Synthesis and analgesic effects of 1-[1-(2-methylphenyl)(cyclohexyl)]-3-piperidinol as a new derivative of phencyclidine in mice

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Abstract
Phencyclidine (1-[1-(phenylcyclohexyl)piperidinol, CAS 956-90-1, PCP, I) and its derivatives have shown many analgesic effects. In this research, a new derivative of PCP (1-[1-(2-methylphenyl)(cyclohexyl)]-3-piperidinol, PD, II) was synthesized and its analgesic (acute and chronic pains) effects were examined on rats using tail immersion (as a model of acute thermal pain) and formalin (as a model of acute and chronic chemical pain) tests and the results are compared to PCP and control groups.
The results indicated that II produces higher analgesic effects in the tail immersion test compared to the PCP and control groups, with a marked and significant increase in tail immersion latency for the doses 1, 5 and 10 mg/kg. The formalin test showed that PD (II) is not effective in acute chemical pain (phase I, 0–5 min after injection) in all doses but chronic pain (initial-phase II, 15–40 min after injection) is significantly attenuated by this compound compared to PCP and saline (control) in doses of 5 and 10 mg/kg.
It is concluded that II is effective in acute thermal (in all doses) and chronic (doses of 5 and 10 mg/kg) pains; however, it is not effective in acute chemical pain compared to PCP and control.

1. Introduction
Pain, one of the basic clinical symptoms, always exists and consequently there is a worldwide need for effective therapies. Therefore an urgent action for the treatment of acute and chronic pain acceptable to the patients is required. This will be understood as successful and satisfactory treatment of pain. Such treatments are presented in a vast number of scientific works which have recently appeared in the field of applied analgesic or fundamental research into nociception [1].

For example, phencyclidine (1-(1-phenylcyclohexyl)piperidinol, CAS 956-90-1, PCP, I, Scheme 1) derivatives exhibiting analgesic activity are well known for this purpose (2–9).
Experimental evidence suggests that glutamate receptors are involved in pain control and behavior mechanism. Behavioral, electrophysiological and biochemical data indicate that the N-methyl-D-aspartate (NMDA) subtypes of glutamate receptors are essential for the development of phenomena associated with chronic pain [10]. In this treatment, PCP binds to the NMDA receptor complex and blocks NMDA-mediated gating of the calcium conductance channel [11]. The binding of PCP to the NMDA receptor results in blocking of the voltage-sensitive potassium and sodium channels. This causes increased calcium entry into the nerve cell, resulting in increased release of neurotransmitters at the presynaptic nerve ending. The potency of PCP analogues in blocking the potassium channel in vitro highly correlates with their behavioral potency in vivo [12].

Furthermore, various animal models of nociception are used to characterize the specific pain conditions in human beings. For example, tail immersion, tail flick and hot plate experiments evaluated analgesic effects on acute cutaneous thermal pain and intraplantar injections of formalin, zymosan and carrageenan are models of acute and chronic chemical pain. [13].

In this research, 1-[1-(2-methylphenyl)(cyclohexyl)]-3-piperidinol (PD, II, Scheme 1), an analogue of PCP with a methyl group on the aromatic ring (α-position) and hydroxyl group on the piperidine ring (m-position),
was prepared and its analgesic effects were examined on rats using tail immersion (as a model of acute thermal pain) and formalin (as a model of acute and chronic chemical pain) tests. The results are compared with PCP and control groups. As indicated in our previous work on this family [4, 6, 8, 9], incorporation of a methyl group on the aromatic ring of the molecule will generate pronounced effects on electron distribution and dipole moments because of its high electron donating character [14]. In addition, incorporation of a hydroxyl group on the piperidine ring of the molecule was anticipated to have pronounced effects on its hydrophilic properties [3, 15] and increases the polarity and solubility activities of the drug.

2. Materials and methods

2.1 Material
Cyclohexanone, piperidine, 3-piperidinol, bromobenzene, magnesium turnings, diethyl ether, 2-bromotoluene were purchased from Merck Chemical Co. (Darmstadt, Germany). Melting points (uncorrected) were determined using a digital Electro Thermal Melting Point apparatus (model 9100, Electrothermal Engineering Ltd., Essex, UK). ¹H and ¹³C NMR spectra were recorded on a Bruker 300 MHz (AMX model, Karlsruhe, Germany) spectrometer (internal reference: TMS) and IR spectra were recorded on a Thermo Nicolet FT-IR (Nexus-870 model, Nicolet Instrument Corp., Madison, WI, USA) spectrophotometer. Mass spectra were recorded using Agilent Technologies 5973, Mass Selective Detector (MSD) spectrometer (Wilmington, USA). Column chromatographic separations were performed over Acrós silica gel (CAS 7631-86-9, particle size 35–70 micrometer, Geel, Belgium). NMRI mice (Pasteur Institute, Tehran, Iran), weighing 20–30 g, were used for pharmacological testing.

2.2 Chemistry (Scheme 1–3)

2.2.1 1-(1-Phenylcyclohexyl)piperidine (PCP) I
This compound was prepared in 58% yield from 1-piperidino-cyclohexanecarbonitrile (I) and phenyl magnesium bromide according to a known procedure [16]. The hydrochloride salt of I (m.p. 233–234°C) was prepared using 2-propanol and HCl and was recrystallized from 2-propanol [16].

2.2.2 3-Hydroxypiperidinocyclohexylcarbonitrile III
This compound was prepared from 3-piperidinol and cyclohexanone based on the known procedure [17] (m.p.: 98–99°C).

2.2.3 1-[1-(2-Methylphenyl)(cyclohexyl)]-3-piperidinol II
A solution containing 4.26 g (0.0204 mol) of nitrile compound (III) in 50 ml of dry THF and diethyl ether (1:1) was added to 2-methylphenyl magnesium bromide (Grignard reagent; prepared from 28.5 g 2-bromo toluene and 4.1 g of Mg in 120 ml dry ether), refluxed for 8 h, left overnight at ambient temperature and then poured into ice-NH₄Cl. The organic layer was separated and washed with water and the base was neutralized with 10% H₂SO₄, washed with 20% NaOH, reextracted with n-hexane, dried and concentrated. The oily residue obtained was passed through a silica gel column using ethyl acetate-hexane (4:1) as the eluent to afford 1.55 g of II (42.86% yield).

The hydrochloride salt of II (m.p. 163–164°C) was prepared using 2-propanol and HCl and was recrystallized from 2-propanol.

IR (KBr): 3437, 3007, 2967, 2858, 1950, 1450, 1630, 1490, 1540, 1396, 1247, 1109, 1051, 790 cm⁻¹.

¹H NMR (CDCl₃) (ppm): 1.39–3.02 (18H, m), 2.35 (3H, s), 3.23 (1H, m), 6.94–7.17 (4H, m).

¹³C NMR (CDCl₃) (ppm): 18.7, 21.9, 22.9, 28.6, 32.2, 33.1, 40.6, 48.6, 57.9, 71.9, 125.5, 125.9, 126.5, 128.8, 134.4, 149.4.

MS: m/z (regulatory intensity): 273 (6.5).

2.3 Pharmacological methods
NMRI mice (Pasteur’s Institute, Tehran, Iran), weighing 20–30 g at the beginning of the experiment, were randomly housed, three to four per cage, in a temperature-controlled colony room under light/dark cycle. Animals were given free access to water

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**Scheme 1: Structure formulas of PCP (I), PD (II), and carbonyl intermediates I and III.**

**Scheme 2: Synthesis of intermediates I and III.**

**Scheme 3: Synthesis of target compounds I and II.**
and standard laboratory rat chow (Pars Company, Tehran, Iran). All behavioral experiments were carried out between 11 am and 4 pm under normal room light and at 25°C. All animals were injected by one investigator and evaluated by another. This study was carried out in accordance with the guidelines set forth in the Guide for the Care and Use of Laboratory Animals (NIH) and those of the Research Council of Islamic Azad University of Medical Sciences (Tehran, Iran).

2.3.1 Tail immersion test

Acute thermal pain is modeled by the tail immersion test [18–20]. Twenty minutes after an i.p. injection [21] of drugs (PCP and its analogue, 1, 5 and 10 mg/kg) or an equivalent volume of saline (control), the mice (n = 12 in each group) were housed in an animal restrainer. Then, the terminal 5 cm of their tails were first submerged into room temperature water (22–24°C) to check the aversion to water and then immersed in 52°C water. The reaction time between immersing the tail and its removal from heated water was measured. Cut-off latency in 15 s was employed to avoid damaging the tail [21, 22].

2.3.2 Formalin test

Formalin test was introduced by Dubuisson and Dennis [23]. In this test, formaldehyde solution (50 µl, 2.5%) was injected subcutaneously into the plantar surface of the hind paw. Then the animal was placed in a Plexiglas chamber (30 x 30 x 30 cm³), with a mirror at 45° angle underneath for accurate observation. In the treatment groups, the drugs (PCP and PD) were administered intraperitoneally 30 min prior to the formaldehyde injection. Prior to the experiments, all animals were brought to the test chamber 5 times at 5-min intervals to adapt them to the environment. The behavioral pain reactions due to formalin injection were detected and recorded for 1 h. The first 15 min after formalin injection is known as the early (I) or acute phase and the period between 15 and 60 min is known as the second (II) or chronic phase. The chronic phase can be divided into initial (15–40 min) and late (40–60 min) periods.

3. Results

3.1 Chemistry

Phencyclidine (I) and 1-[1-[2-methylphenyl][cyclohexyl]-3-piperidinol (II) were synthesized by reaction of substituted Grignard reagent and carbonitrile compounds. To obtain stronger hydrophilic, polarity and solubility properties, a hydroxyl group was substituted on the piperidine ring and a methyl group was substituted on the aromatic ring of the molecule which gives high electron donating property to this group to achieve more electron distribution and dipole moments. A known procedure was applied for the synthesis of compounds I and III with the appropriate modifications [16, 17].

Spectroscopic data (IR, 1H and 13C NMR, mass) confirmed the structure of the compound II. The melting points of the known compounds also confirmed their identity. The purity of each compound was checked by TLC using ethyl acetate-hexane as the eluent.

![Graph showing tail immersion latency](image)

Fig. 1: Mean tail immersion latency (s) in animals receiving PCP (I) and PD (II) hydrochloride or saline (control) in doses of 1, 5 and 10 mg/kg. The tail immersion test was conducted 20 min after the drug injection. Each point represents the mean ± SEM of tail immersion latency (s) in 12 animals.

* and $ p < 0.05, ** and $$$ p < 0.01, ***, and $$$$ p < 0.001: difference from control and PCP groups, respectively.

3.2 Pharmacology

3.2.1 General consideration

Mortality (number of deaths), morbidity (defined as any abnormal condition or behavior due to a disorder), irritability (a condition of aggressiveness or increased response on handling) and other related abnormal states were observed in experimental animals. However, the motor coordination index (measured by rota-rod apparatus, Harvard, UK) did not indicate any significant differences between control and treated mice.

3.2.2 Analgesic activity of PCP (I) and PD (II) hydrochloride with the tail immersion test

Intraperitoneal injection of PCP, PD and saline (control) in three doses (1, 5 and 10 mg/kg) generated analgesic effects in the tail immersion test. The results indicate that PD can produce stronger analgesic effects on the tail immersion test (as a model of acute thermal pain) in comparison to PCP and control groups, with a significant increase in tail immersion latency in doses of 1, 5 and 10 mg/kg (Fig. 1). Therefore it seems that strong electron donating properties of the methyl group on para position of the phenyl ring and also hydrophilic polarity and solubility properties of the hydroxyl group on the piperidine ring facilitate binding to the NMDA receptor complex and could increase tail immersion latencies in comparison with PCP and control groups, as anticipated. The differences in the tail immersion latencies were evaluated using the analysis of variance method (ANOVA).

3.2.2 Analgesic activity of PCP (I) and PD (II) hydrochloride with the formalin test

PCP and PD were administered intraperitoneally in three doses (1, 5 and 10 mg/kg), 30 min before the formaldehyde injection. The results show that PD is not ef-
effective in acute chemical pain in all doses but chronic pain could be significantly attenuated by this compound compared to PCP and saline (control) groups in doses of 5 and 10 mg/kg (Table 1). The differences in the pain scores were evaluated using ANOVA.

### 4. Discussion

PCP was originally introduced as a general analgesic agent. Because of severe psychomimetic side effects it was subsequently withdrawn from application in humans. However, the focus of recent research on PCP has shifted its use as an anesthetic prescrption drug toward the use as a potential neuropharmacological drug [24] and the interest was Initiated on working on its derivatives or analogues [25].

The NMDA receptor is a ligand gated ion channel which serves as one of the primary substrates for excitatory neurotransmission in the central nervous system (CNS). Excessive or abnormal NMDA receptor activity has been linked with numerous neuropathological conditions including acute neurotoxicity which occurs subsequent to head trauma and ischemic stroke as well as a number of chronic, neurodegenerative disorders including neuropathic pain [26].

In this research, a new derivative of PCP with different substitutions in its phenyl and cyclohexane rings was synthesized. Because of the stronger analgesic effects of some of the synthesized derivatives of PCP bearing methyl, methoxy and hydroxyl groups on phenyl and cyclohexane rings [2, 3, 7], a new derivative of PCP with modifications/substitutions in its phenyl and cyclohexane rings was synthesized and the analgesic effects were studied using tail immersion and formalin tests.

### 5. Conclusion

Similar to our previous findings on the analgesic effects of this family, PD (II) could diminish thermal (all doses in the tail immersion test) but not chemical (all doses in the formalin test) acute pains. Chronic pain could also be significantly attenuated by this new drug in comparison with PCP and saline (control) in doses of 5 and 10 mg/kg.

It can be also concluded that adding a methyl group to the aromatic ring (with high electron donating property) resulted in higher electron distribution and dipole moments of PCP. Moreover, adding a hydroxyl group to the piperidine ring (with hydrophilic property) increased the polarity and solubility activity of this new drug (PD, II).

Therefore, such modifications on the PCP molecule are useful, causing diminished thermal and chronic pain in tail immersion and formalin tests in mice.

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### Conflict of Interest

This research is not part of my normal lecturing, employment and consultation, and no institution will require any right from this work. Also, any patent has not been applied or commercial right has not been given to any company and/or institution, and will not be done later.

### References


