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Synthesis, characterization, anthelmintic and Insilico evaluation of 3, 5-di substituted pyrazole-1-carbothioamide derivatives

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Abstract
A Series of Bioactive compounds 3,5 Diphenyl-1H-pyrazole-1-carbothiioamide (4a), 5-(2-chlorophenyl)-3, phenyl-1H-pyrazole-1-carbothiioamide (4b),5–(3-nitrophenyl)-3phenyl-1H-pyrazole-1-carbothiioamide e (4c), 5-(2-hydroxy phenyl)-3-phenyl-1H-pyrazole-1-carbothiioamide (4d), 5-(4-dimethyl amino phenyl)-3 Diphenyl-1H-pyrazole-1-carbothiioamide (4e), were Synthesized according to the Literature methods. The Synthesized compounds were characterized by NMR, IR & Mass Spectroscopy.
All the compounds have been evaluated for *in vitro* antihelmintic activity and were compared with their corresponding standards.

Keywords: 3, 5-Di substituted pyrazole-1-carbothioamide antihelmintic, Albendazole

1. Introduction
According to the World Health Organization infectious diseases are the main cause of death and the key agents of the afflicting worldwide [1]. Helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of under nourishment, Anaemia, Eosinophilia and pneumonia and its worldwide prevalence lies between 500 million to one billion annually approximately [2]. Ideally an anti-helmintic agent should have a broad spectrum of action, high percentage of cure, free from toxicity to the host & should be cost effective [3]. Etano botanicals are widely used for the anti-helmintic activity in children of ages 5 to 13 years. The helminths which infect the intestine are cestodes Ex; Tape worms [*Taenia solium*] nematodes, e.g hook worm [*Ancylostoma duodenal*], roundworm [*Ascaris lumbricoides*] and trematodes or flukes [*Schistosoma mansoni* and *Schistosoma haematoblium*], The diseases originated from parasitic infections causing severe morbidity include lymphatic filariasis, onchocerciasis and schistosomiasis.
Literature survey revealed that pyrazole heterocyclic ring has proved to be versatile nuclei having a myriad spectrum of pharmacological activities like antihelmintic, antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer and antiviral activities [4]. Keeping in view of these valid observations, it was found significant to produce effective drugs with reduced dosing frequency, side effects and improving meets these requirements for the development of new anti-helmintic agent.
The aim of the present work is to investigate the antihelmintic activity of newly synthesized 3,5Diphenyl-1H- Pyrazole-1-Carbothioamide and its derivatives. Triazolidine-3-ones are very effective against various helminthes in decades. Moreover there are certain helminthes, which are found to be resistant to major classes of antihelminthics such as benzimidazoles, imidazothiazoles and macro cyclic lactones. Therefore a search for these 3,5Diphenyl-1H-Pyrazole-1-Carbothioamide and its derivatives leads to the evaluation of Prototype compound with antihelmintic activity.

2. Materials and Methods
Melting points were determined in open glass capillaries using GallenKamp (MFB-600) melting point apparatus and were uncorrected. IR spectra (KBr discs) Bruker analyzers were confirmed by Shimadzu FT-IR Spectrophotometer using KBr pellets technique, Model No.8400S (Japan). 1H and 13C NMR spectra were recorded on Bruker 400 MHz NMR

spectrometer (Switzerland) using DMSO as solvent. T.L.C. was run on silica gel G plates using ethyl acetate: n-hexane (7:3) as developing solvent to assess the progress of reaction and purity of the compounds. All other chemicals used in the present study were of analytical grade.

2.1 Drugs and chemicals; Benzaldehyde- (MERCK-B. No:SC1S610109), O- Hydroxy Benzaldehyde- (MERCK-B. No: PC/201/16-2), 2- Chloro Benzaldehyde- (PALLAV-B. No: PC/388-2/17-2), m- Nitro Benzaldehyde- (PALLAV- B. No: PC/2186/16-2), 4- Dimethyl Amino Benzaldehyde- (MERCK-B. No: QD5Q650881), Acetophenone- (FINAR-B. No:5065602212BP), Ethanol- (CSS.B.No-110605), Potassium Hydroxide- (FISHER), Silica gel-G- (RSEARCH-LAB FINE CHEM industries-b.No:1317310113), Ethyl acetate- (AVRA), Dimethyl Sulphoxide- (AVRA-B.No:N140122180), Sodium Chloride- (FINAR-B.No:76274020), Thiosemicarbazide- (Sisco-B. No; 5595361)

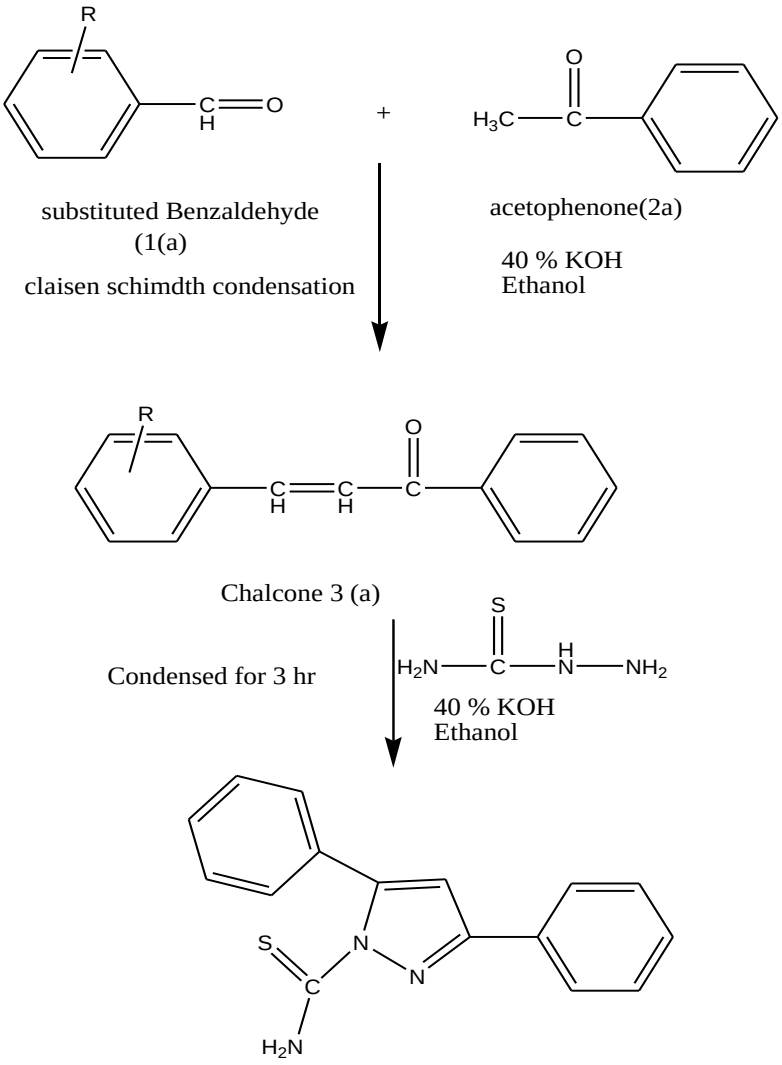
3. Chemical synthesis

Step-1: Synthesis of Chalcones: Acetophenone (0.01mol, 1.2g, Benzaldehyde (0.01mol, 1.06g) were mixed and dissolved in Ethanol (10mL). To this aqueous potassium hydroxide solution (10 ml) was added slowly With constant stirring. The reaction mixture was stirred continuously for 3h at room Temperature. The completion of reaction was confirmed by monitoring TLC using silica gel- G. After completion of the reaction, the reaction mixture was kept in refrigerator Overnight. The product was filtered and washed with cold water till the washings were neutral to Litmus, if necessary acidified with dilute HCl. The product was dried and recrystallized from Rectified spirit to get pale yellow coloured solid chalcones

Step-2: 3, 5 Diphenyl-1H-pyrazole-1-carbothiioamide (4a-4e): Conventional Synthesis

Chalcones (0.01mol, 2.08g), Thiosemicarbazide (0.01mol,0.91g) were mixed and dissolved in ethanol (10ml). To this 40% aqueous potassium hydroxide solution 10ml was added slowly with constant stirring. The Reaction Mixer was refluxed on water bath for 3h. In between TLC was monitored to check the completion of reaction. After completion of reaction, the reaction mixture was cooled to room temperature. And then poured in to ice cold water and neutralized by adding dilute HCl. The Precipitate obtained was filtered, washed with water and dried. The product was recrystallized from rectified spirit. The procedure was illustrated under Scheme 1 and the physical data were tabulated in Table 1.

Scheme 1



5,3-Disubstituted pyrazole-1-carbothioamide (4a-e)

Table 1: Physical Data

code	Compound	M.F	M.W	%yield	C%	H%	O%	N%	Cl%	S%
4a	3,5-diphenyl-1H-pyrazole-1-carbothioamide	$C_{16}H_{13}N_3S$	279.4	63	68.79	4.69	_	15.04	_	11.48
4b	5-(2-chloro phenyl-)3 phenyl-1H-pyrazole-1-carbothioamide	$C_{16}H_{12}ClN_3S$	313.8	60	61.24	3.85	_	13.39	11.30	10.22
4c	5-(4-nitrophenyl) 3 phenyl-1H-pyrazole-1-carbothioamide	$C_{16}H_{12}N_40_2S$	324.4	59	59.25	3.73	9.87	17.27	_	9.89
4d	5-(2-hydroxyphenyl)) 3 phenyl-1H-pyrazole-1-carbothioamide	$C_{16}H_{13}N_3OS$	295.4	65	65.06	4.44	5.42	14.23	_	10.86
4e	5-(4-(dimethylamino)phenyl) 3 phenyl-1H-pyrazole-1-carbothioamide	$C_{18}H_{18}N_4S$	322.4	62	67.05	5.63	_	17.38	_	9.94

Compound [4a]: 3, 5 Diphenyl-1H-pyrazole-1-carbothiioamide: Yield- 80%; **IR Data:** FTIR (γ max, cm-1) 1655 (-***C=C*** stretch), 3040 (=*C-H* stretch), 1068 (=*C-H* bend), 1540 (-C=N), 1200 (N-C=S); ^{1}H NMR (400MHZ, CDCl3): *δ* 7.22, 7.32, 7.48 (Ar-H), *δ* 6.9 (C-H), *δ* 10.238 (H2N-C=S); ^{13}C NMR (400MHZ, CDCl3): *δ* 127.5,128.8,129.3,133.1 (C-H), *δ* 133.1,146.3 (C), *δ* 132 (C=C), *δ* 164 (C=N), *δ* 176.9 (S=C-NH2).

Compound [4b]: 5-(2-chlorophenyl)- 3, phenyl-1H-pyrazole-1-carbothiioamide: Yield- 70%, **IR Data:** FTIR (γ max, C m-1) 1625 (-C=C stretch), 3010 (=C-H Stretch), 1020 (=C-H Bend), 1560 (-C=N),, 1210 (N-C=S); 770 (C-Cl) ^{1}H NMR (400MHZ,CDCl3): *δ* 7.16, 7.20, 7.22, 7.32,7.33,7.42,7.48 (Ar-H), *δ* 6.8 (C-H), *δ* 2.0 (NH2-C=S) ; ^{13}C NMR (400MHZ,CDCl3): *δ* 127.4, 127.5, 128, 129.3, 130 (C-H), *δ* 132.3, 133.1 144.7, 146.3 (C), *δ* 132 (C=C), *δ* 163 (C=N), *δ* 176.2 (S=C-NH2), ***δ*** 132.3 (Ar-Cl).

Compound [4c]: 5–(3-nitrophenyl)-3phenyl-1H-pyrazole-1-carbothiioamide: Yield- 80%, IR Data: FTIR (γmax,cm-1) 1640 (-C=C stretch), 2900 (=C-H stretch), 990 (=C-H bend), 1520 (-C=N) 1540 (Asymmetric Stretch -NO2), 1340 (Symmetric Stretch R-NO2), 1200 (N-C=S); ^{1}H NMR (400MHZ,CDCl3): *δ* 7.22, 7.32, 7.48, 7.74, 8.25 (Ar-H), *δ* 6.8 (C-H), *δ* 2,0 (H2N-C=S), *δ* 0.66 (C=N) ; ^{13}C NMR (400MHZ,CDCl3): *δ* 103.4, 121.6, 127.5, 128.4, 128.8, 129.3 (C-H), *δ* 133.1, 139.2, 144.7, 146.3 (C), *δ* 132 (C=C), *δ* 163 (C=N), *δ* 176.9 (S=C-NH2), *δ* 148.4 (-C-NO2).

Compound [4d]: 5-(2-hydroxy phenyl)-3-phenyl-1H-pyrazole-1-carbothiioamide: Yield- 60%, IR Data: FTIR (γmax,cm-1) 1660 (-C=C stretch), 3010 (=C-H stretch), 924 (=C-H bend), 1530 (-C=N), 3290 (-OH Strech), 1200 (N-C=S) ; ^{1}H NMR (400MHZ,CDCl3): *δ* 6.79, 6.88, 7.05, 7.22, 7.32, 7.48 (Ar-H), *δ* 6.8 (C-H), *δ* 2.0 (H2N-C=S), *δ* 5.0 (C-OH); ^{13}C NMR (400MHZ,CDCl3): *δ* 116.4,, 121.5, 127.9, 128.9, 129.3,133.1 (C-H), *δ* 120.1, 146.3, 155.3 (C), *δ* 132 (C=C), *δ* 163 (C=N), *δ* 176.9 (S=C-NH2), *δ* 155.3 (-C-OH).

Compound [4e]: 5-(4-dimethyl amino phenyl)-3 Diphenyl-1H-pyrazole-1-carbothiioamide: Yield- 80%, IR Data: FTIR (γmax,cm-1) 1640 (-*C=C* stretch), 3080 (=*C-H* stretch), 1025 (=*C-H* bend), 1520 (-C=N) 1200 (N-*C=S*), 1280 (-C-N-); ^{1}H NMR (400MHZ, CDCl3): *δ* 6.65,7.30, 7.32, 7.48 (Ar-H), *δ* 6.8 (C-H), *δ* 2.0 (H2N-C=S) ; ^{13}C NMR (400MHZ, CDCl3): *δ* 114.8, 127.5, 128.4, 128.8, 129.3 (AC-H), *δ* 133.1, 144.3, 146.7 (C), *δ* 133 (C=C), *δ* 164 (C=N), *δ* 176.9 (S=C-NH2), *δ* 40.3 (C-N-CH3).

4. Antihelmintic Activity [5].

Indian adult earthworms of the genus and species, *Pheretima posthuma* (family: Megascolecidae), were used to study the antihelmintic activity. The earthworms were collected from the water logged areas of soils in Nuzvid, Andhra Pradesh, India were washed with normal saline to remove all the fecal matter and waste surrounding their body. The earth worms (*Pheretima posthuma*) 5-8 cm in length and 0.2-0.3 cm width weighing 0.8–3.04 g were used for all experiment protocols. The earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence were used to study the antihelmintic activity.

Procedure

Gum acacia solution (1%) was prepared in normal saline. Test solutions were prepared by using this solution. Sample were taken petriplates and adult healthy earth warms (n=6) were introduced in to them. Observations were made for the time taken to paralyze and time taken for death of the organism. Paralysis was said to occur when the worms do not review even in normal saline. Death was concluded when worms lost their motility followed by fading away of the body colour, and the values are summarized in table

Table 2: Antihelmintic activity of Pyrazole derivatives

Sl. No.	Compounds	Dose	Paralysis time in minutes	Death time in minutes
			Mean ± S.E.M	Mean ± S.E.M
1	4a	100mg/ml	6±0.5	24±0.416
2	4b	100mg/ml	5.48±0.529	10±0.36
3	4c	100mg/ml	7± 0.763	11±0.611
4	4d	100mg/ml	3.45±0.76	19±0.8
5	4e	100mg/ml	3.08±0.36	6±0.72
6	3a	100mg/ml	5±1.2	22±0.781
7	Control (Normal saline)		-------	------
8	Albendazole	100mg/ml	2.51±1.1	18±2.1

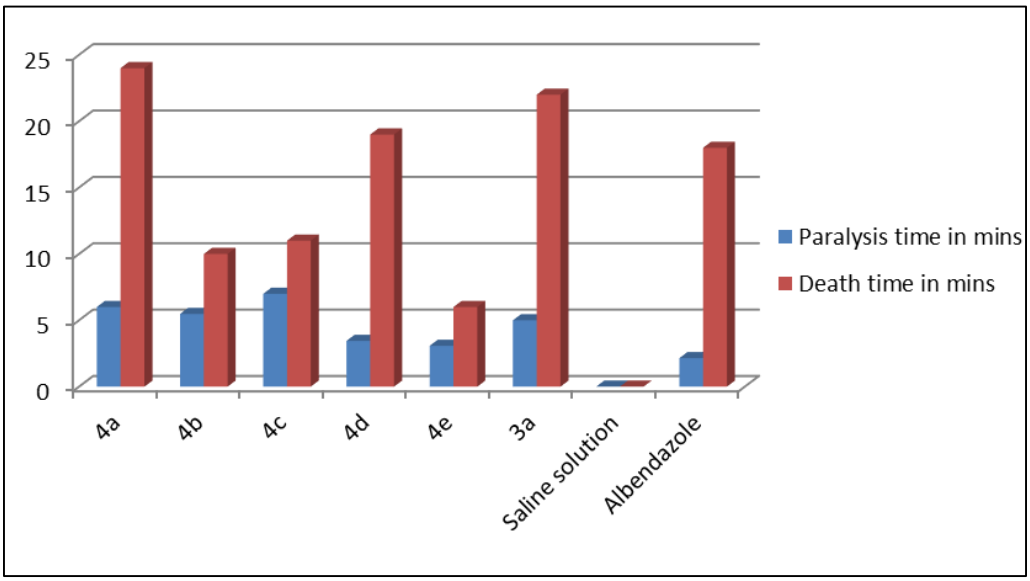


Fig 1: Antihelmintic activity (Paralysis & death times) of pyrazole derivatives

In silico evaluation for drug-likeness and toxicity predictions [6-7]

Currently, in this work three cheminformatics programmes were used to evaluate the drug likeness of compounds, toxicity predictions, to assess the inhibition of the derivatives against 5 subtypes of cytochrome P450. Open source program OSIRIS Property Explorer was used to predict the fragment-based drug-likeness of title compounds and comparing them with Fluconazole and tetracycline, to assess the occurrence frequency of each fragment in the
individual structure. The program estimated the risks of side effects, such as mutagenic, tumorogenic, irritant and reproductive effects, as well as drug-relevant properties including cLogP, LogS (solubility), MW and drug-likeness. Molinspiration cheminformatics used for calculation of important molecular properties like logP, Polar surface area, Number of hydrogen bond donors, Number of hydrogen bond acceptors, Number of rotatable bonds, Volume, Number of violations from rule of five. It was also used to predict bioactive scores for the most important drug targets like GPCR ligand, Kinase inhibitors, Ion channel modulators, nuclear receptors, Protease inhibitors, Enzyme inhibitors.

Table 3: Toxicity risks molecular properties calculation

Compound	Toxicity Risks				Molecular Properties Calculation				
	MUT	TUMO	IRRI	REP	M.W	CLP	logS	DL	DS
4a					279	3.56	-3.04	3.63	0.49
4b					313	4.16	-3.78	3.95	0.43
4c					293.0	3.9	-3.39	2.64	0.45
4d					295	3.21	-2.75	3.44	0.5
4e					322.0	3.21	-2.75	3.44	0.5
Tetracycline					444.4	-1.33	-1.83	5.43	0.81
Fluconazole					322	-0.11	-2.17	1.99	0.87

MUT: Mutagenic; **TUMO:** Tumorigenic; **IRRI:** Irritant; **REP:** Reproductive Effective; **CLP:** CLogP; **Log s:** Solubility mol/lit; **DL:** Drug-Likeness; **DS:** Drug-Score. **MW:** Molecular weight

Table 4: Molinspiration Drug Likeness Properties

Compound code	Compound IUPAC Names	Log P	Polar Surface Area	H-Bond Acceptors	H-Bond Donor	Volume
4a	3,5-diphenyl-1H-pyrazole-1-carbothioamide	3.55	43.85	3	2	247.22
4b	5-(2-chloro phenyl-)3 phenyl-1H-pyrazole-1-carbothioamide	4.18	43.85	3	2	260.76
4c	5-(4-nitrophenyl) 3 phenyl-1H-pyrazole-1-carbothioamide	3.51	89.67	6	2	270.56
4d	5-(2-hydroxyphenyl)) 3 phenyl-1H-pyrazole-1-carbothioamide	3.07	64.08	4	3	255.24
4e	5-(4-(dimethylamino)phenyl) 3 phenyl-1H-pyrazole-1-carbothioamide	3.65	47.09	4	2	293.13

Table 5: Molinspiration Bioactive Scores

Compound	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
4a	-0.41	-0.32	-0.16	-0.42	-0.59	-0.28
4b	-0.32	-0.30	-0.09	-0.35	-0.53	-0.32
4c	-0.46	-0.34	-0.24	-0.41	-0.58	-0.37
4d	-0.31	-0.25	-0.07	-0.20	-0.50	-0.20
4e	-0.28	-0.31	-0.04	-0.28	-0.45	-0.27
Tetracycline	-0.15	-0.24	-0.53	-0.09	-0.04	0.52
Fluconazole	0.04	0.01	-0.09	-0.23	-0.09	0.03

The Online Chemical Modelling Environment (OCHEM) a unique and a web-based platform which supports all the steps required to create a predictive model: one such model developed was cytochrome P450 with 5 subtypes the compounds were evaluated to assess their Inhibition on the subtypes of cytochrome P450

Table 6: O-CHEM (Online Chemical Modeling Environment)

Compound	Aqueous solubility	LogIGC50	AMES	CYP3A4	CYP2D6	CYP2C19	CYP2C9	CYP1A2
4a	5.1	0.67	Inactive	-	-	+	+	+
4b	5.6	0.75	Inactive	-	-	+	+	+
4c	6.3	0.31	Active	-	-	+	+	+
4d	4.9	0.73	Inactive	-	-	+	+	+
4e	6.2	0.2	Inactive	-	-	+	-	+
Tetracycline	3.31	0.23	Inactive	-	-	-	-	-
Fluconazole	1.8	0.15	Inactive	-	-	-	-	-

+ Inhibitor, - Non inhibitor, AQ-aqueous, IGC 50-Environmental toxicity

Results and Discussion

Physicochemical and analytical data were tabulated for synthesized compounds 4a-4e as shown in Table 1. The Structures of the compounds were characterised through IR and 1H and 13C NMR Spectral data, whereas results of antihelmintic studies tabulated in Table 2 reveals that compounds 4e, 4b and 4c were found to be the most potent Compounds in the series which when compared to the standard, compounds 4e, 4b and 4c showed high activity, other compounds 4a, 4d, and 3a showed significant antihelmintic activity.

The derivatives synthesised were evaluated by two online softwares- Osiris, Molinspiration, Ochem. OSIRIS results predicts that the compounds 4d, 4e, had drug scores 0.5. compounds 4a,4b,4c had drug scores in the range 0.43-0.49. toxicity predictions inferred that compounds 4a,4b,4c,4d,4e reproductive, compound 4e is tumorogenic effects whereas the others are safe. From the OCHEM results, all the synthesised compounds were found to inhibit the subtype CYP1A2 of cytochromeP450. Molinspiration results inferred that all the derivatives satisfy Lipinski rule of five so as to behave as a drug and found to have kinase and enzyme inhibition properties.

Conclusion

A series of 3, 5-Di Substituted Pyrazole-1-Carbothioamide Derivatives prepared by a novel method and their ability to paralyze and cause death of Indian earthworms. Though the mechanisms underlying this process remain to be fully elucidated detailed mechanistic studies and lead optimization of these 3, 5-Di Substituted Pyrazole-1-Carbothioamide Derivatives are under investigation. It is intended that the results from these studies will assist in elucidating their precise mechanism of action and provide an approach to develop new potent antihelmintic prototypes for further optimization and development to get new leads in the treatment of helminth infestations.

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