Synthesis and study of anticonvulsant effect of 1-[1-(4-methoxyphenyl) (cyclohexyl)] 4-piperidinol as a new derivative of phencyclidine in PTZ-induced kindling model in male mice

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A B S T R A C T

Background and Objective: Epilepsy is a common disease in communities. Since there is no cure for it and current treatments are not effective for every patient, new method for medical treatment of epileptic patients is necessary. As NMDA receptors antagonists are the most prominent anti-epileptic drugs, in this study we synthesized and investigated anti-epileptic effect of a new piperidine derivate 1-[1-(4-methoxyphenyl) (cyclohexyl)] 4-piperidinol as a new NMDA receptors antagonist in chemical kindling model.

Materials and Methods: In this study, 48 male mice (NMRI), weighting 25-30 g, were selected and randomly divided into 4 groups (n=12 in each group). 1: PTZ 2: 1-[1-(4-Methoxyphenyl) (Cyclohexyl)] 3: piperidinol and 4: valproic acid (positive control). Chemical kindling was induced by PTZ (35 mg/kg, i.p.) injection 11 times one other days (for 22 days). In challenge dose at day 24, PTZ was applied at 75 mg/kg to the animals. Thirty minutes after PTZ injection, the animals were followed for convulsion scores (0-5). Finally, the mean of convulsion phases, threshold and duration of 2 and 5 phases were considered as data and the statistical analysis was done.

Results: Data analysis showed that administration of the new piperidine derivate Methoxy-PCP has a prominent anticonvulsant effect than PCP, especially in reduction of phase 5 duration.

Conclusion: The results suggest that administration of the new piperidine derivate, 1-[1-(4-Methoxyphenyl) (Cyclohexyl)] 4-piperidinol could yield a prominent anticonvulsant effect in epilepsy. Regarding changes in conformation of the new drug as a non-competitive antagonist, it may potentially block the NMDA receptors than other piperidine derivates.

Key Words:
Convulsion
Piperidine
Chemical kindling
Pentylenetetrazole

1. Introduction

Prevalence of epilepsy is about 0.5-2 % in the world, and it may occur in all of the ages. Epilepsy is due to CNS malfunction in which some regions of brain will be activated spontaneously (1). Epilepsy is an unusual neurologic state which has influences on psycological, emotional and educational parameters. More than 50% of epileptic patients suffer from some kind of cognitive problem

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with abnormal behavior (2). Although control of seizure attacks has benefits, but the drugs may have side effects on the patients' cognition (3, 4).

Therefore, searching for new drugs that inhibit epileptic seizures and also improve the cognitive state of the patients is important. Chemical kindling is a method to study epilepsy. In this method, animals will be stimulated gradually and repeatedly for seizure by chemical drugs (5). Seizure may emerge as shaking movements or other forms of neurologic activities such as sensational, cognitive or emotional dysfunctions (6). Drugs which are used for treatment of epilepsy are not able to cure seizures. Therefore, it seems that trying to find new drugs for treatment of epilepsy is very important (7, 35). For this purpose, it is important to know the mechanisms of seizure attacks (8, 9). There are two important mechanisms: 1) mechanisms which reduce the inhibitory factors: a) dysfunction of inhibitory receptors: GABAa & GABAb, b) dysfunction in activation of gabaergic neurotransmitters (10, 2) mechanism which increases the excitatory factors, i.e. increase activities of NMDA receptors.

Phencyclidine is a derivative of piperidine that works as antagonist of NMDA receptors, and in this way can be used as a treatment of seizure attacks (11, 12). Phencyclidine (1-(1-phenylcyclohexyl) piperidine (PCP) and its analogues are highly potent and widely abused psychotomimetic drugs which influence the central nervous system and display analgesic, stimulant, depressant and hallucinogenic effects because of specific binding sites in the brain (13).

Recently, many analogues of phencyclidine have been synthesized (14-25) and their pharmacological activities have been studied. As part of our efforts to reach selective, non-competitive antagonists at the PCP binding site on NMDA receptor complex, we have prepared 1-[1-(4-Methoxyphenyl) (Cyclohexyl)] 4-piperidinol, as an analogue of PCP with a methoxy group on the aromatic ring (m-position) and a phenyl group with cyclohexane ring (a conjugated cyclic ketone, 1-tetralone) to examine its anticonvulsant effect in PTZ-induced kindling model in mice. The results were also compared to PCP and valproic acid. It was anticipated that incorporation of methoxy group on the aromatic ring of the molecule will produce pronounced effects on electron distribution and dipole moments because of the high electron donating character of this group (14).

2. Materials and Methods

1-Tetralone [1, 2, 3, 4-Tetrahydro-1-naphthalenone], cyclohexanone, piperidine, bromo benzene, magnesium turning, diethyl ether, 3-bromo anizole, and all other chemicals were purchased from Merck chemical Co. (Darmstadt, Germany). Melting points (uncorrected) were determined using a digital electrothermal melting point apparatus (model 9100, Electrothermal Engineering Ltd., Essex, UK). 1H and 13C NMR spectra were recorded on a 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 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 male mice (Razi Institute, Tehran, Iran), weighing 22 -26 g were used for pharmacological testing.

2.1. Synthesis of compounds (Schemes 1 and 2)

(1-(1-phenylcyclohexyl) piperidine (PCP) I

This compound was prepared according to reported method (15) from 1-piperidinocyclohexanecarbonitrile (IV) and phenyl magnesium bromide. The hydrochloride salt of I was prepared using 2-propanol and HCl and was recrystallized from 2-propanol (15).

1-Piperidinotetralylcarbonitrile V

To a solution of containing 0.582 g (0.0068 mol) of piperidine in 0.253 g of HCl (37%) and 1.36 g of cold water, 1 g (0.0068 mol) of 1, 2, 3, 4-tetrahydro-1-naphtalenone (1-tetralone) was added. Then, 0.465 g of KCN in 1.02 ml of water, 50 ml of ethanol and 0.1 g of tetra-n-buthylammonium bromide (0.0003 mol) were added and stirred in ambient temperature (25˚ C).

The progress of reaction was controlled by TLC (7:3 ethyl acetate/n-Hexane). After one week no additional progress was seen, so the reaction was extracted with chloroform (75 ml, 3 times). Then, organic layer was separated, dried and concentrated. The oily residue was obtained, which was passed through a silica gel column using ethyl acetate-hexane (7:3) as the eluent to afford 1.13 g of V (69% yield).

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

1H N.M.R. (CDCl3) (p.p.m.): 1.5-2.85 (16H, m), 6.93-7.01 (4H, m).

13C N.M.R. (CDCl3) (p.p.m.): 25.4, 26.2, 26.8, 31, 37.9, 46.7, 52.7, 117.7, 125.5, 128.1, 139.2.

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

1-[1-(4-Methylphenyl) (Cyclohexyl)] 4-piperidinol 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-methoxylphenyl) magnesium bromide (Grignard reagent) (prepared from 24.77 g 3-bromoanisole and 3.075 g of Mg in 17 ml of dry ether), refluxed for 5 additional h in 65-67 oC, left overnight at ambient temperature (25 oC) and then 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, re-extracted with n-Hexane, dried and concentrated. The oily residue was obtained, which was passed through a silica gel column using ethyl acetate-hexane (7:3) as the eluent to afford 2.28 g of III (42% 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 N.M.R. (CDCl3) (p.p.m.): 1.5-2.85 (16H, m), 3.73 (3H, s), 6.59-7.1 (8H, m).

13C N.M.R. (CDCl3) (p.p.m.): 26.2, 27.5, 31.8, 44.8, 47.4, 56, 63, 111.6, 114, 120.2, 120.7, 125.8, 126.2, 128.8, 130, 139.3, 142.8, 144, 162.5.

MS: m/z (regulatory intensity): 321 [M]+ (100), 322 [M+ H]+(7).

2.2. Experimental procedures

In this experimental research, a total of 60 mice (NMRI), weighing 22-26 g (Razi Institue, Tehran, Iran), were randomly divided into six groups including; 1- control, 2- PTZ, 3- positive control (PTZ and valproate 100 mg/kg; i.p. as an anti-convulsant drug), 4, 5 PCP and its new compound methoxy PCP, respectively. Ten mice were housed in each cage at a temperature of 21±2ºC and 12 h light-dark cycling. The mice had free access to standard food and tap water ad libitum. The experimental protocol was approved by the Ethic Committee of Shahed University.

2.3. Kindling

All animals but control group (group 1) were kindled by a total of 11 period injection of PTZ (35 mg/kg; i.p.). Each administration was carried out every second day for 22 days. The challenge dose of 75 mg/kg of PTZ was injected in kindled mice on day 24 (test day). The challenge dose injection of PTZ produced convulsions (clonic and tonic) and lethality. All kindled mice were tested for PTZ challenge dose (75 mg/kg)-induced seizures and status. However, the exhibited phases of seizure (0-6) were observed and categorized using following scale [18] for 30 minutes after PTZ injection. The scale introduces six phases as follows:

0: no response

1: ear and facial twitching

2: convulsive waves axially through the body

3: myoclonic body jerks

4: generalized clonic convulsions turn over into side position

5: generalized convulsions with tonic extension episode and status epilepticus

6: mortality.

2.4. Statistical analysis

Data were expressed as means ± S.E.M. Statistical analyses was carried out using

repeated measure one way analysis of variance (ANOVA) followed by Tukey post-hoc test and p values less than 0.05 were considered as significant differences.

3. Results

3.1. Chemistry

Phencyclidine (I), and 1-[1-(3-methylphenyl) (tetralyl) piperidine (III) were synthesized by reaction of substituted Grignard reagents and carbonitrile compounds (IV, V). To obtain higher electron distribution and dipole moment properties, a methyl group was substituted on the aromatic ring of the molecule (III). Known procedures were applied for the synthesis of compounds I and IV with the appropriate modifications described previously (26, 27).

Bromobenzene and its m-methoxy (II) derivative were reacted with magnesium to form Grignard reagents, which were then reacted with appropriate piperidinocyclohexanecarbonitrile (IV) and piperidinotetralylcarbonitrile (V). Reaction between the Grignard reagents and the carbonitriles were slow and incomplete. So to overcome this problem, molar ratio of Grignard reagents to carbonitriles were increased (26).

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

3.2. Effect of Methoxy-PCP on the PTZ-induced kindling intensity

Statistical analysis of results (as are shown in figure 1) indicates that there are no significant differences among experimental groups in the seizure intensity till 5th injection. As it is shown in figure 1, PCP injection (5.6 mg/kg) at 9, 11 and specially 12th injection is able to significantly reduce PTZ-induced seizure ($p<0.05$). However, valproate (150 mg/kg) significantly reduced seizure intensity in all periods ($p<0.05$).

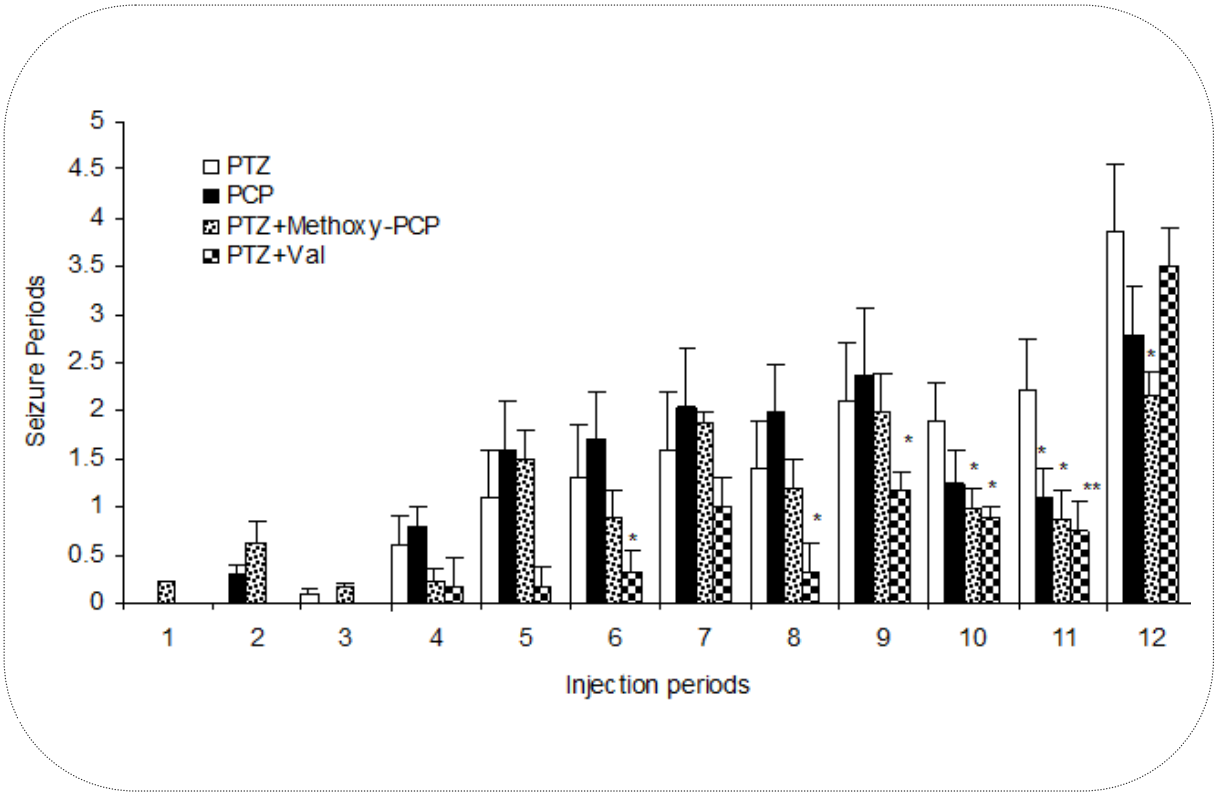

Figure 1. The effect of PCP and Methoxy-PCP pretreatment on the PTZ-induced kindling intensity. *P<0.05 and **P < 0.01 indicate significant differences as compared to PTZ-kindled group

3.3. Effect of Methoxy-PCP on the PTZ-induced kindling factors

As could be seen in Table 1, pretreatment of animals with PCP and methoxy-PCP have significant effect on the duration time of phase 5 ($p<0.05$) and also there was significant difference between valproate150 mg/kg and PCP ($p<0.05$). In addition, Table 2 indicates that only pretreatment of mice with Methoxy-PCP and valproate 150 mg/kg are able to significantly reduce the period that mice remain in phase 5 of seizure ($p<0.05$) and ($p<0.01$).

Table 1. The effect of valproate (150 mg/kg), PCP and methoxy-PCP on the latency of arriving to phase 5 of seizure.

Group test	Phase 5 latency time (s)	Phase 5 duration time (s)	Mortality (%)	Chimney test analysis
				% of mice showing motor impairment
PTZ	3.86 ± 0.70	4.51 ± 0.58	10.20	0
PTZ + VA	3.50 ± 0.60	2.15 ± 0.45 *	0	0
PCP	2.79 ± 0.85	4.15 ± 0.48	12.50	8.33
PTZ + Methoxy-PCP	3.78 ± 0.38	2.66 ± 0.55 *	20	6.33

n=8 in each group.

Table 2. Effect of valproate (150 mg/kg), PCP and Methoxy-PCP on the remaining time in the phases 2 and 5.

Group test	Phase 2 latency time (s)	Phase 2 duration time (s)	Phase 5 latency time (s)	Phase 5 duration time (s)
PTZ	4.41 ± 0.52	27.19 ± 2.19	3.33 ± 0.86	4.11 ± 0.48
PTZ + VA	3.66 ± 1.17	23.50 ± 1.55	2.12 ± 0.60	1.63 ± 0.45 **
PCP	5.81 ± 0.55	19.72 ± 0.74	4.75 ± 0.90	2.58 ± 0.64 *
PTZ + Methoxy-PCP	5.22 ± 1.35	21.98 ± 1.70	4.02 ± 0.48	2.18 ± 0.87 *

n=8 in each group.

4. Discussion

According to the studies on NMDA receptor complex, cationic channels will be opened by the effect of glutamate on its receptor, so Ca2+ and Na+ flow through the channel and the Ca2+ will stimulate the seizure attacks.

Phencyclidine is a non-competitive antagonist of NMDA, so the new piperidine derivative (methoxy-PCP) may work as a non-competitive antagonist of NMDA on the channel and may inhibit epileptic seizures (29).

In one study, it has been demonstrated that the site which PCP will block on the NMDA channel is separate from the known glutamate ligand site, and is different from the channel which will be blocked by Mg2+ ion. Presence of Mg2+ blocks the PCP function and, phencyclidine acts when the channel is not blocked by Mg2+ ion. Furthermore, there is a close interaction between receptors of PCP and NMDA on the channel and sometimes they work together and inhibit the seizure attacks (30). The researches have shown that PCP reduces the activated NMDA channel and in higher concentrations will decrease opening duration of the channel (31). Glycin is a modulator that increases the frequency of opening of activated channels and PCP works oppositely (32). According to the researches, methoxy-PCP acts on NMDA channel and blocks

the flow of Ca2+ through the channel, so decreases the seizures attacks.

According to the earliest studies, the PCP ligand attachment site on NMDA receptors has a high sensitive affinity to the ions with positive charges (33). So, the methoxy-PCP may act as a selective ligand in positive charge environments and has more affinity to the PCP receptor and inhibits the epileptic seizures by this way.

Carter in his study showed that the NMDA receptor has two subunits (NR1, NR2) that will be activated with glutamate and also glycin aminoacid, spermin polyamine and spermidin, will facilitate the glutamate function on the receptors. In fact, they increase NMDA responsibility (34).

In this study, the blocking of NMDA receptors with two types of piperidine derivatives may be by the way of interacting with polyamine sites. The results showed that these two derivatives will antagonize the stimulatory effect of the polyamine and acts on NMDA receptors instead of spermin and spermidin. Therefore, methoxy-PCP might antagonize function of the stimulatory actions of polyamins and in this way blocks the glutamate function and so reduces the seizure attacks and its progression.

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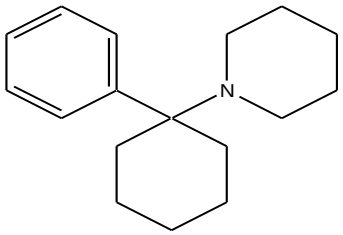
Legends



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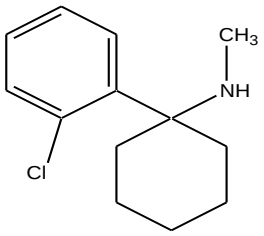

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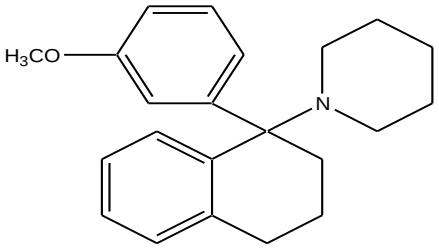

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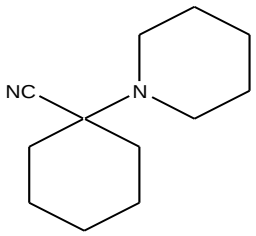

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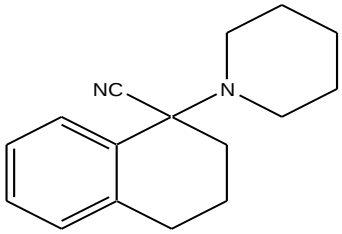
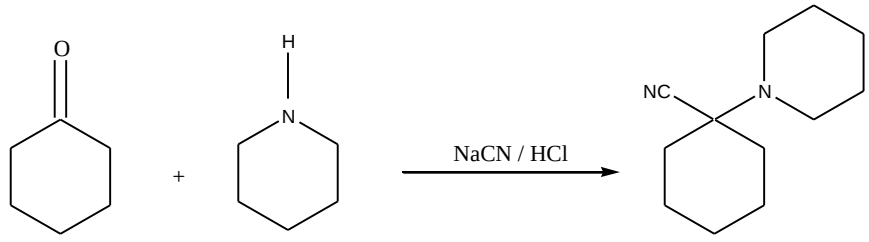


V

Scheme 1. Structure formulas of PCP (I), Ketamine (II), PCP-OCH_3-tetralyl (III) and Carbonitrile intermediates IV and IV.



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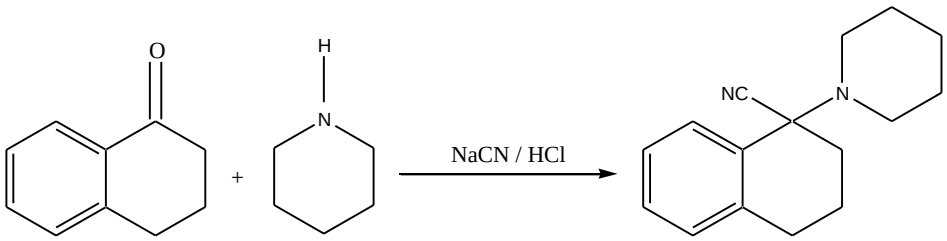
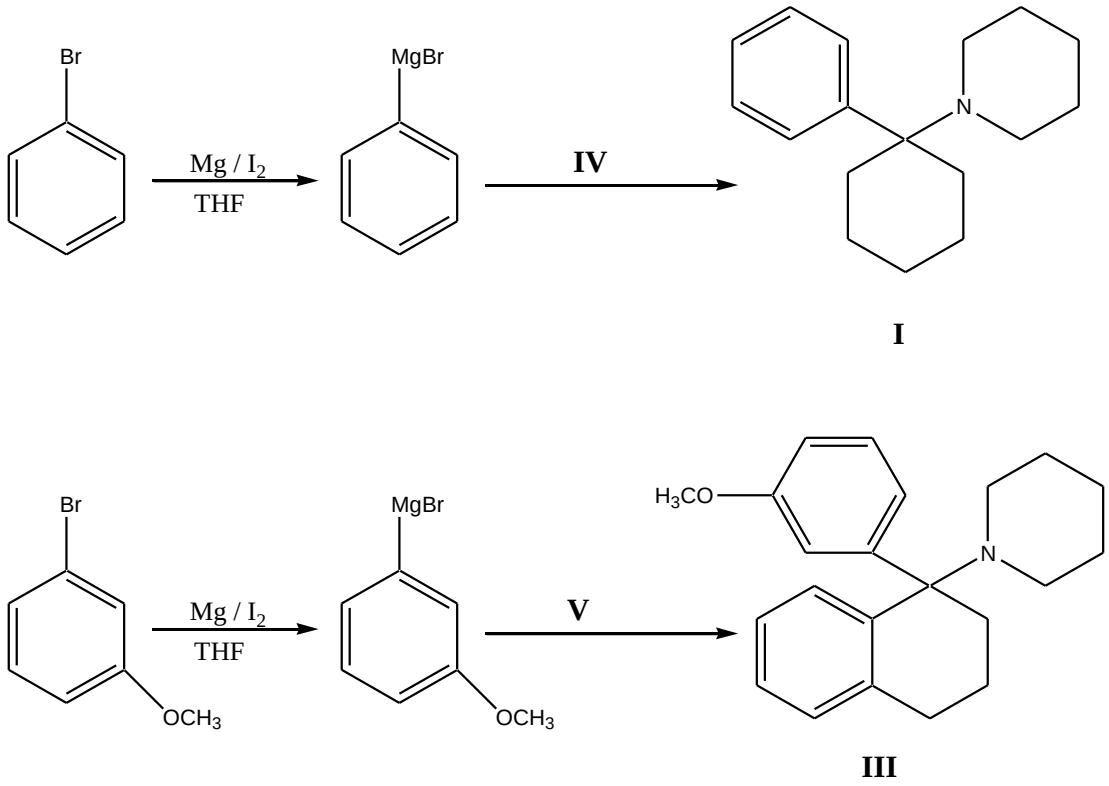


V

Scheme 2. Synthesis of intermediates IV and V.



Scheme 3. Synthesis of compounds I and III.