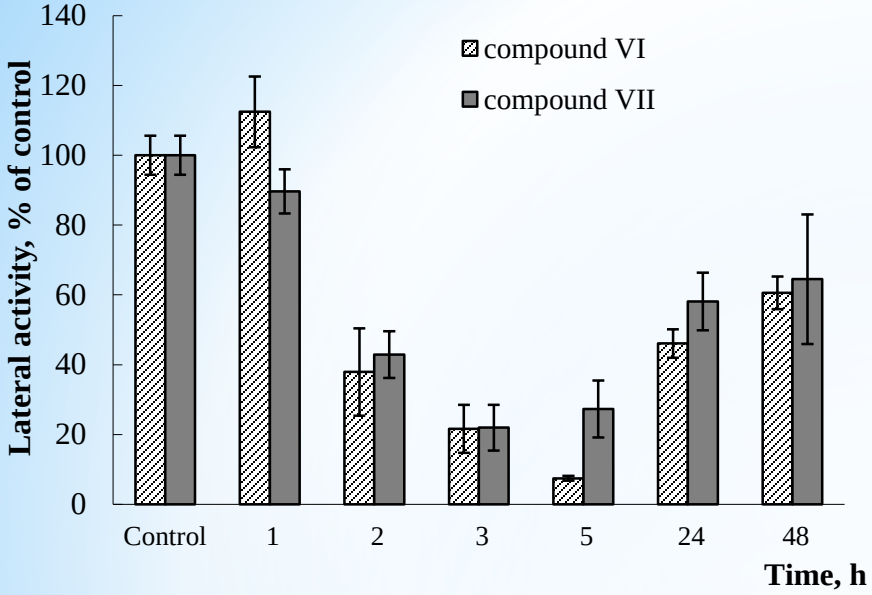
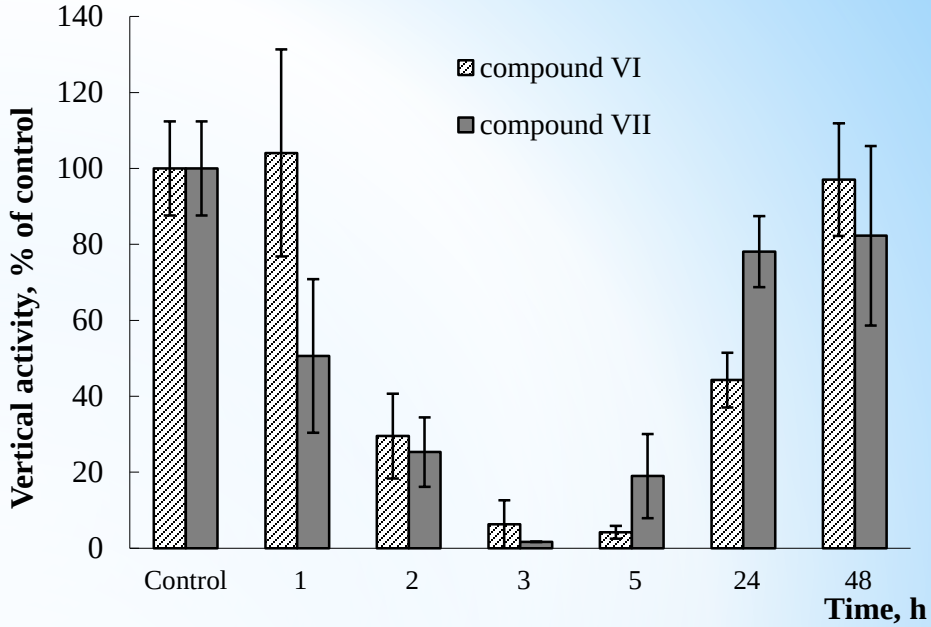


Fig 9. Change in paw thickness. Data are expressed as mean ± standard error.

Sedative activity (OFT test)





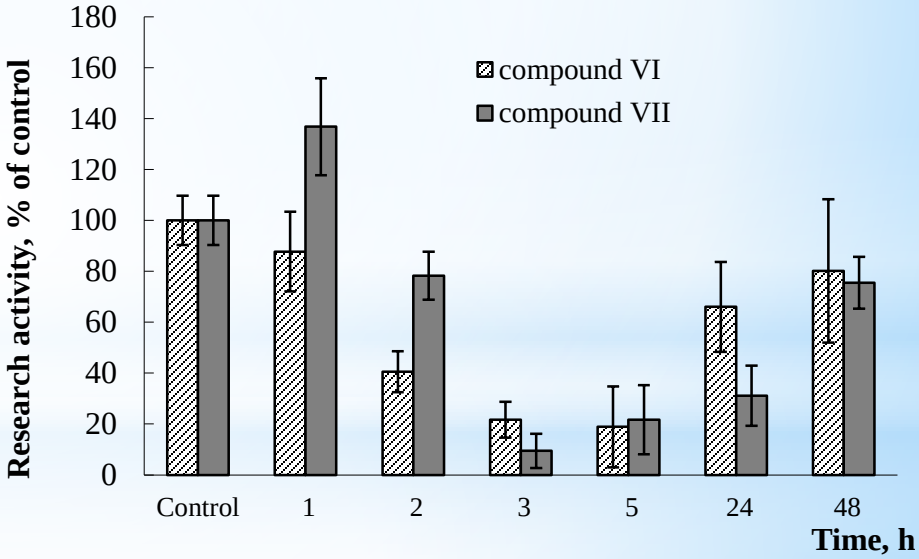


Fig 10. Lateral, vertical and research activities of compounds VI and VII. Data are expressed as mean ± standard error.

Conclusions

In conclusion, the present findings in our study identified menthyl ester of GABA (2-isopropyl-5-methylcyclohexyl 4-aminobutyrate) as a compound with anticonvulsant activity over a wide range of doses: 87-1350 mg/kg whereas menthyl ester of glycine (2-isopropyl-5-methylcyclohexyl 2-aminoacetate) shows significant sedative effect over 6 hours after oral administration at 175 mg/kg.

Complex compounds of $SnCl_4$ with salicyloylhydrazones have demonstrated sedative and anti-inflammatory activities in different models *in vivo*.

All aforementioned compounds can be considered as those that exhibit a combined pharmacological activity due to their simultaneous binding to GABA and TRP receptor types.