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# Synthesis and Analgesic Effects of Methoxy-Pyrrole Derivative of Phencyclidine on Mice

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Phencyclidine, 1-[1-phenylcyclohexyl]piperidine (PCP, **I**) and its derivatives have shown considerable pharmacological effects. In this work, pyrrole derivative of phencyclidine (PCP-pyrrole, 1-[1-phenylcyclohexyl]pyrrole, **II**) and a new derivative (1-[1-[3-methoxyphenylcyclohexyl]pyrrole, **III**) and their intermediates were synthesized, then the acute and chronic pains were examined on mices at 1 mg/kg dosage using tail immersion (as a model of acute thermal pain) and formalin (as a model of acute chemical and chronic pain) tests and the results were compared with phencyclidine and control (DMSO) groups. The results indicated that **III** produced more analgesic effects compared to **II** in tail immersion test and also revealed that the newly synthesized derivative (**III**) significantly reduced chronic pain (especially in initial of phase **II**) in formalin test compared to other groups.

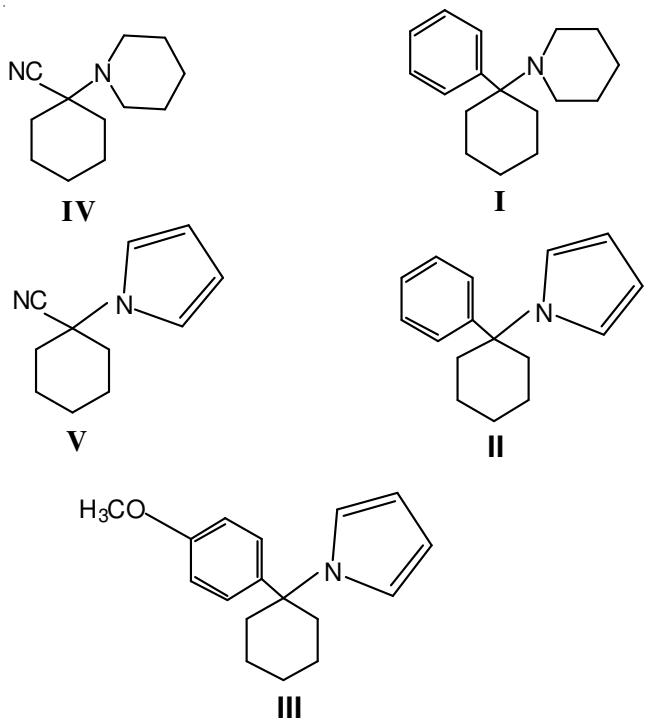
**Key Words: Pyrrole derivatives of phencyclidine, Acute and chronic pains, Tail immersion test, Formalin test.**

## INTRODUCTION

Phencyclidine, 1-[1-phenylcyclohexyl]piperidine (PCP, **I**) (**Scheme-I**) is a synthetic drug that possesses interesting physiological properties. It was initially synthesized in the early 1950s as a potential surgical anaesthetic. Phencyclidine allowed the patient to enter a trance-like state in which the perception of pain could be separated from the sensation, a state that has been termed "dissociative anesthesia"[1].

Phencyclidine and its derivatives performs as non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists[2] that are being investigated as potential therapeutic agents for diseases associated with acute and chronic neuronal excitotoxicity such as focal ischemia, epilepsy and parkinson's disease[3]. They influence the central nervous system and consequently display analgesic, stimulant, depressant and hallucinogenic effects, due to specific binding sites in the brain[4].

Because of pharmacological[5,6] and additional analgesic[7] properties of pyrrole derivatives, a new methoxy analogue (1-[1-[3-methoxyphenylcyclohexyl]pyrrole) (**III**, **Scheme-I**) of **I** and its intermediate (**V**, **Scheme-I**) were synthesized. The analgesic effect of this compound was evaluated on mice using tail immersion (as a model of acute thermal pain) and also formalin (as a model of acute chemical and chronic pain) tests and the results are compared with PCP, PCP-pyrrole (**II**)[7] and control (dimethyl sulfoxide, DMSO) groups.




**Scheme-I:** Structure formulas of PCP (**I**), PCP-pyrrole (**II**), Ome-PCP-pyrrole (**III**) and carbonitrile intermediates **IV** and **V**

As it was revealed in the previous work on this family, the incorporation of methoxy group to the aromatic ring of the molecule will generate pronounced effect on electron distribution and dipole moment because of its high electron donating character[8] and induces higher analgesic effects in the synthesized drugs of this family (**I**)[9,10]. Consequently this group was also added to our new synthesized drug (**III**) to investigate its analgesic performance.

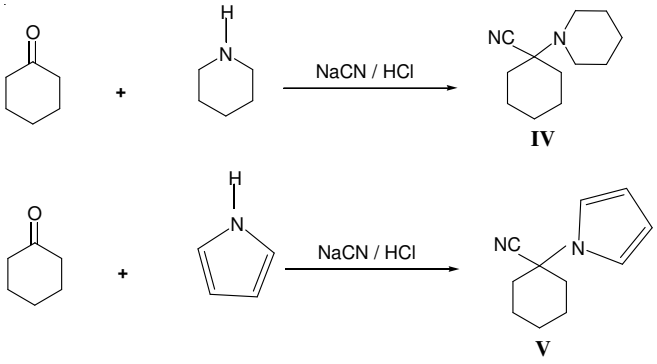
## EXPERIMENTAL

Cyclohexanone, piperidine, pyrrole, bromobenzene, magnesium turnings, diethyl ether, 4-bromoanisole and 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, Electrothermal Engineering Ltd., Essex, UK). $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker 300 MHz (AMX model, Karlsruhe, Germany) spectrometer (internal reference: TMS) and IR spectra was recorded on a Thermo Nicolet FT-IR (Nexus-870 model, 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). NMRI mices (at Pasteur's Institute, Tehran, Iran), weighing 25-30 g were used for pharmacological testing.

### Preparations (Scheme-I-III)

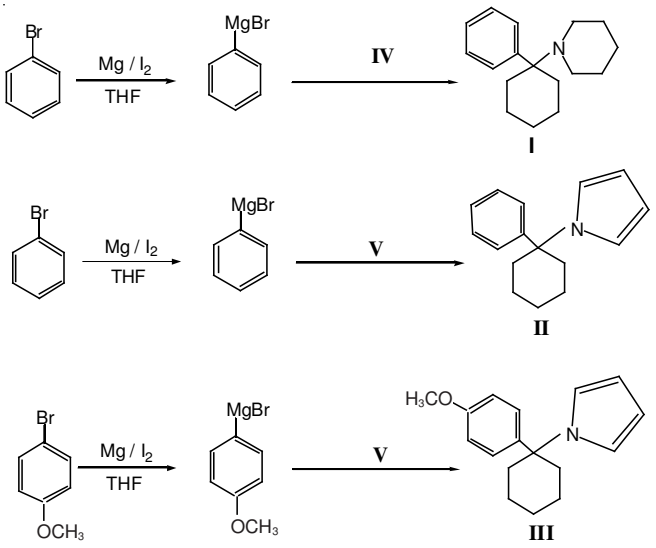
**1-Piperidinocyclohexanecarbonitrile, IV:** This compound was prepared in an organic solvent based on a published method[11] from piperidine, cyclohexanone and KCN with a yield of 77 % (m.p. 113-114 ºC), IR: 2222 $cm^{-1}$.

**1-[1-Phenylcyclohexyl]piperidine (PCP) I:** This compound was prepared in a 58 % yield from 1-piperidinocyclohexanecarbonitrile (**IV**) and phenyl magnesium bromide according to known procedure[12]. The hydrochloride salt of **I** (m.p. 233-234 ºC) was prepared using 2-propanol and HCl and it was recrystallized from 2-propanol.




**Scheme-II:** Synthesis path for synthesizing of intermediates **IV** and **V**

**1-Pyrrolocyclohexylcarbonitrile (V):** This compound was prepared in an organic solvent based on a published method[7] from pyrrole, cyclohexanone and KCN with a yield of 85.5 %.




**Scheme-III:** Synthesis of target compounds **I-III**

**1-[1-Phenylcyclohexyl]pyrrole (PCP-pyrrole) II:** This compound was prepared in a 42.86 % yield from 1-pyrrolocyclohexylcarbonitrile (**V**) and phenyl magnesium bromide according to known procedure[7]. The hydrochloride salt of **I** (m.p. 163-164 ºC) was prepared using 2-propanol and HCl and it was recrystallized from 2-propanol.

**1-[1-[3-Methoxyphenylcyclohexyl]pyrrole (Ome-PCP-pyrrole) III:** A solution containing 3.84 g (0.05 mol) of nitrile (**V**) in a mixture of dry THF and diethyl ether (1:1) was added to a refluxing solution of 3-methoxyphenyl magnesium bromide (Grignard reagent), which was prepared from 9.35 g 3-bromo anisole and 1.22 g of Mg in 40 mL dry ether. It was refluxed for 6 days and was left overnight at ambient temperature (25 ºC), then it was subsequently poured into ice-$NH_4Cl$. The organic layer was separated and washed with water and the base was neutralized with 10 % $H_2SO_4$, washed with 20 % NaOH, re-extracted with *n*-hexane, dried and concentrated. The oily reddish residue obtained, was passed through a silica gel column using ethyl acetate-hexane (4:1) as the eluent to afford 1.46 g of **III** (43 % yield).

The hydrochloride salt of **III** (m.p. 163-164 ºC) was prepared using 2-propanol and HCl and it was recrystallized from 2-propanol. IR (KBr, $\nu_{max}$, $cm^{-1}$): 2925, 1601, 1455, 1267, 1095. $^{1}H$ NMR ($CDCl_3$) (ppm): 1.35-2.35 (10H, m), 3.64 (3H, s), 6.09-6.50 (4H, m), 6.60-7.09 (4H, m). $^{13}C$ NMR ($CDCl_3$) (ppm): 20.9, 27.9, 37.2, 56.2, 58.1, 108.2, 110.9, 111.8, 119.1, 122.3, 128.4, 145.8, 160.3. MS: m/z (regulatory intensity): 255 (11).

**Pharmacological methods:** NMRI mices (Pasteur's Institute, Tehran), weighing 25-30 g at the beginning of the experiment were randomly housed, four per cage in a temperature-controlled colony room under light/dark cycles. Animals were given free access to water and standard laboratory rat chow (supplied by Pars Company, Tehran, Iran). All behavioural experiments were carried out between 11 am and 4 pm under normal room light and at 25 ºC temperature. All animals were injected by one investigator and were 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" (Karaj, Iran).

**Tail immersion test:** The acute thermal pain is modeled by the tail immersion test[13,14]. 20 min after an i.p. injection of drugs (PCP and its analogues, 1 mg/kg, dissolved in 0.2 mL DMSO) or an equivalent volume of DMSO (control), the mices (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 ºC) to check the aversion to water and then immersed in 52 ºC water. The reaction time between immersing the tail and its removal from hot water was measured. Cut-off latency in 15 s was employed to avoid damaging the tail.

**Formalin test:** Formalin test was introduced by Dubuisson and Dennis[15]. In this test, the 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 cm × 30 cm × 30 cm), with a mirror at 45º angle underneath for accurate observation. In the treatment groups, the drugs (PCP and its analogues) were administered intraperitoneally 0.5 h prior to the formaldehyde injection. Prior to the experiments, all animals were brought to the test chamber 5 times at 5 min intervals in order to adapt them to the environment. The behavioural 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 15-60 min is known as the second (II) or chronic phase. However, the chronic phase can be divided into initial (15-40 min) and late (40-60 min) periods.

## RESULTS AND DISCUSSION

**Chemistry:** Phencyclidine (**I**) and the newly synthesized pyrrole derivative (1-[1-[3-methylphenylcyclohexyl]pyrrole, **III**) were synthesized by reaction of substituted Grignard reagent and carbonitrile. To reach higher electron distribution and dipole moments[8], a methoxy group (with high electron donating property) was substituted on the phenyl ring. Also a pyrrole aromatic ring having pharmacological properties[5,6] and analgesic effects[7] was substituted instead of piperidine ring of the molecule. A known procedure was applied for the synthesis of compounds **I**, **II**, **IV** and **V** applying appropriate modifications[7,11,12].

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

### Pharmacology

**General consideration:** Mortality (number of death), morbidity (defined as any abnormal condition or behaviour due to a disorder), irritability (a condition of aggressiveness or increased response on handling) and other related abnormal states were observed in experimental animals. The motor coordination index (measured by Rota-rod apparatus, Harvard, UK) did not indicate any significant differences between control and treated mices.

**Analgesic activity of PCP (I), 1-[1-phenylcyclohexyl] pyrrole (PCP-Pyr, II) and 1-[1-[3-methoxyphenyl cyclohexyl]pyrrole (Ome-PCP-Pyr, III) hydrochloride with tail immersion test:** Intraperitoneally injection of **I-III** and DMSO (control) with the dosage of 1 mg/kg generated analgesic effects in tail immersion test. The results indicated that **III** was effective in tail immersion test (as a model of acute thermal pain) compared to **II** (Fig. 1). The difference in the tail immersion latencies was evaluated using analysis of variance method (ANOVA).

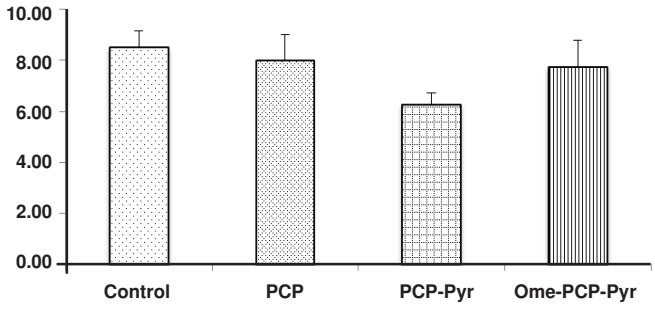



Fig. 1. Mean tail immersion latency (s) in animals receiving PCP (**I**), PCP-pyrrole (**II**), OMe-PCP-pyrrole (**III**) hydrochloride or DMSO (control) in doses of 1 mg/kg. The tail immersion test was conducted 20 min after the drug injection. Each point represents the mean ± SEM of tail immersion latency (s) in 12 animals. ** and $$ $p <$ 0.01 show the difference with control and PCP groups, respectively

**Analgesic activity of PCP (I), 1-[1-phenylcyclohexyl] pyrrole (PCP-Pyr, II) and 1-[1-[3-methoxyphenyl cyclohexyl]pyrrole (Ome-PCP-Pyr, III) hydrochloride with formalin test:** The drugs (**I-III**) and DMSO (control) were administered intraperitoneally with the dosage of 1 mg/kg, 0.5 h prior to formaldehyde injection. The results showed that **III** was not effective for acute chemical pain but significantly reduced chronic pain (especially in initial of phase II) in formalin test compared to other groups (Fig. 2). The difference in the pain scores was evaluated using the analysis of variance method (ANOVA).

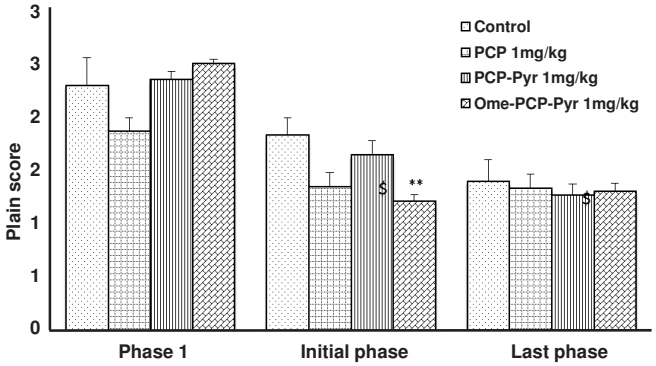



Fig. 2. Comparison between the acute and chronic formalin pains in PCP (**I**), PCP-pyrrole (**II**), OMe-PCP-pyrrole (**III**) hydrochloride (1 mg/kg) and control (DMSO) animal groups. Data show the mean ± SEM of pain score. n = 12, ** $p <$ 0.01 *** $p <$ 0.001 and $ $p <$ 0.05 show the difference with control and PCP groups, respectively

In the recent years, in attempt to reach selective and non-competitive antagonists at the PCP binding site on NMDA

receptor complex, new derivatives of PCP (inserting changes in substitution on the molecule) have been synthesized and their pharmacological performance have been tested[7,9,10,16-22].

In present investigation, new pyrrole derivative of PCP inserting the changes in substitution on its phenyl group and replacing the piperidine with aromatic pyrrole rings were synthesized. As it was indicated in previous work, substitution of methoxy group (high electron donating group with more electron distribution and dipole moment), methoxyphenyl (anisole) instead of phenyl group generates stronger analgesic effects[9,10]. In addition, because of the pharmacological properties of pyrrole derivatives[5,6] such newly developed changes were selected for this family in this work.

The results indicated that substitution of pyrrole instead of piperidine (**II**), decreased the analgesic effects as shown as in previous work[7] because of sharing the non-bonding nitrogen electrons of pyrrole in its aromatic ring as well as decreasing the hydrophilic properties of this atom but strong electron donating properties of the methoxy group on phenyl ring (**III**) could facilitate more binding to NMDA receptor complex and therefore it increase tail immersion latencies and produced more analgesic effects compare to **II**. Although acute chemical pain (in the first phase of formalin test) with a same nervous mechanism[23] and reasons could not attenuate by this new derivative **III** significantly but our results showed that inserting methoxy group on phenyl ring potentiated analgesic effects in the initial phase **II** of chronic pain in formalin test and decreased pain score significantly in comparison with other groups.

## Conclusion

According to the results of this study, it's evident that substitution of pyrrole instead of piperidine in PCP and methoxy group with strong electron donating property on phenyl ring enhances the affinity of this new derivative **III** toward the neural mechanisms that involved in perception of acute thermal and chronic pains in comparison with other groups on mices at 1 mg/kg dosage.

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