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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME NEW PYRAZOLE DERIVATIVES

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ABSTRACT

A new series of pyrazole derivatives have been synthesized by the reaction of substituted pyrazole carbohydrazide and functionalized aromatic aldehydes. All the compounds have been characterized by IR, 1H-NMR and mass spectroscopy. The analgesic activity of the synthesized compound was assessed by tail flick method (for central action) in rat, acetic acid-induced writhing test (for peripheral action) in mice. The effect of the synthesized compounds N'-(2,4-dichlorobenzylidene)-5-methyl-1H-pyrazol-3-carbohydrazide (**5a**), N'-(2,4-dichlorobenzyli-dene)-2-(3,5-dimethyl-1H-pyrazol-1-yl)acetohydrazide(**9**), N'-(4-dimethylaminobenzyli-dene)-5-phenyl-1H-pyrazol -3-carbohydrazide (**5b**) and N'-(2,4-dichlorobenzylidene) -5-phenyl-1H-pyrazol-3-carbohydrazide (**5c**) was also investigated in a battery of behavioural models in mice to assess their sedative effect. Among them, **5b** was found more potent in comparison to 150 mg/kg of acetylsalicylic acid (ASA) with 44.83% of inhibition. In radiant heat tail-flick test the synthesized compounds **5a**, **9**, **5b** and **5c** produced 11.06%, 9.73%, 8.38% and 19.31% ($p<0.01$) elongation of tail flicking time 30 minutes after oral doses of 100 mg/Kg body weight respectively. Further compounds after 60 min, the inhibition of pain were 8.27%, 8.97%, 6% and 12.98% respectively. The synthesized compounds at doses of 50 mg/Kg body weight tested were insignificant when compared with the control. Furthermore the oral administration in mice of compounds at 50 and 100 mg / kg, induces strong sedative effect compared to reference substance Nesdonal and significantly reduced in both the reestablishment time and number of head dips during the traction and hole-board tests, in the rotarod test the pyrazole derivatives significantly reduced the motor coordination of the tested animals**.** From the results the pyrazole derivatives exhibited anti-nociceptive activity by central and peripheral mechanism(s) and possess potent sedative effect.

Keywords: Pyrazole Derivatives, analgesic effect, sedative activity.

INTRODUCTION

Pyrazoles are novel class of heterocyclic compounds possessing wide variety of application in the agrochemical and pharmaceutical industries.[1] Derivatives of pyrazole are found to show good antibacterial [2], analgesic [3], radioprotective [4], anti-convulsant [5], anti-depressant [6], anti-inflammatory [7-8], antifungal [9], herbicidal [10], insecticidal [11], antitumor, anti-HCV [12] and antiviral activity [13]. Sedatives are drugs that decrease activity and have a calming, relaxing effect. At higher doses, sedatives usually cause sleep. Analgesic are drugs that relieves pain, this remedy is difficult to treat and hard to investigate and measure. The large placebo effect, the transient nature of the symptoms, and the

undetermined etiology make the cause of pain remains unclear. Several trials have looked at medications for treating pain. Systematic reviews of studies using synthesized compounds in the treatment of pain found them to be effective and some others have a significant effect on the central nervous system.

Hydrazides, carbohydrazides and similar compounds are well known as useful building blocks for the synthesis of a variety of heterocyclic rings. A large number of heterocyclic carbohydrazides and their derivatives are reported to exhibit significant biological activity [14-15], and the carbohydrazide function represents an important pharmacophoric group in several classes of therapeutically useful substances. [16-19]

In view of these observations and in continuation of our search for biologically active pyrazole derivatives [20], we herein report the synthesis and pharmacological activity of a series of pyrazole carbohydrazide hydrazones **5a-c** and **9**.

RESULTS AND DISCUSSION

Chemistry:

The Synthesis of substituted 1H-pyrazol-3-carbohydrazide hydrazone **5a-c** is outlined in Scheme 1.Treatment of an equimolar amounts of acetone or acetophenone **1** with diethyl oxalate in sodium ethoxide and anhydrous ethanol at room temperature afforded ethyl 4-substituted-2,4-dioxobutanoate **2** which reacted subsequently with hydrazine hydrate in ethanol at reflux condition to afford 5-substituted-1H-pyrazol-3-carboxylate 3 in good yield according to reported literature.[21] Treatment of ester **3** with excess hydrazine hydrate in refluxing ethanol gave the new hydrazide derivatives **4**. The resulting hydrazides were reacted with aromatic aldehydes in ethanol in the presence of glacial acetic acid to give the corresponding hydrazones **5** (Scheme 1).

Scheme 2 illustrates the synthetic route for preparation of *N'-(2,4-dichlorobenzylidene)-2-(3,5-dimethyl-1H-pyrazol-1-yl)acetohydrazide* (**9**). The 3, 5-dimethylpyrazole (**6**) and ethyl 2-(3,5-dimethyl-1H-pyrazol-1-yl) acetate (**7**) were prepared according to the procedure described in the literature.[22-23] The resulting pyrazolylester (**7**) were converted to the corresponding hydrazide intermediate (**8**) by treatment with hydrazine hydrate. Finally, condensing the hydrazide (**8**) with 2,4-dichlorobenzaldehyde at reflux in ethanol to afford the desired N'-(2, 4-dichlorobenzylidene)-3-methyl-1H-pyrazol-5-carbohydrazide (**9**) in good yield.

The structures of the obtained compounds were characterized by usual methods (IR, ^{1}H NMR and Mass spectroscopy).

Pharmacological contribution

Analgesic activity:

The peripheral analgesic activity of the pyrazole derivatives was measured by the acetic acid induced writhing test, the synthesized compounds **5a**, **9**, **5b** and **5c** (50 mg/kg body weight) showed a significant ($p<0.001$) reduction in the number of writhes with 42.83%, 36%, 44.83% and 31,33% of inhibition, respectively (Fig.1). At dose of 100 mg/kg body weight the inhibition were 40.17%, 35.17%, 42.87% and 27.83% for **5a**, **9**, **5b** and **5c** respectively. The constriction response of abdomen produced by acetic acid is a sensitive procedure for peripheral analgesic agents. This response is believed to be mediated by the prostaglandin pathways.[24] In radiant heat tail-flick test the synthesized compounds **5a**, **9**, **5b** and **5c** produced 8.85%, 10.75%, 8;65 %, 9.53 % elongation of tail flicking time 30 minutes after oral doses of 50 mg/kg body weight respectively, the results were insignificant when compared with the control. After 60 minutes the compounds showed 9.65%, 12.23%, 10.88% and 10.22% elongation of tail flicking time. The inhibitions were 11.06%, 9.73%, 8.38% and 19.31% ($p<0.01$) for **5a**, **9**, **5b** and **5c** (100 mg/Kg) respectively after 30 min. Further compounds after 60 min, the inhibition of pain were 8.27%, 8.97%, 6% and 12.98% respectively after 30 and 60 min (Fig.2). In the radiant heat tail-flick test, the compounds prolonged the stress tolerance capacity of the mice, indicating the possible involvement of a higher center.[25] The results obtained in this study indicate that the pyrazole derivatives possesses analgesic properties which are mediated via peripheral and central inhibitory mechanisms.

Sedative activity:

In the traction test pyrazole derivatives given by oral route at 50mg/kg did not significantly alter the reestablishment time; all animals performed normal reestablishment immediately ($P>0.05$). Furthermore, the compounds at the dose of 100mg/kg produced significant sedative effect on the central nervous system (CNS) as indicated by the relatively high time for the reestablishment of the mice. By increasing the doses of the the compounds the average reestablishment time was increased.

The reestablishment time was notably higher than control group ($P<0.001$) (Fig 3). In the fireplace test, we noted in the mice pretreated with pyrazole derivatives (50 and 100 mg/kg) a loss of the initiative, curiosity and a lack of recovery when they are introduced into the chimney. They spent an average time higher than 30 seconds to go up the Pyrex glass tube. Furthermore mice pretreated with Nesdonal could not climb backwards in the tube (Fig

4). Nesdonal which has been chosen as the standard reference drug in this study is central nervous system depressant used in the management of sleep disorders, in fact Nesdonal binds to a specific subunit on the GABAA receptor at a site distinct from the binding site of the endogenous GABA molecule, known as an allosteric site. The GABAA receptor is an inhibitory channel which, when activated, decreases neuronal activity.[26] The result obtained from the rotarod test, showed that **5a**, **9**, **5b** and **5c** at 50 mg/kg (37.67, 11, 26.83 and 9.83%) and 100 mg/kg (21.83, 7.83, 19.66 and 10.16% respectively) significantly reduced the motor coordination of the tested animals compared to positive control with 5,5 %**.** In the hole-board test, the pyrazole derivatives **5a**, **9**, **5b** and **5c** at dose 50 mg/kg body weight decreased the capacity in the mice of the exploration of holes and also th e activity to move in the board (35.33± 1.86), (32.83± 5.87), (31.33± 3.39) and (16.17± 2.50) respectively and compared to the control group, which were statistically highly significant ($P < 0.00$). However, the **5a**, **9**, **5b** and **5c** at dose 100 mg/kg showed highly significant ($P < 0.001$) decrease number in head dipping (25.33 ± 6.80), (23.55 ± 3.27), (17.50 ± 3.33) and (15.83 ± 5.04), compared with the control group [figure], the mice move only in the holes that they are juxtaposed, and eventually loosed the curiosity. It is generally believed that locomotor activity results from brain activation, which is manifested as an excitation of central neurons involving different neuro-chemical mechanismand an increase in cerebral metabolism.

It is possible that the sedative activity of pyrazole derivatives is mediated by GABAergic pathway, since GABAergic transmission can produce profound sedation in mice. [27]

The inhibitory action of GABA consists in the opening of chloride channels to allow hyperpolarizing the membrane, leading to CNS depression and resulting in sedative and hypnosis activity. Glutamate and GABA are quantitatively the most important excitatory and inhibitory neurotransmitters, respectively, in the mammalian brai these data provide pharmacological basis for the therapeutic efficacy of these compounds. [28]

EXPERIMENTAL

Chemistry

Melting points were determined on a Büchi B-545 capillary apparatus and are not corrected Thin-Layer Chromatography (TLC) was carried out on Silica Gel 60 F254 plates (Merck KGaA). IR spectra were recorded with an IR VERTEX 70 FT-IR (BrukerOptics) spectrometer. ^{1}H NMR spectra were recorded on a Bruker Avance 300 (300 MHz) spectrometer, using tetramethylsilane (TMS) as internal standard and DMSO as solvent (CNRST, Rabat, Morocco). Mass spectra were recorded on an API 3200 LC/MS/MS system (CNRST, Rabat, Morocco).

General procedure for the preparation of compounds 3a-b:

The compounds were synthesized according to the literature procedure. [21] To a solution of ethyl 4-substituted-2,4-dioxobutanoate **2** (0,02 mol) in ethanol (20 ml), hydrazine hydrate (0,02 mol) was added. The mixture was refluxed for 10 h. The solvent was evaporated and the solid obtained was recrystallized from petroleum ether.

*Ethyl 5-methyl-1H-pyrazol-3-carboxylat **3a:***

Yield 50% ; M.p. 90-92°C (petroleum ether) ; IR (KBr, ν(cm^{-1})) : 3420-3230 (NH), 1720 (C=O) ; ^{1}H-NMR (300 MHz, DMSO-d_6, δ(ppm)) : δ 1.33 (t, 3H, J=70 Hz), 2.43 (s, 3H), 4.37 (q, 2H, J= 70 Hz), 6.54 (s, 1H) ; MS : m/z = 155,6 ($M-H^+$).

*Ethyl 5-phenyl-1H-pyrazol-3-carboxylate **3b:***

Yield 58% ; M.p. 136-138°C (petroleum ether); IR (KBr, ν(cm^{-1})) : 3138-3104 (NH), 1726 (C=O) ; ^{1}H-NMR (300 MHz, DMSO-d_6, δ(ppm)) : δ 1.29 (t, 3H, J= 71 Hz), 2.44 (s, 3H), 4.29 (q, 2H, J= 71 Hz), 7.29-7.45 (m, 5H), 13.92 (s, 1H) ; MS: m/z = 217.1 ($M-H^+$).

General procedure for the preparation of compounds 4a-b:

To a stirred solution of 1 mmol of 5-substituted-1H-pyrazol-3-carboxylate **3** in ethanol (10 ml), 2 ml of hydrazine monohydrate was added. The mixture was maintained under reflux for 8 hours, until TLC indicated the end of reaction. After cooling, the mixture was poured on ice and the solid formed was collected by filtration, washed with cold water and recrystallized from ethanol.

*5-methyl-1H-pyrazol-3-carbohydrazide **4a**:*

Yield 51% ; M.p. 157-159°C (ethanol); IR (KBr, ν(cm^{-1})) : 3269-3461 (NH, NH2), 1634 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6, δ(ppm)) : δ 2.20 (s, 3H), 4.32 (s, 2H), 6.34 (s, 1H), 9.16 (s, 1H), 12.82 (s, 1H) ; MS: m/z = 141.4 ($M-H^+$).

*5-phenyl-1H-pyrazol-3-carbohydrazide **4b** :*

Yield 66% ; M.p. 207-209°C (ethanol); IR (KBr, ν(cm^{-1})) : 3331-3207 (NH, NH2), 1631 (C=O) ; ^{1}H-NMR (300 MHz, DMSO-d_6, δ(ppm)) : δ 4.44 (s, 2H), 7.10 (s, 1H), 7.32-7.45 (m, 3H), 7.74 (d, 2H, J= 16.5 Hz), 9.49 (s, 1H), 13.58 (s, 1H) ; MS: m/z = 203.3 ($M-H^+$).

General procedure for the preparation of substituted-1H-pyrazol-3-carbohydrazide hydrazone 5a-c:

To a solution of derivatives **4** (1 mmol) in 10 ml of ethanol, it was added an equimolar amount of the appropriate benzaldehyde derivative in the presence of acetic acid. The mixture was maintained under reflux for 2 h, until TLC indicated the end of reaction. Then, the reaction mixture was poured in cold water, and the precipitate formed was filtered out washed with ethanol and recrystallized from methanol/DMF.

N'-(2,4-dichlorobenzylidene)-5-methyl-1H-pyrazol-3-carbohydrazide ***5a*** *:*

Yield 95% ; M.p. 258-260°C (methanol/DMF); IR (KBr, ν(cm^{-1})) : 3326 (NH), 1684 (C=O) ; ^{1}H-NMR (300 MHz, DMSO-d_6, δ(ppm)) : δ 2.27 (s, 3H), 6.49 (s, 1H), 7.35, 7.47 (dd, 1H, J=8.41 and 1.81 Hz), 7.68 (d, 1H, J=1.81 Hz), 7.97 (d, 1H, J=8.41 Hz), 8.86 (s, 1H), 11. 98 (s, 1H), 13.10 (s, 1H); MS: m/z = 297.1 ($M-H^+$).

N'-(4-dimethylaminobenzylidene)-5-phenyl-1H-pyrazol-3-carbohydrazide ***5b:***

Yield 78% ; M.p. 262-264°C (methanol/DMF); IR (KBr, ν(cm^{-1})) : 3219 (NH), 1648 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6, δ(ppm)) : δ 2.95 (s, 6H), 6.72 (s, 1H), 7.16-6.72 (m, 9H), 8.35 (s, 1H), 11.35 (s, 1H), 13.72 (s, 1H) ; MS: m/z = 334.5 ($M-H^+$).

N'-(2,4-dichlorobenzylidene)-5-phenyl-1H-pyrazol-3-carbohydrazide ***5c*** *:*

Yield 62% ; M.p. 234-236°C (methanol/DMF); IR (KBr, ν(cm^{-1})) : 3182 (NH), 1657 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6, δ(ppm)) : δ 7.21 (s, 1H), 7.21-8.04 (m, 8H), 8.91 (s, 1H), 12.11 (s, 1H), 13.81 (s, 1H); MS: m/z = 360.9 ($M-H^+$).

***Procedure for the preparation of 3,5-dimethyl-1H-pyrazole 6*: [22]**

To a solution of acetyl acetone (0.2 mol) in ethanol (20 ml), hydrazine hydrate (0.20 mol) was added. After stirring under reflux for 1 h, the solvent was removed under reduced pressure and the residue obtained was washed with ether. Yield 70%; M.p. 106-108°C (106-109°C petroleum ether).[22] MS: m/z = 97.2 ($M-H^+$).

Procedure for the preparation of ethyl 2-(3,5-dimethyl-1H-pyrazol-1-yl)acetate 7 :

The compounds were synthesized according to the literature procedure.[23] Compound **6** (0.02 mol) was dissolved in acetone (50 ml) and solid K_2CO_3 (0.04 mol) was added. The resulting mixture was refluxed for 18 h, the mixture was filtered, evaporated and the residue was recrystallized from hexane. Yield 35%; M.p. 31-33°C (hexane); IR (KBr, ν(cm^{-1})) : 1730 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6, δ(ppm)) : δ 1.35 (t, 3H, J=70 Hz); 2.23 (s, 6H); 4.40 (q, 2H, J=70 Hz); 4.87 (s, 2H); 6.02 (s, 1H); MS: m/z = 183.2 ($M-H^+$).

Procedure for the preparation of 2-(3,5-dimethyl-1H-pyrazol-1-yl)acetohydrazide 8 :

To a stirred solution of 1 mmol of the ethyl 2-(3,5-dimethyl-1H-pyrazol-1-yl) acetate **7** in ethanol (10 ml), 2 ml of hydrazine monohydrate was added. The mixture was maintained under reflux for 6 h, until TLC indicated the end of reaction. After this time, the reaction mixture was poured on ice and the solid formed was collected by filtration, washed with cold water and recrystallized from ethanol. Yield 51%; M.p. 157-159°C (ethanol); IR (KBr, νcm^{-1}) : 3194-3308 (NH, NH_2), 1636 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6, δ(ppm)) : δ 2.02 (s, 3H), 2.15 (s, 3H), 2.48 (s, 2H), 4.51 (s, 1H), 5.76 (s, 1H), 9.21 (s, 1H); MS: m/z = 169.3 ($M-H^+$).

Procedure for the preparation of N'-(2,4-dichlorobenzylidene)-2-(3,5-dimethyl-1H-pyrazol-1-yl)acetohydrazide 9 :

To a solution of derivative **8** (1 mmol) in 10 ml of ethanol, it was added an equimolar amount of the 2,4-dichlorobenzaldehyde in the presence of acetic acid. The mixture was maintained under reflux for 2 h, until TLC indicated the end of reaction. Then, the mixture was poured in cold water, and the precipitate formed was filtered out washed with ethanol and recrystallized from methanol/DMF. Yield 70%; M.p. 204-206°C (Methanol/DMF); IR (KBr, ν(cm^{-1})) : 3414 (NH), 1684 (C=O); ^{1}H-NMR (300 MHz, DMSO-d6, δ(ppm)) : δ 2.42 (s, 3H), 2.51 (s, 3H), 5,83 (s, 2H), 6.39 (s, 1H), 7.21 (dd, 1H, J_1=8.71 and 2.11 Hz), 7.82 (d, 1H, J=2.11 Hz), 7.89 (d, 1H, J=8.71 Hz), 8.88 (s, 1H), 11.78 (s, 1H); MS: m/z = 325.2 ($M-H^+$).

Study of the analgesic and sedative effects:

The synthesized compounds **5a**, **9**, **5b** and **5c**, were evaluated for their analgesic and sedative effects. The experiments were conducted on male albino mice with a corporal weight between 20-30 g. During the experiments, the animals were fed ad libitum with standard food and water except when fasting was required in the course of the study.

They were kept in a temperature and humidity controlled environment (23 ± 2ºC and 70±5%) with a 12 h light-dark cycle in proper ventilation. The animals were acquired from the animal laboratory of Medicine and Pharmacy Faculty, Mohammed V Souissi University, Rabat. The care of the mice was in compliance with the guidelines of the guide for the care and use of laboratory animals. (Commission on life science, national research council 1996). All efforts were made to minimize animals suffering and

to reduce the number of animals used in the experiments.

Analgesic activity

Acetic-Acid-Induced Writhing Response:

The anagesic activity of all the synthesized compounds were carried out by writhing test, the mice were divided into 10 groups (n = 6). The first group was pretreated with saline 0.9 % (control). Simple groups (Four Synthesized compounds) (50, and 100 mg kg−1, i. p.) and acetylsalicylic acid (150 mg kg^{-1} i. p.) were administered. After 30 min an acetic acid solution (1% v/v i. p.) was injected. After a further 10 min, the number of constrictions was recorded for 10 min. [29]

Central analgesic activity by Tail flick method:

The central analgesic activity of pyrazole derivatives was studied in tail withdrawal assay, as described by D'Amour and Smith.[30] Reaction time of animals to radiant heat was recorded by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail with drawl from the heat (flicking response) is taken as the end point. The measurements of withdrawal time using the tail flick apparatus LE 7106 PANLAB (Harvard Bioscience, Spain), was conducted at 30 and 60 min after administration of synthesized compounds. A cut off period of 15 secs is observed to avoid damage to the tail. Mice weighing 20-30 g were randomly divided into 5 groups of 6 animals each. After recording the baseline latency,

Groups II to IX were administered synthesized compounds at the dose of 50 and 100 mg/kg. Group I (control group) received comparable volume of vehicle (distilled water). Group V received the standard drug morphine hydrochloride (0.1mg/kg bodyweight, i.p.). The tail withdrawal latencies were measured at 0, 30, 60, 90 and 120 min, after the compounds administration.

Sedative activity:

The activity of synthesized compounds on the central nervous system was then studied, using a battery of behavioral tests used in psychopharmacology. For testing sedative activity, the effect of pyrazole derivatives at two doses (50 or 100 mg/kg, i.p.) on mice was qualified in one of the following tests.

Traction Test:

Are considered as subject under a sedative action. When the animals perform normal reestablishment immediately, the reaction is known as positive; otherwise, the reaction is called negative; also, the behaviours of animals were recorded during the period of the experiment. [31-32]

Fireplace Test:

The apparatus used for this test consist of a vertical glass tube 30 cm in length. Mice were individually placed vertically in the glass test tube, a normal mouse typically attempts to escape in thirty seconds, and the mice considered as subject to the sedative effect when performing the rise of cylinder greater than 30 sec. [33]

Rota rod test:

Mice were placed on a rotating rod (2.5 cm diameter divided in six equal compartments, rotating at 12 rpm). Animals remaining on the rod for 2 min in two successive trials (24 h before experiment) were selected for testing.

Groups often mice were treated with: (A) vehicle (0.5 mL/20 g body weight o.v), (B) doses of 50 and 100 mg/kg o.v of synthesized compounds. and (C) dose of 0.5 mg/kg i.p. of Nesdonal. After 60 min of the synthesized compounds treatment, they were placed on the spinning bar of the rota-rod apparatus for 1 min. The time spent (in s) on the rotating rod was recorded. [34]

Hole-Board Test:

This test is used to study the psycholeptic and inhibitory actions in the reaction of exploration. The mice are placed one after one, in the center of a plate of 40x40 cm and 1.8 cm of thick, which are drilled 16 holes 3 cm in diameter, regularly spaced. The board is prepared so that the holes appear bottomless.

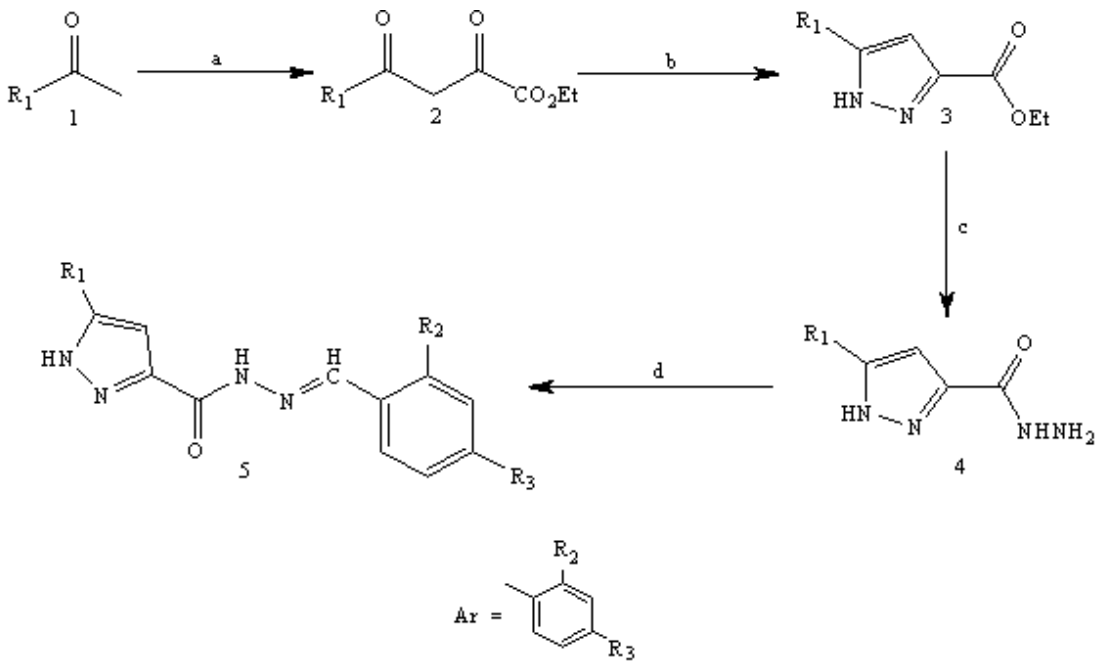
It counts the number of times the mouse plunges his head into a hole. The number of explored holes is noted at the end of 1, 2, 3, 4 and 5 minutes. The averages are calculated for each minute and all 5 minute.

Statistical Analysis

Results of the research are expressed as mean ± S.D (standard deviation) and were tested for statistical significance by one-way ANOVA. Differences were considered statistically significant at the $P < 0.05$ level. The values were obtained by nonlinear regression using the Prism Graph Pad program.

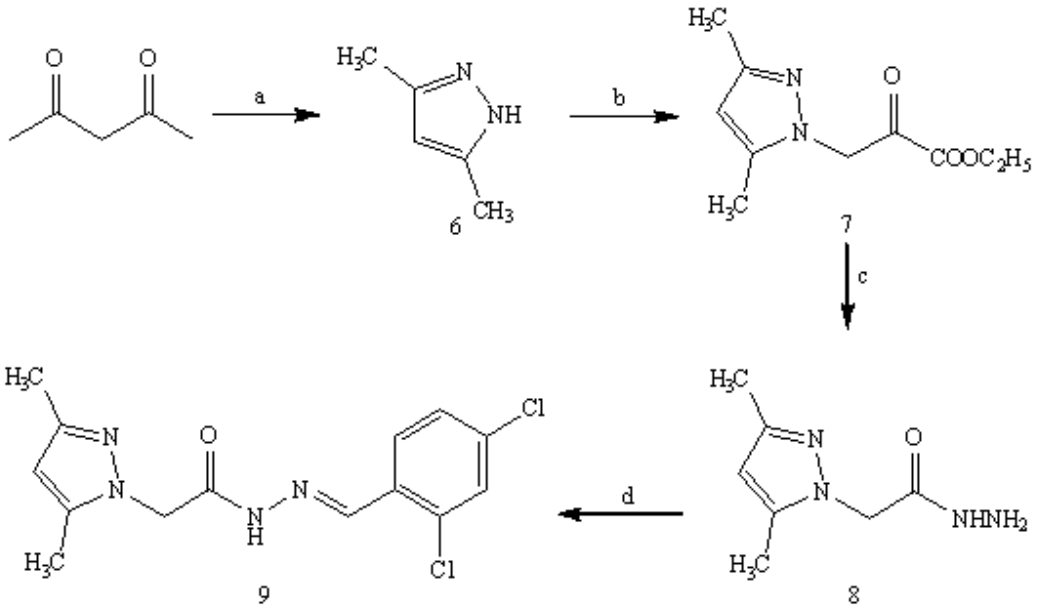
AKNOWLEDGEMENTS

The authors wish to thank all the individuals and institutions who made this survey possible.



Comp	R_1	R_2	R_3
a	CH_3	Cl	Cl
b	C_6H_5	H	$N(CH_3)_2$
c	C_6H_5	Cl	Cl

Scheme 1. Reagents: (a) Diethyl oxalate/NaOEt; (b) H_2NNH_2/H_2SO_4; (c) H_2NNH_2/H_2O/EtOH; (d) ArCHO/EtOH.



Scheme 2. Reagents: (a) H_2NNH_2/H_2O/EtOH; (b) $BrCH_2COOEt/K_2CO_3$/Acétone; (c) H_2NNH_2/H_2O/EtOH; (d) 2,4-ClC_6H_3CHO/EtOH.

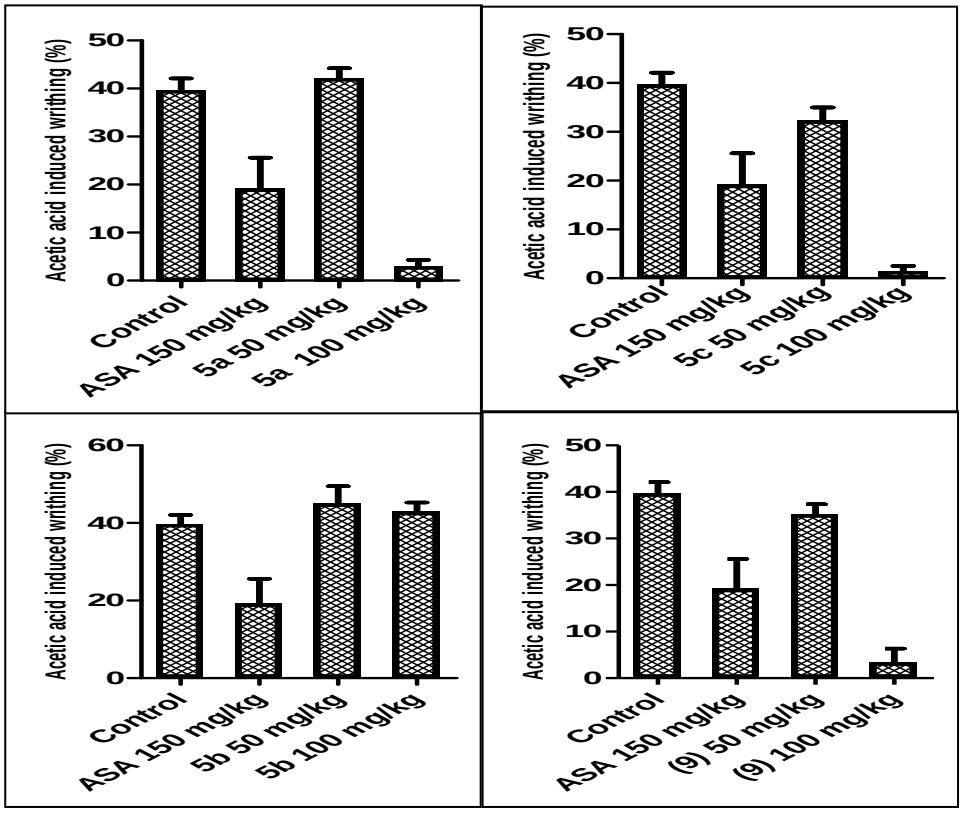


Fig.1: Inhibition of Acetic acid-induced writhing by pyrazole derivatives at different doses. Note that the effect of 1 mg/kg is similar to the effect obtained by 150 mg/kg of Acetylsalicylic acid (ASA).
The difference was evaluated using One-way ANOVA following by Dunnett's multiple comparison tests. P<0.05 was considered significant

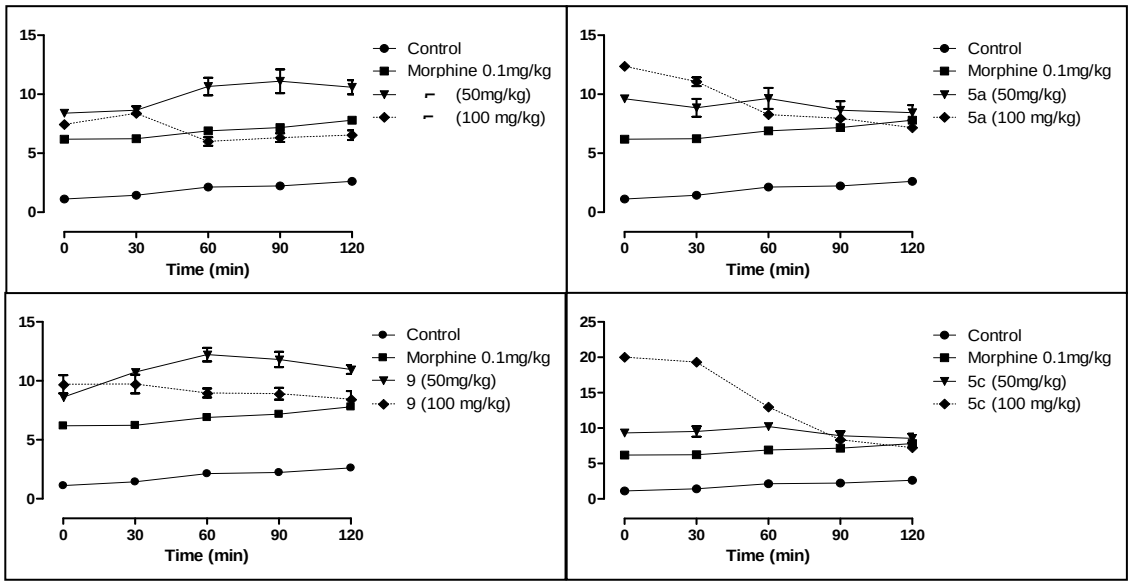


Fig.2 : Reaction Time in mice after administration of pyrazole derivatives and morphine. The effect of pyrazole derivatives is similar to that of morphine. The result is represented as difference (Δ) latency time between stimulation and reaction of mice. The difference was evaluated using two-way ANOVA following by Bonferroni post-tests.

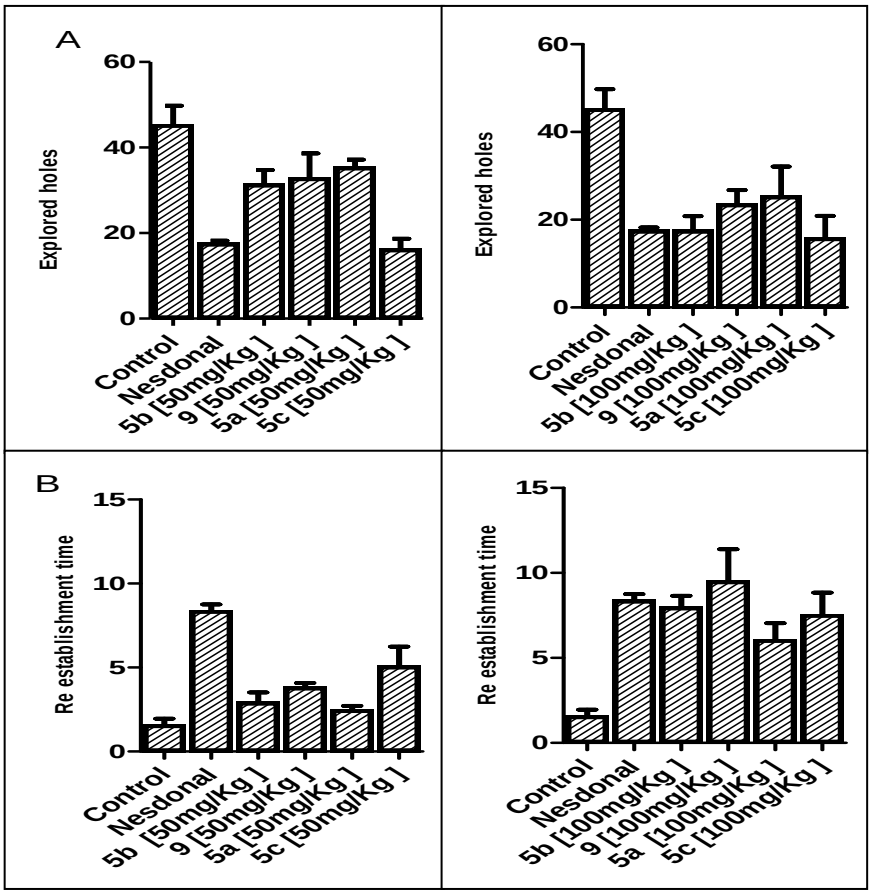


Fig. 3: Sedative activity of pyrazole derivatives using Hole-board and Traction tests. Exploration was measured by (A) the number of holes explored and (B) Re-establishment time.
Data are expressed as mean±SD; P<0.001versus the control group.

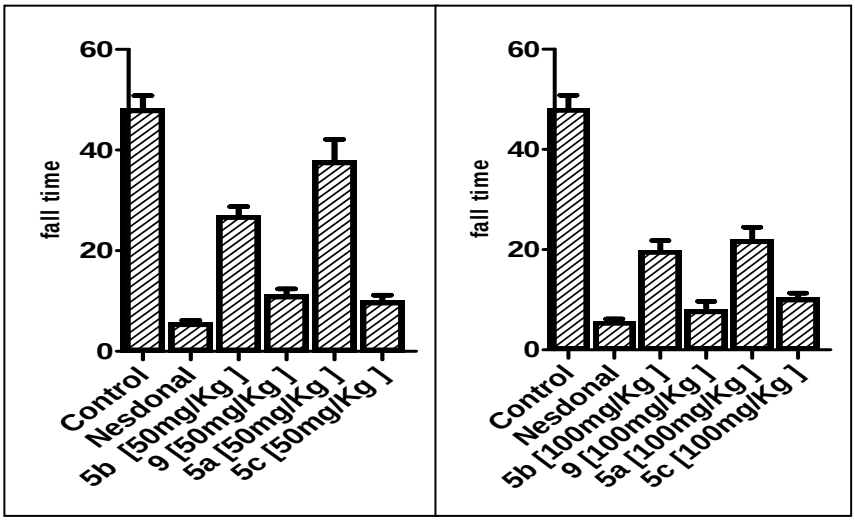


Fig.4: Sedative effect of pyrazole derivatives using *Rota rod* test. The activity was measured by fall time.
Data are expressed as mean±SD; P<0.001versus the control group.

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