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Novel menthone derivatives with anticonvulsant effect

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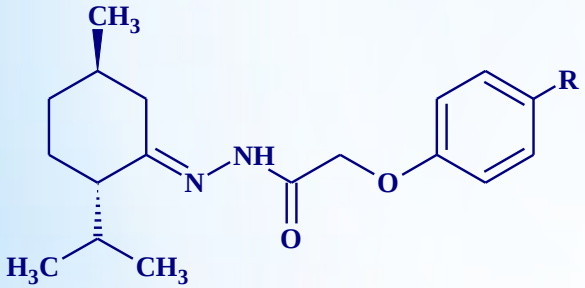
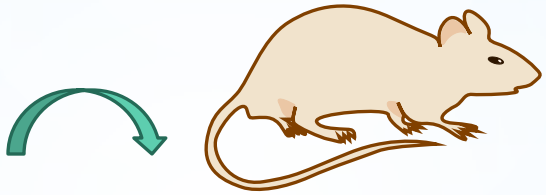
Novel menthone derivatives with anticonvulsant effect

Graphical Abstract



1-5

R = H (**1**), Cl (**2**), Br (**3**), $C(CH_3)_3$ (**4**), $O\text{-}C_6H_5$ (**5**)



Anticonvulsant activity

- pentylenetetrazole-induced convulsion
- maximal electroshock-induced seizures

Anticonvulsant activity

Abstract: Nowadays, a significant number of antiepileptic drugs aimed at influencing the main inhibitory transmitter – gamma-aminobutyric acid (GABA). Compounds with various chemical structures, binding to different $GABA_A$ sites, potentiate the action of amino acid. Recent studies have reported that terpenoids such as *l*-menthone and its derivatives were found to act as modulators of $GABA_A$ receptors, thereby demonstrating anticonvulsant activity. On the other hand, neuroprotective and anticonvulsant potentialities were revealed in phenoxyacetic acid derivatives. Based on the foregoing, the combination of *l*-menthone and phenoxyacetic acid residues into one molecule is feasible for obtaining the pharmacological agents with antiseizure action. In order to achieve the above-mentioned goal, *l*-menthone hydrazones were synthesized via condensation of terpenoid with 4-R-phenoxyacetic acid hydrazides in the presence of a catalytic amount of glacial acetic acid.

The structure of the target compounds has been established by FTIR-ATR, Raman, ^{1}H-NMR and ^{13}C-NMR spectral analysis and EI/FAB/ESI mass spectrometry. Thermal properties of hydrazones were elucidated by DSC and their purity – by HPLC coupled to mass spectrometry. Synthesized compounds were found to exist as *Z*/*E* geometrical isomers about C=N bond and *cis*/*trans* amide conformers. At the present study, the influence of obtained derivatives on the central nervous system was reliably confirmed by evaluating their anticonvulsant activity. The present findings indicate that all aforementioned compounds possess antiseizure action after oral administration on PTZ-induced convulsion and maximal electroshock-induced (MES) seizures.

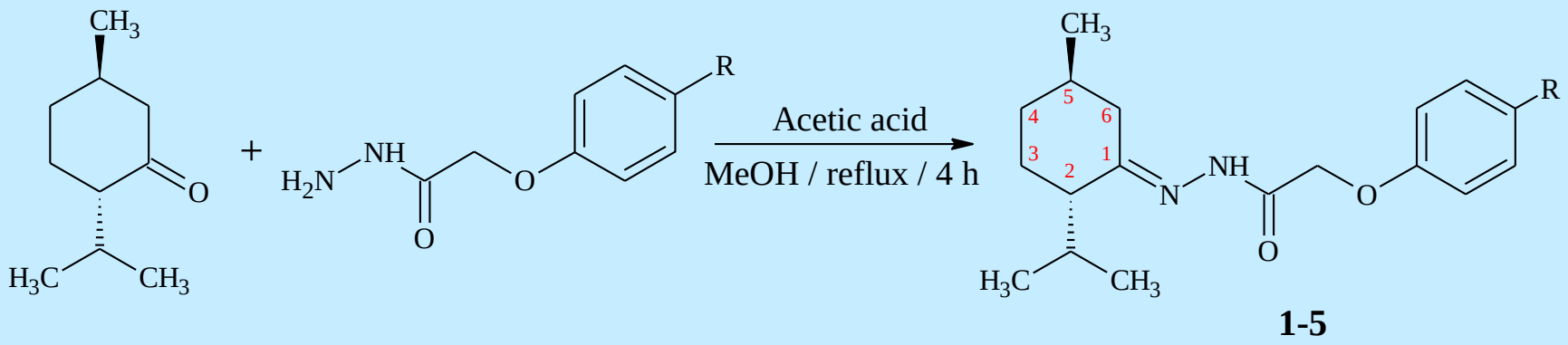
Keywords: hydrazones; l-menthone; anticonvulsant activity; PTZ and MES models; terpenoid

Introduction

Currently, considerable interest in drug development is concentrated on obtaining the drugs, which contemporaneously affect various pharmacological targets exhibiting, thus, the combined action. In this context, particular interest is focused on compounds affecting both the central and peripheral nervous system. Recently, we have embodied this idea by combination of neurotransmitter amino acids with terpenoids capable of binding to the transient receptor potential channels (TRP). The present work is a logical continuation and reveals a strategy for drug development containing residues of cyclic terpene *l*-menthone and *para*-substituted phenoxyacetic acids. Besides binding to TRPM8 channels resulting in pain relief, *l*-menthone was found to act as modulators of $GABA_A$ receptors, thereby demonstrating anticonvulsant activity. Phenoxyacetic acid derivatives in turn also exhibit peripheral nociceptive effects and possess neuroprotective and anticonvulsant potentialities .

Based on the foregoing, the combination of *l*-menthone and phenoxyacetic acid residues into one molecule is feasible for obtaining the drugs, which contemporaneously affect various pharmacological targets. Such a combination might be implemented by synthesis of hydrazones which are principle compounds for drug design due to wide spectrum of pharmacological action. Thus, the current note is devoted to the synthesis, detailed structure determination anticonvulsant activity investigation of hydrazones based on (2*S*,5*R*)-2-isopropyl-5-methylcyclohexanone and *para*-substituted phenoxyacetic acids.

Results and discussion



1-5

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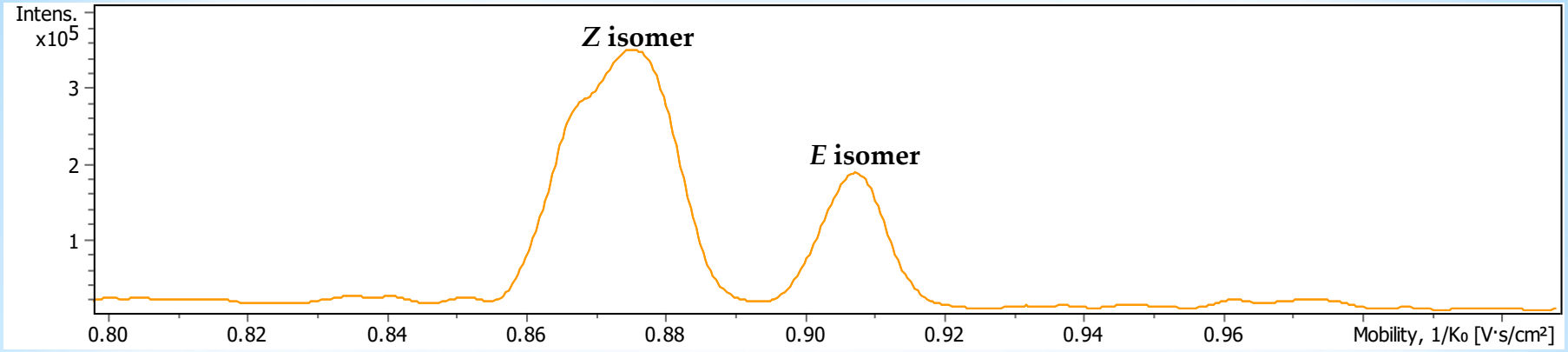
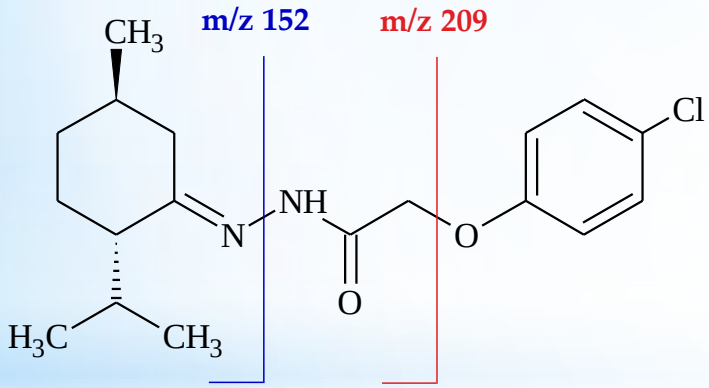
(2*S*,5*R*)-2-Isopropyl-5-methylcyclohexanone hydrazones **3a**-**3e** were synthesized via condensation of *l*-menthone **1** with 4-R-phenoxyacetic acid hydrazides **2a**-**2e** in the presence of a catalytic amount of glacial acetic acid, as shown in Scheme. Synthesized hydrazones were isolated in 76-78% yield as white solid well soluble in organic solvents (chloroform, acetonitrile, benzene, ethyl acetate) and fully characterized by ^{13}C-NMR, ^{1}H-NMR, FTIR-ATR, Raman-spectroscopy and FAB-, EI-, ESI-mass spectrometry.

Thermal behavior of compounds 3a-3e was performed by differential scanning calorimetry (DSC). Additionally, the HPLC analysis was carried out to determine the purity of title compounds.

Results and discussion



Extracted ion mobilogram of compound 3b (*m/z* 337.16) obtained from TIMS-TOF-MS

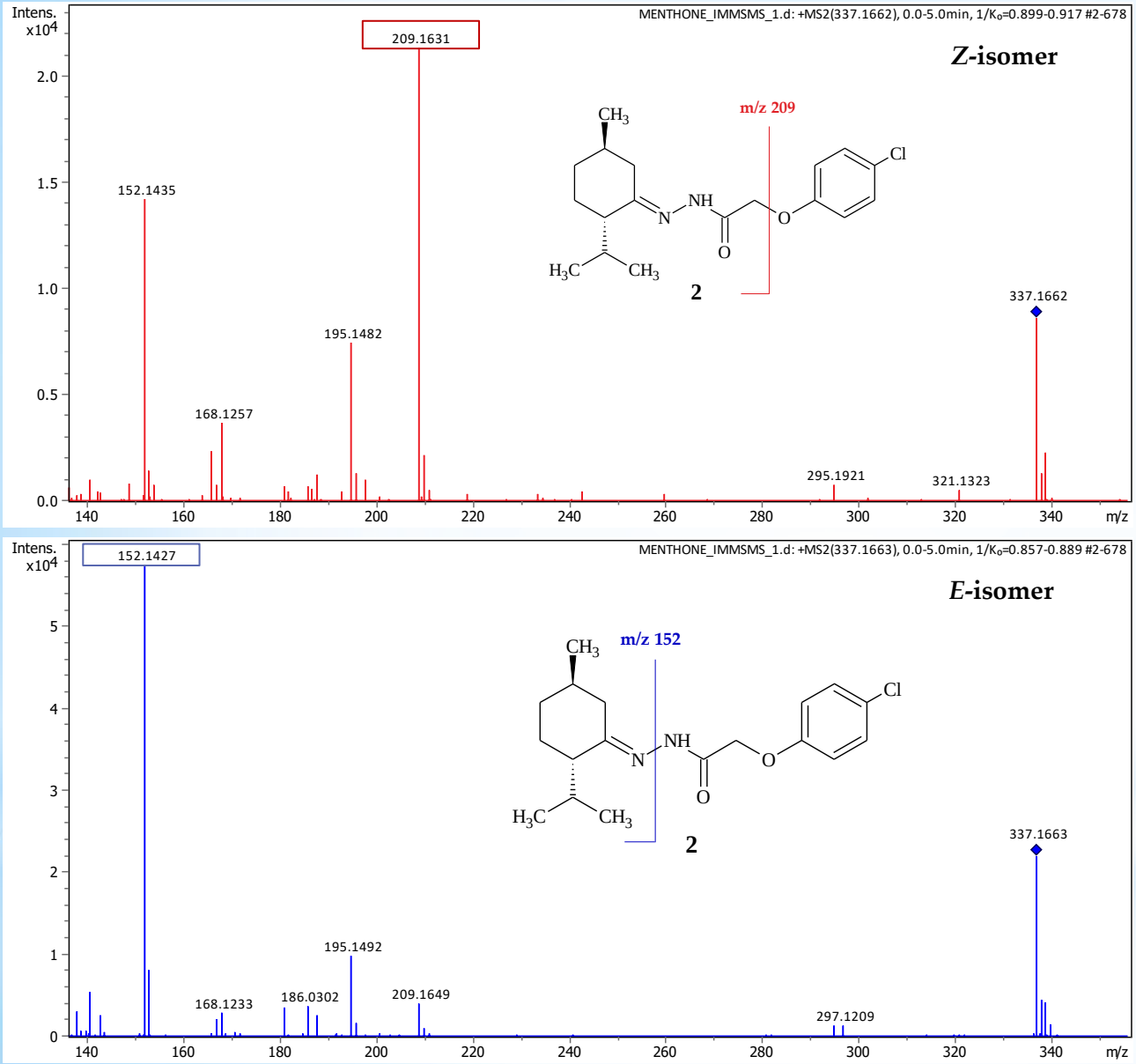


2

In order to distinguish *Z*/*E* geometrical isomers, ion mobility-tandem mass spectrometry (IM-MS/MS) analysis has been applied. Compared to *E* forms, *Z* isomers have less mobility due to steric hindrance of bulky groups that decrease structure compactness

Mechanism of hydrazone 2 fragmentation: in MS/MS mode at the same fragmentation energy, ion at m/z 152 is dominant for *Z*-isomer (blue line), but at m/z 209 is dominant for *E*-isomer (red line).

Results and discussion



MS/MS spectrum of the *Z* isomer and *E* isomer of hydrazone 2.

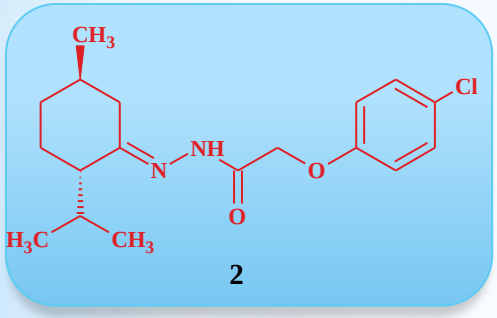
Based on MS/MS spectra of *Z*/*E* forms we may conclude that these isomers have different fragmentation patterns caused by their stability.

For example, in MS/MS spectrum of hydrazone **2** (*Z*-isomer) the most abundant fragment signal is observed at *m*/*z* 152 (cleavage of N–N bond) while for *E* form of this compound – at *m*/*z* 209 (cleavage of C–O bond). Since *Z* isomer is less stable, heavy side chain is leaving easily in order to reduce the stress on the molecule. In contrast, *E* isomer is more relax and small side chain leaves from the molecule forming, thus, fragment ion at *m*/*z* 209.

^{1}H NMR investigation of menthone hydrazones

^{1}H NMR spectra of compounds **1-5** in DMSO-d_6 solution display two sets of singlets related to methylene (CH_2) and imine (NH) protons indicating the presence of *cis*/*trans* conformers. In the ^{1}H NMR spectra the upfield peak of CH_2 group belongs to *trans* conformer whereas downfield peak – to *cis* form. It ought to be pointed out that a similar pattern was observed in the ^{1}H NMR spectra of menthone derivatives **1-5**: two singlets for CH_2 protons (4.58-4.62 ppm and 4.92-4.97 ppm) as well as two singlets for NH protons (10.34-10.41 ppm and 10.51-10.63 ppm).

Thus, we may conclude that obtained hydrazones **1-5** exist in DMSO-d_6 solution as a mixture of *cis*/*trans* conformers.



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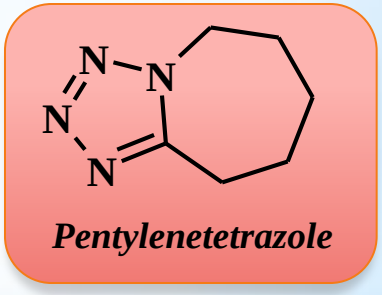
Anticonvulsant activity menthone hydrazones

Pentylenetetrazole-Induced Convulsions in Mice

The anticonvulsant activity of 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 (3 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.



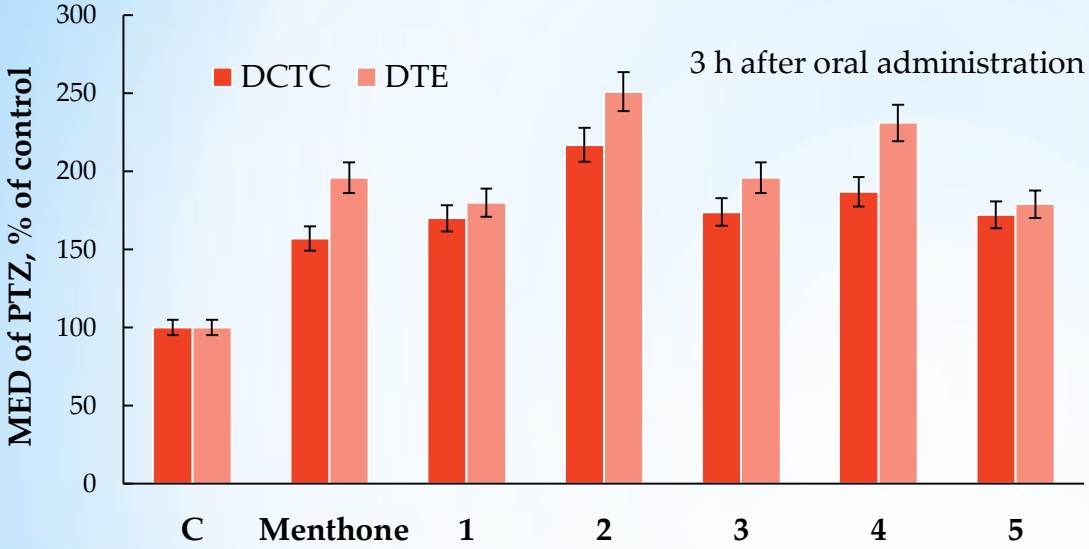
Pentylenetetrazole

Maximal Electroshock Seizure in Mice

Maximal electroshock seizures were induced by the application of corneal electrodes with a current strength of 50 mA (50Hz) for 0.2 sec to mice pre-treated with compounds **1-5** or Tween 80/water emulsion. After electric stimulation, duration of various phases of epileptic attacks along with mortality have been determined.

Anticonvulsant activity of menthone hydrazones

Pentylenetetrazole-Induced Convulsions in Mice



DCTC - dose of pentylenetetrazole for inducing clonic-tonic convulsions;
DTE – dose of pentylenetetrazole for inducing tonic extension

Dose of menthone – 50 mg/kg;
Dose of menthone hydrazones – equimolar to menthone dose

1-5

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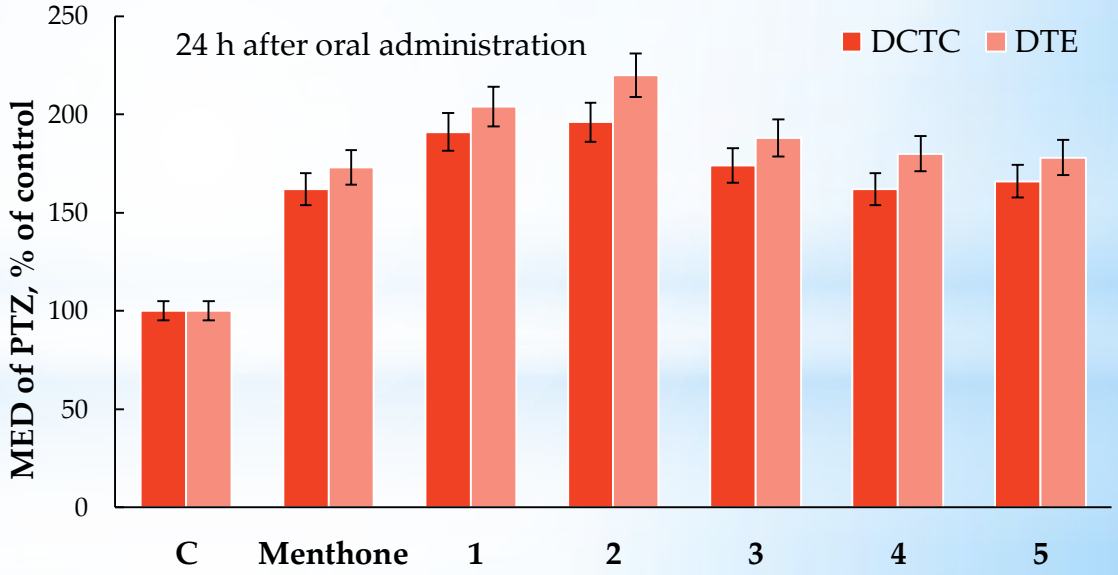
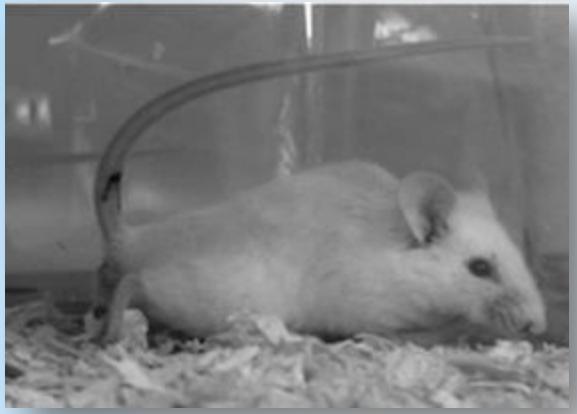
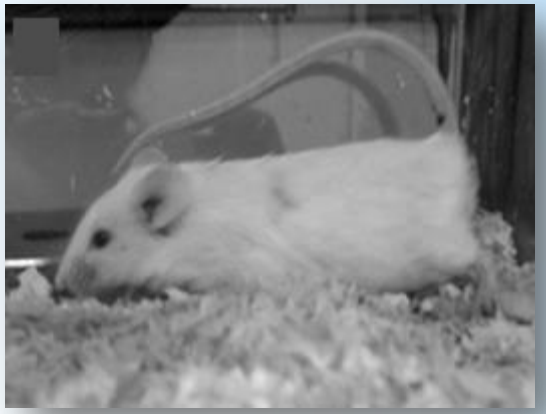


Anticonvulsant activity of menthone hydrazones

Maximal Electroshock Seizure in Mice



Straub reaction in mice



Anticonvulsant effect of compounds 1-5 against maximal electroshock (MES)-induced seizures in mice						
Compound	**1**	**2**	**3**	**4**	**5**	**Control**
	3 h after single oral administration					
% Mortality protection	**60**	**80**	**80**	**40**	**60**	**0**
	24 h after single oral administration					
% Mortality protection	**60**	**60**	**60**	**40**	**40**	**0**

Conclusions

Condensation of (2*S*,5*R*)-2-isopropyl-5-methylcyclohexanone with 4-R-phenoxyacetic acid hydrazides in the presence of a catalytic amount of glacial acetic acid was successfully applied to synthesize the title compounds followed by structure confirmation via FTIR-ATR, Raman, ^{1}H-NMR and ^{13}C-NMR spectral analysis and mass spectrometry.

Based on our experimental data, we may conclude that menthone hydrazones of *para*-substituted phenoxyacetic acids possess anticonvulsant activity both in PTZ and MES test at short and long time period (3 h and 24 h, accordingly).