Research Article

Mariia Nesterkina*, Dmytro Barbalat, Iryna Kravchenko

Design, synthesis and pharmacological profile of (−)-verbenone hydrazones

Abstract: A series of novel (−)-verbenone hydrazones was designed and synthesized via condensation of terpenoid with hydrazides derived from phenoxyacetic acid. The structure of target compounds was confirmed by 1H-NMR and 13C-NMR analysis, Raman and FT-IR spectroscopy, electrospray ionization method and fast atom bombardment (FAB) mass spectrometry. Thermal properties of (−)-verbenone hydrazones 3a–3e were estimated by differential scanning calorimetry and their purity by HPLC coupled to mass spectrometry. Verbenone hydrazones were revealed to exist as Z/E geometrical isomers about C=N bond and cis/trans amide conformers. Verbenone derivatives were estimated as potential anticonvulsant agents after their oral administration against pentylenetetrazole and maximal electroshock convulsions in mice. Analgesic effect of hydrazones was studied by topical application on models of allyl isothiocyanate and capsaicin-induced pain. The present findings indicate that verbenone hydrazones contribute to seizure protection both at short (6 h) and long (24 h) time periods by blocking chemical- and electroshock-induced convulsions. Binding of compounds 3a–3e to TRPA1/TRPV1 ion channels was suggested as a feasible mechanism explaining their significant analgesic activity.

Keywords: verbenone, hydrazones, phenoxyacetic acid, analgesic activity, anticonvulsant effect

1 Introduction

Essential oils as well as their individual constituents possess both significant analgesic activity and effect on central nervous system-related disorders [1]. The results of investigation revealed the pronounced pain relief action of essential oils isolated from Rosmarinus officinalis and Verbena officinalis containing the dominant component bicyclic monoterpenic verbenone [2–4]. Among other findings, a rodent study demonstrated the anticonvulsant activity of verbenone due to direct or indirect impact on the GABAergic system [5]. Moreover, terpenes were found to improve the delivery of drugs through the skin by co-administration with pharmaceutical compounds or through the use of microemulsions for the transdermal absorption of drugs [1,6]. Terpenes and terpenoids may also serve as a transmembrane carrier via their conjugation with hydrophilic compounds [7]. Consequently, terpenoids are unique scaffold that might be used for further chemical modification aimed at synthesizing the compounds simultaneously affecting the central and peripheral nervous systems.

In this context, substantial attention is concentrated on hydrazones comprising their structure azomethine group –NH–N=C– and undergoing hydrolytic reactions both in vitro and in vivo [8,9]. To date, hydrazones based on menthone and camphor were found to prevent seizures spread in the maximal electroshock (MES) test showing simultaneously low neurotoxicity [10,11].

In this study, bicyclic terpenoid (−)-verbenone was used as a base for hydrazone synthesis via its condensation with hydrazides of para-substituted phenoxyacetic acid. As known earlier, phenoxyacetic acid derivatives also possess the peripheral nociceptive action and anticonvulsant effect [12,13]. Taking into account the foregoing, a series of novel (−)-verbenone hydrazones were designed and synthesized, and their spectral characteristics have been described followed by their investigation as potential analgesic and anticonvulsant agents using different experimental models in vivo.
2 Materials and methods

2.1 General

The following chemicals were obtained from commercial sources and used without further purification: (−)-verbenone, 4-chlorophenol, 4-phenoxyphenol, 4-bromophenol, 4-tert-butylphenol and phenol (TCI, USA). Compounds 2a–2e were synthesized in three steps starting from para-substituted phenols as previously reported [14]. The products were characterized by 1H-NMR spectroscopy on AVANCE DRX 500 (500 MHz) instrument and by 13C-NMR spectroscopy on Varian-Mercury 400 spectrometer with the use of DMSO-d$_6$ as a solvent and tetramethylsilane as an internal standard.

FAB mass spectra were recorded on a VG 70-70EQ mass spectrometer equipped with Xe ion gun (8 kV); the samples were mixed with m-nitrobenzyl-alcohol matrix. High-resolution mass spectrometry (HRMS) was carried out on a 6530 Accurrate Mass quadrupole time-of-flight (Q-TOF) spectrometer by means of electrospray ionization method (ESI) coupled to an Agilent 1260 Infinity HPLC system. Chromatographic separation was performed on Agilent ZORBAX Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 μm) at 35°C. Elution was executed at a flow rate of 1.0 mL/min, and the injection volume was 1μL. IR spectra were recorded on PerkinElmer Frontier FT-IR spectrometer with samples in KBr disks. Raman spectra were obtained on Thermo Fisher Scientific DXR Raman Microscope. Differential scanning calorimetry (DSC) was carried out on Q2000 Differential Scanning Calorimeter with the use of aluminum crucibles containing 2 mg of compounds 3a–3e under dynamic nitrogen atmosphere and the heating rate of 1°C min$^{-1}$ at temperature ranging from 20 to 200°C.

2.2 General procedure to the synthesis of (−)-verbenone hydrazones (3a–3e)

To solution of (−)-verbenone (2.0 g, 13.3 mmol) in propanol (50 mL), the equimolar amount (13.3 mmol) of compounds 2a–2e and 2–3 drops of CH$_3$COOH (glacial) were added. The reaction mixture was refluxed for 12 h, then cooled to room temperature and poured into the ice. The obtained precipitate was filtered and dried under vacuum followed by the recrystallization from methanol.

2.2.1 2-Phenoxy-N′-[4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ylidene]acetohydrazide (3a)

Yield 78%; white solid. 1H-NMR (500 MHz, DMSO-d$_6$) δ: 0.83 (s, 3H, H-8), 1.42 (s, 3H, H-9), 1.60 (d, J = 8.8 Hz, 1H, H-7$_{endo}$), 1.91 (t, 3H, H-10), 2.27 (dd, J = 6.0 Hz, J = 1.8 Hz, 1H, H-5), 2.63–2.67 (m, 1H, H-1), 4.61 (s, 1H, CH$_2$), 4.96–4.98 (m, 1H, CH$_2$), 5.82 (d, J = 9.6 Hz, 1H, H-3), 6.85 (d, J = 7.8 Hz, 1H, Ar–H), 6.93 (t, 1H, Ar–H), 6.99 (d, J = 8.5 Hz, 1H, Ar–H), 7.28 (t, 2H, Ar–H). 13C-NMR (100 MHz, DMSO-d$_6$) δ: 169.7; 167.3 (C=O), 158.7 (Ar–C), 157.5 (C-2), 155.4 (C-4), 130.0 (Ar–C), 129.8 (Ar–C), 121.7 (Ar–C), 115.0 (Ar–C), 114.9 (Ar–C), 110.5 (C-3), 66.5; 65.2 (CH$_2$), 48.8 (C-1), 47.8 (C-6), 43.6 (C-5), 42.9 (C-7), 26.3 (C-9), 23.2 (C-8), 22.5 (C-10). FT-IR (v$_{max}$, cm$^{-1}$): 3.468 (N–H), 319 (C–H, Ar), 2.970–2.934 (C–H), 1.697 (C=O), 1.661 (C=N), 1.599 (C–C, Ar), 1.388–1.688 (C=C, Ar). Raman (v$_{max}$, cm$^{-1}$): 3.073 (N=O), 3.030 (C–H, Ar), 2.993–2.837 (C=C), 1.686 (C=C), 1.640 (C=N). MS (FAB) m/z: 299 [M + H]$^+$. HRMS (ESI-TOF) calculated for C$_{18}$H$_{22}$O$_{2}$N$_{2}$ [M$^+$]: 298.379, found 298.385. HPLC: $t_c$ = 4.9 min; 5.3 min. M.p. (DSC) onset: 145.52°C, peak max: 149.22°C (first peak); onset: 154.91°C, peak max: 155.89°C (second peak).

2.2.2 2-(4-Bromophenoxy)-N′-[4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ylidene]acetohydrazide (3b)

Yield 82%; white solid. 1H-NMR (500 MHz, DMSO-d$_6$) δ: 0.83 (s, 3H, H-8), 1.41 (s, 3H, H-9), 1.58 (d, J = 8.2 Hz, 1H, H-7$_{endo}$), 1.90 (t, 3H, H-10), 2.27 (dd, J = 6.0 Hz, J = 1.8 Hz, 1H, H-5), 2.64 (dd, J = 5.9 Hz, J = 1.7 Hz, 1H, H-1), 4.63 (d, J = 7.7 Hz, 1H, CH$_2$), 4.98 (d, J = 7.7 Hz, 1H, CH$_3$), 5.81 (d, J = 16.6 Hz, 1H, H-3), 6.84 (d, J = 8.2 Hz, 1H, Ar–H), 6.91–6.93 (m, 1H, Ar–H), 7.41 (d, J = 8.2 Hz, 1H, Ar–H), 7.44 (d, J = 7.7 Hz, 1H, Ar–H). 13C-NMR (100 MHz, DMSO-d$_6$) δ: 169.1; 166.8 (C=O), 160.4 (Ar–C), 158.1 (C-2), 155.1 (C-4), 132.6 (Ar–C), 132.4 (Ar–C), 119.0 (Ar–C), 117.5 (Ar–C), 117.2 (Ar–C), 110.5 (C-3), 66.9; 65.5 (CH$_2$), 51.3 (C-1), 49.1 (C-6), 43.6 (C-5), 42.9 (C-7), 26.3 (C-9), 23.1 (C-8), 22.1 (C-10). FT-IR (v$_{max}$, cm$^{-1}$): 3.418 (N–H), 3.161 (C–H, C=C–H, Ar), 2.977–2.937 (C=O), 1.698 (C=O), 1.669 (C=N), 1.489 (C–C, Ar), 0.817–0.606 (C=C, Ar). Raman (v$_{max}$, cm$^{-1}$): 3.090 (N–H), 3.072 (C–H, Ar), 2.993–2.805 (C–H), 1.680 (C=O), 1.640 (C=N). MS (FAB) m/z: 377 [M$^+$]. HRMS (ESI-TOF) calculated for C$_{18}$H$_{22}$BrN$_{2}$O$_{2}$ [M$^+$]: 377.276, found 378.274. HPLC: $t_c$ = 9.1 min; 9.9 min. M.p. (DSC) onset: 167.86°C, peak max: 170.91°C (first peak); onset: 174.64°C, peak max: 176.29°C (second peak).
2.2.3 2-(4-Chlorophenoxyl)-N'-(4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ylidene)acetohydrazide (3c)

Yield 84%; white solid. $^1$H-NMR (500 MHz, DMSO-d$_6$): δ: 8.02 (s, 3H, H-8), 1.42 (s, 3H, H-9), 1.59 (d, $J$ = 8.2 Hz, 1H, H-7enol), 1.90 (t, 3H, H-10), 2.27 (dd, $J$ = 6.0 Hz, $J$ = 1.8 Hz, 1H, H-5), 2.65 (dd, $J$ = 5.9 Hz, $J$ = 1.7 Hz, 1H, H-1), 4.62 (d, $J$ = 7.7 Hz, 1H, CH$_2$), 4.97 (d, $J$ = 7.7 Hz, 1H, CH$_2$), 5.82 (d, $J$ = 16.6 Hz, 1H, H-3), 6.85 (d, $J$ = 8.2 Hz, 1H, Ar–H), 6.94–6.96 (m, 2H, Ar–H), 7.26 (d, $J$ = 8.2 Hz, 1H, Ar–H).

$^{13}$C-NMR (100 MHz, DMSO-d$_6$): δ: 169.1; 167.8 (C=O), 160.8 (Ar–C), 158.6 (C-2), 155.3 (C-4), 132.9 (Ar–C), 132.4 (Ar–C), 119.0 (Ar–C), 117.7 (Ar–C), 117.2 (Ar–C), 110.5 (C-3), 66.9; 65.6 (CH$_3$), 51.3 (C-1), 49.1 (C-6), 43.6 (C-5), 42.9 (C-7), 26.3 (C-9), 23.1 (C-8), 22.1 (C-10). FT-IR (v$_{max}$ cm$^{-1}$): 3,466–3,414 (N–H), 3,205 (C–H, Ar), 2,979–2,938 (C–H), 1,697 (C=O), 1,671 (C=N), 1,594 (C=C, Ar), 1,106–1019 (C–H, Ar). HPLC: $t_c$ = 8.0 min; 87 min. M.p. (DSC) onset: 117.3°C, peak max: 140.76°C (first peak); onset: 163.02°C, peak max: 171.38°C (second peak).

2.2.4 2-(4-tert-Butylphenoxy)-N'-(4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ylidene)acetohydrazide (3d)

Yield 70%; white solid. $^1$H-NMR (500 MHz, DMSO-d$_6$): δ: 0.82 (s, 3H, H-8), 1.24 (s, 3H, CH$_3$), 1.41 (s, 3H, H-9), 1.59 (d, $J$ = 8.3 Hz, 1H, H-7enol), 1.91 (t, 3H, H-10), 2.27 (dd, $J$ = 6.0 Hz, 1H, H-5), 2.64 (dd, $J$ = 5.9 Hz, $J$ = 1.7 Hz, 1H, H-1), 4.58 (d, $J$ = 7.2 Hz, 1H, CH$_2$), 4.92 (d, $J$ = 6.5 Hz, 1H, CH$_2$), 5.81 (d, $J$ = 10.9 Hz, 1H, H-3), 6.76–6.86 (m, 2H, Ar–H), 7.25–7.28 (m, 2H, Ar–H). $^{13}$C-NMR (100 MHz, DMSO-d$_6$): δ: 169.5; 164.4 (C=O), 160.3 (Ar–C), 156.5 (C-2), 154.9 (C-4), 143.2 (Ar–C), 126.5 (Ar–C), 126.4 (Ar–C), 114.4 (Ar–C), 114.3 (Ar–C), 110.5 (C-3), 66.4; 65.2 (CH$_3$), 51.3 (C-1), 49.1 (C-6), 43.5 (C-5), 42.8 (C-7), 34.2 (C CH$_3$), 31.8 (CH$_3$), 26.3 (C-9), 23.1 (C-8), 22.1 (C-10). FT-IR (v$_{max}$ cm$^{-1}$): 3,552–3,414 (N–H), 3,232 (C=C, Ar), 2,961–2,863 (C–H), 1,702 (C=O), 1,638 (C=N), 1,616–1,596 (C=C, Ar), 828–611 (C–H, Ar). Raman (v$_{max}$ cm$^{-1}$): 3,075 (N–H), 3,038 (C=C, Ar), 2,990–2,832 (C–H), 1,677 (C=O), 1,642 (C=N). MS (FAB) m/z: 355 [M + H$^+$]. HRMS (ESI-TOF) calculated for C$_{19}$H$_{29}$N$_2$O$_2$ [M$^+$]: 390.474, found 389.950. HPLC: $t_c$ = 15.8 min; 17.3 min. M.p. (DSC) onset: 87.15°C, peak max: 92.29°C (first peak); onset: 122.70°C, peak max: 132.31°C (second peak).

2.3 Anticonvulsant activity

2.3.1 Pentylentetrazole (PTZ)-induced seizures

The anticonvulsant effect of hydrazones 3a–3e was evaluated by intravenous injection of 1% aqueous solution of PTZ into a tail vein as described in Ref. [15]. Pure (–)-verbenone at a dose of 50 mg/kg in Tween 80/ water emulsion has been administered to mice orally. Hydrazones 3a–3e were used in equimolar to verbenone doses, and Tween 80/water emulsion served as a vehicle control and valproic acid (VPA, 400 mg/kg) – as a reference drug [16]. PTZ doses that induced clonic–tonic convulsions (DCT) and tonic extension (DTE) in experimental animals were calculated with respect to control. The anticonvulsant effect of compounds was assessed at different time points (6 and 24 h) from the increase of PTZ minimum effective dose (MED) in comparison to the control group. MED (in %) was determined according to the following formula:

$$MED = V/m \times 10^4,$$

taking into account the volume of PTZ solution (in mL) and the weight of each animal (in g): 10$^{-4}$ – conversion factor for animal weight (g to kg, 10$^3$) and dose of PTZ (mL of PTZ solution to mg of PTZ, 10).
2.3.2 MES-induced seizures

In this method, corneal electrodes (50 mA, 50 Hz) have been applied to induce seizures in experimental animals (mice) pretreated with compounds 3a–3e as described in Section 2.3.1. The percent of mortality was considered as an index of seizure protection.

2.4 Analgesic action

The analgesic activity of verbenone hydrazones 3a–3e was estimated by their topical application as 2% w/w ointments. Pain in experimental animals was induced by chemical stimuli, that is, by subcutaneous injection of TRPV1 and TRPA1 selective agonists – capsaicin and allyl isothiocyanate (AICT) with subsequent identification of pain index as described in our previous study [17]. The study was approved by the Animal Ethics Committee (agreement no. 04/2020) of Odessa National Polytechnic University (Ukraine).

2.5 Statistical analysis

All results are expressed as mean ± standard error mean (SEM). One-way analysis of variance was used to determine the statistical significance of the results followed by Tukey’s post hoc comparison. p < 0.05 was considered as statistically significant.

3 Results and discussion

3.1 Chemistry

The synthesis of (−)-verbenone hydrazones 3a–3e was performed via condensation of 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one 1 with hydrazides of 4-R-phenoxycetic acid (2a–2e) using a catalytic amount of CH3COOH (glacial), according to Scheme 1.

Here, we have to notice that condensation of bicyclic terpenoid verbenone with hydrazides requires more harsh reaction conditions (reflux for 12 h in n-PrOH), whereas monocyclic terpenoids (such as menthone or carvone [18,19]) react under mild conditions (reflux for 4 h in MeOH).

Hydrazones 3a–3e were isolated in 70–84% yield after recrystallization from methanol as white solids soluble in organic solvents such as acetonitrile and chloroform (Figure 1).

The formation of verbenone derivatives was elucidated by mass spectrometry – molecular ion peaks of target compounds 3a–3e correspond to their molecular formulas (FAB and ESI). The FT-IR spectra of verbenone hydrazones exhibit absorption bands of C=O and C=N groups, N–H bonds and alkyl and aromatic C–H. In addition, the formation of C=N bond of hydrazones was confirmed by Raman spectroscopy as intense peak at 1,640–1,644 cm⁻¹. Chemical shift, multiplicity and coupling constants observed in 1H-NMR and 13C NMR spectra prove the proposed structure of compounds 3a–3e. Phase transitions (melting points) of derivatives 3a–3e have been studied by DSC. The purity of (−)-verbenone derivatives was estimated by HPLC analysis using a mixture of acetonitrile and formic acid aqueous solution.

![Scheme 1: Synthesis of (−)-verbenone hydrazones. Reagents and conditions: (i) AcOH (glacial), n-PrOH, reflux, 12 h.](image)

![Figure 1: Structures of (−)-verbenone hydrazones 3a–3e.](image)
(0.01%) in 50:50 ratio as the eluent system. According to the HPLC analysis, two chromatographic peaks were revealed identifying the presence of Z/E geometrical isomers of verbenone hydrazones about C=\(\equiv\)N bond that was additionally supported by the MS data. Retention time recorded for these peaks ranges from 4.9 to 21.3 min (first peak) and from 5.3 to 23.3 min (second peak).

Along with Z/E geometrical isomers, hydrazones were proven to exist in solution as a mixture of cis/trans conformers caused by hindered rotation on the C–N amide bond [20]. The existence of verbenone hydrazones as cis/trans conformers was reliably corroborated by \(^1\)H NMR analysis – the protons of methylene group (CH\(_2\)) appear as two sets of signals. As described, in DMSO-d\(_6\) solutions of hydrazones, both cis and trans amide conformers are formed; nevertheless, cis form is dominated due to the formation of dimers that are stabilized by hydrogen bonds between CO and NH groups [20,21]. The upfield peak of the methylene group corresponds to trans conformer, while downfield peak corresponds to cis form [20]. We have to emphasize that \(^1\)H NMR spectral data of compounds 3a–3e displayed the similar pattern – two singlets for protons of methylene group (4.58–4.63 and 4.92–4.98 ppm). \(^{13}\)C NMR spectra of hydrazones 3a–3e contain also two resonance signals of methylene carbons (66.4–66.9 and 65.2–65.8 ppm) along with two signals of carbonyl carbons (166.5–169.7 and 164.2–167.8 ppm) (Table 1). In this case, upfield signals of carbonyl carbons and downfield lines of methylene carbons refer to amide trans conformer, while downfield lines of carbonyl carbons and upfield signals of methylene carbons are assigned to cis conformer [22]. Thus, we may conclude that according to \(^1\)H NMR and \(^{13}\)C NMR analysis target verbenone derivatives 3a–3e present in DMSO-d\(_6\) solution both in cis and trans form.

| Compound | Conformer | \(^1\)H NMR –CH\(_2\)= (\(\delta\), ppm) | \(^{13}\)C NMR –CH\(_2\)= (\(\delta\), ppm) | \(^{13}\)C NMR –C=\(\equiv\)N (\(\delta\), ppm) |
|----------|----------|---------------------------------|---------------------------------|-----------------|
| 3a       | cis      | 4.97                            | 65.2                            | 169.7           |
|          | trans    | 4.61                            | 65.5                            | 167.3           |
| 3b       | cis      | 4.98                            | 66.5                            | 169.1           |
|          | trans    | 4.63                            | 66.9                            | 168.8           |
| 3c       | cis      | 4.97                            | 65.6                            | 169.1           |
|          | trans    | 4.62                            | 66.9                            | 167.8           |
| 3d       | cis      | 4.92                            | 65.2                            | 169.5           |
|          | trans    | 4.58                            | 66.4                            | 164.4           |
| 3e       | cis      | 4.96                            | 65.8                            | 166.5           |
|          | trans    | 4.62                            | 66.9                            | 164.2           |

### 3.2 Anticonvulsant activity

Considering the antiseizure effect of pure terpenoids and taking into account the high reactivity of pharmacophore group –\(\equiv\)CO–NH–N\(=\)CH\(_2\), significant interest is focused on hydrazones based on terpenoids as scaffold [8,23].

Antiseizure action of verbenone hydrazones 3a–3e was elucidated both on models of chemically and electrically induced seizures at 6 and 24 h after their single oral administration. As illustrated in Figure 2, starting terpenoid along with derivatives 3a–3e demonstrated protective effect against PTZ-induced seizures at 6 h after administration as corroborated by increasing of DCTC and DTE values compared with control (100%).

At this time point, compound 3b (with bromine atom at the para position of benzene ring) was shown to possess maximal anticonvulsant activity with the average values: 278% for DCTC and 303% for DTE. Interestingly, reference anticonvulsant drug VPA (400 mg/kg, p.o.) is inferior in action to hydrazones 3a–3d (\(p < 0.01\)).

Bearing in mind the enzymatic cleavage of C=\(\equiv\)N double bond in hydrazone molecules [24] and, consequently, their possible prolonged action, anticonvulsant estimation was additionally performed at 24 h after administration (Figure 3).

In the PTZ-induced convulsion model, antiseizure action was manifested in experimental animal groups treated with verbenone hydrazones 3a–3e at 24 h after oral administration demonstrating DCTC and DTE values on average 212% and 214%, accordingly, in comparison with the control data (100%) that point out to a significant prolonged action. Furthermore, compounds 3a–3e exhibited higher anticonvulsant potency at long time period versus VPA (\(p < 0.01\)) that might be interpreted by enzymatic cleavage of labile bonds (N–N or C–N amide bond) in hydrazone molecules followed by...
gradual release of active ingredients. Moreover, the prolonged presence of verbenone derivatives in the body may also be realized due to their lipophilicity (log $P$ for 3a–3e ranges from 3.66 to 5.35, log $P$ for VPA is 2.72; ACD/Labs software). Low activity of pure verbenone (log $P$ 1.97) at 24 h after administration (130% for DCTC and 138 for DTE) is explained, in turn, by its excretion at this time point.

Anticonvulsant potency of verbenone hydrazones was additionally assessed in the MES test that is considered as generalized tonic–clonic seizure model and reflects the ability of compounds to prevent seizure spread throughout the brain [25]. According to the data of the MES test, electrical stimuli induced the rigid extension of the hind limbs in 100% of control animals (Table 2).

As mentioned earlier, hydrazones 3a–3d substantially prevented the mortality of animals at 6 h after administration and showed 80–100% protection, which is comparable to the effect of VPA (80%), while moderate antiseizure action was observed for compound 3e and starting verbenone (60%). Interestingly, the activity of the aforementioned hydrazones is maintained for a long time period (24 h) with 60–100% of mortality protection. In turn, pure verbenone administered 24 h prior to the test produced weak action in mice with only 20% of protection. We have to emphasize that seizures in the MES test were characterized by Straub tail when the mice

Figure 2: Anticonvulsant activity of compounds 3a–3e at 6 h after oral administration. Values are given as mean ± SEM, $n = 5$ mice; for all groups $p < 0.01$ compared with control; **$p < 0.01$ compared with VPA.

Figure 3: Anticonvulsant activity of compounds 3a–3e at 24 h after oral administration. Values are given as mean ± SEM, $n = 5$ mice; for all groups $p < 0.01$ compared with control; **$p < 0.01$ compared with VPA.
were orally pretreated with compounds 3a–3e. This phenomenon consisted of the mouse tail becoming rigid and erected across the back of the animal in an S-shaped curve [26]. Straub reaction in mice is described as one of the techniques for indicating analgesic and addicting properties of novel compounds [27]. Although Straub tail response is thought to be mediated by activation of the opioid receptor mechanism, this reaction can also be caused by dopaminergic, α-2 noradrenergic and nicotinic acetylcholine receptor agonists [28,29].

### 3.3 Analgesic action

Bearing in mind the aforementioned Straub reaction and molecular targets of terpenoids (transient receptor potential [TRP] channels), verbenone hydrazones 3a–3e were examined as potential analgesic agents by their topical application using agonists of TRP channels to induce the pain. Analgesic activity of compounds 3a–3e was comparable with the standard drug benzocaine (BZC) used as a topical pain reliever that activates both TRPV1 (comparable with the standard drug benzocaine

| Compound  | 3a | 3b | 3c | 3d | 3e | Verbenone | VPA | Control |
|-----------|----|----|----|----|----|-----------|-----|---------|
| 6 h after single oral administration | % Mortality | 80 | 80 | 80 | 100 | 60 | 60 | 80 | 0 |
| Protection | | | | | | | | | |
| 24 h after single oral administration | % Mortality | 80 | 100 | 60 | 60 | 80 | 20 | 60 | 0 |

As presented in Table 3, after the treatment with the ointment comprising either verbenone or its derivatives 3a–3e, the reaction time varied from 31 to 46 s, reflecting that these derivatives significantly attenuate AITC-induced acute pain and possess an analgesic action comparable with that in BZC treatment. Given the results of analgesic trials, TRPA1 and TRPV1 ion channels might be proposed as one of the molecular targets for compounds 3a–3e.

### 4 Conclusion

Hydrazones based on bicyclic terpenoid (−)-verbenone were synthesized as promising compounds simultaneously affecting both the central and peripheral nervous system. Verbenone derivatives 3a–3e have been demonstrated to protect against electroshock- and PTZ-induced seizures at different time points—6 and 24 h after oral administration. Binding of verbenone hydrazones to TRPA1/TRPV1 ion channels has been suggested as a feasible mechanism for explanation of significant analgesic activity of obtained compounds.

#### Abbreviations

- AITC: allyl isothiocyanate
- BZC: benzocaine
- DCTC: dose inducing clonic–tonic convulsions
- DSC: differential scanning calorimetry
- DTE: dose inducing tonic extension
- ESI: electrospray ionization method
- GABA: gamma-aminobutyric acid
- HRMS: high-resolution mass spectrometry
- MED: minimum effective dose
MES maximal electroshock
PTZ pentylenetetrazol
SEM standard error mean
TOF time-of-flight
TRP transient receptor potential
VPA valproic acid

Conflict of interest: No potential conflict of interest was reported by the authors.

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