Synthesis, characterization and pharmacological evaluation of pyrazole derivatives as potent CNS agents

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The present research article describes the synthesis, characterization and pharmacological estimation of pyrazole derivatives with antianxiety and skeletal muscle relaxant properties, which are used for treatment of anxiety and depression in humans as well as animals. A series of pyrazole derivatives (AS1-AS7) have been synthesized, out of which, compounds AS1 and AS7 have been found to have high skeletal muscle relaxant and anti-anxiety properties.

Anxiety is a multifaceted sentimental reaction marked by sensations of tension, anxious thoughts, and physical transformation like elevated blood pressure. While it is a typical stress response, it can become problematic when it is overwhelming and long-lasting, leading to anxiety disorders. These disorders can greatly hinder a person's daily functioning and standard of life[1].

Anxiety is a complex emotional reaction that involves sensations of tension, anxious thoughts, and physical changes like elevated heart rate, sweating, and muscle tension. Although it serves as a natural alarm response to stress, anxiety can become problematic when it is overwhelming and long-lasting, resulting in anxiety disorders. Anxiety is a mood state focused on future events and involves preparing for potential negative outcomes, while fear is an immediate response to current or looming danger, whether real or perceived. This perspective on human fear and anxiety mirrors the animal predatory imminence continuum, where anxiety parallels an animal's condition during a potential threat and fear aligns with its state during actual or imminent predator contact. There are many symptoms of anxiety which may be seen during the any state of anxiety feeling nervous, restless or tense, having a sense of impending danger, panic or doom, having an increased heart rate, breathing rapidly (hyperventilation), sweating, trembling, feeling weak or tired, trouble concentrating or thinking about anything other than the present worry, having trouble sleeping,

experiencing gastrointestinal (GI) problems, having difficulty controlling worry, having the urge to avoid things that trigger anxiety[2-4].

Ludwig Knorr coined the term "Pyrazole" in 1883 (Ref. 5). Pyrazole chemistry has been well studied compounds in the past. Pyrazoles, commonly known as azoles, are heterocyclic compounds having five members that ring structure and two nitrogen atoms positioned adjacently[6]. A five-membered heterocyclic organic molecule with two neighbouring nitrogen atoms can be found in the center core[7]. Pyrazole has been extensively studied and has demonstrated a great potential for the creation of useful medications[8]. They are specifically utilized as insecticidal, antiviral, anti-parasitic, anticancer, and antibacterial and antifungal drugs. Additionally, some of these substances have anaesthetic, analgesic, anti-inflammatory, and anti-diabetic qualities[9-16]. This five-membered ring can be found in several well-known medications that fall into several categories and have a range of therapeutic effects. Because of the broad range of pharmacological properties of pyrazolines of nicotinic acid hydrazide, such as anti-HIV, antimalarial, antibacterial, and other activities, they have been explored. Among the many biological functions of the pyrazole nucleus are a number of commercially available medications. Derivatives of pyrazoles have also been used extensively in agrochemicals, dyes, luminous materials, and other fields (Fig. 1). Thus, pyrazole chemistry continues to be of interest[17-19].
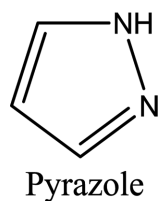
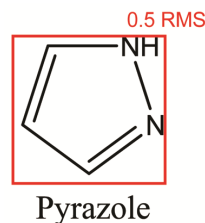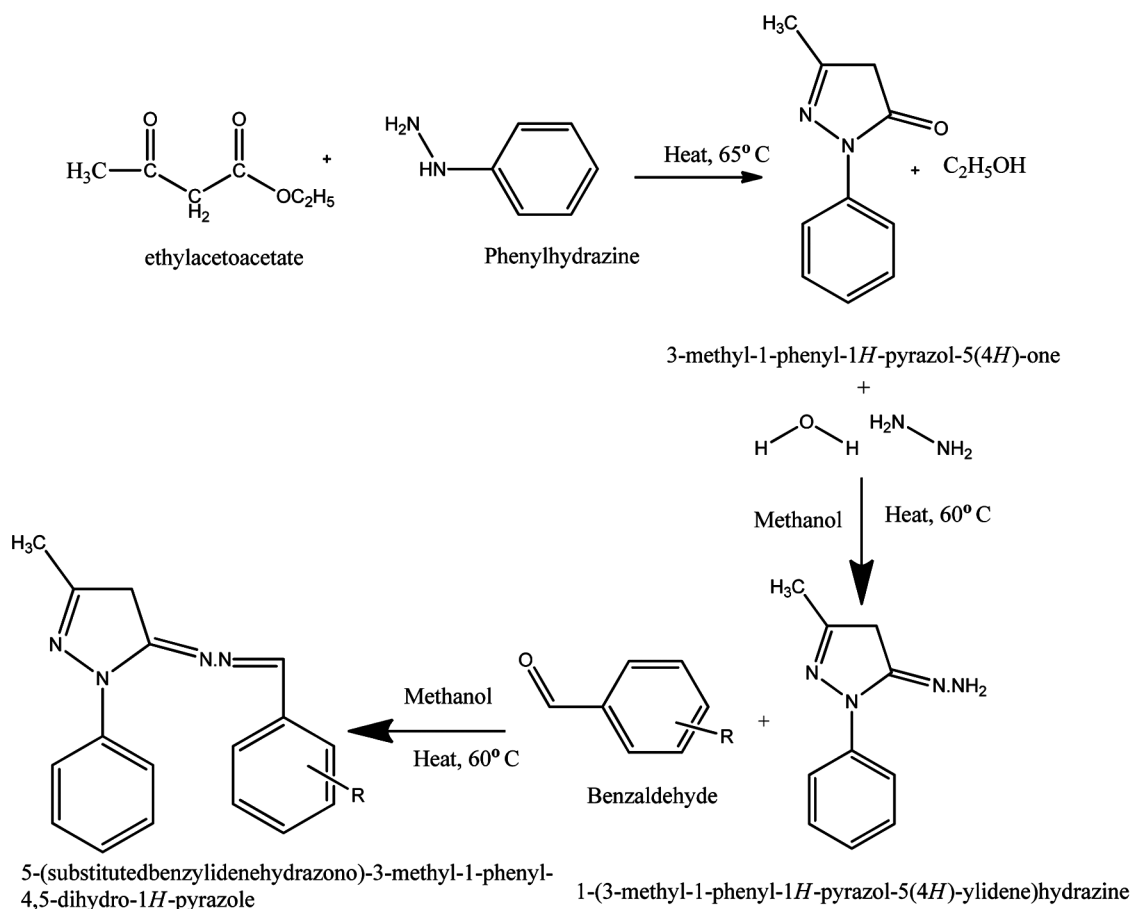Fig. 1 — Structure of pyrazole molecule

Fig. 2 — Root Mean Square Deviation structure of pyrazole molecule

Experimental Section

In this research paper, we discuss the preparation of pyrazole derivatives. In this experimental work many reagents are very costly due to their uses in various type of synthesis. As a result the research work also focused on simplicity to synthesized pyrazole derivatives. In last few years we have showed pyrazole derivatives are being widely used for treatment and mitigation of anxiety. We have synthesized 7 derivatives and 1 initial compound all the derived 7 compounds were found to as our interested area (Fig. 2, Fig. 3). Whenever they are evaluated for their CNS activity they showed minimal to potent activity due to their differ functional groups.

Materials and Methods

All the reagents were procured from Elam Pharma, Shree Ji Pharma international, CDH Ltd., New Delhi

Where R = p-Chlorobenzaldehyde, 4-Bromobenzaldehyde, m-Hydroxybenzaldehyde, m-Nitrobenzaldehyde, p-Dimethylaminobenzaldehyde, 4-Methylbenzaldehyde, 2-Nitrobenzaldehyde

Reagents and conditions: (i) Ethyl acetoacetate (ii) Phenyl hydrazine (iii) Hydrazine hydrate (iv) Different benzaldehydes; stir 70°C, 8-10 h

Fig. 3 — Synthesis of 5-(substitutedbenzylidenehydrazono)-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazole

all the reagents were LR Grade and all the glasswares were procured from Borosil Glass Work Ltd., Ikon Industries., Delhi. The preparation of the compound was started from the chemical reaction b/w Ethyl Acetoacetate and Phenyl Hydrazine. The synthesized compound was further reacted with hydrazine hydrate by using methanol as a solvent. Different Benzaldehydes was used as substituents. The substituted Benzaldehyde was collected from the store room of the University. All the compounds was determine by the ^{1}H NMR (Nuclear Magnetic Resonance), IR and Mass Spectroscopy. Reaction progress was determined by TLC (Thin Layer Chromatography).TLC plate was prepared by using Silica gel G and it was activated for 40 min at 110°C temperature. Solvent system was ethyl acetate: n-hexane (1:1) and spots were analysed in the iodine chamber.

Synthesis of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one

A mixture of phenyl hydrazine (3.65 mL) and ethyl acetoacetate (4.9 mL) was taken in a 250 mL round bottom flask and heated on boiling water bath for 3-4 hours at 65°C. The reaction mixture was stirred continuously with the help of a glass rod. After completion of reaction the reaction mixture was cooled to RT and then 20 mL ether was added with continuous stirring for 5 minute. The solid product was obtained which was filtered and washed with petroleum ether. The product was the recrystallize with dilute ethanol (1:1). The purified product of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one was obtained which was used to continue next step, as shown in Fig. 3 (Table 1)

Synthesis of 1-(3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-ylidene)hydrazine

In a 250 mL RBF 0.01 mol. of 3-methyl-1-Phenyl pyrazole -5-one (0.87gm) was taken in 10 mL of methanol and placed over a water bath and 0.01 mol. an another beaker was taken to prepare a solution

0.4 mL Hydrazine Hydrate (0.4 mL) in 5 mL of Methanol. This mixture was added to the mixture of Hydrazine Hydrate drop by drop. When the reaction was completed the mixture was allowed to get cooled and then washed with petroleum ether. The solid was obtained which was filtered and recrystallized to get the product of 1-(3-methyl-1-phenyl-1*H*-pyrazole)-5(4*H*)-ylidene) hydrazine, as shown in Fig. 3 (Table 1).

Synthesis of 5-(substitutedbenzylidenehydrazono)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazole

1-(3-Methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-ylidene) hydrazine (1.881 g) was taken in 10 mL of 1,4 Dioxan in a 100 mL of Beaker and stirred continuously with the help of magnetic stirrer. 0.01 mol. of substituted Benzaldehydes were taken in 5 mL of 1,4 Dioxan. The mix of benzaldehyde and 1,4 Dioxan was added drop by drop in a solution of -(3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-ylidene) hydrazine and 1,4 dioxan. After completion of reaction the mixture was poured into crushed ice water whereby, precipitate was formed. The precipitate was collected with the help of filter paper and dried, as shown in Fig. 3 (Table 1).

Data for synthesized compounds

5-((4-Chlorobenzylidene)hydrazono)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazole

IR (KBr): 2932 (str, C-H arom), 2953 (str, C-H alip), 1181 (str, C-N arom), 1223 (str, C=N alip), 1628 (str, C=N alip), 1457 cm^{-1} (str, C=C arom); ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.50 (s, 1H, CH$_2$), 8.71 (s, N=CH) 4.95 (s, 1H, N=C-CH$_3$,), 7.20-7.99 (m, 9H, Ar-H (C-H)). Anal. Calcd for C$_{17}$H$_{15}$ClN$_4$ (310.78): C, 65.70; H, 4.86; N, 18.03%.

5-((4-Bromobenzylidene)hydrazono)-3-methyl-1-phenyl-4,5-dihydro-1*H*-pyrazole

IR (KBr): 2951 (str, C-H arom), 2940 (str, C-H alip), 1160 (str, C-N arom), 1218 (str, C=N

Table 1 — Physical and analytical data for synthesized compound

Compd	R	Yield (%)	m.p. (°C)	Mol. Formula	Mol. Wt.	Elemental Analysis		
						C	H	N
AS1	*p*-Chloro benzaldehyde	82	90	C$_{17}$H$_{15}$ClN$_4$	310.78	65.70	4.86	18.03
AS2	4-Bromo benzaldehyde	77	110	C$_{17}$H$_{15}$BrN$_4$	355.23	57.48	4.26	15.77
AS3	*m*-Hydroxy benzaldehyde	84	100	C$_{17}$H$_{16}$N$_4$O	292.34	69.85	5.52	19.17
AS4	*m*-Nitro benzaldehyde	85	120	C$_{17}$H$_{15}$N$_5$O$_2$	321.33	63.54	4.71	21.79
AS5	*p*-Dimethylamino benzaldehyde	78	135	C$_{19}$H$_{21}$N$_5$	319.40	71.45	6.63	21.93
AS6	4-Methyl benzaldehyde	79	134	C$_{18}$H$_{18}$N$_4$	290.36	74.46	6.25	19.30
AS7	2-Nitro benzaldehyde	76	130	C$_{17}$H$_{15}$N$_5$O$_2$	321.33	63.54	4.71	21.79

alip), 1624 (str, C=N alip), 1462 cm^{-1} (str, C=C arom); ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.50 (s, 1H, CH$_2$), 8.70 (s, N=CH) 4.91 (s, 1H, N=C-CH$_3$,), 7.17-7.90 (m, 9H, Ar-H (C-H)). Anal. Calcd for C$_{17}$H$_{15}$BrN$_4$ (355.23): C, 57.48; H, 4.26; N, 15.77%.

3-(((3-Methyl-1-phenyl-1H-pyrazol-5(4H)-ylidene)hydrazono)methyl phenol

IR (KBr): 2937 (str, C-H arom), 2948 (str, C-H alip), 1180 (str, C-N arom), 1241 (str, C=N alip), 1670 (str, C=N alip), 1425 cm^{-1} (str, C=C arom); ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.50 (s, 1H, CH$_2$), 8.71 (s, N=CH) 4.95 (s, 1H, N=C-CH$_3$,), 7.20-7.99 (m, 9H, Ar-H (C-H)), 5.23 (s, Ar-OH). Anal. Calcd for C$_{17}$H$_{16}$N$_4$O (292.34): C, 69.85; H, 5.52; N, 21.79%.

3-Methyl-5-((3-nitrobenzylidene)hydrazono)-1-phenyl-4,5-dihydro-1H-pyrazole

IR (KBr): 2955 (str, C-H arom), 2943 (str, C-H alip), 1162 (str, C-N arom), 1252 (str, C=N alip), 1658 (str, C=N alip), 1458 cm^{-1} (str, C=C arom); ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.50 (s, 1H, CH$_2$), 8.92 (s, N=CH) 4.19 (s, 1H, N=C-CH$_3$,), 7.20-7.92, (m, 9H, Ar-H (C-H)). Anal. Calcd for C$_{17}$H$_{15}$N$_5$O$_2$ (321.33): C, 63.54; H, 4.71; N, 21.79%.

N,N-Dimethyl-4-(((3-methyl-1-phenyl-1H-pyrazol-5(4H)-ylidene)hydrazono)methyl)aniline

IR (KBr): 2937 (str, C-H arom), 2943 (str, C-H aliph), 1156 (str, C-N arom), 1247 (str, C=N alip), 1669 (str, C=N alip), 1464 cm^{-1} (str, C=C arom); ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.50 (s, 1H, CH$_2$), 8.49 (s, N=CH) 4.19 (s, 1H, N=C-CH$_3$,), 7.12-7.96, (m, 9H, Ar-H (C-H)), 3.56, (s, 1H, Ar-N-CH$_3$), 3.12 (s, 1H, Ar-N-CH$_3$). Anal. Calcd for C$_{19}$H$_{21}$ClN$_5$ (319.40): C, 71.45; H, 6.63; N, 21.93%.

3-Methyl-5-((4-methylbenzyylidene)hydrazono-1-phenyl-4,5-dihydro-1H-pyrazole

IR (KBr): 2897 (str, C-H arom), 2925 (str, C-H alip), 1170 (str, C-N arom), 1239 (str, C=N alip), 1665 (str, C=N alip), 1442 cm^{-1} (str, C=C arom); ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.61 (s, 1H, CH$_2$), 8.23 (s, N=CH) 4.25 (s, 1H, N=C-CH$_3$), 7.18-7.76 (m, 9H,Ar-H (C-H)). Anal. Calcd for C$_{18}$H$_{18}$N$_4$ (290.36): C, 74.46; H, 6.25; N, 19.30%.

3-Methyl-5-((2-nitrobenzylidene)hydrazono)-1-phenyl-4,5-dihydro-1H-pyrazole

IR (KBr): 2887 (str, C-H arom), 2932 (str, C-H alip), 1190 (str, C-N arom), 1230 (str, C=N alip),

1648 (str, C=N alip), 1448 cm^{-1} (str, C=C arom); ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.60 (s, 1H, CH$_2$), 8.59 (s, N=CH) 4.29 (s, 1H, N=C-CH$_3$,), 7.22-7.66 (m, 9H, Ar-H (C-H)). Anal. Calcd for C$_{17}$H$_{15}$N$_5$O$_2$ (321.33): C, 63.54; H, 4.71; N, 21.79%.

Docking Studies

The final derivatives **AS1-AS7** were subjected to docking studies to uncover the mechanisms of drug interaction with receptors, utilizing Auto Dock vina software. The target protein structure was sourced from previous research. Energy minimization was performed using the MMFF94 force field, and pdb files were creat to produce stable 3 dimensional structures. The rigid receptor docking protocol was followed, with derivatives being docked into the GABA receptor.

Multiple docking poses with negative binding affinity were observed. Structure elegance was conducted using GBVI/WSA dG. The expected interactions and molecular docking images are presented in Fig. 4, Fig. 5, Fig. 6, Fig. 7, Fig. 8, Fig. 9 and Fig. 10 and the docking scores are provided in Table 2.

These interactions can be analysed using various biochemical and computational methods (Fig. 11, Fig. 12, Fig. 13, Fig. 14, Fig. 15, Fig. 16, Fig. 17).

Computational Studies

Virtual screening for a series of compounds was perform by molecular docking for the prediction of binding affinity of preferred orientation of the compounds and the physicochemical properties (MW, MR, CAA, CMA, CSEV, Ovality, Log P, Number of
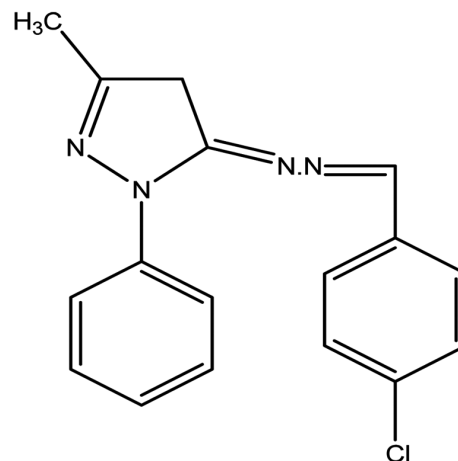

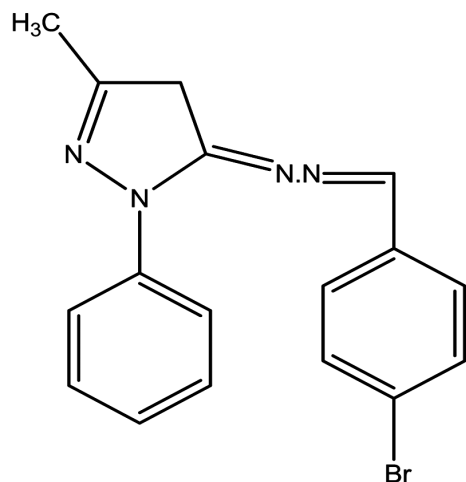Fig. 4 — 5-((4-chlorobenzylidene)hydrazono)-3-methyl-1-phenyl-4,5-dihydro-1H-pyrazole

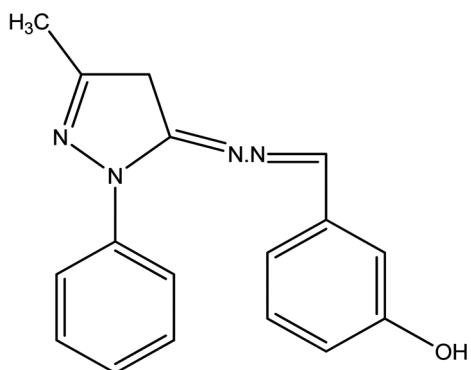Fig. 5 — 5-((4-bromobenzylidene)hydrazono)-3-mehyl-1-phenyl-4,5-dihydro-1*H*-pyrazole

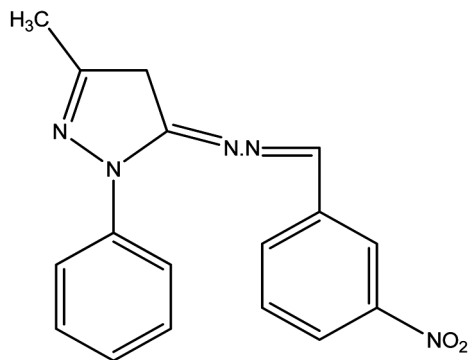Fig. 6 — 3-(((3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-ylidene) hydrazono)methyl phenol

Fig. 7 — 3-methyl-5-((3-nitrobenzylidene)hydrazono)-1-phenyl-4,5-dihydro-1*H*-pyrazole

Fig. 8 — N,N-dimethyl-4-(((3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-ylidene)hydrazono)methyl)aniline

Fig. 9 — 3-methyl-5-((4-methylbenzyylidene)hydrazono-1-phenyl-4,5-dihydro-1*H*-pyrazole

Fig. 10 — 3-methyl-5-((2-nitrobenzylidene)hydrazono-1-phenyl-4,5-dihydro-1*H*-pyrazole

rotable bond, Hydrogen bond, gastrointestinal absorption, synthetic accessibility, Abbott Bioavailability score, Lipinski Filter) of the target compound (AS1-AS7) and standard drug (Diazepam) were calculated using chem 3D Ultra version 12.0 and Swiss ADME free software program (Table 1, Table 3,
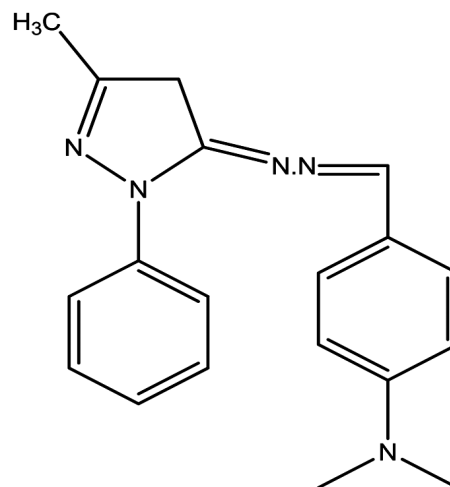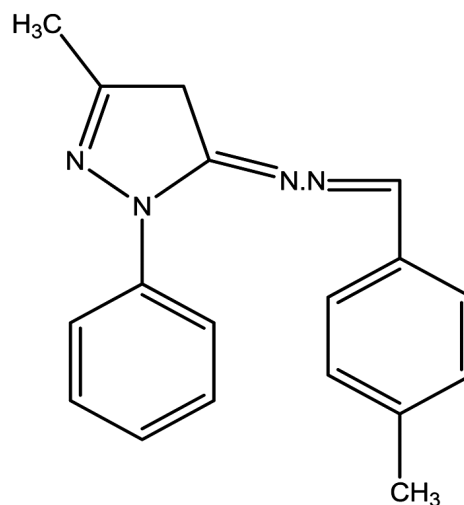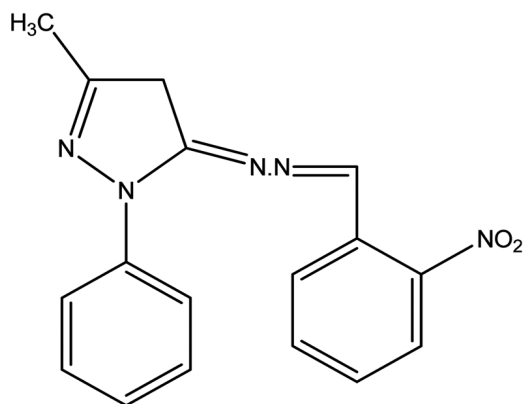
Table 2 — Docking scores of compounds

Compd	Binding affinity (Kcal/mol) against 2z5x
AS1	−9.6
AS2	−7.3
AS3	−8.8
AS4	−7.6
AS5	−7.3
AS6	−7.8
AS7	−9.0

Table 4, Table 5). The comparison of physicochemical characterstics was done and calculated the percentage similarity of target compound with compare to reference drug.

Similarity Calculation

The physical and chemical similarities of all tested compounds, in comparison to the standard drug diazepam, were calculated based on four
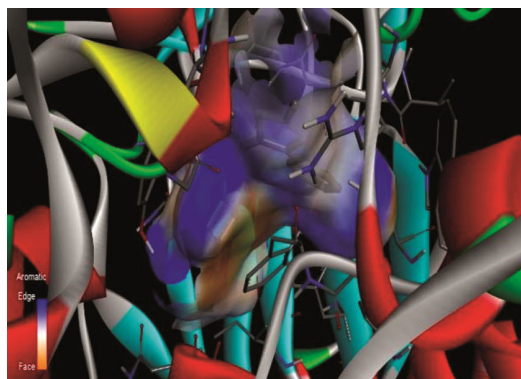

Fig. 11 — (a) The binding and interaction mode of the docked compound AS1 within the HMO structure (PDB ID: 2Z5X). (b) The interactions between compound AS1 and the MAO-A active site involve binding to the enzyme and forming complexes. These interactions can be analyzed using various biochemical and computational methods.
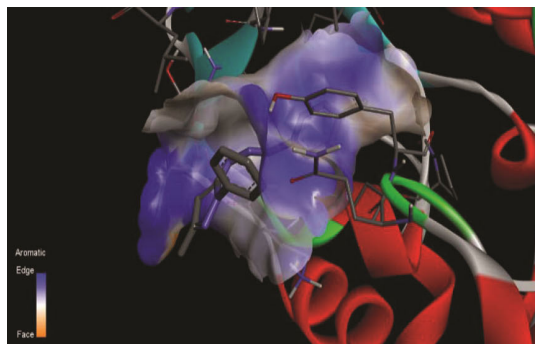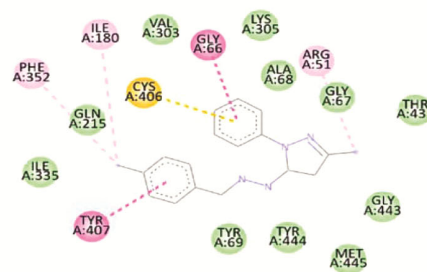

Fig. 12 — (a) The binding and interaction mode of the docked compound AS2 within the HMO structure (PDB ID: 2Z5X). (b) The interactions between compound AS2 and the GABA active site involve binding to the enzyme and forming complexes. These interactions can be analyzed using various biochemical and computational methods.
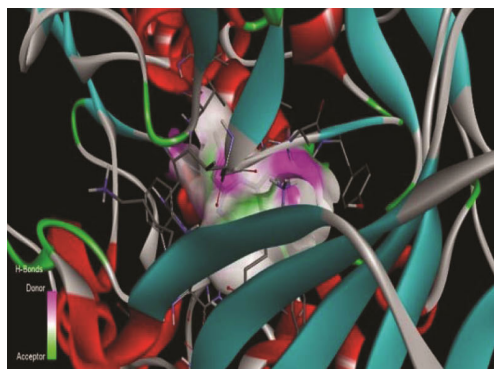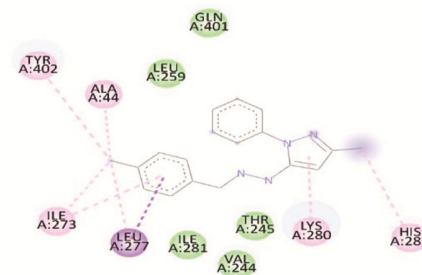

Fig. 13 — (a) The binding and interaction mode of the docked compound AS3 within the HMO structure (PDB ID: 2Z5X). (b) The interactions between compound AS3 and the GABA active site involve binding to the enzyme and forming complexes. These interactions can be analyzed using various biochemical and computational methods.

Fig. 14 — (a) The binding and interaction mode of the docked compound AS4 within the HMO structure (PDB ID: 2Z5X). (b) The interactions between compound AS4 and the GABA active site involve binding to the enzyme and forming complexes. These interactions can be analyzed using various biochemical and computational methods.
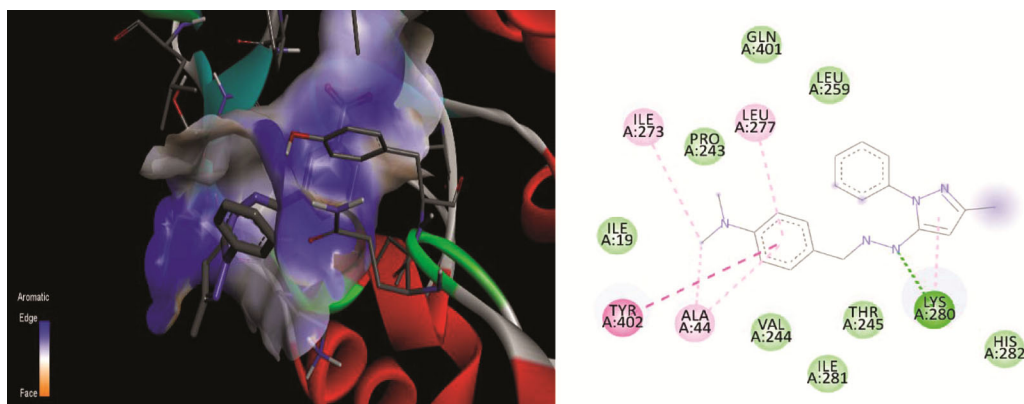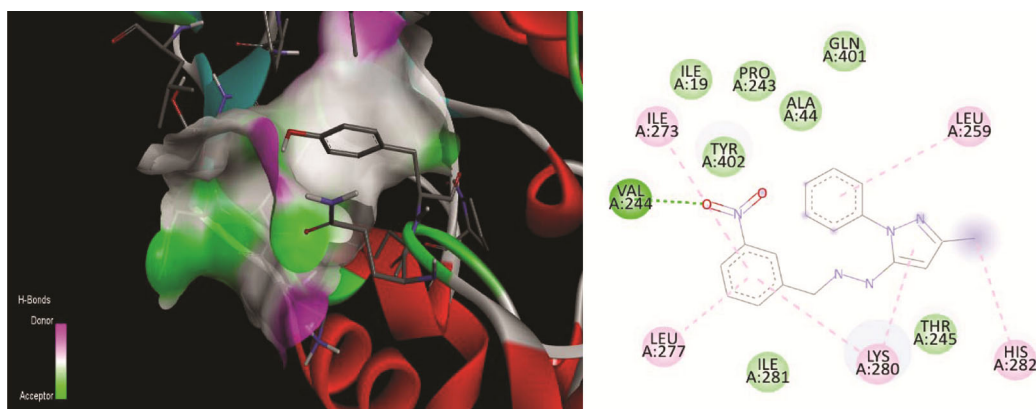

Fig. 15 — (a) The binding and interaction mode of the docked compound AS5 within the HMO structure (PDB ID: 2Z5X). (b) The interactions between compound AS5 and the GABA active site involve binding to the enzyme and forming complexes. These interactions can be analyzed using various biochemical and computational methods.

physicochemical properties using software programs, as shown in Table 6. Initially, the distance di of a specific target compound j to the drug molecules, such as diazepam, was determined using the provided formula.

$$di^2 = \sum_{J=1}^{n} \left(\frac{1 - X_{i,j}}{X_{i,std}} \right)^2$$

Where, $X_{\{i,j\}}$ represents the value of molecular parameters 'i' for compound 'j', and $X_i^{\{STD\}}$ denotes the same molecular parameter for the standard drug, such as Diazepam. The similarity of compound 'j' to the standard drug was then calculated using the formula: Similarity (%) = (1 – R) × 100. Here, (R) is the quadratic mean (root mean square) of (d^2), a measure of central tendency (Fig. 2). The target compounds exhibited good similarity to the standard drug, ranging from 63% to 74%.

Pharmacological Evaluation

The targeted compounds were checked for their pharmacological activity. In which two types of activity was performed. (a)-Skeletal muscle relaxant activity and (b)-Antianxiety Activity in rat by using following model:

➢ Rota rod test

➢ Elevated plus maze method test

Skeletal Muscle Relaxant Activity

Albino rates were weighing 200-250 g using weighing balance and then they are used for activity. The animals were kept in standard conditions and supplied with proper nutrition for a 7-day acclimatization period before the experiment began. Water was provided ad libitum under clean and Sanitary condition. Standard dug was Diazepam.

Rotarod Test for Skeletal muscle relaxant activity

The features of rota rod apparatus, a metal rod with the rubber coating, operating at 25 rotations per minute. Albino rats were positioned on a horizontal wooden rod rotating at the same rotation. Mice that managed to stay on the Rotating rod for 5 minutes during 3 consecutive Attempts were chosen for the test. Albino rats were partitioned into four groups (n = 5). Stock solutions of all test samples and the standard were made using Tween 80 as the suspending agent. Group I distributed as the control group and water. Animals in Group II were given the
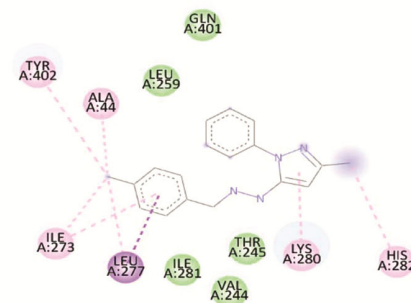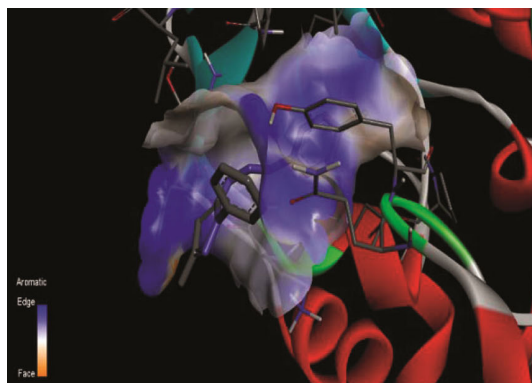

Fig. 16 — (a) The binding and interaction mode of the docked compound AS6 within the HMO structure (PDB ID: 2Z5X). (b) The interactions between compound AS6 and the GABA active site involve binding to the enzyme and forming complexes. These interactions can be analyzed using various biochemical and computational methods.
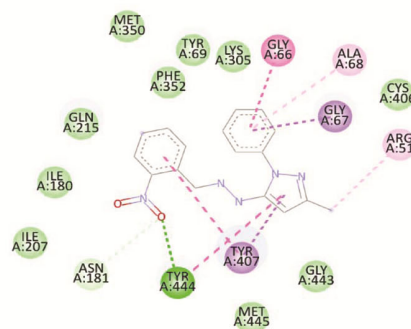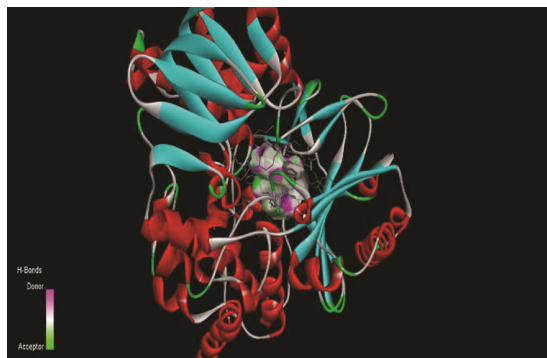

Fig. 17 — (a) The binding and interaction mode of the docked compound AS7 within the HMO structure (PDB ID: 2Z5X). (b) The interactions between compound AS7 and the GABA active site involve binding to the enzyme and forming complexes (Table 3, Table 4, Table 5)

			Table 3 — Drug–likeness properties of derivatives				
Compd	miLogP	Mol. Wt.	TPSA	MR	nON (HBA)	nOHNH(HBD)	CLogP
AS1	3.92	310.78	43.57	99.77	5	0	4.09
AS2	4.63	355.23	40.33	102.46	4	0	4.63
AS3	4.50	292.34	40.33	96.78	4	0	4.36
AS4	4.27	321.33	40.33	103.58	4	0	4.29
AS5	3.31	319.40	60.56	108.97	5	1	3.41
AS6	2.90	290.36	40.33	99.72	4	0	2.21
AS7	3.35	321.33	86.15	103.58	7	0	2.21

				Table 4 — Pharmacokinetic parameter			
Compd	GI abs	BBB permeation	Pgp substrate	CYP2C19	CYP3A4	Lipinski Violation	Ghose Violation	Bioavailability Score
AS1	High	Yes	No	Yes	No	0	Yes	0.55
AS2	High	Yes	No	Yes	No	0	Yes	0.55
AS3	High	Yes	No	Yes	No	0	Yes	0.55
AS4	High	Yes	No	Yes	No	0	Yes	0.55
AS5	High	Yes	No	Yes	No	0	Yes	0.55
AS6	High	Yes	No	Yes	No	0	Yes	0.55
AS7	High	Yes	No	Yes	No	0	Yes	0.55

standard drug Diazepam was administered at a dose of 10 mg/kg Test samples at 10 mg/kg were administered orally to Groups III and IV (Table 7). Each assembly of animals was placed on the rod at 30-minute intervals. Animals who were failed to stay on the rod for more than one minute in more than one trial were considered to have passed the test[20-26], as shown in Table 7.

Anxiety-relieving effect

The albino rats, each weighing between 200-250 grams, were weighed using a balance before being utilized in the experiment. They were maintained under standard conditions and given adequate nutrition for a 7-day acclimatization period before the experiment commenced. Clean and hygienic conditions ensured that water was available to them at all times. Diazepam was used as the standard drug.

Table 5 — PASS–study of compounds (AS1-AS7)

Compd	Pharmacological Activity	Activity Value
AS1	Anxiolytic	0.562
AS2	Anxiolytic	0.581
AS3	Anxiolytic	0.533
AS4	Anxiolytic	0.577
AS5	Anxiolytic	0.541
AS6	Anxiolytic	0.558
AS7	Anxiolytic	0.528

Table 6 — Similarity for all compounds with respect to standard drug (Diazepam)

Compd	Similarity[a,b] (in%) to Diazepam
Diazepam (standard drug)	74
AS1	67
AS2	64
AS3	61
AS4	70
AS5	63
AS6	66
AS7	71

Plus maze method for Anti-anxiety activity

Albino rats weighing 200-250 g were Chosen from the stock colony of the animal facility and given free availability to food and water. The albino rats were kept in an air-conditioned room at $25 \pm 20°C$ within pure daylight. All compounds (10 mg/kg) was freshly prepared as a suspension in 1% Tween 80. Solutions were recently prepared on the day of test and administered orally at volume of 0.25 mL/200-250 g weight of body of the albino rats. The testing animals received diazepam (10 mg/kg) and the testing compounds (10 mg/kg), 60 minutes ahead of maze evaluation. The control group was received water. The plus maze apparatus featured two open arms (16×5 cm²) and two closed arms ($16 \times 5 \times 12$ cm³) facing each other with an open roof area, raised to a height of 25 cm, the test group mice were individually monitored for 5 minutes in the apparatus. Each mouse was placed on the central platform facing an open arm. The number of entries into open and closed arms and the time spent in open arms were recorded during the 5-minute observation (Table 8). The percentage of entries into open arms [(open/open + closed)×100] was calculated for each mouse[27-31], as shown in Table 8.

Statistical Analysis

The expected value ± SEM was determined for every parameter, and statistical analysis was conducted using ANOVA. A highly substantial difference was noted between control and treated clusters, with (***$p<0.001$, **$p<0.01$, *$p<0.05$).

Results and Discussion

Pyrazole derivatives were synthesized using many substituted Benzaldehydes. Firstly ethyl acetoacetate was reacted with phenyl hydrazine in the existence of methanol as solvent, compound A (3-methyl-1-phenyl-1*H*-pyrazole-5(4*H*)-one) was formed. This

Table 7 — The drug's impact on the duration spent on the rota rod was evaluated

Groups	Dose (mg/kg)	Before Drug Add (sec)	30 m after drug Add (sec)	Change in activity (%)
Control	Blank	212±6.23	213±7.24	0.5%
Diazepam	10	209±7.24	36±1.45	85.2%
AS1	10	201±6.45	92.5±7.23*	58.2%
AS2	10	203±5.34	92.33±6.34	57.2%
AS3	10	215±7.54	33±1.25**	70.3%
AS4	10	217±3.43	92.13±6.34	56.1%
AS5	10	219±4.65	113.5±4.23	50.7%
AS6	10	202±5.45	31±0.25**	68.5%
AS7	10	218±6.25	106.6±9.01	50.7%

Mean ± SEM of six animals per group. Statistical significance compared to the standard: ***$p<0.001$, **$p<0.01$, *$p<0.05$.

Table 8 — Antianxiety activity of synthesized compounds

Groups	Dose (mg/kg)	No. of input in open arms	The average duration spent in the open arms (sec)
Control	Blank	2.45±0.23	9.44±0.14
Diazepam	10	12.09±1.24	30.22±0.55
AS1	10	21.20±0.23*	48.32±0.50
AS2	10	24.49±0.43	53.18±0.90
AS3	10	13.21±0.24**	58.21±0.12
AS4	10	29.79±0.13	46.8±0.30
AS5	10	32.12±1.13	121.98±0.70
AS6	10	12.61±0.54**	60.21±0.22
AS7	10	42.39±0.33	147.98±1.70

Mean ± SEM of six animals in each group. Statistical significance *versus* standard (***$p<0.001$, **$p<0.01$, *$p< 0.05$)

compound (A) is further reacted with hydrazine hydrate in the existence of methanol, a compound B (1-(3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-ylidene) hydrazine) was formed. This compound is further treated with different Benzaldehydes as substituents. The % yield of the compounds was almost in high amount. All the prepared compounds (AS1-AS7) were clarified by recrystallization using ethanol. The reaction processing of all compounds were determined by thin layer chromatography. The structure of all the compounds were detected by using IR and ^{1}H NMR spectroscopy and the molecular weight of the prepared compounds was determined by mass spectroscopy. The activities of all the synthesized compounds were checked for the skeletal muscle relaxant activity and anti-anxiety activity. Most of the compounds have showed significant interval between the control and treated group. The data shows that most of the compounds possess good skeletal muscle relaxant and anti-anxiety property.

Conclusion

A number of pyrazole derivatives were prepared by using starting material ethyl acetate and phenyl hydrazine, all the synthesized derivatives were identify by many spectral analysis. Physical properties of all synthesized derivatives were determined by experimental work, chemdraw and some other online software. After the identification all the derivatives were checked for skeletal muscle relaxant property and antianxiety property. Most of the compounds were showed potent activity, when they were compared to standard drug Diazepam. The purpose of this research paper (synthesis and pharmacological evaluation of pyrazole derivatives), could be used as template for the upcoming development through modification or derivatization to design more potent therapeutic agents.

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