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Skin permeability and analgesic activity effects of verbenone hydrazones

Mariia Nesterkina [1*], Sergii Smola [2], Iryna Kravchenko [1]

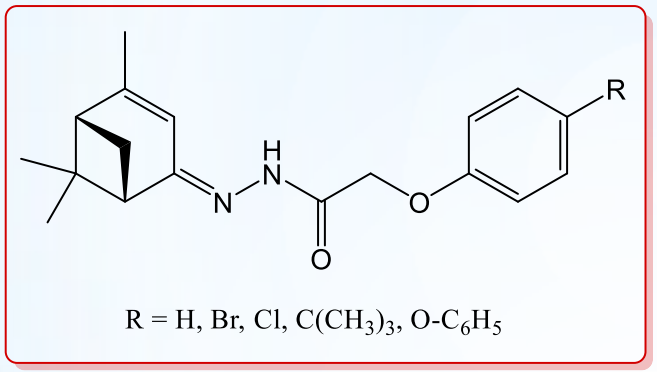
[1]Department of Organic and Pharmaceutical Technologies, Odessa National Polytechnic University, Odessa, Ukraine

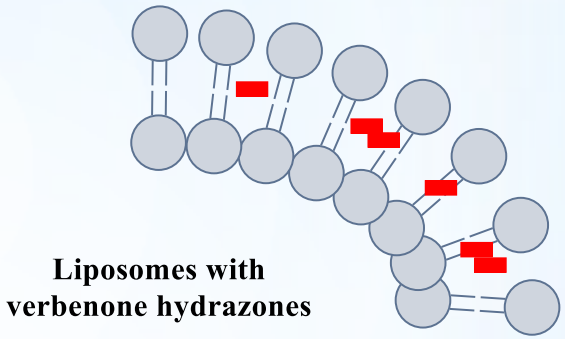
[2]A.V. Bogatsky Physico-Chemical Institute, National Academy of Sciences of Ukraine, Odessa, Ukraine

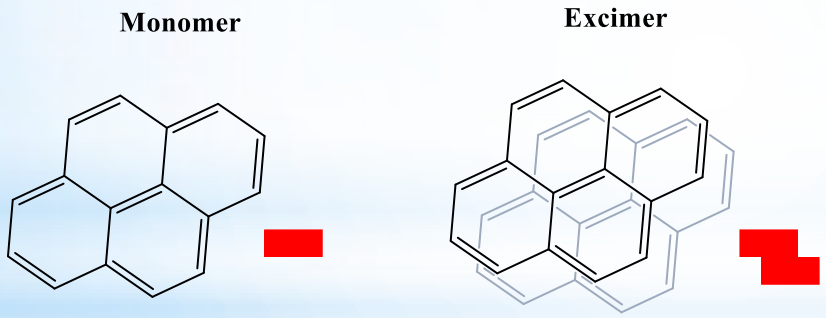
* Corresponding author: mashaneutron@gmail.com

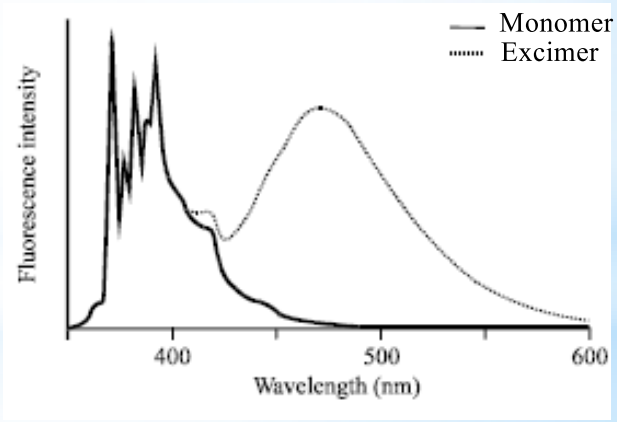
Skin permeability and analgesic activity effects of verbenone hydrazones

Graphical Abstract









Abstract: The present study aims to the development and synthesis of novel potential skin permeability enhancers based on derivatives of bicyclic terpenoid – verbenone. For this purpose, a series of hydrazones has been obtained via (–)-verbenone condensation with hydrazides of *para*-substituted phenoxyacetic acids. The structure of synthesized compounds was characterized by ^{13}C-NMR, ^{1}H-NMR, FT-IR and mass spectrometry.

The action mechanism of verbenone hydrazones on phospholipids of artificial membranes and lipids isolated from the rat stratum corneum was studied by fluorescence and FT-IR spectroscopy. When applying the fluorescent method, excimer/monomer emission intensity (I_E/I_M) ratio was calculated by measuring the relative intensities of pyrene excimer and monomer forms at 394 nm and 475 nm, respectively. According to our data, inclusion of verbenone hydrazones in phospholipid liposomes leads to growth of excimer to monomer ratio (I_E/I_M) indicating a decrease of membrane microviscosity. The disruption of hydrogen-bonded network formed by polar lipid groups was suggested as mechanism of action for verbenone derivatives confirmed by FT-IR analysis.

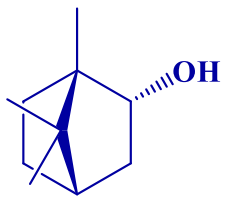
Given the above, (–)-verbenone hydrazones was estimated after transdermal delivery as potential analgesic agents via chemical-induced pain models using capsaicin and allyl isothiocyanate (AITC) as algogens. All the tested compounds were found to suppress painful sensation produced by noxious stimuli which indicates TRP receptors as one of the pharmacological targets of verbenone hydrazones.

Keywords: analgesic affect; hydrazones; skin permeability; verbenone

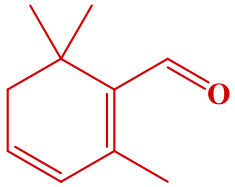
Mechanism and targets of topically applied compounds

Chemical enhancers

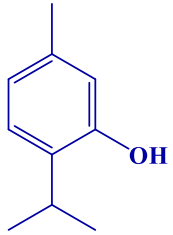
special attention is attracted to terpenes and their derivatives due to their high efficiency and low skin irritation



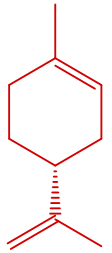
Borneol



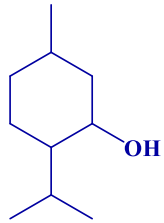
Safranal



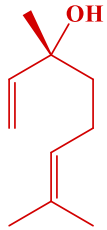
Thymol



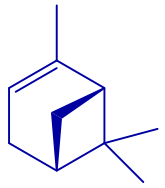
Limonene



Menthol



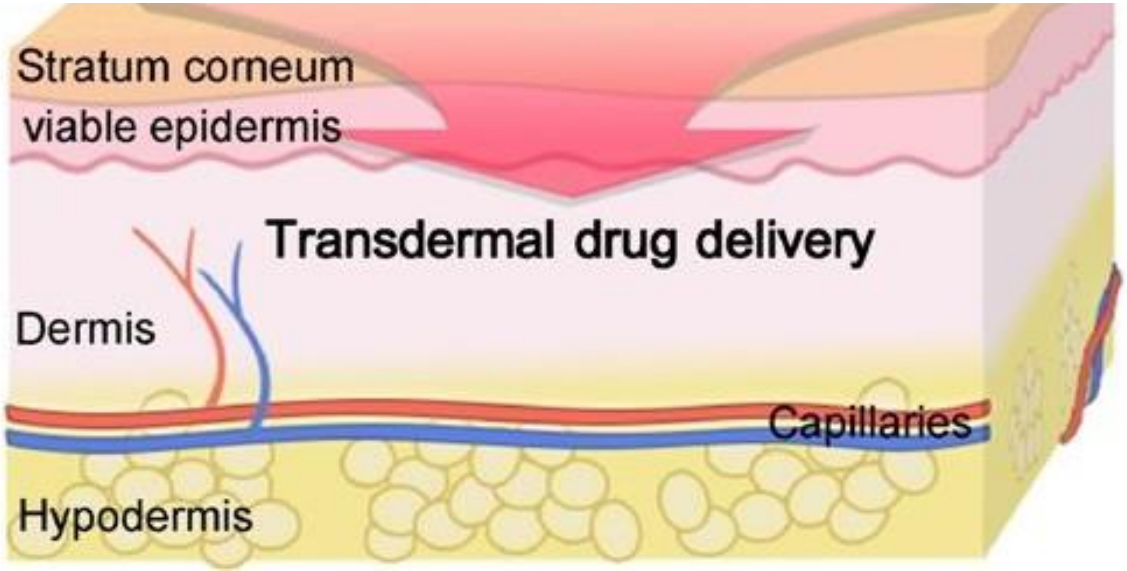
Linalool



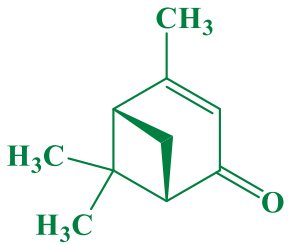
Pinene

Stratum corneum

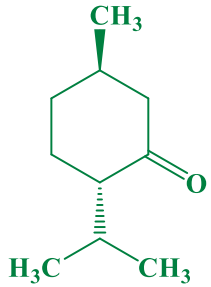
the outermost layer of the epidermis



Synthesis of verbenone hydrazones

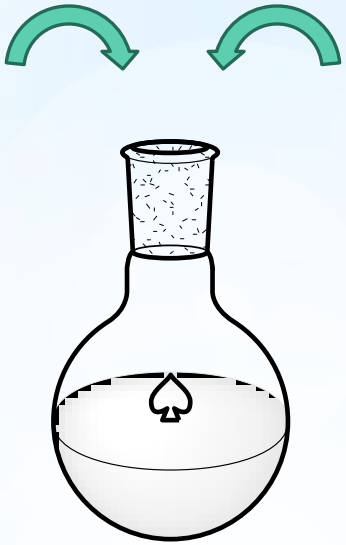


(-)-Verbenone

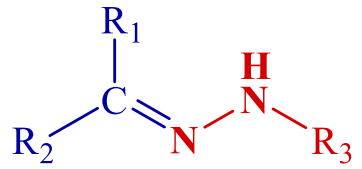


(-)-Menthone

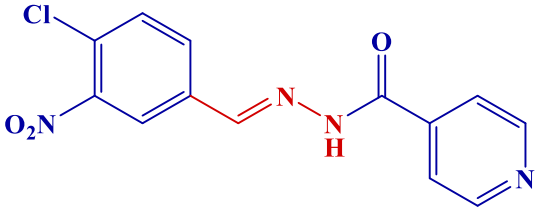
Terpenoid



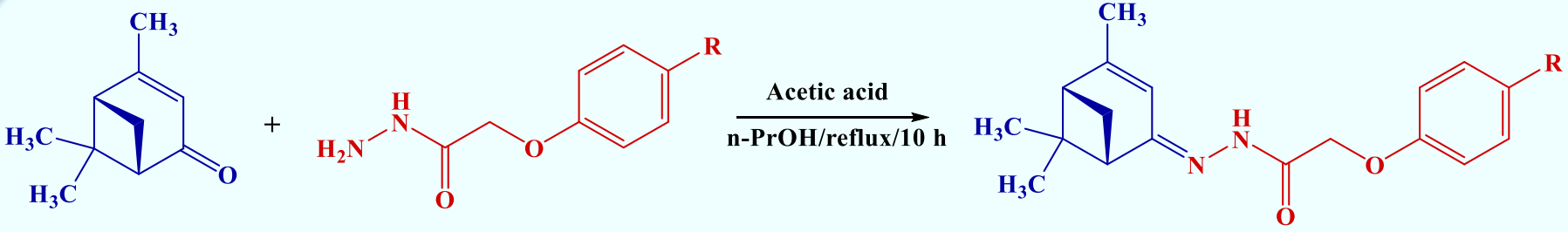
Hydrazide



Pharmacophore group



Anti-inflammatory activity under topical application



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

Mechanism and targets of topically applied compounds

JOURNAL OF LIPOSOME RESEARCH
https://doi.org/10.1080/08982104.2018.1538238

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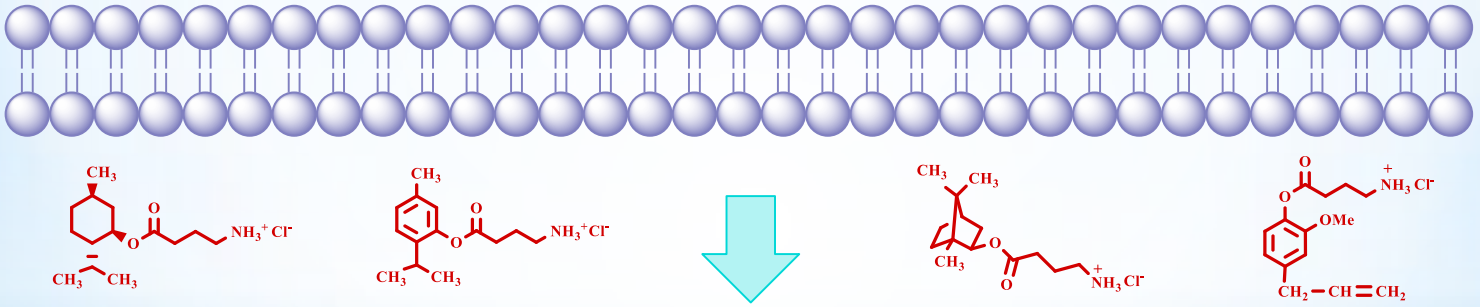
Effect of esters based on terpenoids and GABA on fluidity of phospholipid membranes

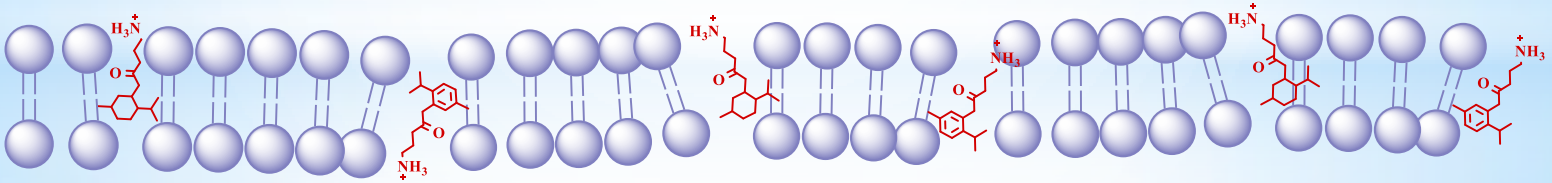
Mariia Nesterkina[a,b], Sergii Smola[c] and Iryna Kravchenko[a,b]

[a]Department of Organic and Pharmaceutical Technology, Odessa National Polytechnic University, Odessa, Ukraine; [b]Department of Pharmaceutical Chemistry, I.I. Mechnikov Odessa National University, Odessa, Ukraine; [c]A.V. Bogatsky Physico-Chemical Institute, National Academy of Sciences of Ukraine, Odessa, Ukraine

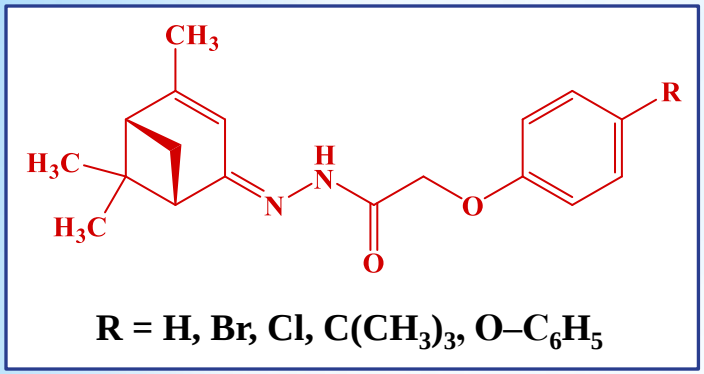
Ordered lipid membranes



Disordered lipid membranes



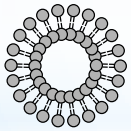
Stages of the study



R = H, Br, Cl, C(CH$_3$)$_3$, O–C$_6$H$_5$

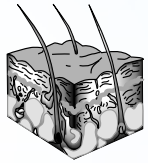
Synthesis

Fluorescence probe



Liposome preparation containing verbenone hydrazones and pyrene

FT-IR spectroscopy



Isolation of lipids from rat stratum corneum

Pharmacological investigation

Investigation of analgesic activity

Investigation of hydrazones' influence on membrane permeability using method of fluorescence probe

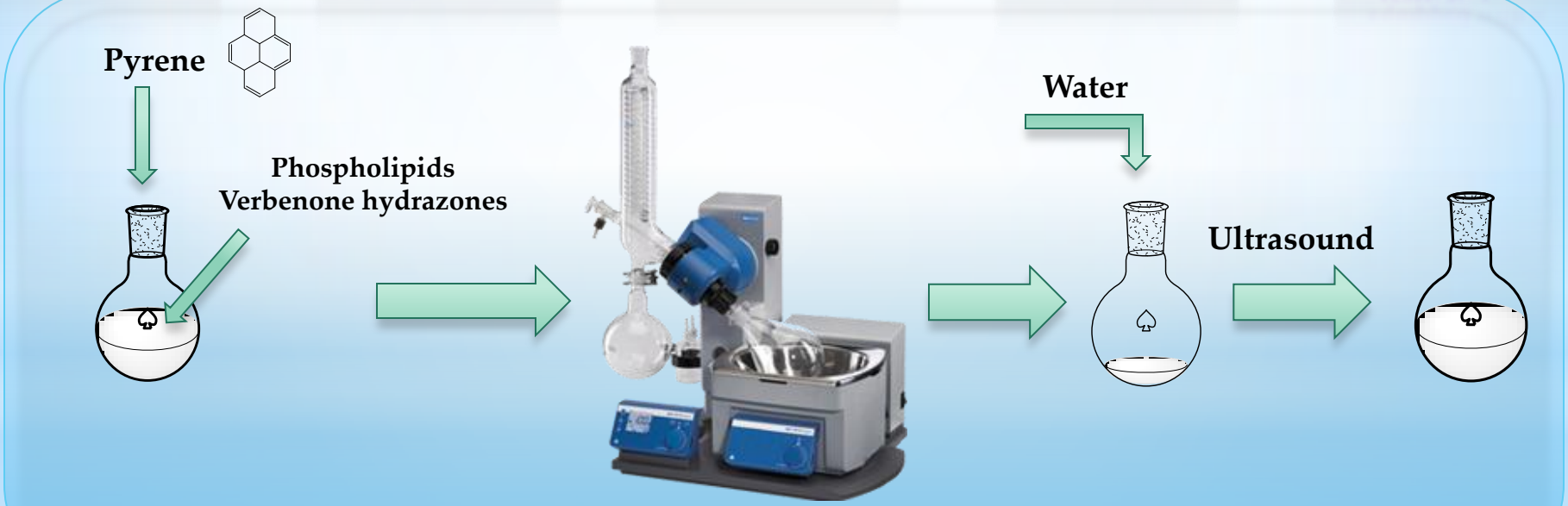
Chloroform solution of pyrene, verbenone hydrazones and lecithin were mixed in a molar ratio 1:10:100

The solvent was removed by slow evaporation under vacuum at 40 °C

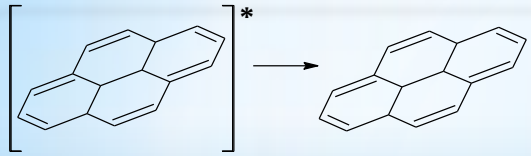
The dried mixture was resuspended in 25 ml deionized water and vigorously stirred for 10 min

The resulting emulsion was then sonicated for 10 min at 22 kHz frequency

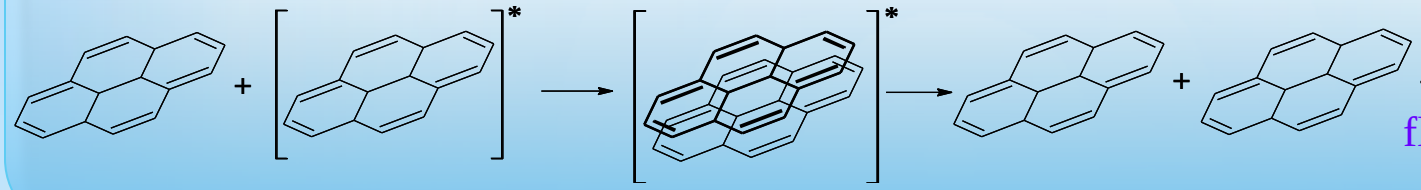
Steady-state fluorescence spectra of samples containing pyrene were recorded on a Horiba Jobin-Yvon Fluorog-FL 3-22 spectrophotometer equipped with a 450W Xe lamp



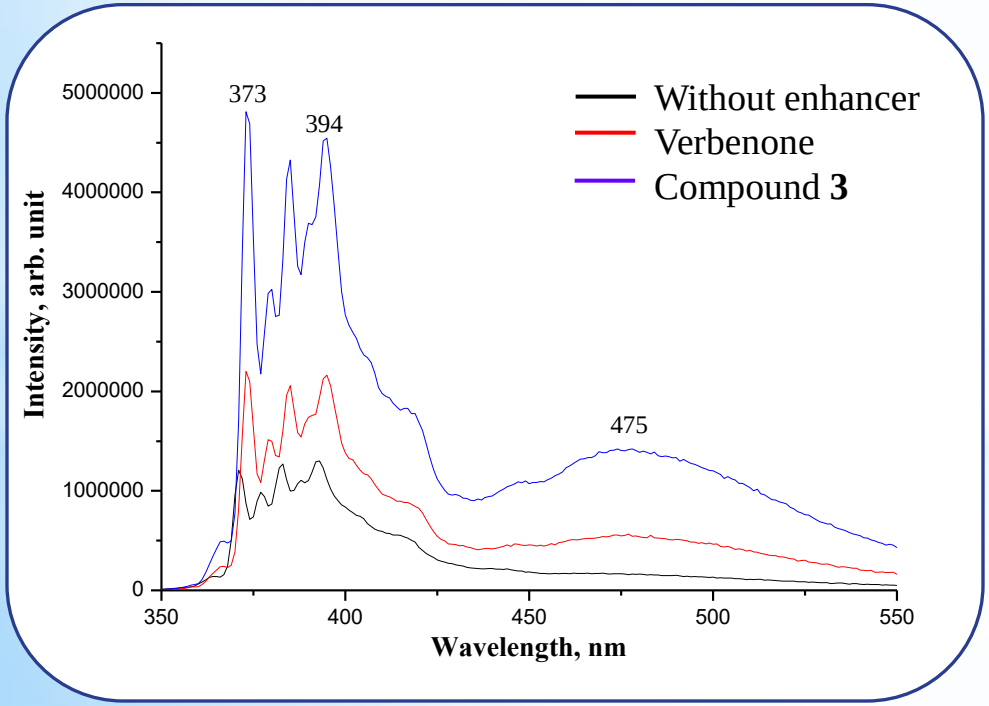
The influence of verbenone and its hydrazones on membrane microviscosity and polarity

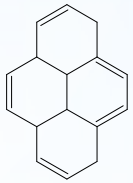


+ $h\nu_M$ - monomer fluorescence (370-395 mn)

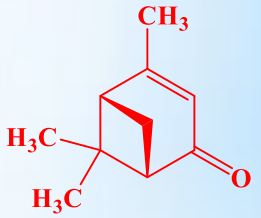


+ + $h\nu_E$ - excimer fluorescence (470-480 nm)

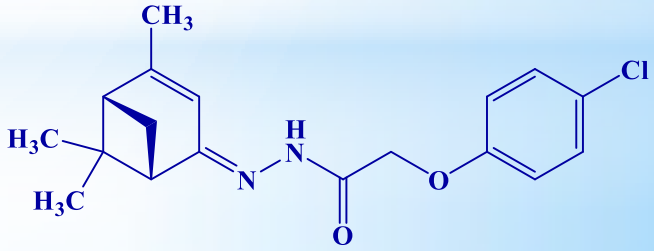




Pure phospholipids with pyrene (without enhancer)

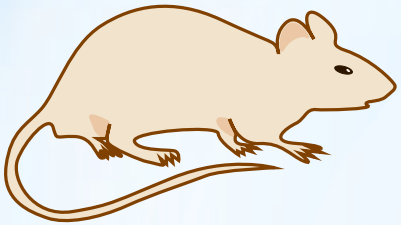


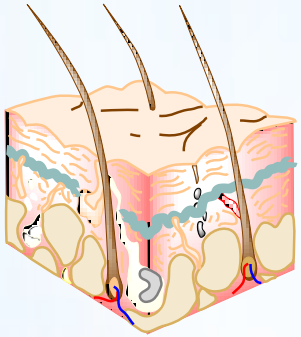
Verbenone

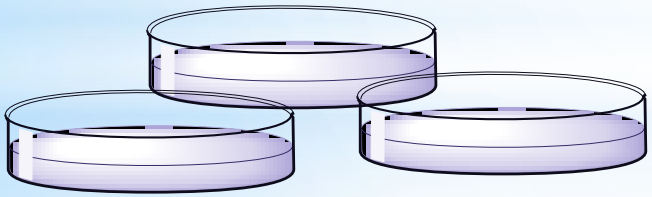


Compound 3

Isolations of lipids from rat stratum corneum







Stratum corneum

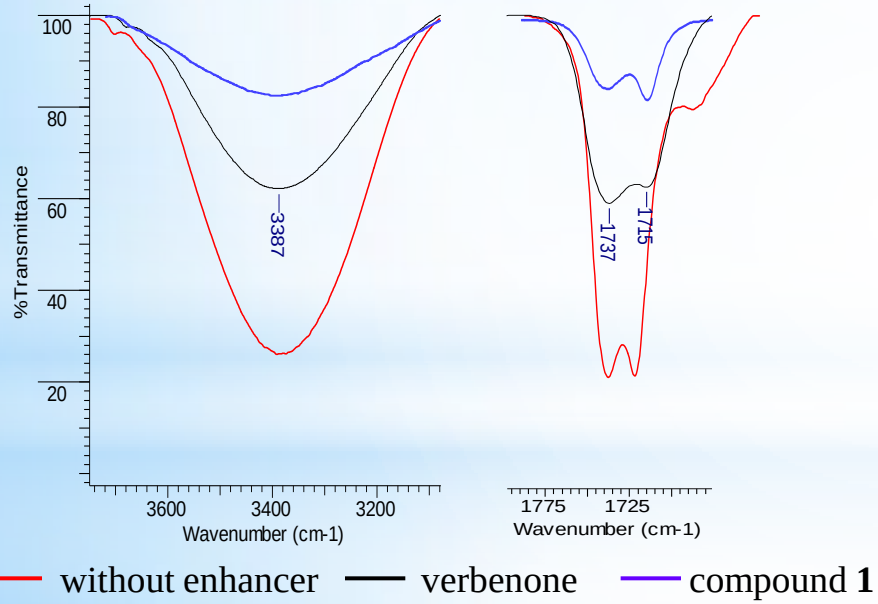
The male Wistar rats (150–180 g) were used for skin preparation

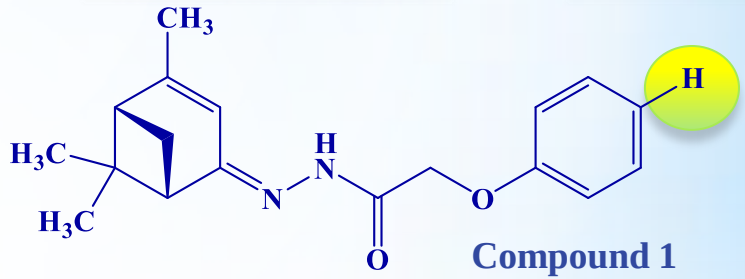
The rats were anesthetized followed by shaving and surgically removing of abdominal and back regions.

SC was separated from the epidermis by incubating in trypsin solution (0.15% in PBS buffer, pH 7.4) during 24 h at 4°C and thereafter 4 h at 37°C. SC was then mechanically separated and carefully washed with a solution of trypsin inhibitor, deionized water and dried.

Investigation of hydrazones' influence on lipids of stratum corneum using FT-IR spectroscopy

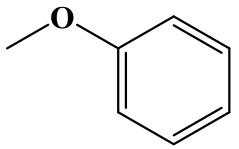
Preparation of stratum corneum → The SC was dipped into chloroform: methanol (2:1) solution and kept in the dark for 72 h → Then the extract was washed twice with distilled water and the lower organic layer was evaporated under vacuum below 40 °C → The samples for FT-IR study have been prepared by dissolving the obtained lipids in carbon tetrachloride (CCl_4) with subsequent addition of hydrazones (10% relative to lipids' mass) → FT-IR spectra were recorded for films

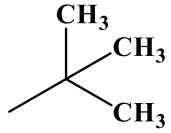




Compound 1

-H -Cl -Br





Decrease of influence on lipids

Pharmacological investigation of verbenone hydrazones

central nervous system

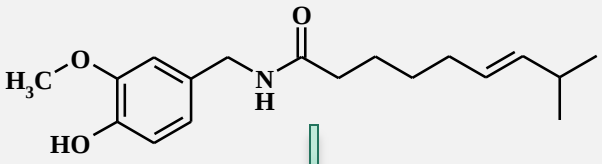
peripheral nervous system

- Pharmacological activity
  - Anticonvulsant
    - Pentylenetetrazole test
    - Maximal electroshock seizures
  - Analgesic
    - Chemical-induced pain
    - Thermal-induced pain

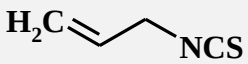
Experimental methods of pain induction

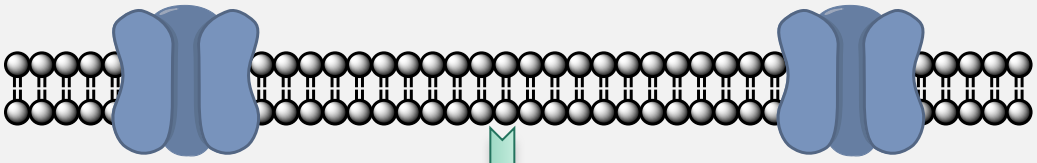
***Dosage form*: 2% ointment** **_Base_: PEG – PEO – 1,2-Propyleneglycol**

Capsaicin



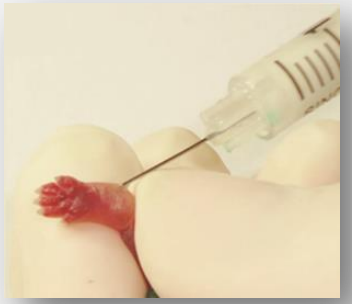
Allyl isothiocyanate (AITC)





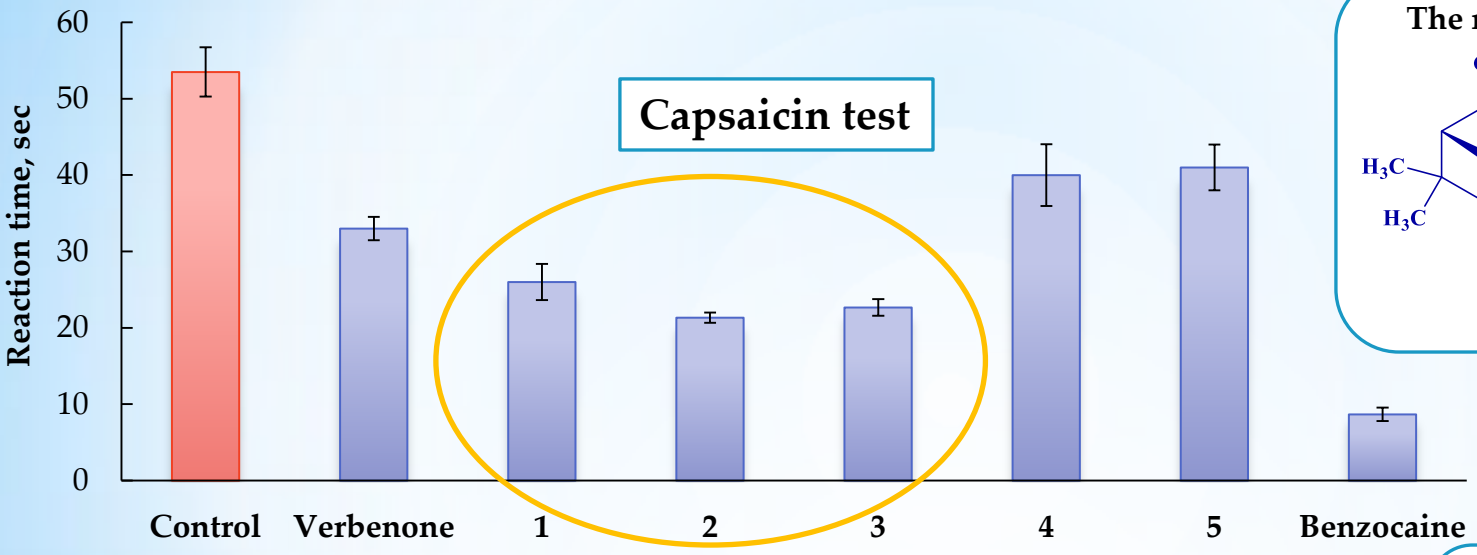
TRPV1 **TRPA1**

Pain

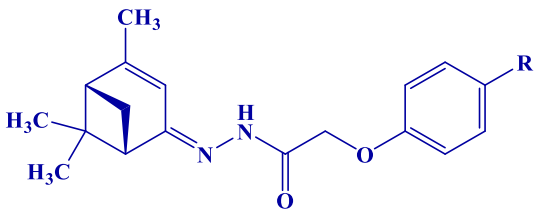


The animal then was placed in an individual plexiglass cage. The time spent licking the injected paw was measured during 5/10 min after capsaicin/AITC administration and was considered as an indicator of pain response.

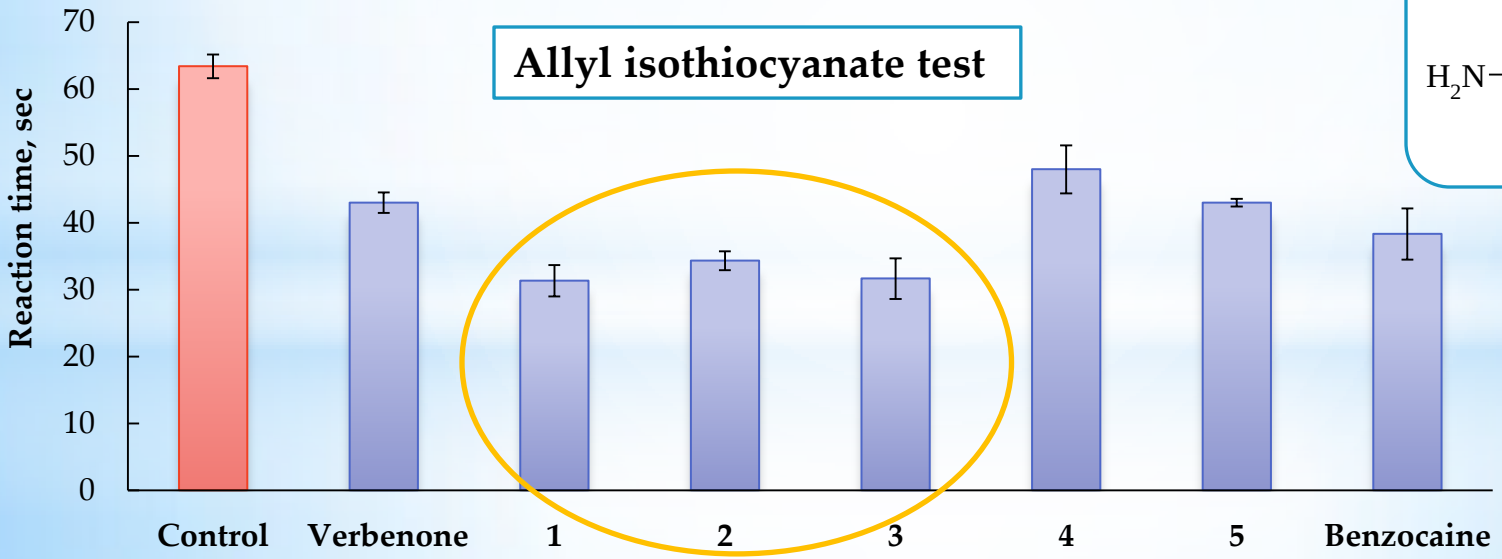
Analgesic activity of verbenone hydrazones



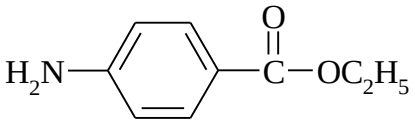
The most perspective structure



R = H, Cl or Br



Reference drug



Benzocaine

Summary

- Condensation of verbenone 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.

- In this study, the interaction of verbenone hydrazones with artificial membranes and lipids isolated from rat SC was investigated with fluorescence and FT-IR spectroscopy.

- Based on our experimental data, we may conclude that verbenone hydrazones demonstrate analgesic action by their topical application in models of chemically-induced pain.

THANK YOU FOR YOUR ATTENTION!

We are open for cooperation