Synthesis and analgesic effects of new pyrrole derivatives of phencyclidine in mice

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Abstract
Phencyclidine (1-(1-phenylcyclohexyl)piperidine, CAS 956-90-1, PCP, I) and many of its analogues have shown some pharmacological effects. In this study, new pyrrole derivatives of I (1-(1-phenylcyclohexyl)pyrrole, II and 1-(1-(4-methylphenyl)cyclohexyl)pyrrole, III) and their intermediates were synthesized and the acute and chronic pains were examined on mice 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 were compared with the PCP and control groups.

The results indicated that III generated higher analgesic effects in the tail immersion test compared to the PCP and control (dimethyl sulfoxide, DMSO) groups, demonstrating a marked and significant increase in tail immersion latency, but this effect was not observed for II in the dose of 1 mg/kg. The formalin test showed that III was effective in acute chemical pain (phase I, 0–5 min after injection), but was not effective for II at the same dosage compared to the PCP and control groups. Also chronic pain will be significantly attenuated by III but II was not effective as compared to the other groups.

It is concluded that substitution of the aromatic pyrrole ring instead of piperidine in the PCP molecule will not be effective alone in tail immersion and formalin tests but the addition of a methyl group (with high electron donating and dipole moments) on the phenyl group plus substitution of the aromatic pyrrole ring can be effective in acute and chronic pain compared to the PCP and control groups.

1. Introduction
Phencyclidine (1-(1-phenylcyclohexyl)piperidine, PCP, I, Scheme 1) was originally introduced as a general anesthetic drug, but it was subsequently withdrawn from prescription for humans due to generating severe psychomimetic side effects [1]. Consequently, the focus of research on PCP has shifted from its application as an anesthetic compound toward potential applications as a neuroporpharmaceutical [2]. Furthermore, the search on noncompetitive N-methyl-d-aspartate (NMDA) receptor antagonists was substantially assisted through the development of a reliable binding assay and to date a considerable number of PCP derivatives have been synthesized and assayed as substrates for the PCP binding site [3, 4].

Because of pharmacological properties of pyrrole derivatives [10, 11] and to investigate the substitution of aromatic-nitrogen ring instead of non-aromatic-nitrogen ring (piperidine), new pyrrole derivatives (1-(1-phenylcyclohexyl)pyrrole, II and 1-(1-(4-methylphenyl)cyclohexyl)pyrrole, III, Scheme 1) of I and its intermediates (IV and V, Scheme 1) with modification on phenyl and piperidine rings were synthesized. The analgesic effects of the compounds were evaluated on mice using the tail immersion test (as a model of acute thermal pain) and the formalin test (as a model of acute chemical and chronic pain) and the results were compared with the PCP and control (dimethyl sulfoxide, DMSO) groups.

It has been observed from our previous work [5–8] that substituting a methyl group (with high electron distribution and dipole moments [9]) on the phenyl ring can impart higher analgesic effects in the synthesized drugs. Therefore this group was also added to our new and initially synthesized drug (I) to produce a second compound (III).
2. Materials and methods

2.1 General

All chemicals; cyclohexanone, piperidine, pyrrole, bromobenzene, magnesium turnings, diethyl ether, 4-bromotoluene 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). 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, DE, USA). Column chromatographic separations were performed over Acros silica gel (No.7631-86-9 particle size 35–70 micrometer, Geel, Belgium). NMRI mice (at Pasteur’s Institute, Tehran, Iran), weighing 25–30 g, were used for pharmacological testing.

2.2 Preparations (Scheme 1–3)

2.2.1 1-piperidinocyclohexanecarbonitrile, IV

This compound was prepared in an organic solvent based on a published method [12] from piperidine, cyclohexanone and KCN with a yield of 77 % (m.p.: 113–114 °C), IR: 2222 cm⁻¹, C=C, str.

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

This compound was prepared in a 58% yield from 1-piperidinocyclohexanecarbonitrile (IV) and phenyl magnesium bromide according to a known procedure [13]. The hydrochloride salt of I (m.p. 233–234 °C) was prepared using 2-propanol and HCl and it was recrystallized from 2-propanol.

2.2.2 1-Pyrrolocyclohexylcarbonitrile V

A mixture containing 35 g, 39 % NaHSO₃ and 10.6 g (0.12 mol) cyclohexanone was stirred in an ice-bath and a stirred mixture of 7.86 g (0.12 mol) KCN and 7.38 g (0.11 mol) pyrrole was added to it, then stirring was continued for 264 h. The organic layer was extracted with diethyl ether and washed with water, 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 17.47 g of V (85.5% yield).

IR: 2864, 2238, 1532, 1454, 1095, 735 cm⁻¹.

1H NMR (CDCl₃) (ppm): 1.42–2.19 (10H, m), 5.87–6.50 (4H, m).

13C NMR (CDCl₃) (ppm): 19.7, 26.4, 30.8, 47.6, 108.5, 121.3, 122.8.

MS: m/z (regulatory intensity): 174 (34).

2.2.1 1-(1-Phenylcyclohexyl)pyrrole (PCP-pyrrole) II

A solution containing 3.84 g (0.02 mol) of nitrite compound (V) in the mixture of dry THF and diethyl ether (1:1) was added to a refluxing solution of phenyl magnesium bromide (Grignard reagent), which was prepared from 7.85 g bromo benzene and 1.22 g of Mg in 20 ml dry ether. It was refluxed for 7 additional hours and was left overnight at ambient temperature (25 °C) and then 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, and 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).

Scheme 1: Structure formulas of PCP (I), PCP-Pyrrole (II), Me-PCP-Pyrrole (III) and Carbonitrile intermediates IV and V.

Scheme 2: Synthesis of intermediates IV and V.

Scheme 3: Synthesis of target compounds I–III.
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): 2928, 2962, 2856, 1448, 1261, 1095 cm⁻¹.

¹H NMR (CDCl₃) (ppm): 1.42–2.19 (10H, m), 5.98–6.55 (4H, m), 7.05–7.18 (5H, m).

¹³C NMR (CDCl₃) (ppm): 20.4, 27.9, 37.2, 57.4, 108.2, 121.7, 125.6, 126.8, 128.3, 144.8.

MS: m/z (regulatory intensity): 225 (22).

2.2.3 1-[1-(4-Methylphenyl)(cyclohexyl)]pyrrole (Me-PCP-pyryl) III

A solution containing 3.84 g (0.05 mol) of nitrile compound (V) in the mixture of dry THF and diethyl ether (1:1) was added to a refluxing solution of 4-methylphenyl magnesium bromide (Grignard reagent), which was prepared from 8.55 g 4-bromo toluene and 1.22 g of Mg in 40 ml dry ether. It was refluxed for additional 36 h and was left overnight at ambient temperature (25 °C) and then it was poured into ice-NH₂Cl. 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, and 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 III (42.86% yield).

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

IR (KBr): 2856, 1606, 1447, 1260, 1095 cm⁻¹.

¹H NMR (CDCl₃) (ppm): 11.42–2.19 (10H, m), 2.34 (3H, s), 5.98–6.55 (4H, m), 6.95–7.05 (4H, m).

¹³C NMR (CDCl₃) (ppm): 20.4, 20.9, 27.9, 37.2, 57.4, 108.2, 121.7, 126.7, 128.7, 134.7, 141.9.

MS: m/z (regulatory intensity): 239 (35).

2.3 Pharmacological methods

NMRI mice (from 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 allowed free access to water and standard laboratory rat chow (supplied by Pars Company, Tehran, Iran). All behavioral experiments were carried out between 11 a.m. and 4 p.m. under normal room light and at 25 °C 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” (Tehran, Iran).

2.3.1 Tail immersion test

The acute thermal pain is modeled by the tail immersion test [14, 15]. Twenty minutes 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 mice (n = 12 in each group) were kept in an animal restrainer. Then, the terminal 5 cm of their tails were first submersed 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. A cut-off latency of 15 s was employed to avoid damaging the tail.

2.3.2 Formalin test

The formalin test was introduced by Dubuisson and Dennis [16]. 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 × 30 × 30 cm³) with a mirror at 45° angle underneath for accurate observation. In the treatment groups, the drugs (PCP and its analogues) 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 in order for them to be adapted 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 15–60 min is known as the second (II) or chronic phase. The chronic phase is further divided into initial (15–40 min) and late (40–60 min) periods.

3. Results

3.1 Chemistry

Phencyclidine (I) and newly synthesized pyrrole derivatives (1-[1-(phenylcyclohexyl)]pyrrole, II and 1-[1-(4-methylphenyl)(cyclohexyl)]pyrrole, III) were synthesized by reaction of substituted Grignard reagent and carbonitrile compounds. To reach more electron distribution and dipole moments, a methyl group (with high electron donating property) was substituted on the phenyl ring. Also a pyrrole aromatic ring was substituted instead of piperidine ring to investigate the substitution of aromatic-nitrogen ring instead of non-aromatic-nitrogen ring (piperidine) as well as its pharmacological properties [10, 11]. A known procedure was applied for the synthesis of compounds I and IV with the appropriate modifications [12, 13].

Spectroscopic data (IR, ¹H and ¹³C NMR, Mass) confirmed the structure of the newly synthesized compounds (II, III and V). 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.

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. 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 The analgesic activity of PCP (I), 1-(1-phenyl-cyclohexyl)pyrrole (PCP-Pyr, II) and 1-[1-(4-methyl-phenyl)(cyclohexyl)]pyrrole (Me-PCP-Pyr, III) hydrochloride with the tail immersion test

Intraperitoneal injection of compounds I, II, III and DMSO (control) with the dose of 1 mg/kg generated an-
3.2.3 The analgesic activity of PCP (I), 1-(1-phenylcyclohexyl)pyrrole (PCP-Pyr, II) and 1-(1-(4-methylphenyl)cyclohexyl)pyrrole (Me-PCP-Pyr, III) hydrochloride with the formalin test

The drugs (I–III) and DMSO (control) were administered intraperitoneally with the dose of 1 mg/kg, 30 min prior to formaldehyde injection. The results showed that II was not effective against acute chemical and chronic pain but these kinds of pain could be significantly attenuated by III compared with the PCP and DMSO (control) groups at a similar dose (Fig. 2) as explained for the tail immersion test results. The difference in the pain scores was evaluated using the analysis of variance method (ANOVA).

4. Discussion

PCP and its derivatives influence the central nervous system and consequently display analgesic, stimulant, depressant and hallucinogenic effects, due to specific binding sites in the brain [17]. Knowing its non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist action, PCP has been demonstrated to produce psychotomimetic effects in humans, so it is a widely abused drug [18, 19].

In recent years, to obtain selective, non-competitive antagonists at the PCP binding site on the NMDA receptor complex, new derivatives of this compound (inserting changes in substitution on the molecule) have been synthesized and their pharmacological activities have been tested [5–8, 18–20].

In the present study, two new pyrrole derivatives of PCP with changes in substitution on PCP’s phenyl group and replacing the piperidine with aromatic pyrrole rings were synthesized. As indicated in our previous work on substitution of the methyl group (high electron donating group with more electron distribution and dipole moment), methylphenyl (tolyl) instead of phenyl group generates stronger analgesic effects [7, 8]. In addition because of the existence of pharmacological properties of pyrrole derivatives [10, 11] and investigation on substituting aromatic pyrrole instead of nonaromatic piperidine group, such newly developed changes were selected in our work. The results indicated that substitution of pyrrole instead of piperidine decreased the analgesic effects of this new drug (II) due to sharing of the non-bonding nitrogen electrons of pyrrole in the aromatic ring as well as decreasing the hydrophilic properties of this atom and drug (II). In such condition, the non-bonding nitrogen electrons of

Fig. 1: Mean tail immersion latency (s) in animals receiving PCP (I), PCP-pyrrole (II), Me-PCP-pyrrole (III) hydrochloride or DMSO (control) in doses of 1 mg/kg. The tail immersion test was conducted 20 min after drug injection. Each point represents the mean ± S.E.M. of tail immersion latency (s) in 12 animals. ***p < 0.001 when compared with the control and ****p < 0.001 when compared with the PCP group.

Fig. 2: Comparison between acute and chronic formalin pain in PCP (I), PCP-pyrrole (II), Me-PCP-pyrrole (III) hydrochloride (1 mg/kg) and control (DMSO) animal groups. Data show the mean ± S.E.M of pain score. n = 12, *p < 0.05, ***p < 0.001 when compared with the control and †p < 0.05 when compared with the PCP group.
piperidine are free and this atom will contribute to the hydrophilic properties more than pyrrole. However, the strong electron donating properties of the methyl group on para position of the phenyl ring (III) facilitate binding to the NMDA receptor complex and could increase tail immersion latencies. Acute chemical and chronic pain could also be significantly attenuated by this compound in comparison with the other groups, as anticipated in our previous work [7, 8].

5. Conclusion

Similar to our previous findings on the analgesic effects of this family of compounds, substitution of the methyl group with high electron donating and dipole moment can be influential in tail immersion and formalin tests with aromatic or non-aromatic nitrogen ring. However, substituting aromatic pyrrole instead of non-aromatic piperidine alone could not relief pain in tail immersion and formalin tests compared to PCP and control groups in mice at 1 mg/kg dose.

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Conflict of Interest

This research is not a part of our normal lecturing, employment, consultation, and involvement; and no institution will require any rights from this work. In addition, no patent has been applied nor any commercial right has been given to any company and/or institution, nor is it envisaged.

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