Synthesis and determination of acute and chronic pain activities of 1-[1-(3-methylphenyl)(tetralyl)]piperidine as a new derivative of phencyclidine via tail immersion and formalin tests

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Key words
- CAS 956-90-1
- CAS 1867-66-9
- Ketamine
- Phencyclidine
- 1-Tetralone derivatives, analgesic effects, formalin test, tail immersion test

Abstract
Phencyclidine (1-(1-phenylcyclohexyl)piperidine, CAS 956-90-1, PCP, I) and ketamine (2-O-chlorophenyl-2-methylaminocyclohexan, CAS 1867-66-9, II) revealed some analgesic effects. Some of their derivatives have been synthesized for biological properties studies. Utilizing 1-tetralone as a starting material, 1-[(3-methylphenyl)(tetralyl)]piperidine, (PCP-CH2-tetralyl, III) was synthesized and its analgesic effects were studied on rats via tail immersion (as a model of acute thermal pain) and formalin (as a model of acute chemical and chronic pain) tests and compared with those of ketamine and PCP. The results indicated a marked anti-nociception 2–25 min after ketamine injection, but this analgesic effect lasted for 40 min following PCP-CH2-tetralyl application in the tail immersion test. However, the data obtained from the formalin test showed that chronic pain could be significantly attenuated by ketamine, PCP and PCP-CH2-tetralyl.

1. Introduction
Phencyclidine (1-(1-phenylcyclohexyl)piperidine, CAS 956-90-1, PCP, I, Scheme 1) was originally introduced as a general anesthetic agent [1–3]. This compound and its analogues showed many biological properties including highly potent and widely abused psychotomimetic properties which affect the central nervous system [4]. They also display analgesic, stimulating, depressive and hallucinogenic effects on the specific binding sites in brain [2]. PCP binds to N-methyl-D-aspartate (NMDA) receptor complex and blocks NMDA-mediated gating of the calcium channel conductance [5, 6]. The analgesic effect of ketamine (2-O-chlorophenyl-2-methylaminocyclohexan, CAS 1867-66-9, II, Scheme 1), the other PCP analogue, was first described by Domino et al. in 1965 [7]. It is widely known as an NMDA receptor antagonist and its interactions with other receptors are being increasingly recognized. Even though glutamate receptors play essential roles in synaptogenesis and neuronal survival [8, 9], they also influence cell death induced by excitotoxicity [9–11]. Ketamine is a low-affinity, use-dependent, non-competitive antagonist of NMDA receptors [12–14]. The blockade is use-dependent in which the rates of onset and the recovery from blockade are increased upon applying NMDA agonists [15]. Ketamine concentration-based differences in the mechanism of NMDA receptor blockade also have clinical implications, in which the analgesic properties of ketamine appears at low levels and the anesthetic effects appear at much higher concentrations [15].

Up to now, many analogues of phencyclidine have been synthesized [16–22] and their pharmacological activities have been investigated. Therefore as part of our objectives to obtain selective, non-competitive antagonists at the PCP binding site on NMDA receptor complex, 1-[(3-methylphenyl) (tetralyl)]piperidine (PCP-CH2-tetralyl, III, Scheme 1), was produced as an analogue of I with a methyl group on the aromatic ring (m-position) and a phenyl group on the cyclohexane ring.
2. Materials and methods

2.1 General

1-Tetralone (1,2,3,4-tetrahydro-1-naphthalenone), cyclohexane, piperidine, bromobenzene, magnesium turnings, diethyl ether, 3-bromotoluene and all other chemicals, were purchased from Merck Chemical Co. (Darmstadt, Germany). Melting points (uncorrected) were determined using a digital Electro Thermal Melting Point apparatus (model 9100, Electro thermal Engineering Ltd., Essex, UK). $^1$H and $^{13}$C NMR spectra were recorded on a Bruker 300 MHz (model AMX, Karlsruhe, Germany) spectrometer (internal reference: TMS) and IR spectra were recorded on a Thermo Nicolet FT-IR (model Nexus-870, Nicolet Instrument Corp, Madison, Wisconsin, USA) spectrophotometer. Mass spectra were recorded using Agilent Technologies 5973, Mass Selective Detector (MSD) spectrometer (Wilmington, USA). Column chromatographic separations were performed over Acros silica gel (No. 7631-86-9 particle size 35–70 micrometer, Geel, Belgium). Adult female Wistar rats (Pasteur Institute, Tehran, Iran), weighing 250–300 g, were used for pharmacological testing.

2.2 Synthesis of compounds (Scheme 1 and 2)

2.2.1 (1-(1-Phenylcyclohexyl)piperidine (PCP) I

This compound was prepared according to a reported method [24] from 1-piperidinocyclohexane-carbonitrile (IV) and phenyl magnesium bromide. The hydrochloride salt of I was prepared using 2-propanol and HCl and was recrystallized from 2-propanol [24].

2.2.2 1-Piperidinotetralycarbonitrile V

1 g (0.0068 mol) 1-tetralone was added to a solution containing 0.582 g (0.0068 mol) of piperidine in 0.253 g HCl (37 %) and 1.36 g cold water. Then, 0.465 g KCN in 1.02 ml water, 50 ml ethanol and 0.1 g tetra-n-buthylammonium bromide (0.0003 mol) were added and the resulting mixture was stirred at ambient temperature (25°C). The progress of the reaction was controlled using TLC (thin layer chromatography) (7:3 ethyl acetate/n-hexane). After one week, no further progress was observed. Thus, the product was extracted with chloroform (75 ml, 3 h). Then, the organic layer was separated, dried and concentrated. An oily residue was obtained, which was passed
through a silica gel column using ethyl acetate-hexane (7:3) as the eluent to produce 1.13 g of V (69 % yield).

IR (KBr): 3066, 2941, 2560, 1454, 1436, 1324, 1287, 1225, 764 cm⁻¹.

¹H NMR (CDCl₃) (ppm): 1.5–2.85 (16H, m), 6.93–7.01 (4H, m).

¹³C NMR (CDCl₃) (ppm): 25.4, 26.2, 26.8, 31, 37.9, 46.7, 52.7, 117.7, 125.3, 128.1, and 139.2.

MS: m/z (relative intensity): 240 [M]⁺ (76), 241 [M+ H]⁺ (15).

2.2.3 1-[1-(3-Methylphenyl) (tetryl)] piperidine III

A solution containing 4 g (0.016 mol) of nitrile compound (V) in 10 ml of dry THF was added to a refluxing solution of (3-methylphenyl) magnesium bromide (Grignard reagent) prepared from 22.65 g 3-bromotoluene and 3.075 g of Mg in 17 ml of dry ether, and the mixture was refluxed for 5 further hours at 65–67 °C and then left overnight at ambient temperature (25 °C). After this period, it was 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. An oily residue was obtained, which was passed through a silica gel column using ethyl acetate/n-hexane (7:3) as the eluent to produce 2.10 g of III (40 % yield).

The hydrochloride salt of III was prepared using 2-propanol and HCl and was recrystallized from 2-propanol.

IR (KBr): 3066, 2941, 1602, 1483, 1454, 1436, 1324, 1287, 1225, 764 cm⁻¹.

¹H NMR (CDCl₃) (ppm): 2.35 (3H, s), 6.88–7.09 (8H, m).

¹³C NMR (CDCl₃) (ppm): 21.2, 26.2, 27.5, 31.8, 44.8, 47.4, 63, 125.4, 125.8, 126.2, 126.7, 128.8, 128.9, 129.1, 138.2, 139.3, 142.8, 142.9.

MS: m/z (relative intensity): 305 [M]⁺ (100), 306 [M+ H]⁺ (12).

2.3 Pharmacological tests

Adult female Wistar rats (Pasteur Institute, Tehran), weighing 250–300 g at the beginning of the experiment were randomly selected and each group of three to four were housed in a cage at a temperature-controlled colony room under light/dark cycles. Animals were given free access to water and standard laboratory rat chow (Pars Company, Tehran, Iran). All behavioral experiments were carried out between 11 a.m. and 4 p.m. under normal room light and temperature (25 °C). All animals were injected by one investigator and evaluated by another one. This study was carried out in accordance with the policies set forth in the Guide for the Care and Use of Laboratory Animals (NIH) and the Research Council of Shahed University of Medical Sciences (Tehran, Iran).

2.3.1 Tail immersion test

Acute thermal pain modeled by the tail immersion test was used [25–27], 2, 5, 10, 15, 20, 25, 30, 35 and 40 min after an intraperitoneal (i.p.) injection of either drug (6 mg/kg [17, 18, 21], and under LD₅₀ limit dosage of the drugs [18]) (I, II, III) or an equivalent volume of saline (control), the rats (n = 7–9 in each group) were housed in an animal restrainer. Then, the last 5 cm of their tails was first submerged in room temperature water (22–24 °C) to check for an aversion to water and afterwards was immersed in 52 °C water. The reaction time between immersing the tail and its removal out of the heated water was measured. A cut-off latency of 20 s was employed to avoid damaging the tail.

2.3.2 Formalin test

The formalin test introduced by Dubuisson and Dennis [28] was used. In this method, a formaldehyde solution (50 μl, 2.5 %) was injected subcutaneously into the plantar surface of the hind paw and then the animal was placed in a plexiglass chamber (30 × 30 × 30 cm³), with a mirror at 45° angle underneath for accurate observation. In the treatment groups, the drugs (ketamine, PCP and PCP-CH₃-tetralyl (III)) were administered intraperitoneally 30 min prior to the formaldehyde injection. Prior to the experiments, all animals were brought to the test chamber 5 h with 5 min intervals in order to adapt them to the testing environment. The behavioral pain reactions due to the 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 15–60 min is the second (II) or chronic phase. However, the chronic phase could be divided into initial (15–40 min and late (40–60 min periods).

Scheme 3: Synthesis of compounds I and III.
3. Results

3.1 Chemistry
Phencyclidine (I) and 1-[1-(3-methylphenyl) (tetracyl) piperidine (III) were synthesized by reaction of substituted Grignard reagents and carbonitrile compounds (IV, V) (Scheme 3). A methyl group was substituted on the aromatic ring of the molecule (III) to obtain more electron distribution and dipole moment’s properties. Common procedures were applied for the synthesis of compounds I and IV with the appropriate modifications described previously [24].

Bromobenzene and its m-methyl (II) derivative were reacted with magnesium to form Grignard reagent, which was then reacted with the appropriate piperidino-cyclohexanecarbonitrile (IV) and piperidinotetralylcarbonitrile (V). The reaction between the Grignard reagent and the carbonitrile was slow and incomplete; to overcome this deficiency, the molar ratio of Grignard reagent to carbonitrile was increased [29].

Spectroscopic data (IR, $^1$H and $^{13}$C NMR, Mass) confirmed the structure of the new compounds III and V. The melting points of known compounds also confirmed their identity. The purity of each compound was checked by TLC using ethyl acetate/n-hexane as the eluent.

3.2 Pharmacology
3.2.1 General consideration
Mortality (number of death), 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, comparison of the motor coordination index (measured by rota-rod apparatus, Harvard, UK) indicated no significant differences between control and treatment rats.

3.2.2 Analgesic activity of PCP (I), ketamine (II) and 1-[1-(3-methylphenyl) (tetracyl) piperidine (III) hydrochlorides with tail immersion and formalin tests
Intraperitoneal injection of PCP (I), ketamine (II) and PCP-CH$_3$-tetralyl (III) hydrochloride (6 mg/kg) (dissolved in saline) generated a significant analgesic effect in the tail immersion test. Comparison of the analgesic effect of these drugs showed a marked anti-nociception 2–25 min after ketamine injection. However, this analgesic effect lasted for 40 min following PCP-CH$_3$-tetralyl application (Fig. 1). The data obtained from the formalin test showed that chronic pain could be significantly attenuated by ketamine, PCP and PCP-CH$_3$-tetralyl (Fig. 2). The difference in the tail immersion latencies and pain scores were evaluated using analysis of variance (ANOVA).

It seems that strong electron donating properties of the methyl group on meta position of the phenyl ring and also incorporation of an extra aromatic and flat phenyl group on the cyclohexane ring (a conjugated cyclic ketone, 1-tetralone) (III) increase the binding tendency to NMDA receptor complex and tail immersion latencies in comparison with ketamine and PCP with higher half-life.

4. Discussion
Phencyclidine (PCP) was originally developed as a human anesthetic, but its utilization in human medicine was discontinued soon afterwards because of its serious psychological side effects [30]. Therefore, it is no more than a pharmacological tool interesting at least through its derivatives or analogues [31]. Electrophysiological and binding studies have revealed that various antagonists of NMDA receptors, including phencyclidine, ketamine and MK-801 bind to PCP-site mainly when the channels are in the open or activated state [18]. Previous studies suggested that ketamine may interact with the

Fig. 1: Mean tail immersion latency (s) in animals receiving ketamine, PCP and PCP-CH$_3$-tetralyl hydrochloride in saline (control). The tail immersion test was conducted 2, 5, 10, 15, 20, 25, 30, 35 and 40 min after the drug injection. Each point represents the mean ± S.E.M. of tail immersion latency (s) in 7–9 animals.
NMDA receptor at two potentially distinct sites: one site located within the channel pore and a second site associated with the hydrophobic domain of the protein. The binding of the agonist to the receptor is assumed to modify the binding of ketamine to both sites [32]. Ketamine is formulated as a highly water soluble hydrochloride salt but under physiological conditions, a large fraction of the drug exists in the lipid-soluble form. Therefore, the concentration of ketamine in the lipid phase is several orders of magnitude greater than that in aqueous phase [33]. Ketamine gains access to a blocker site associated with the lipid membrane of the lipid protein interface. On the other hand, the predominance of closed-channel blockade suggests that ketamine's analgesic properties might result from closed-channel rather than open-channel blockade [33, 34].

Because of stronger analgesic effects of some of the synthesized derivatives of PCP with methyl, methoxy, hydroxyl groups on phenyl and cyclohexane rings [17, 21], higher electron distribution and dipole moments of the methyl group [16] and for decreasing in conversion of conformation isomers of the drug [23], a new derivative of PCP (III) and its carbonitrile intermediate with different substitutions in its phenyl and cyclohexane rings were synthesized and the analgesic effects were studied.

Comparison of the tail immersion and formalin tests data indicates that PCP-CH$_3$-tetranyl diminished thermal but not chemical (formalin) acute pain. It indicates that different mechanisms are involved in the thermal and chemical acute pain which cause different responses. However, the long lasting effect for PCP-CH$_3$-tetranyl in comparison with ketamine and PCP could be related to the longer half-life of III. The analgesic effect of ketamine and PCP-CH$_3$-tetranyl in initial and late phases of formalin chronic pain (phase II) showed that these anti-nociceptive effects occur via the central nervous system (CNS). In this regard, chronic formalin pain (phase II) is reported to be mediated by inflammatory mediators and due to CNS activation [35].

**Acknowledgement**

The authors would like to thank Mrs. Fariba Ansari for her assistance with the pharmacological tests and Dr. Ahmad Jahan Latibiari for his assistance in the English revision of this paper. Karaj Islamic Azad University is acknowledged for its financial support of this work.

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