Send Orders for Reprints to reprints@benthamscience.net

Mini-Reviews in Medicinal Chemistry, **2014***, 14,* **000-000**

Synthesis and Pain Perception of New Analogues of Phencyclidine in NMRI Male Mice

Abbas Ahmadi[*a], Mohsen Khalili[b], Sara Marami[a], Afsane Ghadiri[a] and Babak Nahri-Niknafs[a]

[a]*Department of Chemistry, Faculty of Science, Islamic Azad University, Karaj Branch, Karaj, Iran;* [b]*Neurophysiology Research Center, Shahed University, Tehran, Iran*

Abstract: Phencyclidine (PCP, **I**) and many of its derivatives have demonstrated many pharmacological effects. They interact with a number of neurotransmitter systems within the central nervous system. For example, Phencyclidine is a noncompetitive antagonist of the N-methyl-d-aspartate (NMDA) subtype of the glutamate receptor, and it causes the release and inhibits the reuptake of monoaminergic neurotransmitters, including dopamine, serotonin and norepinephrine. In this study, new thienyl (TCP, **II**), as well as benzothiophen (BTCP, **III**) derivatives (**IV**-**VII**) were synthesized. The acute and chronic pain activities of these drugs were studied using the tail immersion and formalin tests on mice and the results were compared with PCP, TCP and control groups at dosage of 10 mg/kg. The results indicated that the drug **VII** produced more analgesic effects on acute chemical pain in formalin test compared with other drugs. In addition, this analgesic effect was remarkably seen for drugs **II**, **VI** and **VII** in chronic pain in the mentioned test in comparison with other drugs. Also, the results showed that acute thermal pain could be diminished by drugs **VI**, **II** and **I** compared with other drugs in tail immersion test. It can be concluded that more analgesic effects of new BTCP analogues (**VI** and **VII**) may be concerned with antinociception activities of benzothiophene group and also with binding to cocaine site on the dopamine transporter receptor which seems to be more potent than PCP receptor in decreasing pain.

Keywords: Formalin test, NMDA and dopamine receptors, Pain activities, Phencyclidine, Tail Immersion Test, TCP and BTCP derivative.

1. INTRODUCTION

Phencyclidine was originally introduced as a general anesthetic drug but it was subsequently withdrawn from prescription for humans due to generating severe psychotomimetic side effects. Consequently, the focus of research on PCP has shifted from its application as an anesthetic compound toward its potential applications for neuropharmaceutical effects [1, 2]. PCP and its analogues appear to bind to the NMDA receptor and the binding results in blocking of the voltage-sensitive potassium and sodium channels. This causes increasing calcium entry into the nerve cell, to releasing neurotransmitters into pre-synaptic nerve endings. The potency of these analogues in blocking the potassium channel *in vitro* highly correlates with their behavioral potency *in vivo* [3, 4].

The NMDA subclass of glutamate receptors are composed of an ion channel which possesses multiple sites for agonist and antagonist bindings. There is much experimental evidence suggesting that these receptors are involved in the mechanisms controlling the acquisition and expression of pain-related behaviors [5]. Behavioral effects of phencyclidine and its derivatives can vary by dosage. Low doses produce numbness in the extremities, characterized by staggering, unsteady gait and loss of balance. Moderate doses produce analgesia and anesthesia while high doses may lead to hallucination and convulsion [6, 7].

So far, many PCP derivatives (with changes in substitution on the molecule) have been synthesized and their pharmacological activities have been tested [8-12]. In this study, piperidine ring in this molecule was replaced by 2, 2, 6, 6-tetra methyl piperidine or 2,2,6,6-tetra methyl-4-piperidinol with four methyl and hydroxyl groups in newly synthesized compounds which can generate higher analgesic effects compounds [9, 11, 12]. Secondly, with replacing the phenyl group in PCP by a bioisosteric 2-thienyl (Thienylcyclidine, TCP, **II**) which could be caused to the reports on specific high binding affinity, selectivity for the PCP site in NMDA receptors, as well as neuroprotective potential properties [8, 13-15], current researchers synthesized the new analogues (**IV** and **V**). Moreover, because of reported a broad range of important biological activities including antibacterial, antimicrobial, analgesics, anti-inflammatory, antifungal and etc., for benzothiophene compounds [16, 17], other new analogues (**VI** and **VII**) of **I** with replacement of phenyl by 3-benzothiophen group for producing new BTCP (1-[1-(2-benzo[b] thiophenyl) cyclohexyl] piperidine, **III**) derivatives were synthesized in this work. The analgesic activities of these drugs were measured on mice using the tail immersion (as a model of acute thermal pain) and the formalin (as a model of acute chemical and chronic pain) tests and the results were compared with control, PCP and TCP groups.

*Address correspondence to this author at the Department of Chemistry, Faculty of Science, Islamic Azad University, Karaj Branch, P.O. Box: 31485-313, Karaj, Iran. Tel/Fax: +98-26-34403575;
E-mails: a-ahmadi@kiau.ac.ir; and ahmadikiau@yahoo.com

1389-5575/14 $58.00+.00

2. MATERIALS AND METHOD

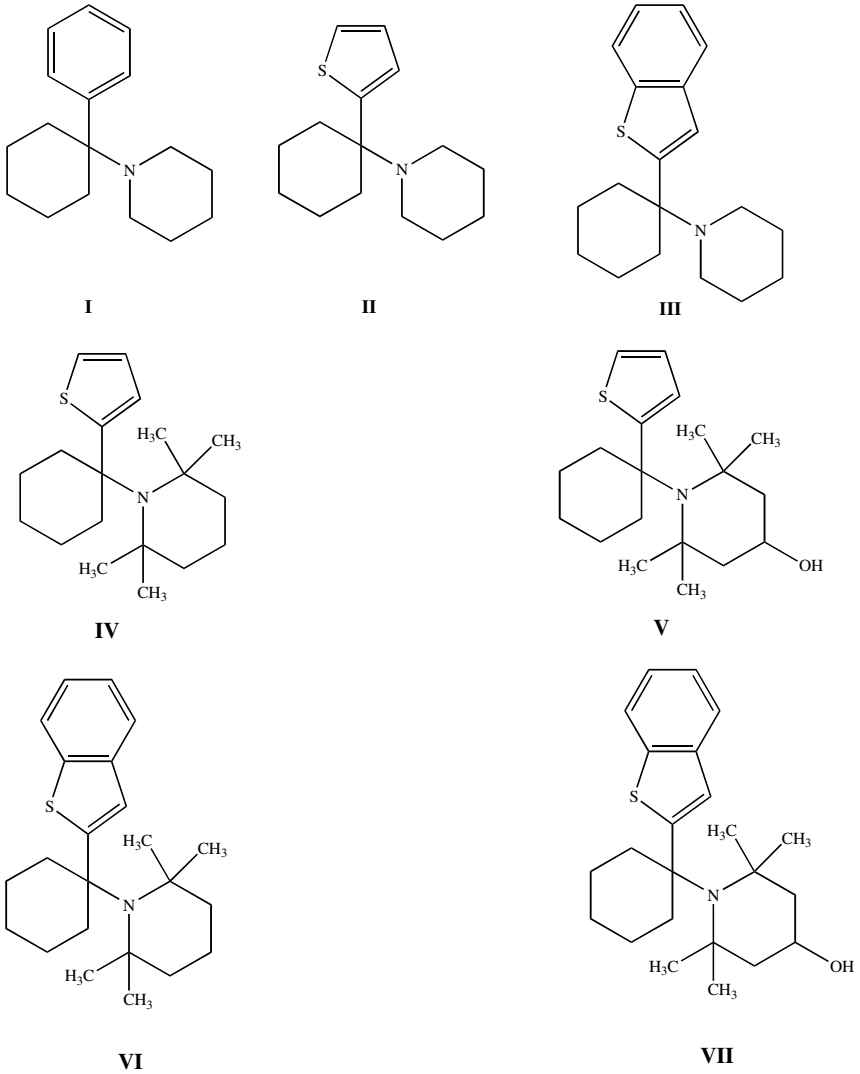
2.1. General

All chemicals and reagents were purchased from Merck Chemical Co. (Darmstadt, Germany). Melting points (uncorrected) were determined with a digital Electrothermal melting point apparatus (*model 9100*, *Electrothermal Engineering Ltd*., Essex, *UK*). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 300 MHz (model AMX, Karlsruhe, Germany) spectrometer (internal reference: TMS). IR spectra were recorded on a Thermo Nicolet FT-IR (model *Nexus-870,* Nicolet Instrument Corp., Madison, Wisconsin, U.S.A.) spectrometer. Mass spectra were recorded on an Agilent Technology-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). Phencyclidine (PCP, **I**) and Thienylcyclidine (TCP, **II**) were prepared based on the published methods [8, 18].

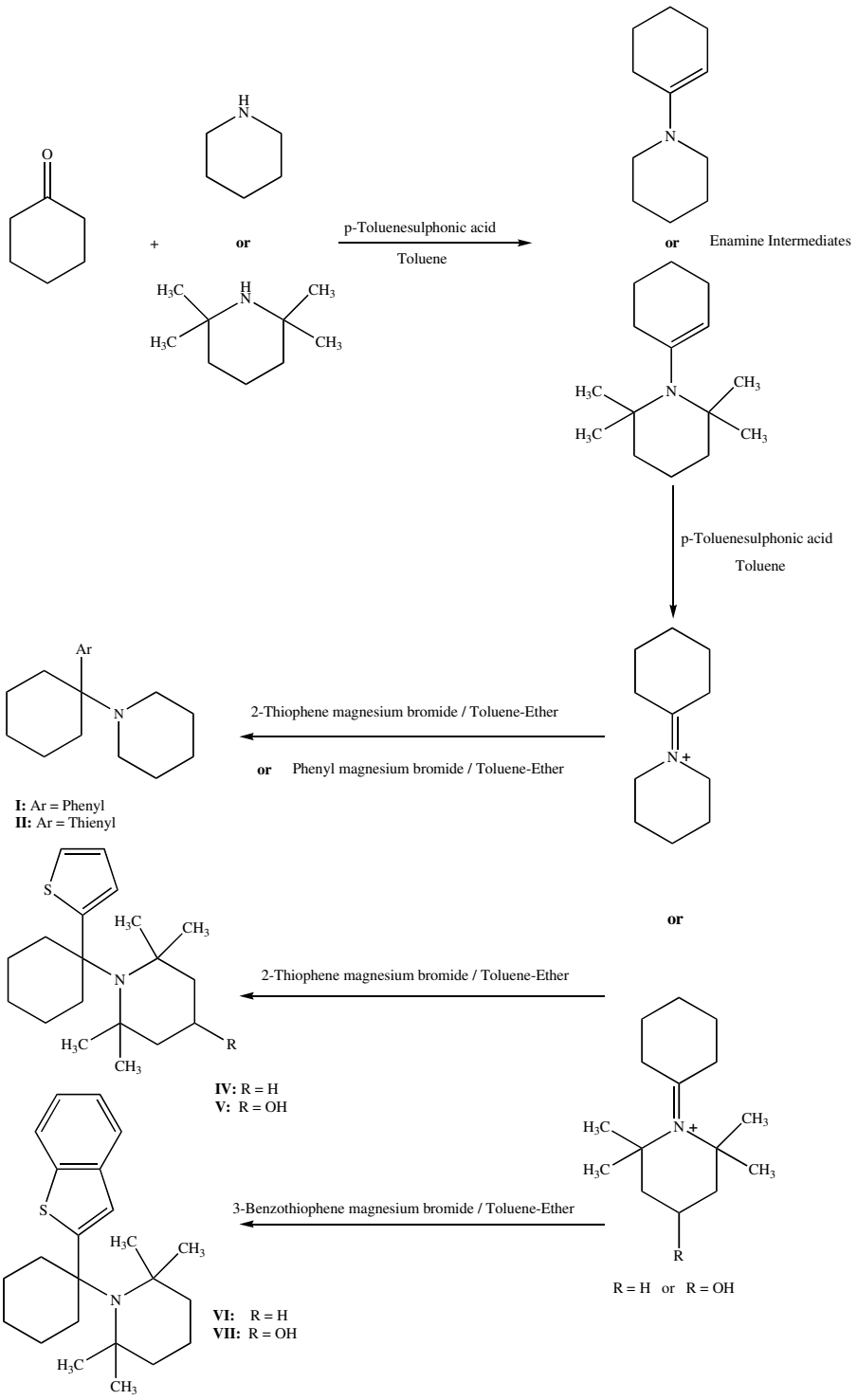
2.2. Preparations (Scheme 1 and 2)

2.2.1. 2,2,6,6-tetramethyl-1-(1-(thiophen-2-yl)cyclohexyl)piperidine (IV) and 2,2,6,6-tetramethyl-1-(1-(thiophen-2-yl)cyclohexyl)piperidin-4-ol (V)

These compounds were prepared by adding a solution of 2-thiophen magnesium bromide (Grignard reagent, prepared from refluxing 18 h of 6.28 g 2-bromothiophen and 0.96 g of Mg in 40 ml of dry ether) to the imine intermediate solutions [prepared by adding 5.7 g azeotropic distillation of dried *p*-toluenesulfonic acid in 50 ml toluene] to the cooled enamine solutions (prepared by 20 h heating under a dean stark trap of 2,2,6,6-tetramethyl piperidine or 2,2,6,6-tetra methyl-4-piperidinol (0.047 mol), cyclohexanone (0.04 mol) and *p*-toluenesulfonic acid (0.08 g) in 50 ml toluene)]. These mixtures were stirred for 3 additional days at ambient temperature (25 ^{o}C) and were subsequently poured into ice-NH_4Cl and NH_4OH. The organic layers were separated and water-washed. The bases were neutralized with 10% of



Scheme (1). Structure formulas of PCP (**I**), TCP (**II**), BTCP (**III**), 2,2,6,6-tetramethyl-1-(1-(thiophen-2-yl)cyclohexyl)piperidine (**IV**), 2,2,6,6-tetramethyl-1-(1-(thiophen-2-yl)cyclohexyl)piperidin-4-ol **(V),** 1-(1-(benzo[b]thiophen-3-yl)cyclohexyl)-2,2,6,6-tetramethylpiperidine (**VI**) and 1-(1-(benzo[b]thiophen-3-yl)cyclohexyl)-2,2,6,6-tetramethylpiperidin-4-ol **(VII)**.



Scheme (2). Synthesis of intermediates (enamines and imines) and final compounds (**IV-VII**).

H_2SO_4, washed with 20% of NaOH, re-extracted with ether, dried and concentrated. The pale solid compounds were obtained which were passed through a silica gel column using ethyl acetate-hexane (95:5) as the eluent to afford **IV** (0.07 g, dark brown) or **V** (0.19 g, dark brown).

IV: IR (KBr): 2923, 1676, 1412, 1237, 1198, 1050 cm^{-1}.^{1}H-NMR ($CDCl_3$) (ppm): 1.03-2.34 (28H, m), 6.8-7.7 (3H, m).^{13}C-NMR ($CDCl_3$) (ppm): 25.7, 26, 32.6, 51.3, 56.1, 67.3, 75.4, 112, 120.7, 126.9, 127.5, 134.5, 161.2. Anal. Calcd. for $C_{19}H_{31}NS$: C, 74.69; H, 10.23; N, 4.58; Found: C, 74.72; H, 10.25; N, 4.55. MS: m/z (regulatory intensity): 305 (25), 247 (40), 225 (22), 166 (46), 141 (24), 83 (25), 82 (24).

V: IR (KBr): 3439, 3109, 1680, 1497, 1416, 1234, 1207, 1042, 827 cm^{-1}.^{1}H-NMR ($CDCl_3$) (ppm): 0.84-2.37 (26H, m), 2.49

(1H, s), 3.32 (1H, s), 6.91-7.26 (3H, m).^{13}C-NMR ($CDCl_3$) (ppm): 25.7, 26, 32.6, 51.3, 56.1, 67.3, 75.4, 112, 120.7, 126.9, 127.5, 134.5, 161.2. Anal. Calcd. for $C_{19}H_{31}NOS$: C, 70.98; H, 9.72; N, 4.36; Found: C, 71.02; H, 9.75; N, 4.33. MS: m/z (regulatory intensity): 320 (8), 253 (46), 246 (7), 239 (14), 167 (27), 158 (60), 83 (25), 82 (24).

2.2.2. 1-(1-(benzo[b]thiophen-3-yl) cyclohexyl)-2, 2, 6, 6-tetramethylpiperidine (VI) and 1-(1-(benzo[b]thiophen-3-yl) cyclohexyl)-2, 2, 6, 6-tetramethylpiperidin-4-ol (VII)

These compounds were prepared from adding a solution of 3-benzothiophene magnesium bromide (Grignard reagent, prepared from refluxing 25 h of 8.52 g 3-bromo benzothiophen and 0.96 g of Mg in 40 ml of dry ether) to the imine intermediate solutions [prepared by adding 5.7 g azeotropic distillation of dried *p*-toluenesulfonic acid in 50 ml toluene to the cooled enamine solutions (prepared by 20h heating under a dean stark trap of 2,2,6,6-tetramethyl piperidine or 2,2,6,6-tetra methyl-4-piperidinol (0.047 mol), cyclohexanone (0.04 mol) and *p*-toluenesulfonic acid (0.08 g) in 50 ml toluene)]. These mixtures were stirred for 5 additional days at ambient temperature (25 ^{o}C) and were subsequently poured into ice-NH_4Cl and NH_4OH. The organic layers were separated and water-washed. The bases were neutralized with 10% of H_2SO_4, washed with 20% of NaOH, re-extracted with ether, dried and concentrated. The pale solid compounds were obtained, which were passed through a silica gel column using ethyl acetate-hexane (95:5) as the eluent to afford **VI** (0.22 g, red) or **VII** (0.9 g, dark red).

VI: IR (KBr): 2923, 2340, 1626, 1455, 1423, 1382, 1251, 1017, 827 cm^{-1}.^{1}H-NMR ($CDCl_3$) (ppm): 1.17-1.62 (28H, m), 7.3-7.9 (5H, m).^{13}C-NMR ($CDCl_3$) (ppm): 25.7, 26, 32.6, 51.3, 56.1, 67.3, 75.4, 112, 120.7, 126.9, 127.5, 134.5, 161.2.Anal. Calcd. for $C_{23}H_{33}NS$: C, 77.69; H, 9.35; N, 3.94; Found: C, 77.72; H, 9.38; N, 3.90.MS: m/z (regulatory intensity): 355 (6), 296 (40), 221 (63), 134 (57), 82 (26).

VII: IR (KBr): 3435, 2919, 1643, 1586, 1453, 1429, 1278, 1061, 729 cm^{-1}.^{1}H-NMR ($CDCl_3$) (ppm): 1.08-1.76 (26H, m), 2.49 (1H, s), 3.36 (1H, s), 7.04-8.10 (5H, m).^{13}C-NMR ($CDCl_3$) (ppm): 25.7, 26, 32.6, 51.3, 56.1, 67.3, 75.4, 112, 120.7, 126.9, 127.5, 134.5, 161.2. Anal. Calcd. for $C_{23}H_{33}NOS$: C, 74.34; H, 8.95; N, 3.77; Found: C, 74.38; H, 8.99; N, 3.74. MS: m/z (regulatory intensity): 370 (11), 272 (17), 215 (7), 170 (14), 161 (100), 140 (17), 134 (38), 82 (11).

2.3. Pharmacological Methods

2.3.1. Animals

Eighty-four NMRI male mice (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 12 h light/dark cycle. Animals were given free access to water and standard laboratory mice chow (Pars Company, Tehran, Iran). All behavioral experiments were carried out between 9 am and 4 pm under the normal room light and at 25^{o}C. 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 Shahed University of Medical Sciences (Tehran, Iran).

2.3.2. Pain Assessment

2.3.2.1. Formalin Test

In this test, formaldehyde solution (20 µl, 3%) was subcutaneously injected into the plantar surface of control mice hind paw. Then, the animals were placed in a Plexiglas chamber (30×30×30 cm^3) with a mirror at 45^{o} angle underneath for accurate observation. In the treatment and control groups (n=12 per group), the drugs (**I, II, IV-VII**, 10 mg/kg dosage) and saline were administered intraperitoneally 30 minutes before formaldehyde injection. However, prior to the experiments, all animals were habituated to the observation chamber for 30 minutes prior to the experimental sessions 5 times at 5 minutes intervals to adapt them with the environment. The behavioral pain reactions i.e, the licking time and frequency were detected and record in each 5 min intervals during 45 min period after formalin injection. The first 10 minutes after formalin injection is known as the acute neurogenic pain and the period between 15-45 minutes is known as chronic inflammatory pain [19]. Separate groups of animals received various derivatives of Phencyclidine (10 mg/kg), 30 min prior to being injected with formalin.

2.3.2.2. Tail Immersion Test

The acute thermal pain was normally modeled by the tail immersion test. Thirty minutes after an intraperitoneal injection of drugs (**I-VII**, 10 mg/kg dosage) and an equivalent volume of saline (control), the mice (n = 12 in each group) were housed in an animal restrainer. Then, the terminal 3cm of their tails was first submerged into room temperature water (22~24 ^{o}C) to check their aversion to water and then immersed in 52^{o}C water. The reaction time between immersing the tail and its removal from heated water was measured and recorded as pain threshold. The record of pain threshold was repeated in 5 minute intervals till 45 min after initiation of the test. Cut-off latency in 15 s was employed to avoid damaging the tail [20].

2.3.2.3. Statistical Analysis

Sigma stat 3.5 soft was used for statistical analysis. The measured data were presented as means ± S.E.M. Comparisons were carried out as one way analysis of variance (ANOVA) followed by post-hoc Tukey test with a p-value<0.05 as the level of significance.

2.3.2.4. Experimental Psychomotor Coordination (PMC) Index

This test was done by Rota-rod Treadmill with shock facility apparatus (Harvard model 865) after root or new drugs administration. First, animals were trained by their placing on the rolling bar where they had to walk on it. Then, for 5 times, they were placed in the case with these characteristics: initial speed = 4 rpm, final speed = 30 rpm, initial to final speed time = 4 min, shock intensity = 1.1 mA, shock duration = 0.2-0.8 sec, experimental length time = 5 min, interval between experiments = 2 min. The mean stay time on the rod per trial was taken as a PMC index.

3. RESULTS

3.1. Chemistry

Phencyclidine (PCP, **I**), Thienylcyclidine (TCP, **II**) and their newly synthesized analogues (**IV-VII**) were synthesized via enamine method [18] in this study. This method has the advantage of not involving toxic cyanide (nitrile) intermediates which were obtained in Bruylants method (other famous method for synthesizing of PCP analogues). The first step of this reaction is the dehydration of piperidine, 2,2,6,6-tetra methyl piperidine or 2,2,6,6-tetra methyl 4-piperidinol and cyclohexanone to form enamine compounds. Adding anhydrous *p*-toluenesulfonic acid to these mixtures produces imminium salts in the second step. Finally, the reaction of these salts with phenyl magnesium bromide, 2-thiophen magnesium bromide or 3-benzothiophene magnesium bromide (Grignard reagents, prepared from bromobenzene, 2-bromo thiophen or 3-bromo benzothiophen and Mg in dry ether) yields the desired products (Scheme **1**). For increasing the rate of reaction in final step, molar ratio of Grignard reagents to imminium salts were increased. This higher ratio could also increase the yield of reaction between hydroxyl substituted imminium salts and Grignard reagents to form the desired compounds (**V** and **VII)** without protecting hydroxyl group by trimethylchlorosilane, which was previously applied [21]. Spectroscopic (IR, ^{1}H and ^{13}C NMR, Mass) and elemental (CHN) data confirmed the structure of the newly synthesized compounds (**IV-VII**). 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 not observed in the experimental animals. However, the motor coordination index (measured by Rota-rod apparatus, Harvard, UK) did not indicate any significant difference among the treated rats.

3.2.2. Antinociceptive Activity of the Compounds in Formalin Test

All synthesized drugs were administered intraperitoneally with the dosage of 10 mg/kg, 30 minutes before the formaldehyde injection. Licking time measurement showed that the drug **VII** had the best analgesic action in acute (53.39 %) pain (especially in 1st interval) comparing to the others (Table **1**). Also, the chronic pain was reduced in many interval times by all drugs, but a marked anti-nociceptive effect in most intervals were observed by **II** (especially in 15, 25, 40 and 45 min) **VI** (especially in 20, 30 and 35 min) and **VII** (especially in 35 min) after formalin application. Licking frequency analysis showed that in acute pain (the first interval) the drug **VII** could significantly reduce the pain for 44.60%. Moreover, the obtained data showed that the chronic pain was reduced in many interval times by all drugs, but a marked anti-nociceptive effects in most intervals were observed by **VI** (20, 30-45 min) and **II** (25 and 35 min) after formalin application (Table **2**).

3.2.3. Antinociceptive Activity of the Compounds in tail Immersion Test

The intraperitoneal injection of PCP (**I**), TCP (**II**) and their new synthesized drugs (**IV-VII**) with the dosage of 10 mg/kg generated analgesic effects in all interval times within the tail immersion test were compared to the control group. Marked anti-nociceptive effects in most intervals were observed by **VI** (especially in 5, 15-25 and 45 min), **II** (10, 20, 30 and 35 min) and **I** (40 and 45 min) (Table **3**). Nevertheless, in comparison between the original (**I** and **II**) and newly synthesized compounds (**IV**-**VII**), PCP and **VI** could possibly increase the pain threshold in most intermission times.

Table 1. Comparison of Licking Time Periods in Control and Other Treatment Animal Groups.

		Animal Groups Test						
		Control	**I**	**II**	**V**	**VII**	**IV**	**VI**
Interval test time (min)	5	2.19±0.11	1.64±0.06	1.27±0.13	1.51±0.07	0.66±0.09*	1.19±0.01	1.27±0.15
	10	2.18±0.12	1.43±0.11	0.90±0.19	1.83±0.60	1.35±0.23	1.41±0.30	1.25±0.32
	15	5.60±0.17	3.26±0.41	1.36±0.45*	2.75±0.80	2.36±0.60	2.31±0.50	2.35±0.56
	20	4.11±0.34	2.96±0.13	1.55±0.24*	3.66±0.90	1.21±0.66*	2.89±0.10	1.10±0.10*
	25	4.68±0.35	2.37±0.52	1.55±0.13*	4.58±0.99	2.98±0.50	2.11±0.66	3.10±0.67
	30	4.14±0.26	2.90±0.12	1.15±0.39*	5.50±1.20	1.25±0.11*	2.54±0.56	0.91±0.32*
	35	4.42±0.42	3.00±0.18	1.97±0.14*	5.41±0.35	0.60±0.35*	2.41±0.46	0.70±0.51*
	40	6.11±0.42	4.36±0.17*	2.36±0.32	4.25±0.42	4.25±0.42	3.98±0.31	5.40±0.29
	45	4.66±1.63	3.69±.37	1.67±.56*	4.78±.88	3.90±.77	4.20±.51	4.20±.34

The values of table show mean± SEM of licking time in control, PCP (I), TCP (II) and new synthesized drugs (IV-VII). * $p < 0.05$, n=12.

Table 2. Comparison of Licking Frequency in Control and Other Treatment Animal Groups.

		Animal Groups Test						
		Control	**I**	**II**	**V**	**VII**	**IV**	**VI**
Interval test time (min)	5	10.83±1.6 *	7.16±0.60	12.00±0.40	11.6±0.74 *	6.00±1.10	10.4±0.50	9.50±1.40
	10	4.33±0.94	1.00±0.35	0.50±0.23	0.6±0.60	1.00±0.81	0.50±0.22	0.30±0.30
	15	2.33±0.66	2.66±0.56	1.70±0.39	1.20±0.40	2.33±1.18	2.00±0.94	1.70±0.30
	20	4.50±1.52	1.66±0.67	3.00±0.61	2.50±0.97	3.00±0.70	2.50±0.20	0.60±0.28 *
	25	5.83±1.57	2.00±0.67	1.25±0.61	1.40±0.97	1.66±0.72	1.20±0.20	2.50±0.28
	30	10.66±1.9	5.80±0.37	4.25±1.7	3.50±0.97	3.80±0.47	4.21±0.20	1.50±0.50 *
	35	6.33±1.57	2.00±0.34	1.00±0.57	2.00±0.31	1.33±0.54	0.88±0.39	0.10±0.1 *
	40	7.16±1.3	2.30±0.31	2.50±0.52	1.80±0.91	2.33±0.98	2.50±0.20	0.80±0.20 *
	45	3.50±1.2	2.60±0.50	3.50±0.23	3.40±1.32	3.10±0.10	3.20±0.38	1.10±0.50 *

The values of table show mean± SEM of licking frequency in control, PCP (I), TCP (II) and new synthesized drugs (IV-VII). * $p < 0.05$, n=12.

Table 3. Comparison of Tail Immersion Test Pain Threshold in Control and Other Treatment Animal Groups.

		Animal Groups Test						
		Control	**I**	**II**	**V**	**VII**	**IV**	**VI**
Interval test time (min)	5	3.60±0.53	4.12±0.54	6.62±.92	6.75±0.99	6.00±0.94	6.25±033	7.25±0.90
	10	4.25±0.52	5.22±0.65	7.10±0.73	7.12±0.87	6.25±0.79	6.50±0.28	6.50±0.60
	15	3.80±0.44	5.66±0.18	6.75±0.45	6.00±0.32	5.75±0.61	7.00±0.21	7.25±0.4
	20	4.50±0.32	7.33±0.64	9.12±0.98*	5.50±0.56	6.87±0.81	6.5±0.28	9.00±0.70*
	25	5.00±0.62	8.00±0.44	6.62±0.75	7.00±0.84	6.87±0.58	8.87±0.2	10.12±0.80*
	30	4.75±0.64	9.44±0.44*	9.62±1.57*	4.87±0.99	5.87±1.10	8.12±0.38	7.37±0.66
	35	4.37±0.53	8.33±0.53	8.75±1.57	8.75±0.99	7.00±1.10	6.87±0.38	7.37±0.66
	40	4.00±0.5	9.00±0.22*	6.12±0.47	5.62±0.53	5.75±0.41	6.00±0.35	7.58±0.45*
	45	4.62±0.18	10.22±0.29*	7.50±0.92	6.62±1.10	5.87±1.05	5.37±0.37	11.37±0.99*

The values of table show mean± SEM of pain threshold in control, PCP (I), TCP (II) and new synthesized drugs (IV-VII). * $p < 0.05$, n=12.

4. DISCUSSION

The unique pharmacology of phencyclidine (as an famous drug of aryl cyclohexyl amine family) has been resulted in several structure-activity studies which discussed it in terms of its 3-ring system; an aromatic ring producing an area of negative charge; a basic heterocyclic nitrogen ring bearing a localized positive charge; and cycloalkyl ring which is lipophilic in character [22]. Phencyclidine exerts its pharmacological effects through several neurotransmitter systems. In addition to binding to the NMDA receptor, it also interferes with other brain functions. It blocks muscarinic and nicotinic acetylcholine receptors [23] as well as preventing neuronal depolarization at the aspartate-sensitive glutamate receptor [24]. It also binds to one of the several types of opiate receptors (sigma) on nerve cell membranes [25, 26].

Previous studies showed that thienyl analogue of PCP (TCP) is a potent non-competitive antagonist of glutamate at PCP sites of NMDA receptor and it seems that the TCP analogues are the better candidates than PCP ones for specific neuropharmaceutical activities such as antinociception [8, 27]. Also BTCP (**III**) is a phencyclidine's analogue which has a benzothiophene moiety with many biological properties [16, 17] which is highly selective for the dopamine (DA) neuronal transporter (DAT) like cocaine, when compared to PCP. It binds very poorly to the PCP receptor within the NMDA-gated Ca^{2+} channel. Accordingly, it inhibits DA reuptake, increases striatal levels of extracellular DA and evokes the behaviors related to dopaminergic stimulation [28].

BTCP and some of its analogues also bind to the cocaine site on the dopamine transporter receptor with greater potency than cocaine itself [29, 30], therefore the behavioral and

neurochemical effects of its acute administration are similar to those of cocaine.

The differences between BTCP and cocaine have been observed in chronic treatments. BTCP can mimic some of the behavioral actions of cocaine, but at the same time may perhaps have a lower abuse potential and produce fewer adverse side effects than cocaine. Such a behavioral profile would identify this compound as a possibly suitable candidate for agonist pharmacotherapy of cocaine addiction [31]. Therefore, the major two pharmacological activities of phencyclidine analogues (inhibition of DA uptake and of PCP binding) are clearly separated. Modification of the aryl group of PCP changes the selectivity of the compounds for the two sites: thus, BTCP is specific for the DA uptake site; TCP is specific for PCP sites. Phencyclidine and its derivatives with an unmodified phenyl ring with close affinities for the two sites which are not selective [15].

The results indicated that drug **VII** produce more analgesic effects in acute chemical pain in formalin test compared to other drugs. In addition, this analgesic effect was remarkably seen for drugs **II**, **VI** and **VII** in chronic pain of the mentioned test and in comparison with other drugs. Also the results showed that acute thermal pain could be diminished by drugs **VI**, **II** and **I** compared with other drugs in tail immersion test. Comparing the drugs together, it seems that all the applied drugs generated more analgesic effects compared to control group, while TCP was better than PCP in most times of the two tests. It can be considered with high affinity of this drug to PCP site in NMDA receptor which can produce more analgesic effects. In formalin test, the drugs **VII** and **VI** (new BTCP analogues) could reduce more acute chemical pain comparing to TCP, **IV** and **V** (new TCP analogues). The results for chronic pain also revealed that these new BTCP analogues could reduce more pain relative to new TCP ones as well as TCP alone.

These results confirmed that binding to cocaine site on the dopamine transporter receptor in theses BTCP analogues is responsible for their superior analgesic effects compared to other drugs with high affinity to binding with PCP receptors. In tail immersion test, the drugs **VI** (new BTCP analogues) could reduce more acute thermal pain compared to TCP and its newly synthesized analogues (**IV** and **V**) but this effect was low for other new BTCP derivative (**VII**) with hydroxyl group which may be concerned with higher steric hindrance of this group on the molecule. Also in comparison to similar structural new analogues together, it seems that TCP showed better results for decreasing pain compared to new its synthesized analogues (**IV** and **V**) and this result for **IV** is better than **V**.

These results indicated that adding four methyl and hydroxyl groups on piperidine ring of TCP has not been effective enough for increasing the binding affinity of these new drugs to PCP receptors (for more analgesic properties) and this effect for **V** with hydroxyl group is more than **IV** which can be accounted for the higher steric hindrance of these groups on the molecules.

In comparing **VI** and **VII**, it seems that, there is not so much difference between their analgesic results in most chronic pain times. Therefore, it can be concluded that additional hydroxyl group on piperidine ring of **VII** does not decrease pain compared to **VI,** except in acute chemical pain. The higher observed analgesic effects for phencyclidine compared to TCP and its newly synthesized drugs in acute thermal pain at longer times (40 and 45 min after injection of drugs) in hot water may be concerned with lower binding ability of TCP and its new analogues to PCP receptors in these conditions. Further research is recommended for finding out its mechanism.

5. CONCLUSION

It can be concluded that new BTCP analogues (**VI** and **VII**) showed the best possible effect to diminish acute thermal, chemical and chronic pain in compared with other drugs. This can be accounted for by the analgesic activity of benzothiophen group as well as the binding to cocaine site on the dopamine transporter receptor which seems to be more potent than PCP receptor in decreasing pain. Other findings in this research showed that thienylcyclidine (**II**) had better effects in decreasing acute thermal, chemical and chronic pain compared to its newly synthesized analogues and phencyclidine which might be related to its higher affinity to PCP receptor and probably to high steric hindrance of four methyl groups on piperidine ring in these new analogues. It could be responsible for lower binding to PCP receptor (especially for **V** with additional hydroxyl group) whereas this steric hindrance could not so effective in binding the new BTCP analogues with cocaine receptor.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work was a research project at Islamic Azad University, Karaj Branch, Iran and benefited from the financial supports of INFS (Iran National Foundation Support). The authors would like to express their gratitude to both sponsors. They thank Fariba Ansari for her assistance with the pharmacological tests. They also appreciate Dr. Natasha Pourdana, the certified editor in Journal of Language Education in Asia (LEiA), for proofreading the initial draft of this article.

REFERENCES

[1] Carlson, K.M.; Wagner, G.C. Effects of phencyclidine on schedule-controlled responding following neurotoxic lesions of the striatum. *Life Sciences*, **2005**, 77, 372-385.

[2] Johnson, K.M.; Jones SM. Neuropharmacology of phencyclidine: Basic mechanisms and therapeutic potential. *Annu Rev Pharmacol Toxicol.* **1990**, *39*, 707-750.

[3] Albuquerque, E.; Aguayo, L.; Warnick, J.; Ickowicz, R.; Blaustein, M. Interaction of phencyclidine with ion channels of nerve and muscle Behavioral implications. *Federation Proc*, **1983**, *42*, 2584.

[4] Compton, A.D.; Slemmer, J.E.; Drew, M.R.; Hyman, J.M.; Golden, K.M.; Balster, R.L.; Wiley, J.L. Combinations of clozapine and phencyclidine: effects on drug discrimination and behavioral inhibition in rats. *Neuropharmacology*, **2001**, *40,* 289-297.

[5] Rabben, T.; Skjelbred, P.; Oye, I. Prolonged Analgesic Effect of Ketamine, an *N*-Methyl-Daspartate Receptor Inhibitor, in Patients with Chronic Pain. *J. Pharm. Exp. Ther.,* **1999,** *289*, 1060-1066.

[6] Diaz, J. *How Drugs Influence Behavior*. Englewood Cliffs: Prentice Hall, **1996**.

[7] Mozayani, A. Phencyclidine Effects on Human Performance and Behavior. *Forensic Science Review*, **2003**, *15* (1), 61-74.

[8] Al-deeb O.A.A. New analgesic derived from the phencyclidine analogue thiencyclidine. *Arzneimittelforschung,* **1996**, *46*(I), 505-508.

[9] Ahmadi, A.; Khalili, M.; Abbassi, S.; Javadi, M.; Mahmoudi, A.; Hajikhani, R. Synthesis and Study on Analgesic Effects of 1-[1-[4-methylphenyl][cyclohexyl]-4-piperidinol and 1-[1-[4-methoxyphenyl][cyclohexyl]]-4-piperidinol as Two new Phencyclidine Derivatives. *Arzneimittelforschung,* **2009**, *59*(4), 202-206.

[10] Ahmadi, A.; Khalili, M.; Hajikhani, R.; Naserbakht, M. New morpholine analogues of phencyclidine: Chemical synthesis and pain perception in rats. *Pharmacol. Biochem. Behave.,* **2011***, 98,* 227-233.

[11] Hajikhani, R.; Ahmadi, A.; Naderi, N.; Yaghoobi, K.; Shirazizand, Z.; Rezaee, N. M.; Nahri-Niknafs, B. Effect of Phencyclidine Derivatives on Anxiety-like Behavior Using an Elevated-plus Maze Test in Mice. *Adv. Clin. Exp. Med.* **2012**, *21*, 3, 307–312.

[12] Ahmadi, A.; Kermani, M.; Naderi, N.; Hajikhani, R.; Rezaee, N. M.; Javadi, M.; Nahri-Niknafs, B. Synthesis and antinociceptive behaviors of new methyl and hydroxyl derivatives of phencyclidine. *Cur. Med. Chem.* **2012**, *19*(5),763-769.

[13] Vignon, J.; Chicheportiche, R.; Chicheportiche, M.; Kamenka, J.M.; Geneste, P. A new tool with high affinity to the PCP receptor in rat brain. *Brain Res.*, **1983,** *280*, 194-197.

[14] Chaudieu, I.; Vinon J.; Chicheportiche, M.; Harfi, A.; Kamenka, J.M. Comparaison entre les sites de fixation de la [^{3}H]phencyclidine (PCP) et de la [^{3}H](thiényl-2)-1 cyclohexylpipéridine (TCP) dans le système nerveux central de rat. *Eur. J. Med. Chem*., **1987**, *22*, 359-362.

[15] Chaudieu, I.; Vignon, J.; Chicheportiche, M.; Kamenka, J.M.; Trouiller, G.; Chicheportiche, R. Role of the aromatic group in the inhibition of phencyclidine binding and dopamine uptake by PCP analogs. *Pharmacol. Biochem. Behav.,* **1989** *32*(3), 699-705.

[16] Pawar, P.Y.; Mane, B.Y.; Magar, S.D.; Kasar, V.D.; Tupe, A.P. Synthesis, characterization, Analgesics and Anti-inflammatory activity of Some 4,5,6,7-tetrahydrobenzothiophene Derivatives. *Cur. Pharm. Res.,* **2012**, *2*(4), 649-654.

[17] Patterson, S.; Jones, D.C.; Shanks, E. J.; Frearson, J. A.; Gilbert, I.H.; Wyatt, P. G.; Fairlamb, A.H. Synthesis and Evaluation of 1-(1-(Benzo[b]thiophen-2-yl)cyclohexyl)piperidine (BTCP) Analogues as Inhibitors of Trypanothione Reductase. *Chem. Med. Chem.,* **2009**, *4*, 1341-1353.

[18] Godefroi, E.F.; Maddox, V. H.; Parcell, R F. Process for producing a depressant-like effect on the central nervous system, *U.S. Patent* 3,079,136, **1963**.

[19] Dubuisson, D.; Dennis, S.G. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain,* **1977**, *4*(2), 161-74.

[20] Ramabadran, K.; Bansinath, M.; Turndorf, H.; Puig, M.M. Tail immersion test for the evaluation of a nociceptive reaction in mice. *Methodol. Consider. J. Pharmacol. Methods,* **1989**, *21*(1), 21-31.

[21] Itzhak, Y.; Kalir, A.; Weissman, B.A.; Cohen, S. New analgesic drugs derived from phencyclidine. *J. Med. Chem.,* **1981**, *24*, 496-499.

[22] Ferle-Vidović, A.; Kaštelan, M.; Petrović, D.; Šuman, L.; Kaselj, M.; Škare, D.; Mlinarić-Majerski, K. Synthesis and biological activity of phencyclidine and its adamantylamine derivatives. *Eur. J. Med. Chem.*, **1987,** *28*(3), 243-250.

[23] Albuquerque, E.; Aguayo, L.; Warnick, J.; Ickowicz, R.; Blaustein, M. Interaction of phencyclidine with ion channels of nerve and muscle Behavioral implications. *Federation Proc,* **1983**, *42*, 2584.

[24] Johnson, K.; Snell, L.; Morten, R. N-Methyl-D-aspartate enhanced ^{3}H-TCP binding to rat cortical membranes effects of divalent cations and glycine. *Pharmacol. Biochem. Behav*., **1987**, *28*, 128.

[25] Ishii, A.; Seno, H.; Watanabe-Suzuki, K.; Kumazawa, T.; Matsushima, H.; Suzuki, O.; Katsumata, Y. Ultra sensitive determination of phencyclidine in body fluids by surfaceionization organic mass spectrometry. *Anal. Chem.,* **2000**, *72*, 404.

[26] Jirgensons, A.; Kauss, V.; Kalvinsh, I.; Gold, M.R.; Danysz, W.; Parsons, C.G.; Quack, G. Synthesis and structure-affinity relationships of 1,3,5-alkyl substituted cyclohexylamines binding at NMDA receptor PCP site. *Eur. J. Med. Chem.*, **2000**, *35*, 555.

[27] Michaud, M.; Warren, H.; Drian, M.J.; Rambaud, J.; Cerruti, P.; Nicolas, J.P.; Vignon, J.; Privat, A.; Kamenka, J.M. Homochiral structures derived from 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP) are potent non-competitive antagonists of glutamate at NMDA receptor sites. *Eur. J. Med. Chem*., **1994**, *29*, 869-876.

[28] Florence, E.; Jacques, H.; Hélène, H.; Jacques, V.; Jean-Marc, K. The low affinity PCP sites in the rat cerebellum not only bind TCP-like but also BTCP-like structures. *Eur. J. Med. Chem.,* **2000**, *35*, 323-331.

[29] Xiao, S. H.; Lionel, P. R.; Mariena, V. M.; Mohyee, E.E.; de Costa, B. R. Synthesis and biological evaluation of 1-[1-(2 benzo[b]thienyl) cyclohexyl]piperidine homologs at dopamine-uptake and phencyclidine-, and .sigma.-binding sites. *J. Med. Chem.*, **1993**, *36* (9), 1188-1193.

[30] Jacques, V.; Valérie, P.; Catherine, C.; Jean-Marc, K.; Robert, C. [^{3}H]N-[1-(2-Benzo(b)thiophenyl) cycohexyl]piperidine ([^{3}H]BTCP): a new phencyclidine analog selective for the dopamine uptake complex. *Eur. J. Pharmacol.,* **1988**, *148* (3), 427-436.

[31] Martin-Fardon, R.; Weiss, F. N. N-[1-(2-Benzo[b]Thiophenyl) Cyclohexyl]-Piperidine (BTCP) Exerts Cocaine-Like Actions on Drug-Maintained Responding in Rats. *Neuropsychopharmacology,* **2000**, *23*(3), 316-325.

Received: January 15, 2013 Revised: September 09, 2013 Accepted: October 21, 2013