SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIDIABETIC ACTIVITY OF NOVEL PYRAZOLINE FUSED INDOLE DERIVATIVES

ABSTRACT

Aim: The primary purpose of this research work is to synthesize, characterize and biological evaluation of novel pyrazoline fused indole derivatives lead to creating a new molecular frame work.
Methodology: In the present study, the new series of novel pyrazoline fused indole derivatives were synthesized from from indole and substituted acetophenone by the 4 step process. In the first step indole and dimethyl formamide were coupled by using phosphorous oxychloride and NaOH to prepare the compound 1 Indole-3-aldehyde. In the second step compound 1 was condensed with substituted aetophenone to synthesis the compound 2 chalcones (a-h). In the third step chalcones 2(a-h) were coupled with semicarbazide or thiosemicarbazide to synthesis the compound 3(a-p). In the final step compound 3(a-p) were coupled with indole-3-aldehyde to prepare the final product of R-substitutedN-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide and R-substitutedN-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide 4(a-p).
Results: The chemical structures of the synthesized compounds were characterized by means of IR, Mass and NMR spectroscopy. The compounds were screened for anti-diabetic activity by In-vitro and In-vivo methods. In In-vivo method 4a, 4m have exhibited moderate anti-diabetic activity as that of standard drug, glibenclamide. In In-vitro method 4a, 4e & 4m have shows moderate anti-diabetic activity as that of reference standard, acarbose.
Conclusion: The synthesized novel pyrazoline fused indole derivatives have moderate antidiabetic activity as that of standard drug by In-vitro and In-vivo methods. These compounds can be further exploited to get the potent lead compound.

Keywords: Pyrazoline, Indole, Indole-3-aldehyde and antidiabetic activity

1. INTRODUCTION

Pyrazolines:

Pyrazolines are the reduced form of the pyrazole moiety having one endocyclic double bond. There are 3 isomers of pyrazoline moiety based on the position of the endocyclic double bond. They are the one of the most studied group among the azole family. Pyrazolines have been proved to possess analgesic, anti-inflammatory, antipyretic, antidiabetic, antibacterial, antifungal, antitubercular, anti parasitic, insecticidal, cytotoxic activities.[1] Many methods have been described in the literature for the synthesis of substituted pyrazolines. Some of the methods are synthesis from chalcones, from aryl hydrazines by reacting with 3-butynol, from alkyl dihalides by reacting with primary amine[2], from cyclocondensation of α, β-unsaturated carbonyl compounds with hydrazine or arylhydrazines[3] and subsequent dehydrogenation. In order to develop an efficient synthetic approach to the various 3 and 5 substituted-1H-pyrazolines. Although these methods were found many applications in synthesizing novel pyrazolines, there is still a great deal of work remaining to enable the development of efficient protocols for structurally different

compounds and to make these reactions more practical by using inexpensive and easily available starting materials.

Indole:

Indole is also known as benzopyrrole which contains benzenoid nucleus and has 10 π-electrons (two from lone pair on nitrogen and double bonds provide eight electrons) which makes them aromatic in nature. Similar to the benzene ring, electrophilic substitution occurs readily on indole due to excessive π-electrons delocalization[4]. Indole is an important heterocyclic system that provides the skeleton to lysergic acid diethylamide (LSD), strychnine and alkaloid obtained from plants. Physically, they are crystalline colorless in nature with specific odors. The addition of the indole nucleus to medicinal compounds that is biologically active pharmacophore made it an important heterocyclic compound having broad-spectrum biological activities[5]. Due to this, researchers took interest to synthesize various scaffolds of indole for screening different pharmacological activities. Various natural compounds contain indole as parent nucleus for example tryptophan. Indole-3-acetic acid is a plant hormone produced by the degradation of tryptophan in higher plants. Derivatives of indole are of wide interest because of their diverse biological and clinical applications. Here, we have tried to summarize the important pharmacological activity of indole derivatives[6]. Indole derivatives possess various biological activities, i.e., antiviral[7], anti-inflammatory[8], anticancer[9], antiHIV[10], antioxidant[11], antimicrobial[12], antitubercular[13], antidiabetic[14], antimalarial[15], anticholinesterase activities[16], etc.

Many methods have been described in the literature for the synthesis of substituted indoles. Some of the methods are synthesis from Fischer indole synthesis[17], aryl bromides and allyl alcohols[18], *N*-nitrosoanilines with alkynes[19], *o*-bromonitrobenzenes with various vinyl Grignard reagents[20] and *o*-nitrobenzyl cyanides with boronic acids[21]. Although these methods were found many applications in synthesizing novel indoles, there is still a great deal of work remaining to enable the development of efficient protocols for structurally different compounds and to make these reactions more practical by using inexpensive and easily available starting materials.

2. MATERIAL AND METHODS

All the required chemicals used were obtained from Aldrich and Sd-fine chemicals. All the solvents used were of laboratory grade. Each reaction was monitored by TLC by using appropriate solvent system, which was selected by trial and error method. Precoated TLC plates (0.25mm silica gel) were obtained from E. Merck. All the synthesized compounds were purified by recrystallization. Melting points were determined on Fisher Johns melting point apparatus and they were uncorrected. All the H^1- NMR spectra were recorded on sophisticated multinuclear FT-NMR spectrometer model Avance-II (Bruker) using $CDCl_3$ and DMSO-d_6 as solvents, tetra methyl silane (TMS) as internal standard.

Mass spectra of the compounds were recorded on Mass spectrometer model Agilent 1100 series, and the method used is ESI method. And they were reported in m/z value as molecular ion peak. IR spectra were recorded on Nexus 670 FTIR thermonicolet instrument by KBr disc method.

The final compounds were synthesized as given below:

Scheme:

Step 1:- Synthesis of Indole-3-aldehyde

Step 2:- Synthesis of R-substituted 3-(1H-indol-3-yl)-1-phenylprop-2-en-1-one

Step 3:- Synthesis of R-substituted 5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide and R-substituted 5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide

Step 4:- Synthesis of R-substituted N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide and R-substituted N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide

Step 1:-

Synthesis of Indole-3-aldehyde: In three-necked RBF add 288ml (274 g., 3.74 moles) of freshly distilled dimethylformamide and contents are cooled in an ice-salt bath for about 30 min, and 86 ml. (144 g., 0.94 mole) of freshly distilled $POCl_3$ is subsequently added with stirring to the dimethylformamide over a period of 30 min. Then added a solution of 100 g. (0.85 mole) of indole in 100 ml. (95 g., 1.3 moles) of dimethylformamide to the yellow solution over a period of 1 hour during which time the temperature should not rise above 10°C. The syrup is stirred efficiently at this temperature for 1 hour. At the end of the reaction period, 300 g. of crushed ice is added. This solution is transferred with 100 ml. of water to a three-necked flask containing 200 g. of crushed ice and fitted with an efficient mechanical stirrer and separatory funnel containing a solution of 375 g. (9.4 moles) of NaOH in 1l of water. The aqueous base is added dropwise with stirring until about one-third of it has been added. The remaining two-thirds is added rapidly with efficient stirring and the resulting suspension is heated rapidly to the boiling point and allowed to cool to room temperature, after which it is placed in a refrigerator overnight. The precipitate is collected on a filter and resuspended in 1 l. of water. Most of the inorganic material dissolves, and the product is then collected on a filter, washed with three 300-ml. portions of water and air-dried, to get indole-3-aldehyde, recrystallized from ethanol if desired.

Step 2:-

Synthesis of R-substituted 3-(1H-indol-3-yl)-1-phenylprop-2-en-1-one: In a 500ml of bolt head flask take a solution of 22gm of sodium hydroxide in 200ml of water and 100gm (122.5ml) rectified spirit. Immerse the flask in a bath of a crushed ice, then add (0.43mol) of freshly distilled acetophenone, start the stir and then add (0.43mol) of pure Indole-3-carboxaldehyde. Keep the temperature of the mixture at about 25°C (limits are 15 -30°C) and stir vigorously until the mixture is so thick that stirring is no longer effective (2-3hours). Remove the stirrer and leave the reaction mixture in an ice-chest or refrigerator overnight. Filter the product with suction on a Buchner funnel or a sintered glass funnel and wash with cold water until the washings are neutral to litmus and then with 20ml of ice-cold rectified spirit. Recrystallise from rectified spirit warmed to 50°C (about 5ml per gram).

Step 3:-

Synthesis of R-substituted 5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide and R-substituted 5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide: To the solution of chalcone derivatives (0.01mol) and semicarbazide or thiosemicarbazide (0.012mol) in 25 ml of ethanol, a solution of sodium hydroxide (0.025mol) in 5ml of water was added and refluxed for 8 hour. The products were poured into crushed ice and the solid mass which separated out was filtered dried and recrystallized from appropriate solvents.

Step 4:-

Synthesis of R-substituted N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide and R-substituted N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide: To the solution of pyrazole derivatives (0.01mol) and Indole-3-carboxaldehyde (0.01mol) in 25 ml of ethanol and add 2-5ml of glacial acetic acid and refluxed for 2 hour. The products were poured into crushed ice and the solid mass which separated out was filtered dried and recrystallized from appropriate solvents.

Anti-diabetic activity:

In vivo anti-diabetic activity:

Experimental Animals: Male Wistar rats (170–220 g) were used to study the antidiabetic activity. Animals were housed in standard laboratory conditions (temperature 22 ±2^{0}C and humidity 45 ± 5^{O}C with 12h day: 12h night cycle).

Acute toxicity studies:

The acute toxicity study of synthesized compounds was performed as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The synthesized compounds were administrated to rats by gavage using a stomach tube in increasing dose levels of 100, 500 and 1000 mg/kg b.wt, respectively[22]. All animals were observed for gross behavioral, neurological, autonomic and toxic effects at short intervals of time for 5 h after administration and then for next 24 h. Food consumption and body weights were recorded daily for 21 days.

Induction of diabetes:

Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg b.wt), freshly prepared in 0.1M sodium citrate buffer (pH 4.5) after overnight fasting[23]. Animals were fed with 5% glucose solution for 12 h to avoid hypoglycaemia. On the 4th day of STZ administration, blood glucose level was measured through Glucometer and the rats with moderate diabetes, having hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic and were employed in the study.

Experimental design:

A total of 30 rats (6 normal; 24 STZ-diabetic rats) were assigned to the study. The rats were randomly divided into five groups of six animals each. Group 1: Normal control rats (NC) received vehicle only (1% CMC; 2 ml/kg b.wt), Group 2: Diabetic control rats (DC), Group 3 and 4: Diabetic rats received synthesized compounds 4a and 4g at the dose of 50 mg/kg b.wt, respectively, Group 5: Positive control received a reference standard drug Glibenclamide (5 mg/kg b.wt). All treatments were given orally after

the 4th day of STZ administration (except normal control) for 21 days. The body weight was recorded initially and after the end of the treatment. Blood was withdrawn from the tail vein each time blood glucose level was measured by Glucometer (one touch, Johnson & Johnson) on 0, 7, 14 and 21 day of the study.

Estimation of blood glucose and glycosylated hemoglobin (HbA1c):

After the completion of treatment, the rats were fasted overnight and blood samples for fasting blood glucose and glycosylated hemoglobin (HbA1c) were obtained from the tail vein under mild ether anesthesia. Fasting blood glucose was measured by Glucometer (one touch, Johnson & Johnson) and glycosylated hemoglobin (HbA1c) was estimated using the method of Nayak and Pattabiraman[24].

In vitro anti-diabetic activity:

In vitro α-amylase inhibition assay: The α-amylase inhibitory activity was determined by using soluble starch as a substrate in a colorimetric reaction by the method of Bernfield [23]. α-amylase was dissolved in phosphate buffer saline (PBS, 0.02 mol/L, pH 6.8) at a concentration of 0.1 mg/mL. Various concentrations of sample solutions (0.25 mL) were mixed with α-amylase solution (0.25 mL) and incubated at 37 °C for 5 min. Then the reaction was initiated by adding 0.5 mL 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37 °C for 3 min, the reaction was stopped by adding 0.5 mL DNS reagent (1% Dinitrosalicylic acid, 0.05% Na_2SO_3 and 1% NaOH solution) to the reaction mixture and boiling at 100 °C for 5 min. After cooling to room temperature, the absorbance (Abs) at 540 nm was recorded by a spectrophotometer[25].

In vitro α- glucosidase inhibition assay:

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of control (Acarbose std. drug) and the synthesized compound(s) for 5 min at 37°C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1µg/ml) to it followed by incubation for 40 min at 35°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of the colour was measured at 540nm[25].

The percentage inhibition of α-amylase and α-glucosidase was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

3. RESULTS AND DISCUSSION

The synthesis and biological evaluation of novel pyrazoline fused indole derivatives lead to creating a new molecular frame work. Sixteen novel pyrazoline fused indole derivatives from indole and substituted acetophenone were prepared by following steps. In the first step indole and dimethyl formamide were coupled by using phosphorous oxychloride and NaOH to prepare the compound 1. In the second step compound 1 was condensed with substituted aetophenone to synthesis the compound 2(a-h). In the third step chalcones 2(a-h) were coupled with semicarbazide or thiosemicarbazide to synthesis the compound 3(a-p). In the final step compound 3(a-p) were coupled with indole-3-aldehyde to prepare the final product

of R-substitutedN-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide and R-substitutedN-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide 4(a-p).

SCHEME:

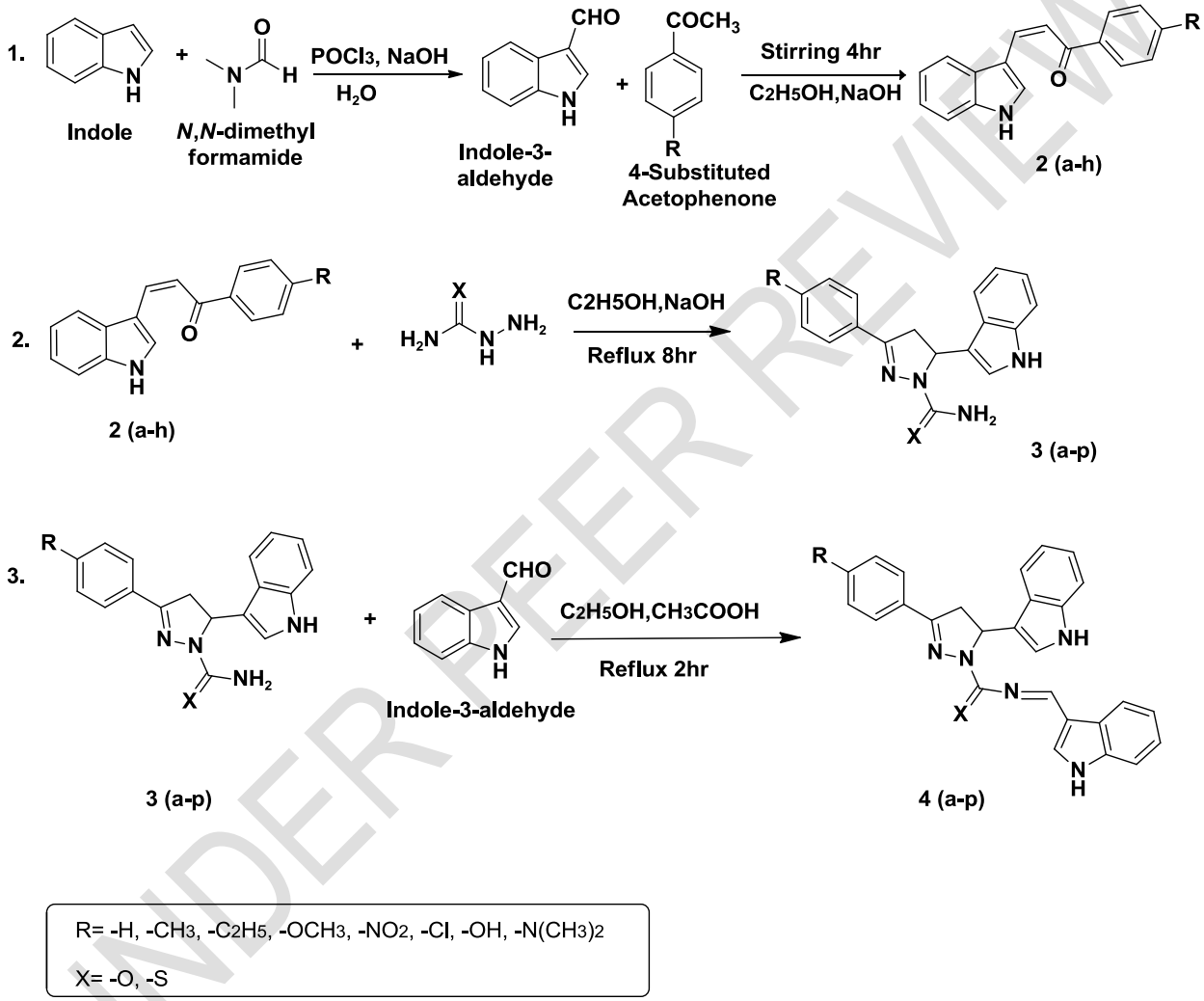

Table-1. List of synthesized compounds 4 (a-p):

Comp	-X	-R	IUPAC Name
4a	-O	-H	N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide
4b	-O	-CH_3	N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-(p-tolyl)-4,5-dihydro-

			1H-pyrazole-1-carboxamide
4c	-O	$-C_2H_5$	N-((1H-indol-3-yl)methylene)-3-(4-ethylphenyl)-5-(1H-indol-3-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide
4d	-O	$-OCH_3$	N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide
4e	-O	$-NO_2$	N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide
4f	-O	-Cl	N-((1H-indol-3-yl)methylene)-3-(4-chlorophenyl)-5-(1H-indol-3-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide
4g	-O	-OH	N-((1H-indol-3-yl)methylene)-3-(4-hydroxyphenyl)-5-(1H-indol-3-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide
4h	-O	$-N(CH_3)_2$	N-((1H-indol-3-yl)methylene)-3-(4-(dimethylamino)phenyl)-5-(1H-indol-3-yl)-4,5-dihydro-1H-pyrazole-1-carboxamide
4i	-S	-H	N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide
4j	-S	$-CH_3$	N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide
4k	-S	$-C_2H_5$	N-((1H-indol-3-yl)methylene)-3-(4-ethylphenyl)-5-(1H-indol-3-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide
4l	-S	$-OCH_3$	N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide
4m	-S	$-NO_2$	N-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide
4n	-S	-Cl	N-((1H-indol-3-yl)methylene)-3-(4-chlorophenyl)-5-(1H-indol-3-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide
4o	-S	-OH	N-((1H-indol-3-yl)methylene)-3-(4-hydroxyphenyl)-5-(1H-indol-3-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide
4p	-S	$-N(CH_3)_2$	N-((1H-indol-3-yl)methylene)-3-(4-(dimethylamino)phenyl)-5-(1H-indol-3-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide

Table-2. Physical data of R-substitutedN-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamideandN-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide

Comp	-X	-R	Mol formula	Mol.wt	M.P (^{0}C)	Yield (%)

4a	-O	-H	$C_{27}H_{21}N_5O$	431	212-214	92
4b	-O	$-CH_3$	$C_{28}H_{23}N_5O$	445	204-206	89
4c	-O	$-C_2H_5$	$C_{29}H_{25}N_5O$	459	225-228	90
4d	-O	$-OCH_3$	$C_{28}H_{23}N_5O_2$	461	247-249	93
4e	-O	$-NO_2$	$C_{27}H_{20}N_6O3$	476	229-231	88
4f	-O	-Cl	$C_{27}H_{20}ClN_5O$	465	236-239	86
4g	-O	-OH	$C_{27}H_{21}N_5O_2$	447	221-223	82
4h	-O	$-N(CH_3)_2$	$C_{29}H_{26}N_6O$	474	224-246	84
4i	-S	-H	$C_{27}H_{21}N_5S$	447	239-242	91
4j	-S	$-CH_3$	$C_{28}H_{23}N_5S$	461	248-251	86
4k	-S	$-C_2H_5$	$C_{29}H_{25}N_5S$	475	237-239	88
4l	-S	$-OCH_3$	$C_{28}H_{23}N_5OS$	477	242-244	81
4m	-S	$-NO_2$	$C_{27}H_{20}N_6O_2S$	492	245-247	82
4n	-S	-Cl	$C_{27}H_{20}ClN_5S$	482	236-238	80
4o	-S	-OH	$C_{27}H_{21}N_5OS$	463	239-242	85
4p	-S	$-N(CH3)_2$	$C_{29}H_{26}N_6S$	490	242-245	87

Table-3. Spectral data of R-substitutedN-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamideandN-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide

Comp	-X	-R	IR Spectra	Mass spectra(m/z)	^{1}H NMR spectra (DMSO)
4a	-O	-H	N-H peak at 3350cm^{-1},C=O peak at 1580cm^{-1}, Ar C-H peak at 3150cm^{-1}, C=N peak at 1670cm^{-1}, C-N peak at 1560cm^{-1}, N-N peak at 1385cm^{-1}	432 (M+1)	δ 9.91 (d, *J* = 6.8 Hz, 1H), 9.41 (s, 1H), 8.21 – 8.16 (m, 1H), 7.86 (d, *J* = 6.9 Hz, 1H), 7.67 – 7.61 (m, 2H), 7.50 – 7.34 (m, 5H), 7.31 – 7.25 (m, 1H), 6.30 (dddt, *J* = 10.7, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.3, 10.2, 8.2, 1.7, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.1, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.33 (td, *J* = 5.7, 1.7 Hz, 1H), 4.59 – 4.53 (m, 1H), 4.24 (tddd, *J* = 7.0, 4.4, 1.8, 0.9 Hz, 1H), 3.62 (dd, *J* = 13.6, 5.7 Hz, 1H), 3.37 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.10 – 3.02 (m, 1H)
4b	-O	$-CH_3$	N-H peak at 3400cm^{-1},C=O peak at 1560cm^{-1}, Ar C-H peak at	446 (M+1)	δ 9.91 (d, *J* = 6.8 Hz, 1H), 9.41 (s, 1H), 8.21 – 8.16 (m, 1H), 7.86 (d, *J* = 6.9 Hz, 1H), 7.58 – 7.52 (m, 2H),

			3250cm^{-1}, methyl C-H peak at 2800 cm^{-1}, C=N peak at 1630cm^{-1}, C-N peak at 1550cm^{-1}, N-N peak at 1371cm^{-1}		7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.28 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 7.19 (dq, *J* = 7.9, 0.8 Hz, 2H), 6.30 (dddt, *J* = 10.7, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.3, 10.2, 8.2, 1.7, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.1, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.33 (td, *J* = 5.7, 1.7 Hz, 1H), 4.59 – 4.53 (m, 1H), 4.28 – 4.20 (m, 1H), 3.62 (dd, *J* = 13.6, 5.7 Hz, 1H), 3.37 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.06 (dddt, *J* = 8.3, 5.5, 2.9, 1.7 Hz, 1H)
4c	-O	-C_2H_5	N-H peak at 3406cm^{-1},C=O peak at 1542cm^{-1}, Ar C-H peak at 3031cm^{-1}, ethyl C-H peak at 2950 cm^{-1}, C=N peak at 1700cm^{-1}, C-N peak at 1580cm^{-1}, N-N peak at 1368cm^{-1}	460 (M+1)	δ 9.91 (d, *J* = 6.8 Hz, 1H), 9.41 (s, 1H), 8.21 – 8.16 (m, 1H), 7.86 (d, *J* = 6.9 Hz, 1H), 7.58 – 7.52 (m, 2H), 7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.24 (m, 3H), 6.30 (dddt, *J* = 10.7, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.3, 10.2, 8.2, 1.7, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.1, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.33 (td, *J* = 5.7, 1.7 Hz, 1H), 4.59 – 4.53 (m, 1H), 4.24 (ddddd, *J* = 7.0, 6.0, 4.4, 1.8, 0.9 Hz, 1H), 3.62 (dd, *J* = 13.6, 5.7 Hz, 1H), 3.37 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.10 – 3.02 (m, 1H), 2.69 (qt, *J* = 7.3, 0.9 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 3H)
4d	-O	-OCH_3	N-H peak at 3300cm^{-1},C=O peak at 1580cm^{-1}, Ar C-H peak at 3250cm^{-1}, methyl C-H peak at 2850 cm^{-1}, C-O peak at 1243 cm^{-1}, C=N peak at 1680cm^{-1}, C-N peak at 1596cm^{-1}, N-N peak at 1385cm^{-1}	462 (M+1)	δ 9.91 (d, *J* = 6.8 Hz, 1H), 9.41 (s, 1H), 8.21 – 8.16 (m, 1H), 7.86 (d, *J* = 6.9 Hz, 1H), 7.65 – 7.59 (m, 2H), 7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.25 (m, 1H), 6.95 – 6.89 (m, 2H), 6.30 (dddt, *J* = 10.7, 8.1, 1.9, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.2, 10.3, 8.2, 1.7, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.1, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.33 (td, *J* = 5.7, 1.7 Hz, 1H), 4.59 – 4.53 (m, 1H), 4.24 (ddddd, *J* = 7.0, 6.0, 4.4, 1.8, 0.9 Hz, 1H), 3.80 (s, 2H), 3.62 (dd, *J* = 13.6, 5.7 Hz, 1H), 3.37 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.06 (dddt, *J* = 7.3, 4.7, 1.8, 1.1 Hz, 1H)
4e	-O	-NO_2	N-H peak at 3350cm^{-1},C=O peak at 1530cm^{-1}, Ar C-H peak at 3031cm^{-1}, C=N peak at 1730cm^{-1}, C-N peak at 1570cm^{-1}, N-O peak at 1550 cm^{-1}, N-N peak at 1379cm^{-1}	477 (M+1)	δ 9.91 (d, *J* = 6.8 Hz, 1H), 9.41 (s, 1H), 8.21 – 8.15 (m, 3H), 7.90 – 7.83 (m, 3H), 7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.25 (m, 1H), 6.30 (dddt, *J* = 10.6, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.3, 10.2, 8.2, 1.7, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.1, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H),

					5.33 (td, *J* = 5.7, 1.7 Hz, 1H), 4.59 – 4.53 (m, 1H), 4.24 (ddddd, *J* = 7.9, 6.0, 4.4, 1.8, 0.9 Hz, 1H), 3.62 (dd, *J* = 13.6, 5.7 Hz, 1H), 3.37 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.10 – 3.02 (m, 1H)
4f	-O	-Cl	N-H peak at 3400cm^{-1},C=O peak at 1570cm^{-1}, Ar C-H peak at 3100cm^{-1}, C=N peak at 1700cm^{-1}, C-N peak at 1596cm^{-1}, C-Cl peak at 850 cm^{-1},N-N peak at 1385cm^{-1}	466 (M+1)	δ 9.91 (d, *J* = 6.8 Hz, 1H), 9.41 (s, 1H), 8.21 – 8.16 (m, 1H), 7.86 (d, *J* = 6.9 Hz, 1H), 7.63 – 7.57 (m, 2H), 7.50 – 7.44 (m, 1H), 7.44 – 7.38 (m, 2H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.25 (m, 1H), 6.30 (dddt, *J* = 10.7, 8.1, 1.9, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.3, 10.2, 8.2, 1.7, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.1, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.33 (td, *J* = 5.7, 1.7 Hz, 1H), 4.59 – 4.53 (m, 1H), 4.28 – 4.20 (m, 1H), 3.62 (dd, *J* = 13.6, 5.7 Hz, 1H), 3.37 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.10 – 3.02 (m, 1H)
4g	-O	-OH	N-H peak at 3450cm^{-1}, OH peak at 3200 cm^{-1}, C=O peak at 1600cm^{-1}, Ar C-H peak at 3050cm^{-1}, C=N peak at 1750cm^{-1}, C-N peak at 1520cm^{-1}, N-N peak at 1368cm^{-1}	448 (M+1)	δ 9.91 (d, *J* = 6.8 Hz, 1H), 9.41 (s, 1H), 8.21 – 8.16 (m, 1H), 7.86 (d, *J* = 6.9 Hz, 1H), 7.61 – 7.55 (m, 2H), 7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.28 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 6.88 – 6.82 (m, 2H), 6.30 (dddt, *J* = 10.7, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.2, 10.3, 8.2, 1.7, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.0, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.33 (td, *J* = 5.7, 1.7 Hz, 1H), 4.59 – 4.53 (m, 1H), 4.24 (ddddd, *J* = 7.0, 6.0, 4.4, 1.8, 0.9 Hz, 1H), 3.62 (dd, *J* = 13.6, 5.7 Hz, 1H), 3.37 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.10 – 3.02 (m, 1H)
4h	-O	-N(CH_3)$_2$	N-H peak at 3300cm^{-1},C=O peak at 1580cm^{-1}, Ar C-H peak at 3100cm^{-1}, methyl C-H peak at 2850 cm^{-1}, C=N peak at 1700cm^{-1}, C-N peak at 1596cm^{-1}, N-N peak at 1380cm^{-1}	475 (M+1)	δ 9.91 (d, *J* = 6.8 Hz, 1H), 9.41 (s, 1H), 8.21 – 8.16 (m, 1H), 7.86 (d, *J* = 6.9 Hz, 1H), 7.65 – 7.59 (m, 2H), 7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.25 (m, 1H), 6.77 – 6.72 (m, 2H), 6.30 (dddt, *J* = 10.7, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.2, 10.3, 8.2, 1.7, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.1, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.33 (td, *J* = 5.7, 1.7 Hz, 1H), 4.59 – 4.53 (m, 1H), 4.24 (ddddd, *J* = 7.0, 6.0, 4.4, 1.8, 0.9 Hz, 1H), 3.62 (dd, *J* = 13.6, 5.7 Hz, 1H), 3.37 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.06 (dddt, *J* = 7.3, 4.6, 1.8, 1.1 Hz, 1H), 2.92 (s, 5H)
4i	-S	-H	N-H peak at 3450cm^{-1},N-C=S	448	δ 9.97 (d, *J* = 6.9 Hz, 1H), 9.83 (s, 1H), 8.21 – 8.16 (m, 1H), 7.85 (d, *J* =

			peak at 2050cm^{-1}, Ar C-H peak at 3150cm^{-1}, C=N peak at 1780cm^{-1}, C-N peak at 1560cm^{-1}, N-N peak at 1371cm^{-1}	(M+1)	6.7 Hz, 1H), 7.67 – 7.61 (m, 2H), 7.50 – 7.34 (m, 5H), 7.31 – 7.25 (m, 1H), 6.30 (dddt, *J* = 10.6, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.3, 10.3, 8.4, 1.8, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.1, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.23 (td, *J* = 6.0, 1.8 Hz, 1H), 4.63 – 4.58 (m, 1H), 4.29 – 4.20 (m, 1H), 3.60 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.35 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.06 (dddt, *J* = 7.3, 5.3, 2.6, 0.9 Hz, 1H)
4j	-S	-CH_3	N-H peak at 3350cm^{-1},N-C=S peak at 2100cm^{-1}, Ar C-H peak at 3000cm^{-1}, C=N peak at 1700cm^{-1}, methyl C-H peak at 2800 cm^{-1}, C-N peak at 1596cm^{-1}, N-N peak at 1385cm^{-1}	462 (M+1)	δ 9.97 (d, *J* = 6.9 Hz, 1H), 9.83 (s, 1H), 8.21 – 8.16 (m, 1H), 7.85 (d, *J* = 6.7 Hz, 1H), 7.58 – 7.52 (m, 2H), 7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.25 (m, 1H), 7.22 – 7.16 (m, 2H), 6.30 (dddt, *J* = 10.6, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.3, 10.3, 8.4, 1.8, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.0, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.23 (td, *J* = 6.0, 1.8 Hz, 1H), 4.63 – 4.58 (m, 1H), 4.29 – 4.20 (m, 1H), 3.60 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.35 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.06 (dddq, *J* = 8.0, 4.4, 1.8, 0.9 Hz, 1H)
4k	-S	-C_2H_5	N-H peak at 3400cm^{-1},N-C=S peak at 2130cm^{-1}, Ar C-H peak at 3150cm^{-1}, C=N peak at 1750cm^{-1}, ethyl C-H peak at 2850 cm^{-1}, C-N peak at 1585cm^{-1}, N-N peak at 1368cm^{-1}	476 (M+1)	δ 9.97 (d, *J* = 6.9 Hz, 1H), 9.83 (s, 1H), 8.21 – 8.16 (m, 1H), 7.85 (d, *J* = 6.7 Hz, 1H), 7.58 – 7.52 (m, 2H), 7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.24 (m, 3H), 6.30 (dddt, *J* = 10.6, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.3, 10.3, 8.4, 1.8, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.0, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.23 (td, *J* = 6.0, 1.8 Hz, 1H), 4.63 – 4.58 (m, 1H), 4.29 – 4.20 (m, 1H), 3.60 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.35 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.06 (dddq, *J* = 8.0, 4.4, 1.9, 0.9 Hz, 1H), 2.69 (qt, *J* = 7.3, 0.9 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 3H)
4l	-S	-OCH_3	N-H peak at 3350cm^{-1},N-C=S peak at 2100cm^{-1}, Ar C-H peak at 3150cm^{-1}, C-O peak at 1240 cm^{-1}, C=N peak at 1698cm^{-1}, methyl C-H peak at 2800 cm^{-1}, C-N peak at 1596cm^{-1}, N-N peak at 1379cm^{-1}	478 (M+1)	δ 9.97 (d, *J* = 7.0 Hz, 1H), 9.83 (s, 1H), 8.21 – 8.16 (m, 1H), 7.85 (d, *J* = 6.8 Hz, 1H), 7.65 – 7.59 (m, 2H), 7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.25 (m, 1H), 6.95 – 6.89 (m, 2H), 6.30 (dddt, *J* = 10.6, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.4, 10.3, 8.4, 1.8, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.1, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.23 (td, *J* = 6.0, 1.8 Hz, 1H), 4.63 – 4.58 (m, 1H), 4.29 – 4.20

					(m, 1H), 3.80 (s, 2H), 3.60 (dd, *J* = 13.5, 6.0 Hz, 1H), 3.35 (dd, *J* = 13.7, 6.0 Hz, 1H), 3.06 (ddddd, *J* = 8.4, 4.6, 2.8, 1.7, 1.0 Hz, 1H)
4m	-S	$-NO_2$	N-H peak at 3350cm^{-1},N-C=S peak at 2150cm^{-1}, Ar C-H peak at 3100cm^{-1}, C=N peak at 1720cm^{-1}, C-N peak at 1570cm^{-1}, N-O peak at 1550 cm^{-1}, N-N peak at 1371cm^{-1}	493 (M+1)	δ 9.97 (d, *J* = 6.9 Hz, 1H), 9.83 (s, 1H), 8.21 – 8.15 (m, 3H), 7.90 – 7.82 (m, 3H), 7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.25 (m, 1H), 6.30 (dddt, *J* = 10.6, 8.1, 1.9, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.3, 10.3, 8.4, 1.8, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.0, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.23 (td, *J* = 6.0, 1.8 Hz, 1H), 4.63 – 4.58 (m, 1H), 4.29 – 4.20 (m, 1H), 3.60 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.35 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.06 (dddt, *J* = 7.3, 5.3, 2.6, 0.9 Hz, 1H)
4n	-S	-Cl	N-H peak at 3400cm^{-1},N-C=S peak at 2050cm^{-1}, Ar C-H peak at 3050cm^{-1}, C=N peak at 1780cm^{-1}, C-N peak at 1585cm^{-1}, C-Cl peak at 800 cm^{-1}, N-N peak at 1385cm^{-1}	483 (M+1)	δ 9.97 (d, *J* = 6.9 Hz, 1H), 9.83 (s, 1H), 8.21 – 8.16 (m, 1H), 7.85 (d, *J* = 6.7 Hz, 1H), 7.63 – 7.57 (m, 2H), 7.50 – 7.44 (m, 1H), 7.44 – 7.38 (m, 2H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.25 (m, 1H), 6.30 (dddt, *J* = 10.6, 8.1, 1.9, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.3, 10.3, 8.4, 1.8, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.0, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.23 (td, *J* = 6.0, 1.8 Hz, 1H), 4.63 – 4.58 (m, 1H), 4.25 (dtdd, *J* = 7.9, 5.9, 1.7, 0.9 Hz, 1H), 3.60 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.35 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.06 (dddt, *J* = 7.3, 5.4, 2.6, 0.9 Hz, 1H)
4o	-S	-OH	N-H peak at 3250cm^{-1},N-C=S peak at 2150cm^{-1}, OH peak at 3400 cm^{-1}, Ar C-H peak at 3150cm^{-1}, C=N peak at 1700cm^{-1}, C-N peak at 1590cm^{-1}, C-Cl peak at 800 cm^{-1}, N-N peak at 1375cm^{-1}	464 (M+1)	δ 9.97 (d, *J* = 6.9 Hz, 1H), 9.83 (s, 1H), 8.21 – 8.16 (m, 1H), 7.85 (d, *J* = 6.7 Hz, 1H), 7.61 – 7.55 (m, 2H), 7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.28 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 6.88 – 6.82 (m, 2H), 6.30 (dddt, *J* = 10.6, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.3, 10.3, 8.4, 1.8, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.1, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.23 (td, *J* = 6.0, 1.8 Hz, 1H), 4.63 – 4.58 (m, 1H), 4.29 – 4.20 (m, 1H), 3.60 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.35 (dd, *J* = 13.7, 6.0 Hz, 1H), 3.06 (dddq, *J* = 8.2, 4.6, 2.0, 1.0 Hz, 1H)
4p	-S	$-N(CH_3)_2$	N-H peak at 3300cm^{-1},N-C=S peak at 2100cm^{-1}, Ar C-H peak at 3150cm^{-1}, C=N peak at 1750cm^{-1}, methyl C-H peak at 2800 cm^{-1}, C-	491 (M+1)	δ 9.97 (d, *J* = 7.0 Hz, 1H), 9.83 (s, 1H), 8.21 – 8.16 (m, 1H), 7.85 (d, *J* = 6.7 Hz, 1H), 7.65 – 7.59 (m, 2H), 7.50 – 7.44 (m, 1H), 7.37 (td, *J* = 7.4, 1.1 Hz, 1H), 7.31 – 7.25 (m, 1H), 6.78 – 6.72 (m, 2H), 6.30 (dddt, *J* =

			N peak at 1600cm^{-1}, N-N peak at 1379cm^{-1}		10.6, 8.1, 1.8, 0.9 Hz, 1H), 6.12 (ddddd, *J* = 12.4, 10.4, 8.4, 1.8, 0.8 Hz, 2H), 5.96 (dddd, *J* = 10.1, 6.0, 1.8, 0.8 Hz, 1H), 5.76 (dq, *J* = 5.3, 1.7 Hz, 1H), 5.23 (td, *J* = 6.0, 1.8 Hz, 1H), 4.63 – 4.58 (m, 1H), 4.29 – 4.20 (m, 1H), 3.60 (dd, *J* = 13.5, 6.0 Hz, 1H), 3.35 (dd, *J* = 13.6, 6.0 Hz, 1H), 3.06 (ddddd, *J* = 8.3, 4.6, 2.8, 1.7, 1.0 Hz, 1H), 2.92 (s, 5H)

Anti-diabetic activity:

In vivo anti-diabetic activity:

Effect of synthesized compounds on body weight:

Table 4 presents the effect of synthesized compounds and glibenclamide on changes in body weight. In diabetic control rats, there was a significant decrease (22.57%) in final body weight when compared to normal control rats. Treatment with synthesized compounds showed significant ($P < 0.05$) increases in body weight of diabetic rats. Change in body weight was minimal for positive control (1.02%) whereas synthesized compounds treatments showed moderate improvement in body weight (1.50–2.51%).

Effect of synthesized compounds on glycosylated hemoglobin (HbA1c):

Fig. 1 shows the effect of synthesized compounds and glibenclamide on HbA1c level in normal and experimental rats. STZ treated rats showed a significant elevation in HbA1c level (5.25%) as compared with normal control. Following synthesized compounds and glibenclamide administration to diabetic rats caused a significant reduction ($P < 0.05$) in HbA1c level (~3–5%) as compared to diabetic control rats.

Effect of synthesized compounds on blood glucose:

The blood glucose level was measured in normal and experimental groups at 0 days, 7th day, 14th and 21st day of treatment. STZ administration showed a significant increase ($P < 0.05$) in the blood glucose level when compared to normal control group. There was dramatically reduction in blood glucose level from 221 mg/dL to 188 mg/dL after 21 days of treatment with synthesized compound 4a at the dose of 50 mg/kg b.wt. (Table 5)

Table 4: Effect of synthesized compounds and glibenclamide on body weight and glycosylated hemoglobin content in STZ-induced diabetic rats

Groups	**Body Weight (g)**		**% Change in body weight**	**HbA1c (%)**
	Initial	**Final**		
Group-I, Normal Control	198 ± 4.20	204 ± 3.42	2.51	4.90 ± 0.22
Group-II, Diabetic control	196 ± 5.65	151 ± 4.59a	−22.57	10.23 ± 0.27
Group-III, Diabetic + synthesized	192 ± 4.80	195 ± 3.62b	1.52	6.34 ± 0.20

compound 4a ,(50 mg/kg b.wt)				
Group-IV,Diabetic + synthesized compound 4m,(50 mg/kg b.wt)	194 ± 2.30	198 ± 3.30b	2.51	7.92 ± 0.18
Group-V,Diabetic+Glibenclamide (5 mg/kg b.wt)	195 ± 4.90	197 ± 4.35b	1.025	6.49 ± 0.16

Data represented as mean ± SEM (n=6).

a = (P < 0.05) statistically significant difference when compared with Normal control.

b = (P < 0.05) statistically significant difference when compared with Diabetic control

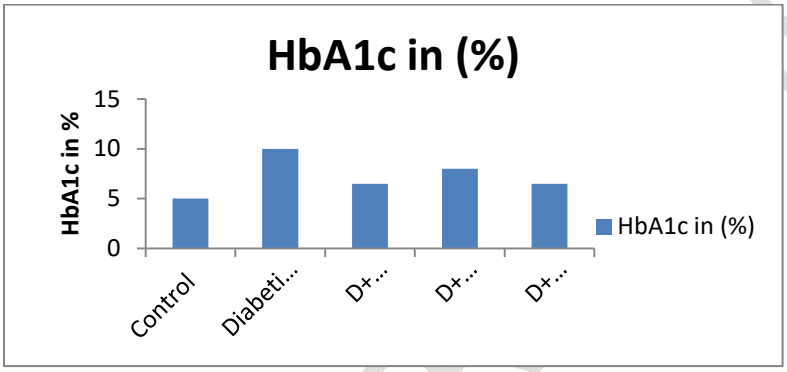

Fig 1: Effect of synthesized compounds and glibenclamide on Glycosylated hemoglobin (%) in STZ-induced diabetic rats

Table 5: Effect of synthesized compounds and glibenclamide on blood glucose level in STZ-induced diabetic rats

Groups	Blood Glucose Level (mg/dL) Time Intervals (Days)			
	0	**7**	**14**	**21**
Group-I Normal Control	80	79	81	78
Group-II, Diabetic control	276	310	320	315
Group-III, Diabetic + synthesized compound 4a ,(50 mg/kg b.wt)	272	250	221	188
Group-IV, Diabetic + synthesized compound 4m ,(50 mg/kg b.wt)	274	258	236	211
Group-V, Diabetic+Glibenclamide ,(5 mg/kg b.wt)	276	248	215	175

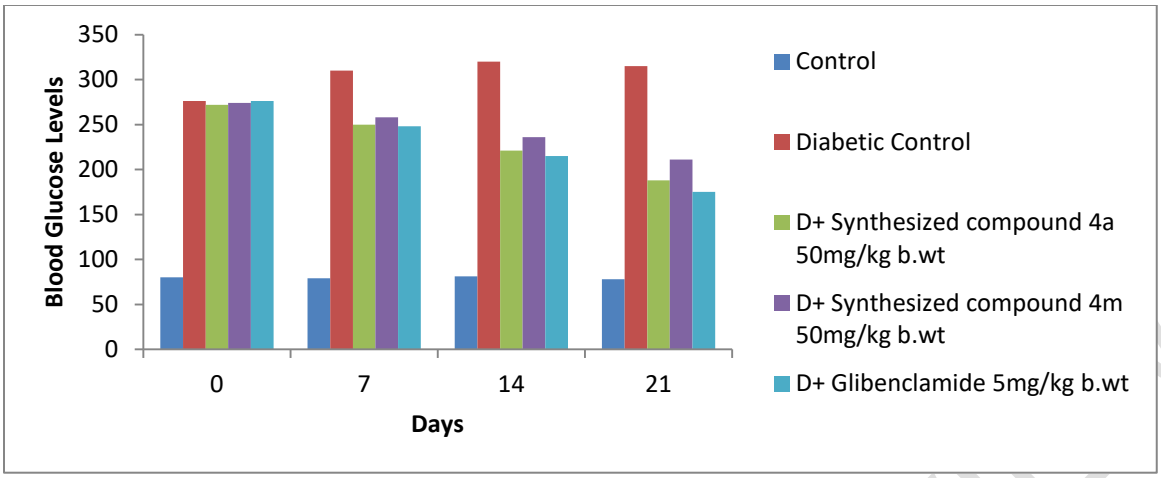

Fig 2: Effect of synthesized compounds and glibenclamide on blood glucose level in STZ-induced diabetic rats. Data represented as mean ± SEM (n=6). Statistically significant differences were observed between Control to DC: $^{a}p<0.05$ and DC to drug treated groups: $^{b}p < 0.05$

In vitro anti-diabetic activity:

In vitro α-amylase inhibition assay:

In the present study, synthesized compounds showed a significant inhibition of α- amylase enzyme activity in a concentration dependent manner. Synthesized compounds 4a, 4e and 4m at the concentrations 20, 40, 60, 80 and 100 µg/ml gives different % inhibition of α-amylase enzyme activity. The acarbose used as a reference standard at the same concentrations showed variable inhibition of α-amylase activity. (Table. 6)

Table.6: Effect of Synthesized compounds and acarbose in the in vitro α -amylase inhibition model

S. No	Synthesized compound	% inhibition of α-amylase enzyme activity Concentration of sample (µg/ml)				
		20	40	60	80	100
1	4a	24	46	58	66	70
2	4e	22	42	50	58	68
3	4m	18	39	46	55	60
4	Acarbose	27	51	64	82	87

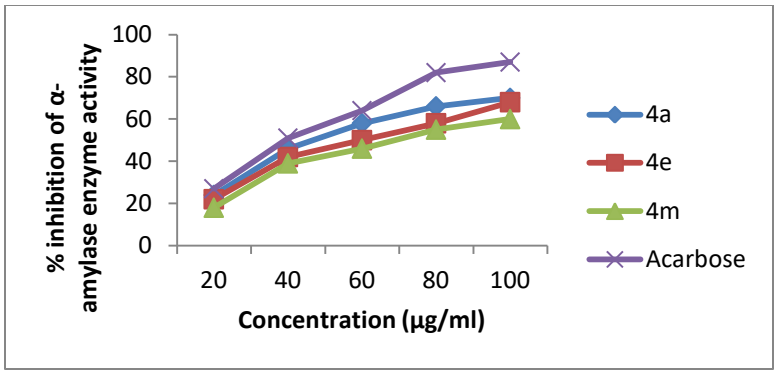

Fig. 3: Effect of Synthesized compounds 4a, 4e, 4m and acarbose in the in vitro α -amylase inhibition model

In vitro α-Glucosidase inhibition assay:

In the present study, synthesized compounds showed a significant inhibition of □-Glucosidase enzyme activity in a concentration dependent manner. Synthesized compounds 4a, 4e and 4m at the concentrations 20, 40, 60, 80 and 100 µg/ml ml gives different % inhibition α-Glucosidase enzyme activity. The acarbose used as a reference standard at the same concentrations showed variable inhibition of α-Glucosidase activity. (Table. 7)

Table.7: Effect of Synthesized compounds and acarbose in the in vitro α-Glucosidase inhibition model

S. No	Synthesized compound	% inhibition of α-Glucosidase enzyme activity Concentration of sample (µg/ml)				
		20	40	60	80	100
1	4a	12	24	36	55	66
2	4e	9	22	33	41	59
3	4m	8	17	26	34	46
4	Acarbose	15	32	48	64	75

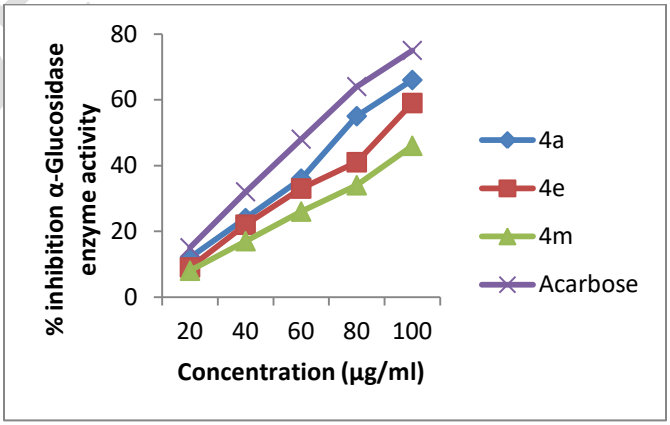

Fig. 4: Effect of Synthesized compounds 4a, 4e, 4m and acarbose in the in vitro □-Glucosidase inhibition model

4. CONCLUSION

The present study was aimed to synthesis, characterization of the pyrazoline fused indole derivatives. The synthesis of novel compounds involves the four steps; in the first step indole was treated with N, N-dimethyl formamide to synthesize the compound 1 indole-3-aldehyde. In the second step compound 1 was treated with 4-Substituted acetophenone to synthesize compound 2(a-h). In the third step chalcones (2a-2h) were treated with thiosemicarbazide or semicarbazide to synthesize compound 3(a-p). In the final step compound 3(a-p) were treated with indole-3-aldehyde to prepare the final product R-substitutedN-((1H-indol-3-yl) methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide and R-substitutedN-((1H-indol-3-yl)methylene)-5-(1H-indol-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide 4(a-p). The chemical structures of the synthesized compounds were characterized by means of IR, Mass and NMR spectroscopy. The compounds were screened for anti-diabetic activity by In-vitro and In-vivo methods. In In-vivo method 4a, 4m have exhibited moderate anti-diabetic activity as that of standard drug, glibenclamide. In In-vitro method 4a, 4e & 4m have shows moderate anti-diabetic activity as that of reference drug, acarbose. These compounds can be further exploited to get the potent lead compound.

REFERENCES

1. Kumar S, Bawa S, Drabu S, Kumar R, Gupta H. Biological activities of pyrazoline derivatives--a recent development. "Recent Pat Antiinfect Drug Discov"[jour] 2009 Nov; 4(3):154-63.doi: 10.2174/157489109789318569
2. Allan and Richmond synthesis of novel heterocyclic substances journal of organic chemistry 2,1998,22
3. Dias LRS, salvador RRS, pyrazole carbahydrazole derivatives of pharmaceutical interest. Pharmaceuticals. 2012, 5:317-24
4. Lakhdar S, Westermaier M, Terrier F, Goumont R, Boubaker T, Ofial AR, Mayr H (2006) Nucleophilic reactivities of indoles. J Org Chem 71:9088–9095
5. Sharma V, Pradeep K, Devender P (2010) Biological importance of the indole nucleus in recent years: a comprehensive review. J Heterocycl Chem 47:491–502
6. Kaushik NK, Kaushik N, Attri P, Kumar N, Kim CH, Verma AK, Choi EH (2013) Biomedical Importance of Indoles. Molecules 18:6620–6662
7. Xue S, Ma L, Gao R, Lin Y, Linn Z (2014) Synthesis and antiviral activity of some novel indole-2-carboxylate derivatives. Acta Pharmaceutica Sinica B 4(4):313–321
8. Pedada SR, Yarla NS, Tambade PJ, Dhananjaya BL, Bishayee A, Arunasree KM, Philip GH, Dharmapuri G, Aliev G, Putta S, Rangaiah G (2016) Synthesis of new secretory phospholipase A2-inhibitory indole containing isoxazole derivatives as anti-inflammatory and anticancer agents. Eur J Med Chem 112:289–297
9. Zhuang SH, Lin YC, Chou LC, Hsu MH, Lin HY, Huang CH, Lien JC, Kuo SC, Huang LJ (2013) Synthesis and anticancer activi ty of 2, 4-disubstituted furo[3,2-b]indole derivatives. Eur J Med Chem 66:466–479
10. Kasralikar HM, Jadhavar SC, Bhusare SR (2015) Synthesis and molecular docking studies of oxochromenyl xanthenone and indolyl xanthenone derivatives as anti-HIV-1 RT inhibitors. Bioorg Med Chem Lett 25:3882–3886
11. Silveira CC, Mendes SR, Soares JR, Victoria FN, Martinez DM, Savegnago L (2013) Synthesis and antioxidant activity of new C-3 sulfenyl indoles. Tetrahedron Lett 54:4926–4929

12. Xu H, Fan LL (2011) Antifungal agents. Part 4: Synthesis and antifungal activities of novel indole[1,2-c]-1,2,4-benzotriazine derivatives against phytopathogenic fungi invitro. Eur J Med Chem 46:364–369
13. Velezheva V, Brennan P, Ivanov P, Kornienko A, Lyubimov S, Kazarian K, Nikonenko B, Majorov K, Apt A (2016) Synthesis and antituberculosis activity of indole–pyridine derived hydrazides, hydrazide–hydrazones, and thiosemicarbazones. Bioorg Med Chem Lett 26(3):978–985
14. Tejasree et al., (2014) Antidiabetic Activity Of 1-(4-(Dimethylamino)Benzylidene)-5-(2-Oxo Indolin-3-Ylidene) Thiocarbohydrazone In Rats. IJPSR, 2014; Vol. 5(7): 2738-2743
15. Luthra T, Nayak AK, Bose S, Chakrabarti S, Gupta A, Sen S (2019) Indole based antimalarial compounds targeting the melatonin pathway: their design, synthesis and biological evaluation. Eur J Med Chem 168:11–27
16. Bingul M, Ercan S, Boga M (2020) The design of novel 4, 6-dimethoxyindole based hydrazide-hydrazones: Molecular modeling, synthesis and anticholinesterase activity. J Mol Struct 1213:128202
17. The Fischer indole synthesis Philip A. Roussel *J. Chem. Educ.* 1953, 30, 3, 122
18. J. Panther, T. J. J. Müller, *Synthesis*, 2016, *48*, 974-986
19. Rhodium(III)-Catalyzed Indole Synthesis Using N–N Bond as an Internal Oxidant by B. Liu, C. Song, C. Sun, S. Zhou, J. Zhu, *J. Am. Chem. Soc.*, 2013, *135*, 16625-16631
20. Total Synthesis of Indoles from Tricholoma Species via Bartoli/Heteroaryl Radical Methodologies by A. Dobbs, *J. Org. Chem.*, 2001, *66*, 638-641
21. Pd-Catalyzed Cascade Reaction for the Synthesis of 2-Substituted Indoles by J. Jadhav, V. Gaikwad, R. Kurane, R. Salunkhe, G. Rahsinkar, *Synlett*, 2012, *23*, 2511-2515
22. M.N. Gosh, Toxicity studies, Fundamentals of Experimental Pharmacology, Scientific Book Agency, Calcutta, 1984, pp. 153–158
23. Gajdosik, A. Gajdosikova, M. Stefek, J. Navarova, R. Hozova, Streptozotocininduced experimental diabetes in male Wistar rats, Gen. Physiol. Biophys. 18 (1999) 54–62
24. S.S. Nayak, T.N. Pattabiraman, A new colorimetric method for the estimation of glycosylated hemoglobin, Clin. Chim. Acta 109 (1981) 267–274
25. A. Kuppusamy, U.Muthusamy, S. Andichetiar Thirumalaisamy,S.Varadharajan,K. Ramasamy, and S. Ramanathan, "*In vitro* (□-glucosidase and □-amylase inhibition) and *in vivo* antidiabetic property of phytic acid (IP6) in streptozotocin-nicotinamideinduced type 2 diabetes mellitus (NIDDM) in rats," *Journal of Complementary and Integrative Medicine*, vol. 8, no. 1, article 9, 2011