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TRP modulators based on glycine and mono-, bicyclic terpenoids – synthesis and pharmacological properties

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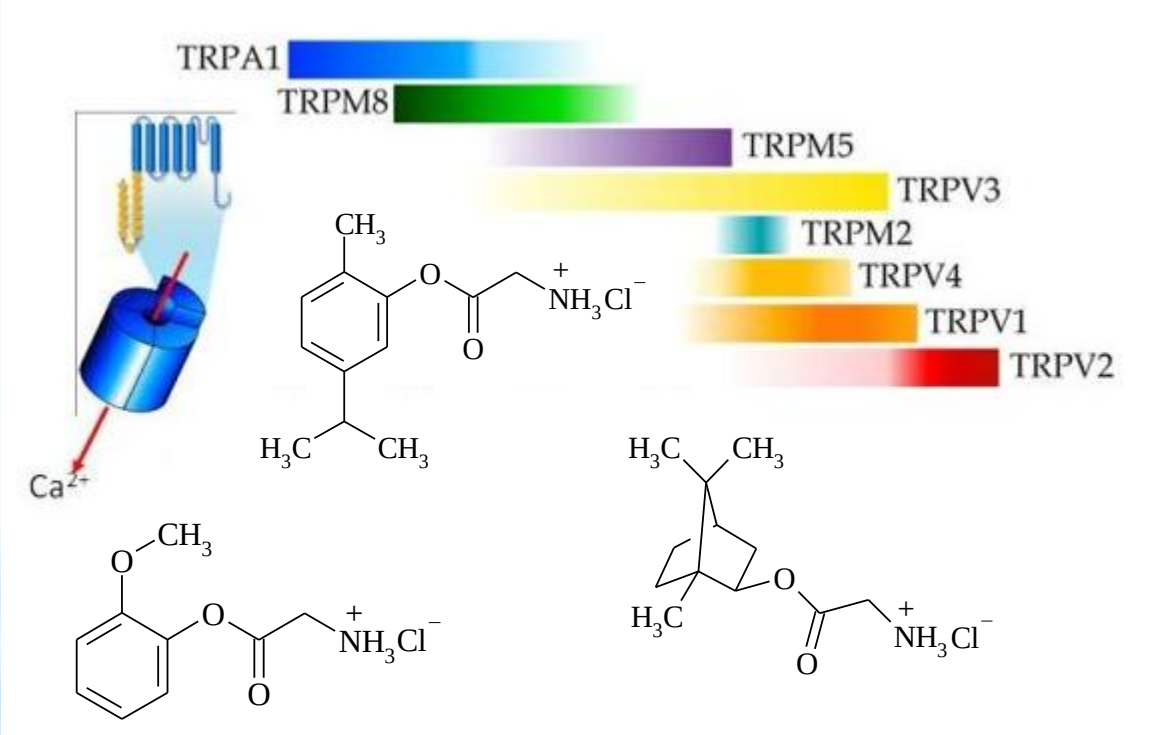
TRP modulators based on glycine and mono-, bicyclic terpenoids – synthesis and pharmacological properties



Abstract:

Currently, significant interest in drug development is focused on obtaining the drugs, which contemporaneously affect various pharmacological targets exhibiting, thus, the combined action.

Herein we demonstrate the possibility of development of novel drugs possessing a wide range of pharmacological activity which are simultaneously able to modulate TRP-channels and bind to glycine receptors. For this purpose esters based on mono- and bicyclic terpenoids (menthol, thymol, carvacrol, guaiacol, eugenol, borneol) with inhibitory amino acid (glycine) were synthesized via Steglich esterification. Their anticonvulsant action was evaluated by a PTZ-induced convulsion model and analgesic effect − by pharmacological models of thermal and chemical stimuli. All studied esters were found to produce antinociceptive effects and attenuate acute pain more than the reference drug benzocaine after their topical application.

The present findings indicate that glycine esters of abovementioned terpenoids are not classical prodrugs and possess their own pharmacological activity. Prolonged antiseizure action of the esters was revealed at 24 h after oral administration. Moreover, orally co-administered gidazepam (1 mg/kg) and glycine esters produce synergistic seizure prevention effects.

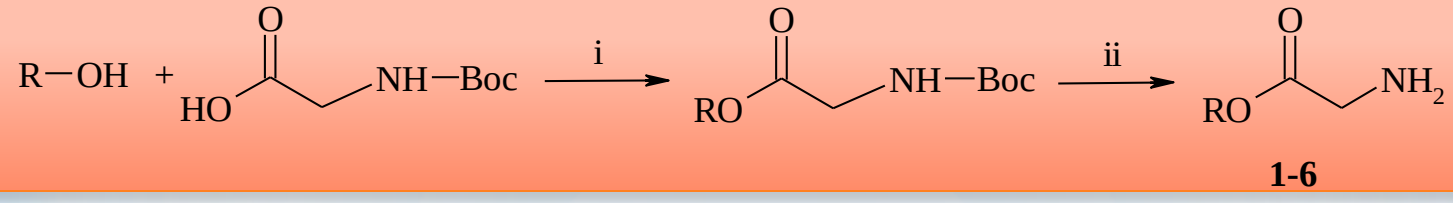
Keywords: terpenoids esters, glycine, TRP channels, pharmacological activity.

Introduction

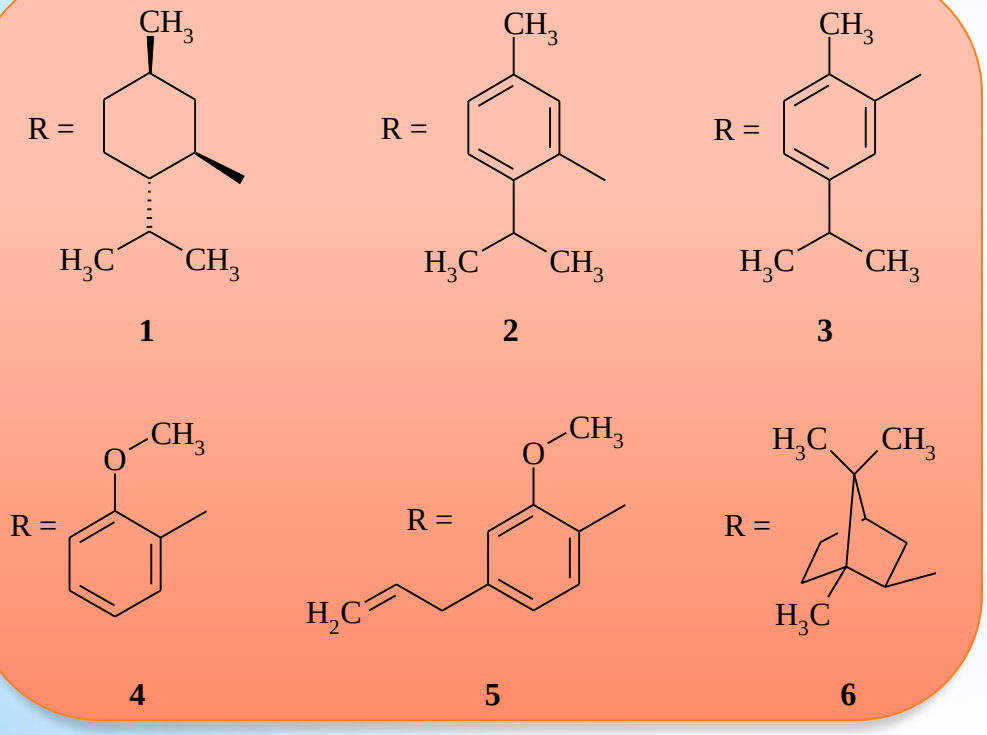
Glycinergic system is the target of a wide range of drugs active on the CNS, including anxiolytics, sedative-hypnotics, general anesthetics and anticonvulsants (Macdonald and Olsen, 1994). Recent studies have reported that cyclic monoterpenes menthol and thymol also have actions within the CNS (Zhang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

In the present study, we have synthesized novel esters of glycine with some monocyclic and bicyclic terpenoids (L-menthol, thymol, carvacrol, guaiacol, eugenol and borneol) and demonstrated their high analgesic and anticonvulsant activities. Prolonged anticonvulsant action was found for these esters; the synthesized compounds additionally produce synergistic seizure prevention effects when co-administered with gidazepam.

Results and discussion



Synthetic pathway of compounds **1**–**6**. *Reagents and conditions*: (i) DMAP, CH_2Cl_2, rt, 10 min; DCC, 0 °C, 30 min; rt, 10 h; (ii) HCl, CH_3COOH. All esters were prepared as hydrochlorides.



Esters based on the corresponding terpenoids (**1**–**6**) were synthesized using DCC/DMAP coupling method followed by deprotection of the amino groups in the HCl/CH_3COOH medium.

Acute toxicity

Acute toxicity of compounds 1-6 determined on white outbred mice by oral administration

Compound	LD_{50}, mg/kg	Compound	LD_{50}, mg/kg
Menthol	3400	**1**	1350
Thymol	640	**2**	> 2000
Carvacrol	471	**3**	> 2000
Guaiacol	621	**4**	1300
Borneol	1059	**5**	3000

Acute toxicity of compounds 1-6 determined on white outbred mice by intravenous administration

Compound	LD_{50}, mg/kg	Compound	LD_{50}, mg/kg
Menthol		**1**	50
Thymol	110	**2**	150
Carvacrol	80	**3**	100
Guaiacol	170	**4**	100
Borneol	56	**5**	50

Anticonvulsant activity of terpenoids esters with glycine

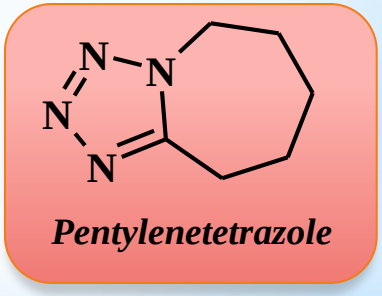
Anticonvulsant activity (PTZ test)

Pentylenetetrazole-Induced Convulsions in Mice

The anticonvulsant activity of tested compounds was evaluated by pentylenetetrazole model (PTZ), which includes the determination of pentylenetetrazole minimum effective doses (MED) inducing clonic-tonic convulsions (CTC) and tonic extension (TE) in test animals upon intravenous infusion of 1% aqueous solution into a tail vein. Doses of pentylenetetrazole for inducing clonic-tonic convulsions (DCTC) and tonic extension (DTE) were calculated relative to control. The anticonvulsant effect of compounds was estimated at certain time points (0.5, 1, 3, 6, 18 and 24 h) from the increase of pentylenetetrazole MED compared with a control group. MED in percent was calculated using the formula:

$$MED\ (\%) = V/m * 10^4$$

where MED—minimum effective dose of PTZ inducing DCTC or DTE; V—volume of PTZ solution, ml; m—animal weight, g.



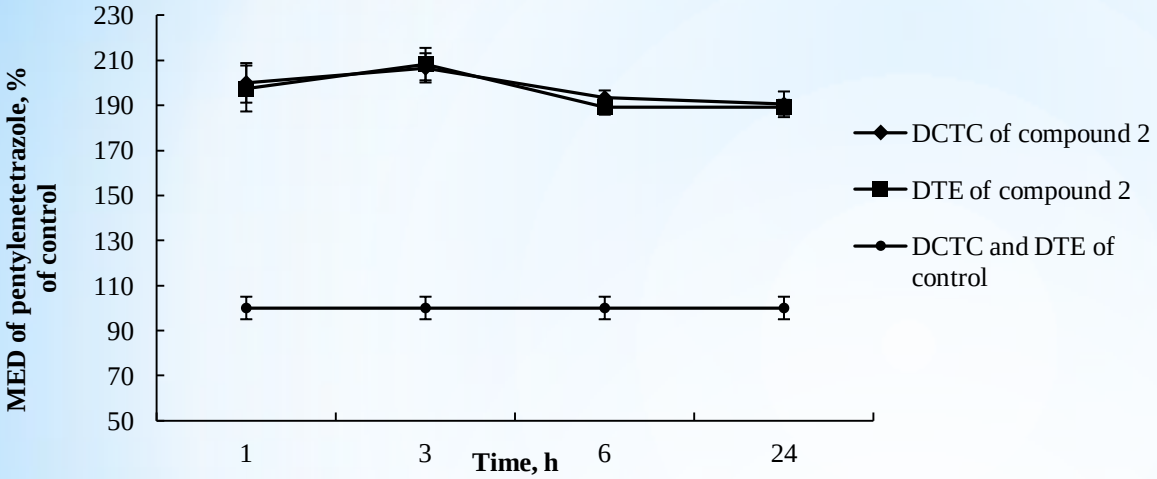
Pentylenetetrazole

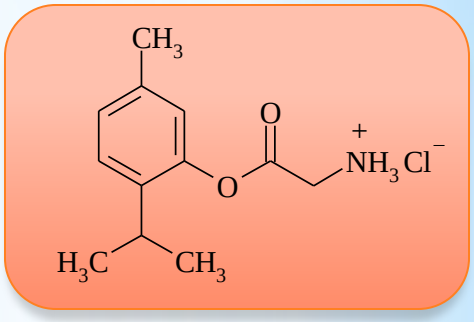
Co-Administration Effect of Gidazepam and GABA Esters **1**–**6**

Mice were distributed into 10 groups of five animals each, treated orally with gidazepam 1 mg/kg (GDZ); glycine esters **1-6** and mixture of GDZ and **1-6**. The anticonvulsant activity of compounds **1**–**6** and GDZ as well as mixtures of GDZ with **1**–**6** was evaluated in model of acute generalized seizures as described above; pharmacological effect of compounds was estimated in 3 h.

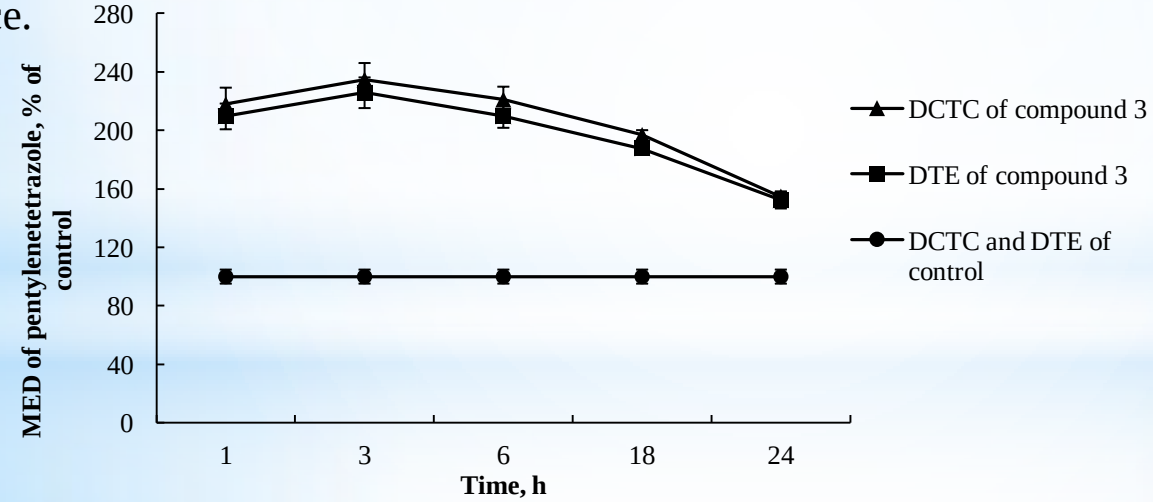
Anticonvulsant activity of terpenoids esters with glycine

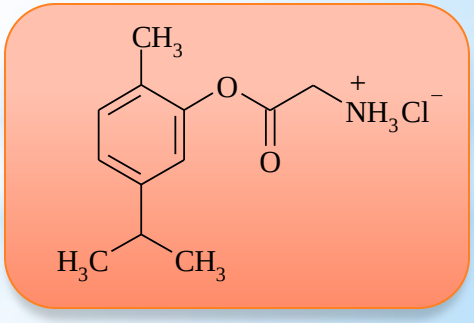
Anticonvulsant activity of compound **2**; time-response relationship. Values are given as mean ± SEM, n = 5 mice.





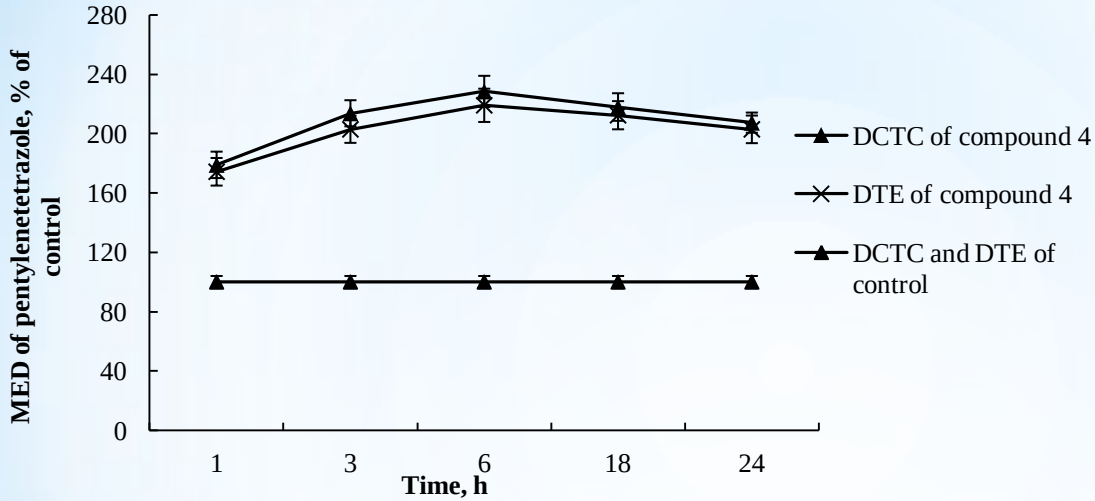
Anticonvulsant activity of compound **3**; time-response relationship. Values are given as mean ± SEM, n = 5 mice.

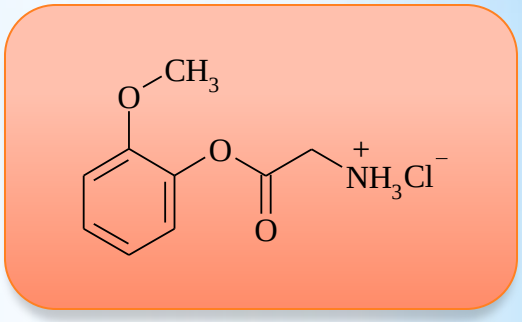




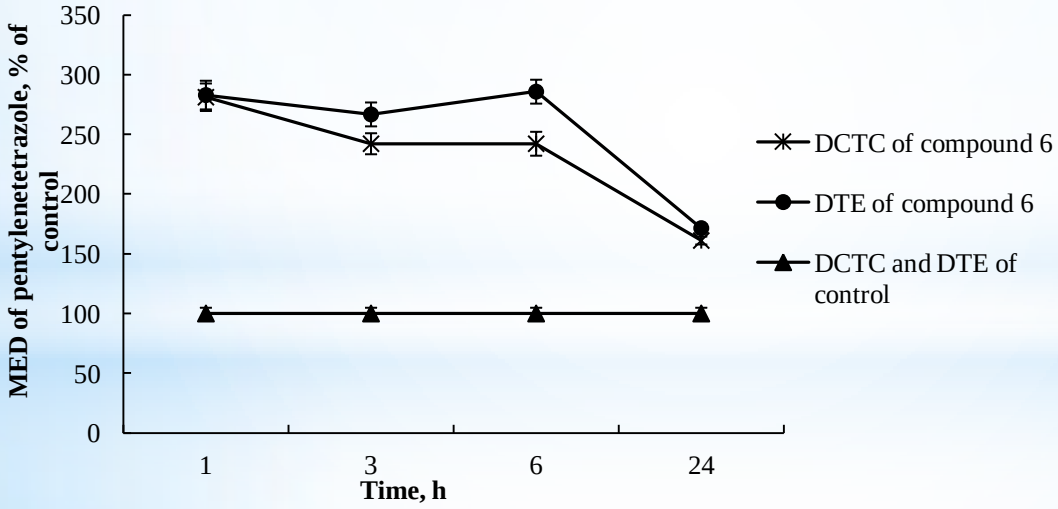
Anticonvulsant activity of terpenoids esters with glycine

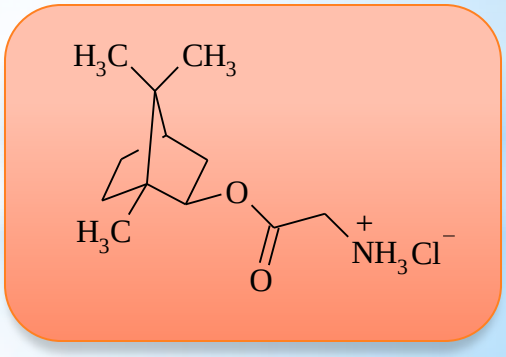
Anticonvulsant activity of compound **4**; time-response relationship. Values are given as mean ± SEM, n = 5 mice.



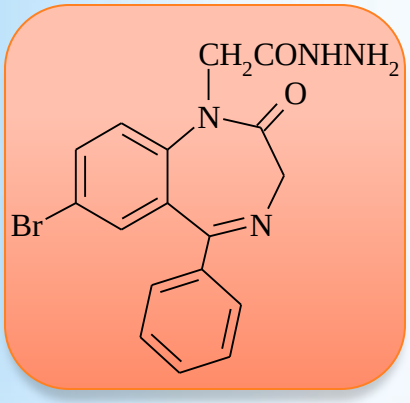


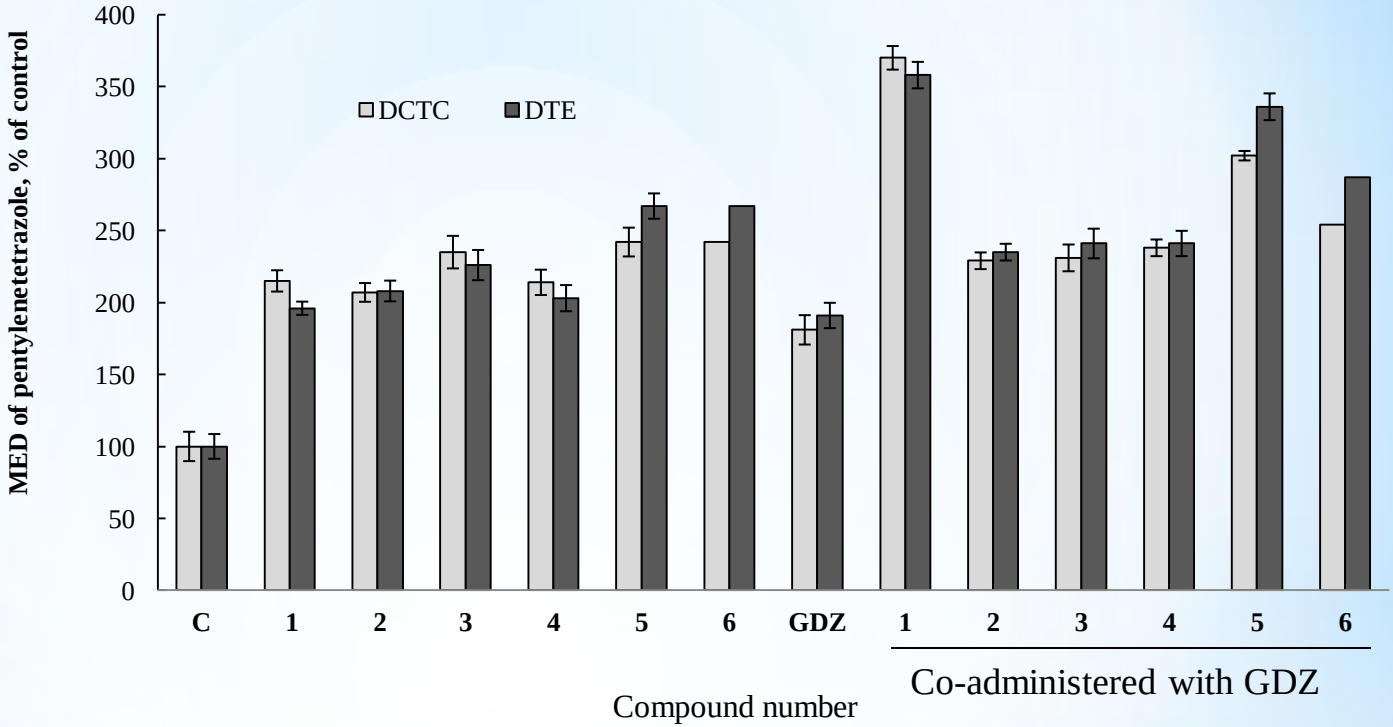
Anticonvulsant activity of compound **6**; time-response relationship. Values are given as mean ± SEM, n = 5 mice.





Synergistic anticonvulsant effect after oral co-administration of gidazepam and esters of terpenoids with glycine





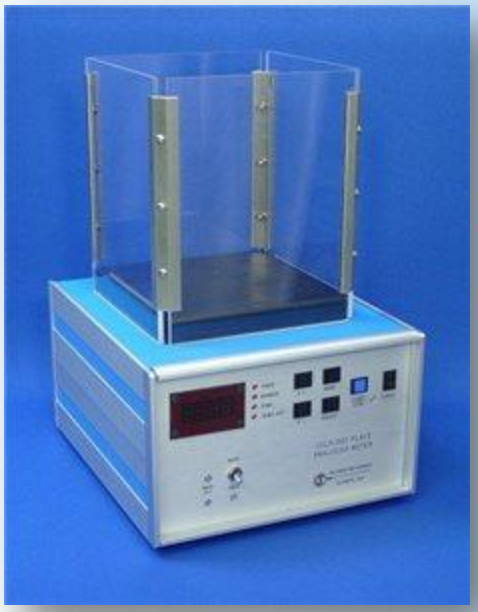
Anticonvulsant activity of compounds 1-6 and their mixture with gidazepam (GDZ)

Our experimental data demonstrate that orally co-administered gidazepam and glycine esters of monoterpenes (L-menthol, thymol, carvacrol, guaiacol and borneol) produce synergistic effect in seizures prevention suggesting that these esters are not acting via the benzodiazepine site.

Analgesic properties of terpenoidsesters

Analgesic activity of compounds **1**–**6** tested by hot plate method in mice (2% w/w ointment).

Compound	Reaction time (in sec)	Compound	Reaction time (in sec)
Menthol	24,2 ± 3,9	**1**	29,3 ± 6,5
Thymol	15,3 ± 0,3	**2**	46,7 ± 1,3
Carvacrol	19,3 ± 1,9	**3**	21,3 ± 2,5
Guaiacol	19,5 ± 0,5	**4**	22,7 ± 2,8
Borneol	26,7 ± 2,8	**5**	49,3 ± 0,9
Benzocaine	18,3 ± 0,9	**Control**	10,3 ± 0,6



Hot plate test: Analgesic activity was measured by hot-plate test as acute pain model. The mice were placed on a hot plate maintained at 55°C one at a time. In this experiment, latency to respond to the heat stimulus was determined by the amount of time (in seconds) it takes for mouse to lick one of its paws. Cut-off time was fixed at 60 sec to minimize the tissue damage that occurs during prolonged contact with heated surface.

Analgesic properties of terpenoidsesters

Antinociceptive effect of compounds **1**–**6** on the formalin (early phase), capsaicin and AITC tests in mice

Compound	Reaction time (in sec)		
	Formalin test	Capsaicin test	AITC test
Control	84.8 ± 6.1	48.0 ± 2.6	67.0 ± 3.6
Benzocaine	36.3 ± 2.4	28.7 ± 6.6	48.0 ± 2.0
1	19.3 ± 3.7	11.0 ± 1.5	16.0 ± 4.6
2	19.3 ± 0.8	16.0 ± 3.2	13.3 ± 0.9
3	29.7 ± 6.9	13.8 ± 0.8	14.8 ± 0.9
4	35.7 ± 1.9	19.7 ± 3.6	29.5 ± 8.3
5	19.3 ± 1.5	15.3 ± 1.3	20.3 ± 1.2
Menthol	24.0 ± 5.6	11.0 ± 2.7	6.8 ± 1.2
Thymol	50.3 ± 5.2	35.3 ± 4.1	25.3 ± 3.8
Carvacrol	38.3 ±3.8	23.3 ± 6.4	34.5 ± 2.2
Guaiacol	54.7 ± 6.8	22.7 ± 2.7	20.7 ± 2.8
Borneol	26.3 ± 4.7	12.3 ± 5.0	8.3 ± 3.5

Formalin-induced licking: After the adaptation period, 20 µl of 2% formalin solution in 0.9% NaCl was injected subcutaneously into the dorsal surface of the right hindpaw.

Capsaicin-induced licking: Following the adaptation to the experimental conditions, 20 µl of capsaicin solution (6 µg/paw) was injected subcutaneously under the skin of the dorsal surface of the right hindpaw.

AITC-induced licking: Following the adaptation to the experimental conditions, 20 µL of 0.5% (*w*/*w*) allyl isothiocyanate (AITC) solution was injected subcutaneously under the skin of the dorsal surface of the right hindpaw.

The animal then was placed in an individual plexiglass cage. The time spent licking the injected paw was measured from 0 to 5 min after formalin/capsaicin/AITC administration and was considered as an indicator of pain response.

Sedative activity of menthyl glycine ester

Open Field Test (OFT test)

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

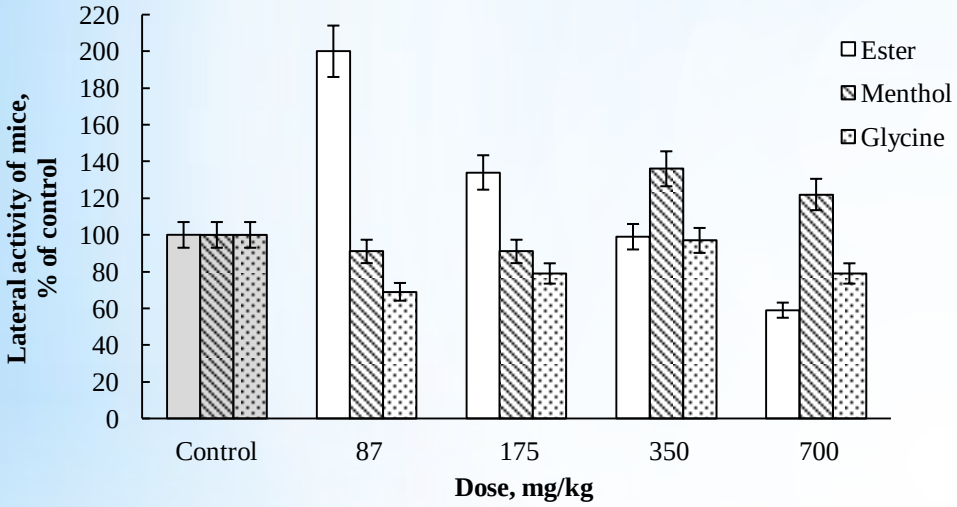


Fig. 1. Comparable lateral activity of mice in 3 h after oral administration of glycine menthyl ester, menthol and glycine (dose-response relationship).

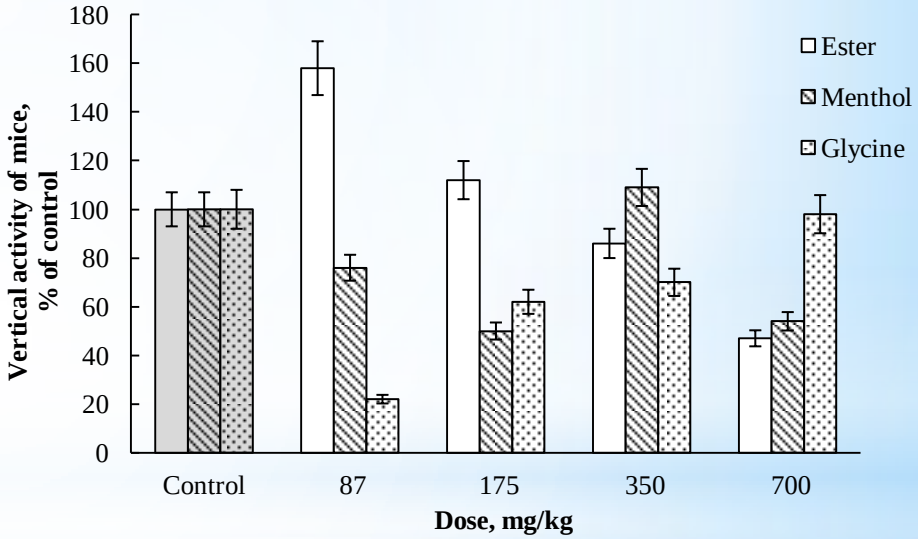


Fig. 2. Comparable vertical activity of mice in 3 h after oral administration of glycine menthyl ester, menthol and glycine (dose-response relationship).

Considering the possible prolonged action of obtained esters, 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

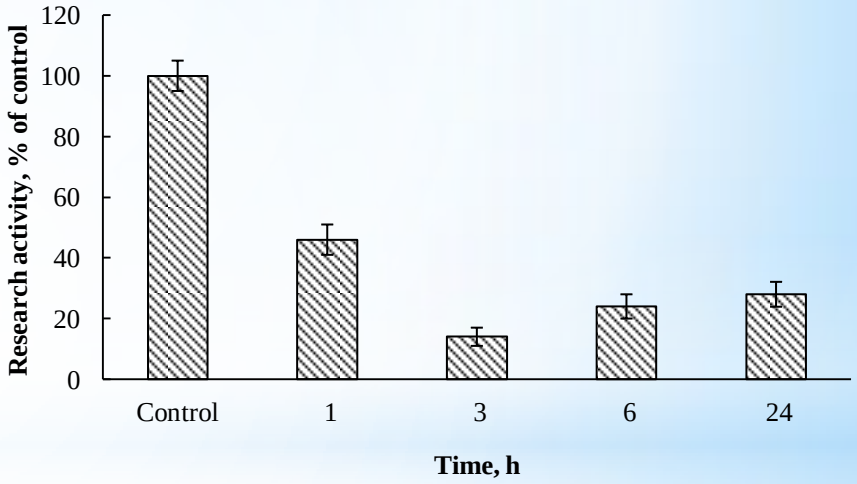


Fig. 4. Research activity of mice after oral administration of glycine menthyl ester at dose 175 mg/kg (time-response relationship).

Conclusions

In conclusion, esters based on mono- and bicyclic terpenoids (menthol, thymol, carvacrol, guaiacol, eugenol, borneol) with inhibitory amino acid (glycine) were synthesized via Steglich esterification. Their anticonvulsant action was evaluated by a PTZ-induced convulsion model and analgesic effect − by pharmacological models of thermal and chemical stimuli. All studied esters were found to produce antinociceptive effects and attenuate acute pain more than the reference drug benzocaine after their topical application.

The present findings indicate that glycine esters of abovementioned terpenoids are not classical prodrugs and possess their own pharmacological activity. Prolonged antiseizure action of the esters was revealed at 24 h after oral administration. Moreover, orally co-administered gidazepam (1 mg/kg) and glycine esters produce synergistic seizure prevention effects.