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Synthesis and Pharmacological Activity of Some Pyrazolone Derivatives

Faruk Alam[1*] and Ruhul Amin[1]

[1]Faculty of Pharmaceutical Sciences, Assam Down Town University, Gandhi Nagar, Panikhaiti, Guwahati, Assam - 781026, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author FA designed the study, performed the chemical synthesis, statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RA also managed the literature searches and the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

A sequence of novel pyrazolone derivative was produced by the reaction of 2, 4-dinitrophenyl hydrazine (0.1 mol) and ethyl acetoacetate with benzaldehydes followed by hydrazine hydrate and finally treated with secondary amine and formaldehyde and the synthesized compounds were characterized by their physical properties (M.P and TLC) and UV, IR, ^{1}HNMR, Mass spectroscopic studies respectively. The entire synthesized complex was tested for their anthelmintic, antimicrobial and haemostatic activity against gram-positive and gram-negative strains of bacteria and *Eudrilus eugenia* and human venous blood. The antimicrobial activity of synthesized pyrazolone derivatives was assessed by agar cup method. All the synthesized complexes were screened for the antimicrobial, athelmintic and haemostatic activity against some gram (+ve), Gram(-ve) organisms, *Eudrilus eugenia* and human venous blood. The complexes exhibited reasonable to upright activity when compared with the standard one.

Keywords: Synthesis; spectral analysis; antimicrobial activity; anthelmintic activity; haemostatic activity.

**Corresponding author: E-mail: faruk_2007a@rediffmail.com;*

1. INTRODUCTION

A systemic literature search conducted in various database of web of science, Pubmed, EMBASE, Science direct and Pubmed, including the methods of synthesis for various pyrazolone derivatives and their structural activity. The derivatives of pyrazolone have been studied in multiple medicinal chemists for their wide range of physiological activity. Pyrazolone structurally is a 5-membered heterocyclic containing 2 adjacent nitrogen atoms. It can be synthesized as a derivative of pyrazole possessing an additional keto (=O) group. The synthesis of pyrazolones was first discovered by Ludwig Knorr in 1883, via a condensation reaction between ethyl acetoacetate and phenyl hydrazine [1]. These compounds showed notable antibacterial, anti-inflammatory, analgesic, antitubercular, antifungal, antioxidant and antitumor activities [2-6]. Due to their easier preparation and potent biological activity, pyrazolone complexes plays a vital role and represents a new prototype for combinatorial and medicinal chemistry. Pyrazolones are pharmacophores of numerous compounds that display pharmacological activities such as analgesic and antipyretic (propylphenazone, phenazone, metamizole etc.) [7], anti-cancer (TELIN) [8], anti-ischemic (edaravone) [9] and anti-anxiolytic [10]. Pyrazolones are gaining significant importance, especially in new drug discovery lineups towards cardiovascular [11] and cerebral ischaemia diseases [12]. In our present study, we have demonstrated the various ability of an important class of synthetic molecules containing a pair of basic moieties like pyrazolone and pyrazoline (5a–5d) as hemostatic, anthelmintic and antimicrobial agents.

2. METHODS OF PREPARATION

The substituted pyrazolone derivatives were prepared by the reaction of a mixture of 2'-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-1*H*,3'*H*-3,4'-bipyrazol-3'-one (0.1 mol), different Secondary amine (0.1 mol), Formaldehyde (0.1 mol) and in ethanol was refluxed for 9 hrs and leave the reaction mixture in an ice chest or refrigerator overnight. The solid separated was filtered, washed with water and dried. The obtained crude product recrystallized from absolute ethanol. The completion of the reaction was monitored by TLC using the suitable solvent system as the mobile phase. Pharmacological evaluation of the molecules reveals that compounds 5a, 5c and 5d exhibited hemostatic, anthelmintic and antimicrobial efficacy nearly similar to the standard.

2.1 Experimental

Melting points were determined by open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Techno search Instrument, M-15 FT-IR spectrophotometer. The Ultraviolet visible spectroscopy analysis has been carried out in UV–Pharma Spec 2060+ Anlytical UV-visible spectrophotometer using the concentration of 0.01% of the synthesized substituted pyrazole compounds in Chloroform as a solvent. ^{1}H FT-NMR analysis spectra were recorded (DMSO-d6) on a Brukur (400 MHz) spectrometer. Chemical shift values are given in δ scales. The mass spectra were recorded on TOF- LC/MS system. The completion of the reaction was checked by thin-layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 f254). Commercial grade solvents and reagents were used without further purification. The entire synthesized compounds were prepared as per the Scheme-1.

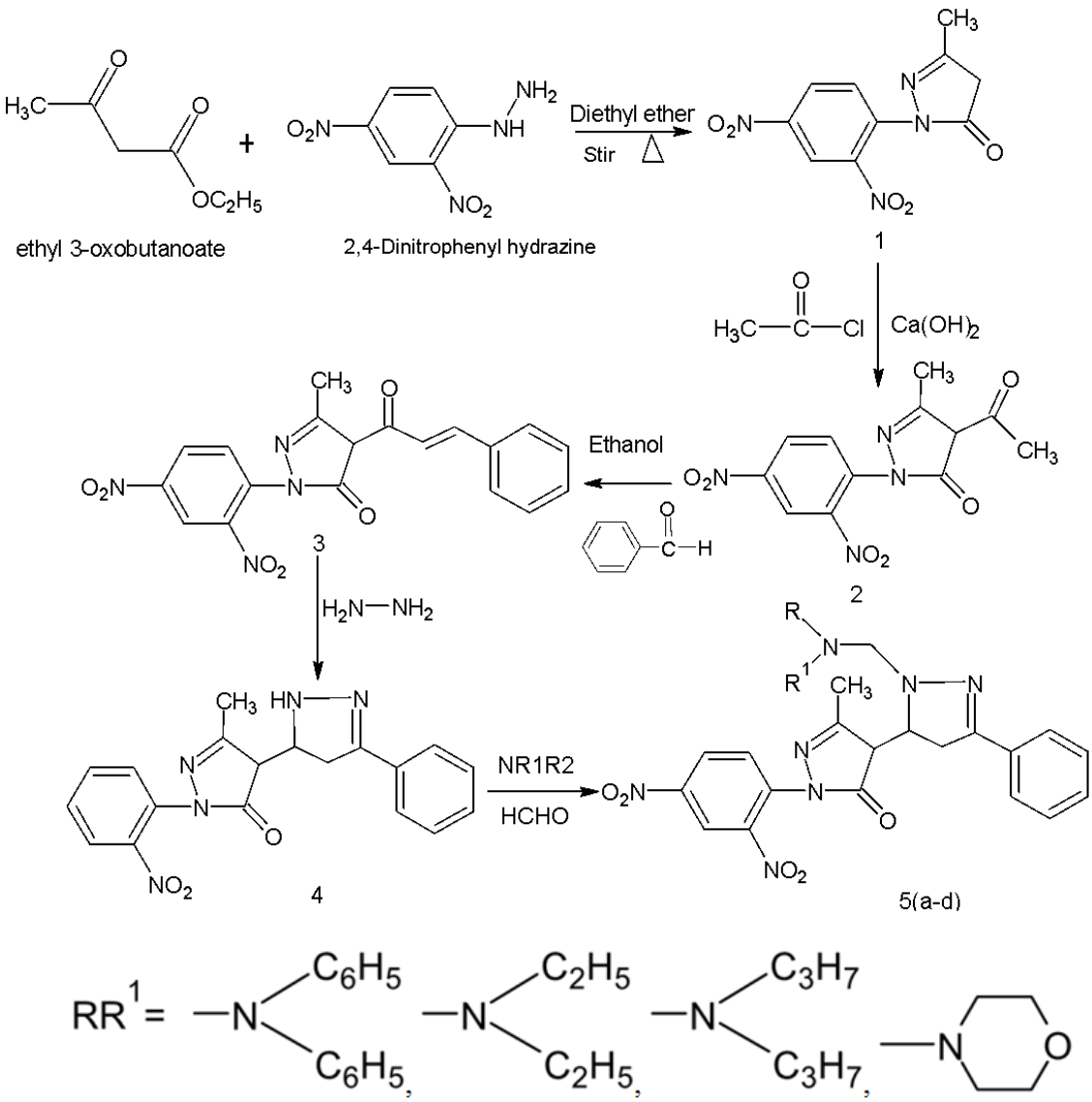
2.2 Chemistry

2.2.1 Synthesis of 2-aryl-5-methyl-2, 4-dihydro-3*H*-pyrazol-3-one[13]

A mixture of 2, 4-dinitrophenyl hydrazine (0.1 mol) and ethyl aceto acetate (0.1 mol) were taken in a clean and dried beaker or evaporating pan. The mixture was heated on boiling water bath for about 2 hour with periodical stirring of contents with a glass rod. After completion of the reaction, the reddish syrup was obtained. It allowed to cool and excess of diethyl ether was added. The contents were stirred vigorously until the product precipitated. The crude pyrazolone weighed after drying in the air. The percentage of yield was found to be 96.57. The product (a dark yellow solid) recrystallized from absolute ethanol, m.p 78°C.

2.2.2 Synthesis of 4-acetyl-2-(2,4-dinitrophenyl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one

A mixture of substituted pyrazolone (0.1 mol), actyl chloride (0.1 mol), calcium hydroxide (0.08 mol) was taken in a clean and dried round bottom flask. The mixture was refluxed on boiling water bath for about 1 hour. The reaction mixture was poured in crushed ice with constant stirring and acidified the contents using 0.1 N HCl.



Scheme-1

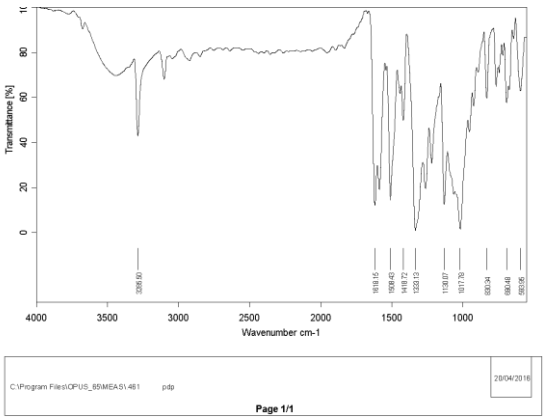
The contents were stirred vigorously until the product precipitated. The crude produt, after drying in the air weighed. The percentage of yield was found to be 81.34. The product (a dark yellow solid) recrystallized from absolute ethanol, m.p 82°C. [14].

2.2.3 Synthesis of 2-(2,4-dinitrophenyl)-5-methyl-4-[3-phenylprop-2-enoyl]-2,4-dihydro-3*H*-pyrazol-3-one

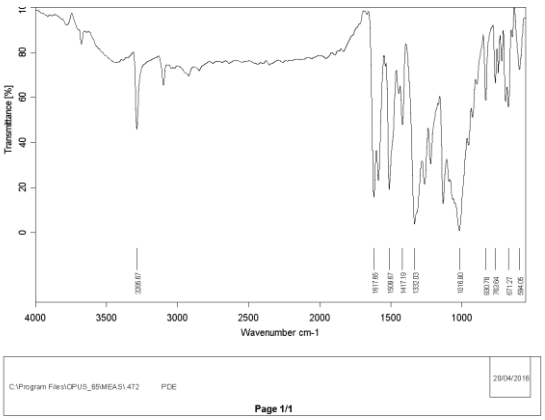
A mixture of 2-aryl-5-(2-oxopropyl)-2, 4-dihydro-3*H*-pyrazol-3-one (0.1 mol) and benzaldehyde (0.1 mol) were taken in ethanol in a clean and dried round bottom flask. The mixture was refluxed on boiling water bath for about 4 hours. The reaction mixture was cooled in an ice bath until the product precipitated. The crude products, after drying in the air weighed. The obtained crude product recrystallized from absolute ethanol. The completion of reaction was monitored by TLC using ethyl acetate: hexane (9:1) as mobile phase.

2.2.4 Synthesis of 2'-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-1*H*,3'*H*-3,4'-bipyrazol-3'-one

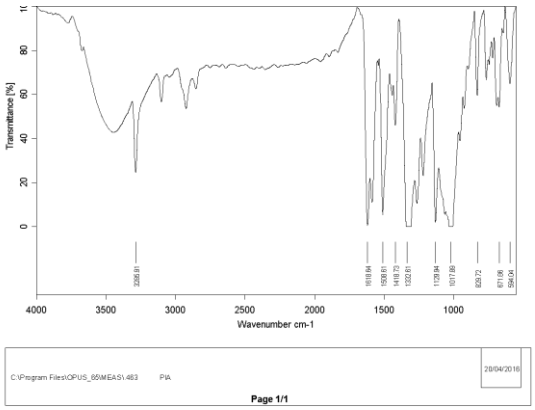
A mixture of substituted pyrazolone (0.1 mol) and hydrazine hydrate(0.1 mol) was taken in 25 ml of dry ethanol in a clean and dried round bottom flask. The mixture was refluxed on boiling water bath for about 8 hours. The reaction mixture was cooled in ice bath until the product precipitated. The crude products, after drying in the air



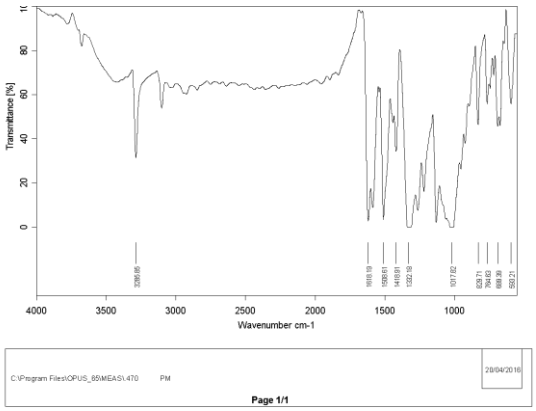
Compound 5A



Compound 5B



Compound 5C



Compound 5D

Fig. 1. IR Spectra of synthesized compounds

weighed. The obtained crude product recrystallized from absolute ethanol. The completion of the reaction was monitored by TLC using ethyl a suitable solvent system as the mobile phase.

2.2.5 Synthesis of alkyl amine substituted bipyrazolone [15,16]

A mixture of bipyrazolone (0.1mol), different Secondary amine (0.1mol), Formaldehyde (0.1 mol), and in ethanol was refluxed for 9 hrs and leave the reaction mixture in an ice chest or refrigerator overnight. The solid separated was filtered, washed with water and dried. The completion of reaction was monitored by TLC using suitable solvent system as mobile phase.

2.2.6 2'-(2, 4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-diphenyl amino-3,4'-bipyrazol-3'-one (5a)

MF $C_{32}H_{27}N_7O_5$; M. Wt 589; Colour: Dark Brown; Nature: Crystalline Powder; mp 245-247°C; yield 55.55%; Rf: 0.60; Solubility: Ethanol, Acetone, Acetic Acid; FTIRcm^{-1}: 3285 (Ar.C-H), 3100 (N-N str), 2845(N-CH_2 str), 1742 (C=O,ester), 1618 (C=N str), 1508,1570 (Ar-NO_2); 1418 (C=C,aromatic), 1017 (-CH_3), 830 (C-H def); mass: *m/z* 589(M^+); ^{1}H FT-NMR (400 MHz, DMSO, *δ* ppm), 2.15 (m, 3H, CH_3), 3.45 (m, 2H, CH_2), 7.49 (m, 3H, ArH), 7.79 (m, 5H, H-Ar.), 8.36-8.85 (m, 5H, H-Ar.); λmax: 453 nm.

2.2.7 2'-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-diethyl amino -3, 4'-bipyrazol-3'-one(5b)

MF $C_{24}H_{27}N_7O_5$; M. Wt 493; Colour: Brick Red: Crystalline Powder; Nature; mp 260-262°C; yield 60.24%; Rf: 0.42; Solubility: Ethanol, Acetone, Acetic Acid; FTIRcm^{-1}: 3286 (Ar.C-H), 3125 (N-N str), 2850,2790 (N-CH_2 str), 1750 (C=O, ester), 1617 (C=N str), 1509,1575 (Ar-NO_2); 1417 (C=C, aromatic); 1016 (-CH_3); 840 (C-H def); mass: *m/z* 493(M^+); ^{1}H FT-NMR

(400 MHz, DMSO, δ ppm), 2.15 (m, 3H, CH_3), 3.45 (m, 2H, CH_2), 7.47 (m, 3H, ArH), 7.79 (m, 5H, H-Ar.), 8.69-8.85 (m,5H, -C_2H_5); λmax: 493nm.

2.2.8 2'-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-isopropyl amino -3, 4'-bipyrazol-3'-one(5c)

MF $C_{23}H_{25}N_7O_5$; M. Wt 479; Colour: Dark Orange; Nature: Crystalline Powder ; mp 250-253°C; yield 56.85%; Rf: 0.57; Solubility: Ethanol, Acetone, Acetic Acid,Chloroform; FTIRcm^{-1}: 3286 (Ar.C-H), 2960 (C-H str), 3120 (N-N str), 2810 (N-CH_2 str), 1619 (C=N str), 1509 (Ar-NO_2), 1419 (C=C, aromatic), 1018 (-CH_3), 830(C-H def); mass: *m/z* 479(M^+); ^{1}H FT-NMR (400 MHz, DMSO, δ ppm), 2.15 (m, 3H, CH_3), 3.45 (m, 2H, CH_2), 7.47 (m, 3H, ArH), 7.80 (m, 5H, H-Ar.), 8.69-8.85 (m, 5H, -CH_3); λmax: 485 nm.

2.2.9 2'-(2, 4-dinitrophenyl)-5'-methyl-5-phenyl-2', 4, 4', 5-tetrahydro-morpholino -3,4'-bipyrazol-3'-one(5d)

MF $C_{24}H_{25}N_7O_6$; M. Wt 507; Colour: Dark Orange; Nature: Crystalline Powder ; mp 238-241°C; yield 58.16%; Rf: 0.61; Solubility: Ethanol, Acetone, Acetic Acid, Chloroform, Benzene ; FTIR cm^{-1}: 3286 (Ar.C-H), 3135 (N-N str), 2845(N-CH_2 str), 1765(C=O, ester), 1618 (C=N str), 1508(Ar-NO_2), 1419 (C=C, aromatic), 1017 (-CH_3), 829 (C-H def); ^{1}H FT-NMR (400 MHz, DMSO, δ ppm), 2.56 (m, 3H, CH_3), 3.55 (m, 2H, CH_2), 7.47 (m, 3H, ArH), 8.09-8.11 (m, 5H, H-Ar.), 8.69-8.85 (m, 8H, CH_2, morpholine); mass: 507$(M)^+$; λmax: 465 nm.

3. ANALYTICAL ASSAY

3.1 Antibacterial Activity

The newly synthesized compounds were screened for their antibacterial activity against gram-positive bacteria *Staphylococcus aureus* (NTCC-6571) and *Bacillus subtilis*-SB and gram-negative bacteria *Escherichia coli* (TG_1-4), *Vibrio choleri* (V_1) and *Pseudomonas aeruginosa* (A_2). The standard strains used for the antimicrobial activity were procured from the Assam down town University, Guwahati, India. Antibacterial activity was carried out by Zone of inhibition (in mm) and serial broth dilution method.

3.2 Zone of Inhibition [17]

Cups were filled with 0.1 ml of the test solution and 0.1 ml of standard solution (100 µg/ml, 250 µg/ml,) and blank (D.M.F) were placed in each cups separately under aseptic condition. Then the Petri dishes uniform diffusion of the drug into the agar medium. All the Petri dishes were then incubated at 37°C for 24 hours and zones of inhibition were measured and results are presented in Table 1.

3.3 Determination of Minimum Inhibitory Concentration (MIC) [18]

The MICs were determined by the standard agar dilution method. The synthesized compounds were dissolved in 10µg/ml of DMF, as they were not fully soluble in water and then diluted by sterile distilled water to make up the solution. Compounds (5a-5d) were screened for their antibacterial activity in triplicate against *Staphylococcus aureus* (NTCC-6571), *Bacillus subtilis*-SB, *Escherichia coli* (TG_1-4), *Vibrio choleri* (V_1) and *Pseudomonas aeruginosa* (A_2) at different concentrations of 25,50, 100 (Table 2). The drugs which were found to be active in primary screening were diluted to obtain required concentrations to get more close result. The growths of bacterial cultures were monitored after 24 and 48 h. The lowest concentration, which showed no growth after spot subculture was considered as MIC for each drug. Also, the highest dilution showing at least 99% inhibition is taken as MIC. The test mixture selected for this assay contained 10^8 cells/ml. The standard drug used for this study was amoxycillin and it showed MIC at 25, 50 and 100 µg/ml against *Staphylococcus aureus* (NTCC-6571), *Bacillus subtilis*-SB, *Escherichia coli* (TG_1-4), *Vibrio choleri* (V_1) and *Pseudomonas aeruginosa* (A_2), respectively.

3.4 Anthelmintic Activity [19,20]

The anthelmintic screening was done on the synthesized novel pyrazole derivatives. Albendazole was used as standard. Adult earthworms *Eudrilus eugenia*, washed with normal saline to remove all the faecal matter, were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites in human beings. Albendazole was diluted with normal saline to obtain 0.075% w/v, 0.150% w/v and 0.225% w/v. as standards and poured into Petri dishes. All the test compounds were prepared in a minimum quantity of DMF and diluted to 15 ml with normal saline to obtain

the same concentration as like as standard and taken into the Petri dishes. Normal saline serves as a control for standard. Six earth worms of nearly equal size were placed in each Petri dish at room temperature. The compounds were evaluated by the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with the standard drug. The time taken by worms to become motionless was noted as paralysis time. To ascertain the death of the motionless worms were frequently applied with

Table 1. Zone of inhibition (IN MM) 250µG/ML against bacteria

Compound code	Zone of inhibition (in mm)				
	B.s	***S.a***	***E.c***	***P.a***	***V*C**
5a	31±0.91	28±0.73	29±0.08	25±0.09	27±0.65
5b	24±0.19	20±0.86	18±0.05	28±0.98	19±0.35
5c	23±0.82	26±0.43	21±0.09	17±0.43	22±0.86
5d	28±0.04	24±0.85	30±0.98	16±0.12	30±0.91
Amoxycilline	31±0.01	36±0.02	39±0.01	38±0.02	35±0.01
DMF	--	--	--	--	--

S .a: Staphylococcus aureus- NTCC-6571; E.c: Escherichia coli-(TG_1)$_4$; B.s: Bacillus subtilis - BS $_4$; P.a: Pseudomonas aeruginosa (A_2); V.C-Vibrio choleri-(V_1);n=3

Table 2. Mic-values of synthesized compounds against various microorganisms

Compound Code	*MIC values in µg/ml				
	B.s	***S.a***	***E.c***	***P.a***	***V.c***
5a	100	100	50	50	100
5b	25	100	50	25	100
5c	50	50	100	50	100
5d	50	100	25	50	100
Amoxycilline	100	100	100	100	100
DMF	---	---	---	---	---

S .a: Staphylococcus aureus- NTCC-6571; E.c: Escherichia coli-(TG_1)$_4$; B.s: Bacillus subtilis - BS $_4$; P.a: Pseudomonas aeruginosa (A_2); V.C-Vibrio choleri-(V_1)

Table 3. Anthelmintic activity

Sl. no.	Product code	Concentration	Time in minutes	
			Paralysis time	Death time
1	Control	---	---	---
2	Albendazole	0.075%	11	20
		0.150%	7	18
		0.225%	5	14
3	5a	0.075%	26	39
		0.150%	21	28
		0.225%	17	24
4	5b	0.075%	21	26
		0.150%	18	21
		0.225%	17	20
5	5c	0.075%	15	23
		0.150%	12	17
		0.225%	10	15
6	5d	0.075%	09	12
		0.150%	06	10
		0.225%	04	08

***Average of six readings; Species of Earth warm:- Eudrilus eugenia*

Table 4. Haemostatic activity

Sl. no.	Product code	Clotting time (Seconds)
1	5a	121±12
2	5b	74±10
3	5c	56±13
4	5d	67±09
5	Control	210±08

n=3

external stimuli, which stimulate and induce movement in the worms, if alive. The mean lethal time and paralysis time of the earthworms for different test compounds and standard drug are tabulated in Table 3.

3.5 Haemostatic Activity [21]

Clotting time of blood in the presence of various synthesized compound was determined in vitro using Lee White's Method as follows:

Human venous blood was collected in a clean, dry and corning glass tube. Clotting time determination in the presence and absence of various compounds was determined and compared. A hundred milligrams of the dried compound was suspended in distilled water and final volume was made to 0.5 ml. This extract preparation was used in experimental sets. One millilitre of freshly withdrawn human venous blood was taken in a clean, grease and detergent free corning glass tube of 1cm diameter containing 0.5ml of various compound preparations. Control determination was performed using 0.5 ml of distilled water instead of solution of compound and results are presented in Table 4.

4. RESULTS AND DISCUSSION

All the synthesized compounds are characterized based on Physical parameters, Melting point measurement, Determination of ⌊max by UV-Visible spectrophotometer, Identification of functional group by FTIR analysis, Identification and position of Hydrogen atom by PMR spectrophotometer, Determination of molecular weight by Mass spectrophotometer.

4.1 Physical Parameters

Physical parameter like physical state (colour, nature), percentage of yield, solubility, molecular weights were determined and molecular formula were elucidated.

4.2 Melting Point Measurement

The melting points of the synthesized compounds were measured and are uncorrected by open capillary and heating by using the Melting point apparatus.

4.3 Determination of λ Max by UV–Visible Spectrophotometer

The λ max of the synthesized compounds was scanned in chloroform medium using chloroform as blank in different concentration in a double beam UV-Visible spectrophotometer.

4.4 Identification of Functional Group by FTIR Analysis

Presences of the different functional groups in the synthesized compounds were identified by interpreting FTIR graph. The graphs were taken by preparing solid sample for IR by KBr pellet method. The thoroughly dried KBr was grounded with a sample in 100:1 (KBr: sample) ratio. The pellets were prepared in a hydraulic KBr press.

4.5 Anti-bacterial Activity

All the synthesized compounds were assayed for their antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Vibrio choleri* by cup plate method. Further, their minimum inhibitory concentration values against these microorganisms were determined by serial dilution method. Nutrient agar plates (37°C, 24 hr) used for the cultivation of bacteria. The results of antibacterial are summarized in the table with standard drugs Amoxycilline for comparison. Most of the synthesized compounds were found to possess a varying degree of anti-bacterial activities as evident from their minimal inhibitory concentration (MIC). Results from the zone of inhibition study clearly show that compounds 2'-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-diphenyl amino -3,4'-bipyrazol-3'-one (5a) was found to be more active against the entire tested microorganisms whereas remaining compounds were found to be moderate or less active against the entire tested microorganism. Compound 2'-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-diphenyl amino -3,4'-bipyrazol-3'-one (5a) are found to be most active among all the synthesized compounds comparable to the amoxycilline taken as a standard drug. It is evident from Anti-

bacterial results that compound showing activity against both gram^{+ve} and gram $^{-ve}$ bacteria.

4.6 Anthelmintic Activity

All the synthesized compounds were assayed for their anthelmentics activity. The assay was performed in vitro using adult earthworm (*Eudrilus eugenia*) owing to its anatomical and physiological resemblance . The substituted Pyrazole derivative was found to be superior to a standard drug with respect to anthelmintic activity. Compounds 2'-(2, 4-dinitrophenyl)-5'-methyl-5-phenyl-2', 4, 4', 5-tetrahydro-morpholino -3,4'-bipyrazol-3'-one(5d) was found to be better active among all the synthesized compounds comparable to Albendazole taken as a standard drug whereas remaining compounds found to be moderate to less active.

4.7 Haemostatic Activity

All the synthesized compounds were subjected for haemostatic activity determined in vitro using Lee White's Method. Human venous blood was collected in a clean, dry and corning glass tube. Clotting time determination in the presence and absence of various compounds was determined and compared. Distilled water has been taken as a control instead of synthesized compound solution. Compounds 2'-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-isopropyl amino -3,4'-bipyrazol-3'-one(5c) was found to be better active among all the synthesized compounds when compared to distilled water taken as control solution whereas remaining compounds found to be moderate to less active.

5. CONCLUSION

The substituted pyrazolone derivatives are gaining importance through their varied biological and pharmacological properties. In continuation of our work on substituted pyrazolone derivatives, we reported the synthesis, analytical study and biological evaluation (anti-bacterial, anthelmintic and haemostatic study) of substituted pyrazolone derivatives. The synthesis of the compounds was described as outlined in the scheme. The substituted pyrazolone derivatives were prepared by the reaction of a mixture of 2'-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-1*H*,3'*H*-3,4'-bipyrazol-3'-one (0.1 mol), different Secondary amine (0.1 mol), Formaldehyde (0.1 mol) and in ethanol was refluxed for 9 hrs and leave the reaction mixture in an ice chest or refrigerator overnight. The solid separated was filtered, washed with water and dried. The obtained crude product recrystallized from absolute ethanol. The completion of the reaction was monitored by TLC using the suitable solvent system as mobile phase. All the compounds have been characterized by their analytical and spectral data (UV IR, NMR and Mass). The synthesized compounds were subjected for antibacterial and anthelmintic study. During the pharmacological study, all the compounds were found to have significant antibacterial as well as anthelimentics activity when compared with Amoxycilline and Albendazole as a standard drug respectively. The compound also having remarkable haemostatic activity when compared with distilled water as a control instead of drug solution. From the above done work it had been known that the synthesized pyrazolone derivatives 2'-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-diphenyl amino -3,4'-bipyrazol-3'-one (5a) have more antibacterial and 2'-(2, 4-dinitrophenyl)-5'-methyl-5-phenyl-2', 4, 4', 5-tetrahydro-morpholino -3,4'-bipyrazol-3'-one(5d) showed high anthelmintic activity. The synthesised compounds like 2'-(2,4-dinitrophenyl)-5'-methyl-5-phenyl-2',4,4',5-tetrahydro-isopropyl amino -3,4'-bipyrazol-3'-one(5c) also have significant haemostatic activity. From this entire research work, we can conclude that this research work can be used for further study to synthesize many useful medicinal compounds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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