

Synthesis and Study the Analgesic Effects of New Analogues of Ketamine on Female Wistar Rats

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Abstract: Ketamine (2-o-chlorophenyl-2-methylaminocyclohexan, CAS 1867-66-9, CI-581, Ketalar, **I**), a potent derivative of Phencyclidine (1-[1-phenylcyclohexyl] piperidine, CAS 956-90-1, PCP, **II**), and many of its analogues have shown anesthetic and analgesic effects. In this research, new derivatives of **I**, (2-[*p*-methoxybenzylamino]-2-[*p*-methoxyphenyl] cyclohexanone, ket-OCH₃, **III**), (2-[*p*-methylbenzylamino]-2-[*p*-methoxyphenyl] cyclohexanone, ket-CH₃, **IV**) and their intermediates (**V-VIII**) were synthesized and the acute and chronic pains of **III** and **IV** were evaluated 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 were compared with ketamine and control (saline) groups. The results indicated that in tail immersion and formalin tests, these new derivatives (**III** and **IV**) were usually effective for decreasing pain on rats.

Keywords: Acute and chronic pains, analgesic effects, CAS 1867-66-9, CAS 956-90-1, formalin test, new derivatives of ketamine, tail immersion test.

INTRODUCTION

Ketamine (2-o-chlorophenyl-2-methylaminocyclohexan, CAS 1867-66-9, CI-581, Ketalar, **I**, Scheme 1) was originally synthesized and developed by Park-Davis Company in 1962. It is a Phencyclidine (1-[1-phenylcyclohexyl] piperidine, CAS 956-90-1, PCP, **II**, Scheme 1), derivative that produces anesthesia and analgesia similar to PCP, but generates a shorter duration of action and less propensity to produce convulsions [1-3]. A dissociative anesthetic with complex actions on central nervous system (CNS), ketamine acts as a non-competitive antagonist of glutamate N-methyl-D-aspartate (NMDA) receptor. It also shows other complex effects on the CNS [4]. Ketamine interacts with multiple binding sites (NMDA and non-NMDA glutamate receptors, nicotinic and muscarinic cholinergic receptors, adrenergic and opioid receptors), however, it is generally believed that NMDA receptor antagonism accounts mostly for its anesthetic and partly for analgesic effects, whereas some of its analgesic effects are mediated through its agonistic effects at opioid receptors within the central nervous system [5]. So far, some of the derivatives of ketamine have been synthesized [6-10] and the pharmacological activities, such as respiratory depression and antinociception [5], acute and chronic pain [11] analgesic effects [12-18] have been studied.

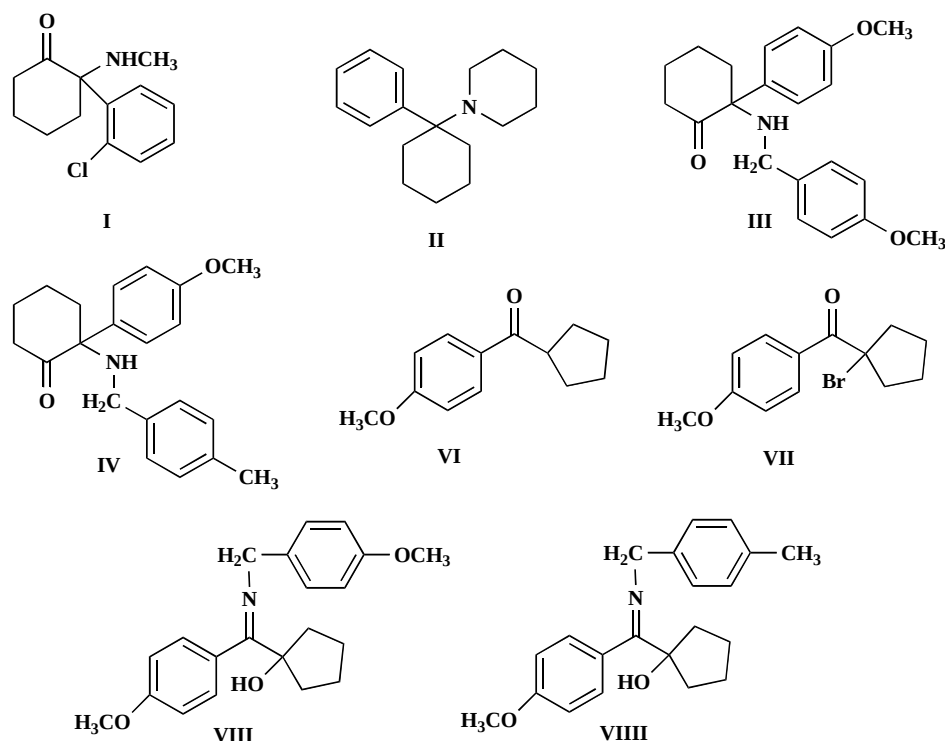
In this work, two new ketamine derivatives (**III** and **IV**) were synthesized and the analgesic effects of these compounds were evaluated on rats using tail immersion (as a model of acute thermal pain) and formalin (as a model of acute chemical and chronic pain) tests whereas the results were compared with ketamine (**I**) and control (saline) groups.

MATERIAL AND METHODS

General

All chemicals, including cyclopentyl bromide, 4-methoxy benzonitrile, bromine, 4-methyl benzylamine, 4-methoxy benzylamine, magnesium turnings and diethyl ether 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). IR spectra were recorded on a Thermo Nicolet FT-IR (Nexus-870 model, Nicolet Instrument Corp, Madison, Wisconsin, U.S.A.) 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). Female Wistar rats (at Pasteur's Institute, Tehran, Iran), ranging from 175g to 230g of weight, were used for pharmacological testing.

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Scheme 1. Structure formulas of Ketamine (**I**), Phencyclidine (**II**), Ket-OCH₃ (**III**), Ket-CH₃ (**IV**), *p*-methoxyphenylcyclopentyl ketone (**VI**), 1-Bromocyclopentyl-(*p*-methoxyphenyl) ketone (**VII**), 1-(α -*p*-methoxybenzylimino) (*p*-methoxybenzyl) cyclopentanol (**VIII**) and 1-(α -*p*-methoxybenzylimino) (*p*-methoxybenzyl) cyclopentanol (**VIII**).

Preparations (Scheme 2)

Cyclopentyl magnesium bromide, V

This compound was prepared from Cyclopentyl bromide and magnesium in diethyl ether following a published method [19].

p-methoxyphenylcyclopentyl ketone (VI)

This compound was prepared at 64% yield from cyclopentyl magnesium bromide (V) and *p*-methoxybenzonitrile according following a known procedure [19].

1-Bromocyclopentyl-(*p*-methoxyphenyl) ketone (VII)

This compound was prepared at 58% yield from VI and bromine in carbon tetrachloride at 0°C following a published method [20].

1-(α -*p*-methoxybenzylimino) (*p*-methoxybenzyl) cyclopentanol (VIII)

A solution containing 8.2g of bromo ketone (**VII**) in 30 ml benzene and 20g (0.15 mol) of *p*-methoxybenzylamine was stirred in room temperature for 5 days. Then, *n*-pentane was added and the reaction mixture was filtered, evaporated and concentrated. The obtained brown oily residue was passed through a silica gel column using ethyl acetate-hexane as the eluent to afford 6g of **VIII** (57 % yield).

IR (KBr): 3430, 2933, 1708, 1608, 1512, 1437, 1248, 1032 cm^{-1} . ^1H N.M.R. (CDCl_3) (*p.p.m.*): 0.92-1.48 (8H, m), 2.02 (1H, m), 3.76 (6H, s), 4.46 (2H, s), 6.52-7.75 (8H, m). ^{13}C N.M.R. (CDCl_3) (*p.p.m.*): 23.1, 37.7, 56.2, 59.4, 79.8,

114.8, 131.1, 131.3, 132.9, 158.8, 163.2, 164.4. *MS*: *m/z* (regulatory intensity): 339 (11).

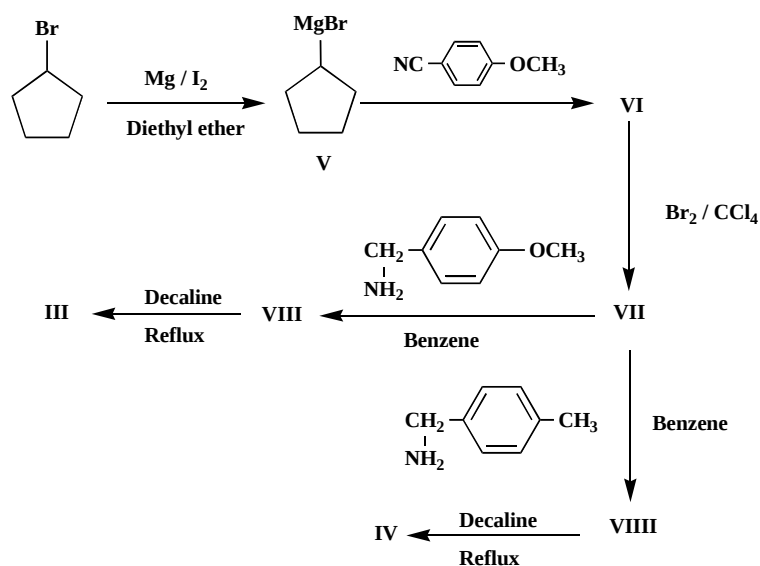
1-(α -*p*-methylbenzylimino) (*p*-methoxyphenyl) cyclopentanol (VIII)

A solution containing 8.2 g of bromo ketone (**VII**) in 50ml of benzene and 20g (0.16 mol) of *p*-methyl benzylamine was stirred in room temperature for 7 days. Then, *n*-pentane was added and the reaction mixture was filtered, evaporated and concentrated. The obtained brown oily residue was passed through a silica gel column using ethyl acetate-hexane as the eluent to afford 4.97g of **VIII** (41 % yield).

IR (KBr): 3380, 3329, 2873, 1619, 1563, 1439, 1333, 1021, 812 cm^{-1} . ^1H N.M.R. (CDCl_3) (*p.p.m.*): 0.94-1.34 (8H, m), 2.02 (1H, m), 2.32 (3H, s), 4.49 (2H, s), 6.92-7.75 (8H, m). ^{13}C N.M.R. (CDCl_3) (*p.p.m.*): 23.1, 24.8, 37.7, 56.2, 59.4, 79.8, 114.8, 129.2, 131.3, 132.9, 133.9, 136.2, 163.2, 164.4. *MS*: *m/z* (regulatory intensity): 323 (11).

2-(*p*-methoxybenzylamino)-2-(*p*-methoxyphenyl) cyclohexanone (Ket-OCH₃) (III)

A solution containing 6 g of **VIII** in 30 ml of decaline refluxed for 4 hours in 190°C. Then the reaction mixture was extracted with diethyl ether. The organic layer was separated and water-washed and the base was neutralized with 10% H₂SO₄, washed with 20% NaOH, re-extracted with *n*-Hexane, dried and concentrated. The obtained oily residue was passed through a silica gel column using ethyl acetate-hexane as the eluent to obtain 3.25g of **III** (54 % yield). The



Scheme 2. Synthesis of intermediates (V-VIII) and final compounds (III and IV).

hydrochloride salt of **III** (m.p.133-136°C, red brownish solid) was prepared using 2-propanol and HCl, and recrystallized from 2-propanol.

IR (KBr): 3383, 3324, 2920, 1615, 1515, 1475, 1382, 1072, 801 cm^{-1} .

^1H N.M.R. (CDCl_3) (p.p.m.): 0.89-1.30 (8H, m), 2.01 (1H, m), 3.79 (6H, s), 3.90 (2H, s), 6.90-7.63 (8H, m). ^{13}C N.M.R. (CDCl_3) (p.p.m.): 23.1, 25.03, 35.82, 37.54, 55.6, 64.8, 77.7, 114.3, 128.8, 132.1, 158.7, 161.4, 207.4. MS: m/z (regulatory intensity): 339 (12).

2-(p-methylbenzylamino)-2-(p-methoxyphenyl) cyclohexanone (KET- CH_3) (IV)

A solution containing 5g of **VIII** in 30ml of decaline was refluxed for 5 hours in 190°C. Then the reaction mixture was extracted with diethyl ether. The organic layer was separated, water-washed and the base was neutralized with 10% H_2SO_4 , washed with 20% NaOH, re-extracted with n-Hexane, and it was dried and concentrated. The oily residue obtained, was passed through a silica gel column using ethyl acetate-hexane as the eluent to afford 1.5g of **IV** (30 % yield).

The hydrochloride salt of **IV** (m.p. 129-130°C, black solid) was prepared using 2-propanol and HCl and it was recrystallized from 2-propanol.

IR (KBr): 3418, 2922, 1687, 1603, 1513, 1450, 1257, 1021 cm^{-1} .

^1H N.M.R. (CDCl_3) (p.p.m.): 0.86-1.96 (8H, m), 2.03 (1H, m), 2.30 (3H, s), 3.79 (3H, s), 3.84 (2H, s), 6.92-7.58 (8H, m). ^{13}C N.M.R. (CDCl_3) (p.p.m.): 19.7, 25.2, 26.7, 32.7, 37.1, 48.9, 55.7, 76.6, 114.7, 129.2, 129.7, 133.9, 162.8, 208.4. MS: m/z (regulatory intensity): 323 (12).

Pharmacological Methods

Female Wistar rats (Pasteur's Institute, Tehran, Iran), weighing 175-230g, were randomly housed in a group of four per cage in a temperature-controlled colony room under

light/dark cycles. Rats were given 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. All the animals were injected by a researcher, and evaluated by another. This study was carried out according to the Guides for the "Care and Use of Laboratory Animals" (NIH) and those of the "Research Council of Shahed University of Medical Sciences, Tehran, Iran".

Tail Immersion Test

The acute thermal pain was modeled by the tail immersion test [21, 22]. Twenty minutes after an intraperitoneal injection of drugs (ketamine and its analogues, 6mg/kg) or an equivalent volume of saline (control), the rats (n = 8 in each group) were housed in an animal restrainer. Then, the terminal 5cm of their tails was first submerged into room temperature water (22~24 °C) to check their 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 15s was employed to avoid damaging the tail.

Formalin Test

The Formalin test was introduced by Dubuissou and Dennis [23]. 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 cm^3) mirrored at 45° angle underneath for accurate observation. In the treatment groups, the drugs (ketamine and its analogues) were administered intraperitoneally 30 minutes prior to the formaldehyde injection. Prior to the experiments, all animals were brought to the test chamber five times at five-minute-intervals in order to adapt them to the environment. The behavioral pain reactions due to formalin injection were detected and recorded for 1 hour. The first 15 minutes after formalin injection was known as the early (I) or acute phase and the period between 15-60

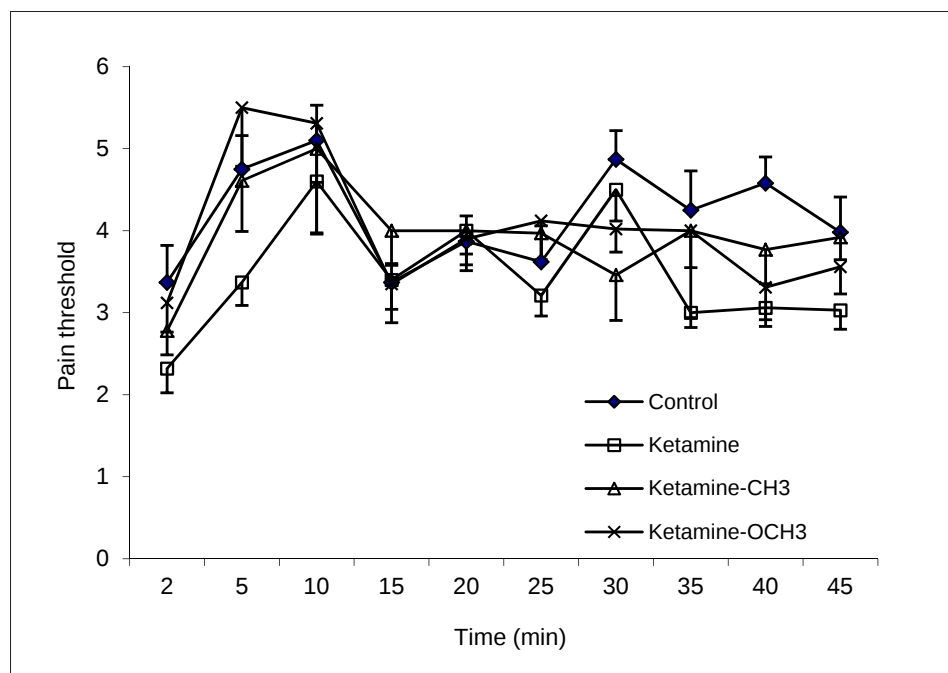


Fig. (1). Mean tail immersion latency (s) in animals receiving Ketamine (I), Ket-OCH₃ (III), Ket-CH₃ (IV) hydrochloride or saline (control) in doses of 6 mg/kg. The tail immersion test was conducted 20 minutes after the drug injection. Each point represents the mean $\pm$ S.E.M. of tail immersion latency (s) in 8 animals.

minutes known as the second (II) or chronic phase. However, the chronic phase could be divided into initial (15-40 min.) and late (40-60 min.) periods.

RESULTS

Chemistry

Ketamine (I) and its newly synthesized derivatives (III and IV) were synthesized by reaction of cyclopentyl magnesium bromide (Grignard reagent, V) with substituted benzonitriles (chlorine, *p*-methoxy and *p*-methyl) compounds. To reach more electron distribution and dipole moments, a methoxy group was substituted in the phenyl ring [24]. Additionally, benzylamines with electron donating groups [24] (III and IV) were caused that the nonbonding nitrogen electrons of the amine group would be more active comparing to methylamine in ketamine. The known procedures were applied to synthesize the compounds V-VII [19, 20]. Spectroscopic data (IR, ¹H and ¹³C NMR, Mass) confirmed the structure of the newly synthesized compounds (III, IV, VIII, and VIII). The purity of each compound was checked by TLC using ethyl acetate-hexane as the eluent.

Pharmacology

General Consideration

Mortality (number of death), morbidity (an 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 the experimental animals. The motor coordination index (measured by Rota-rod apparatus, Harvard, UK) did not indicate any significant differences between control and treated rats.

The analgesic activity of ketamine (I), 2-(p-methoxybenzyl-amino)-2-(p-methoxyphenyl) cyclohexanone (ket-OCH₃, III) hydrochloride with Tail Immersion Test

Intraperitoneal injection of ketamine (I) and 2-(p-methoxybenzylamino)-2-(p-methoxyphenyl) cyclohexanone (ket-OCH₃, III) hydrochloride in dose of 6mg/kg generated analgesic effects in tail immersion test (as a model of acute thermal pain). As indicated in Fig. (1), both compounds (ketamine and its newly synthesized derivative) could produce apparently identical analgesic effects in tail immersion test, comparing to control group, especially in 5-25 minutes after the drugs injection. However, a mild but non-significant difference between analgesic effects of I and III in 5-10 and 25-45 minutes after the drugs application was observed. The difference in the tail immersion latencies was evaluated using method of analysis of variances (ANOVA).

The analgesic activity of ketamine (I), 2-(p-methylbenzyl-amino)-2-(p-methoxyphenyl) cyclohexanone (ket-CH₃, IV) hydrochloride with Tail Immersion Test

Intraperitoneal injection of ketamine (I) and 2-(p-methylbenzylamino)-2-(p-methoxyphenyl) cyclohexanone (ket-CH₃, IV) hydrochloride in dose of 6mg/kg generated analgesic effects in tail immersion test (as a model of acute thermal pain). As indicated in Fig. (1), both compounds (I and IV) could produce apparently identical analgesic effects in tail immersion test, comparing to control group, especially in 15-25 minutes after the drugs injection. However, a mild but non-significant difference between analgesic effects of these compounds (I and IV) in 25-45 minutes after the drugs application was observed. The difference in the tail immersion latencies was evaluated using method of analysis of variances (ANOVA).

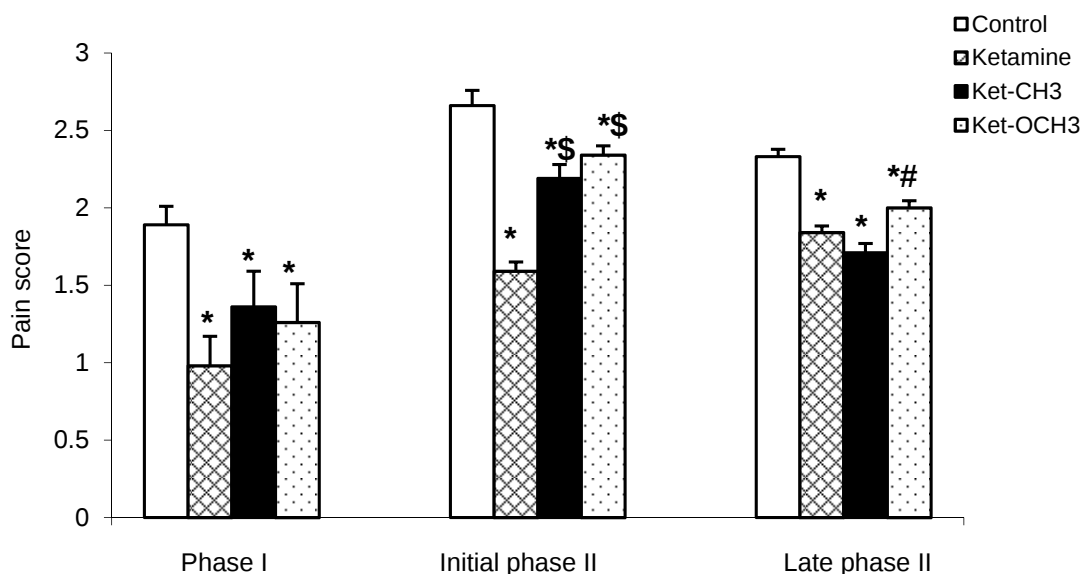


Fig. (2). Comparison between the acute and chronic formalin pains in Ketamine (I), Ket-OCH₃ (III), Ket-CH₃ (IV) hydrochloride or saline (control) in doses of 6 mg/kg. Data show the mean $\pm$ S.E.M of pain score. $n=8$, * $p<0.05$, *** $p<0.001$ when compared with control and \$ $p<0.05$ when compared with Ketamine group.

The analgesic activity of ketamine (I) and 2-(*p*-methoxybenzylamino)-2-(*p*-methoxyphenyl) cyclohexanone (ket-OCH₃, III) hydrochloride with Formalin Test

The drugs (I and III) were intraperitoneally injected in dose of 6 mg/kg, 30 minutes before the formaldehyde injection. Results showed that both compounds (I and III) have significant and similar effects on acute formalin chemical pain (as a model of acute chemical and chronic pain) (Fig. 2) comparing to control group. However, chronic formalin pain (phase I and II) could significantly attenuate with these compounds (I and III), comparing to control group. The difference in the pain scores was evaluated using method of analysis of variances (ANOVA).

The analgesic activity of ketamine (I) and 2-(*p*-methylbenzylamino)-2-(*p*-methoxyphenyl) cyclohexanone (ket-CH₃, IV) hydrochloride with Formalin Test

The drugs (I and IV) were intraperitoneally injected in dose of 6mg/kg, 30 minutes before the formaldehyde injection. Results showed that both compounds (I and IV) have a significant and similar effects on acute formalin chemical pain (as a model of acute and chronic chemical pain) (Fig. 2) comparing to control group. However, chronic formalin pain (phase I and II) could be significantly attenuate with these compounds (I and IV), comparing to control group. The difference in the pain scores was evaluated using method of analysis of variances (ANOVA).

DISCUSSION

The analgesic effect of ketamine was first described by Domino and collaborators [1]. This compound in low sub-anesthetic dosage was considered a reliable analgesic in acute pain [21] acting more selectively as a non-competitive blocker of the NMDA receptor to the PCP recognition site [25]. The analgesic effect of ketamine occurs concentrated within its PCP site occupancy range [26], but at higher concentration, ketamine interacts with sigma sites and opioid,

kappa and delta receptors [27]. In this research, two new ketamine derivatives were synthesized with substitution changes in phenyl (*p*-methoxyphenyl), amine (*p*-methoxybenzylamin, III and *p*-methylbenzylamin, IV) groups. Stated in our previous work, substituting methyl and methoxy groups (high electron donating groups with more electron distributions and dipole moments) in PCP (a well-known ketamine derivative) generated stronger analgesic effects [24, 28]. To have some pharmacological effects of benzylamine derivatives, such newly developed changes were selected in our recent work. Results indicated that in tail immersion test, these new derivatives (III and IV) could be effective more frequently in decreasing pain, comparing to control group. Also lower anti-nociception effects were observed in tail immersion test in 5, 10 and 25 minutes for III; 15, 20 and 25 minutes for IV; 2, 5, 10, 25 and 35-45 minutes for ketamine after injection, comparing to control group. In formalin test, the results showed that the new derivatives (III and IV) and ketamine (I) exhibited significant but apparently identical effects on acute chemical pain, comparing to control group. However, chronic pain (phase I and II) could be significantly attenuated with new compounds (III and IV), comparing to control group.

It was concluded that such changes on ketamine structure might decrease their half-life by higher conjugations in liver or excretions in kidney. The structural disturbance due to these changes might be considered as another cause for their semi-agonistic effects on ketamine receptors. Furthermore, as ketamine mainly produces analgesic effects through its receptors in central nervous system (CNS), it might be concluded that the newly synthesized compounds could not easily be transferred from blood brain barriers as efficiently as ketamine.

CONCLUSION

Introducing a methoxy group with a high electron donation and dipole moment to phenyl ring, and swapping methy-

ketamine with methyl or methoxybenzylamines could generate analgesic effects in tail immersion and formalin tests comparing to a control group of rats at 6 mg/kg dosage.

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