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SYNTHESIS OF SOME NEW 1-(2, 4-DINITROPHENYL)/PHENYL-4-(SUBSTITUTED PHENYL THIOUREIDO) HYDRAZONO-3-METHYL-2-PYRAZOLIN-5-ONES AND THEIR BIOLOGICAL ACTIVITY

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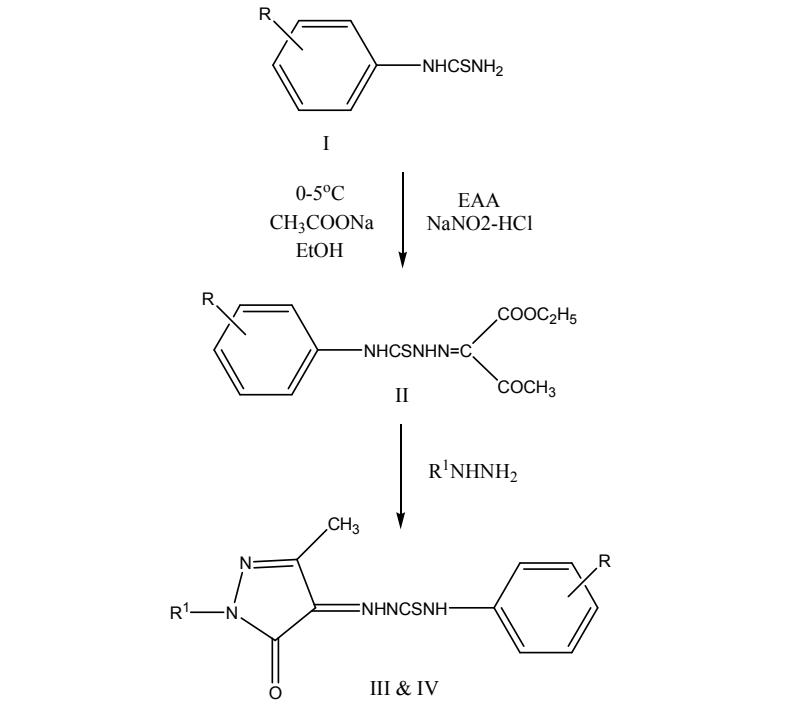
Abstract
In present investigation, we have synthesized substituted phenylthiourea (I) and reacted it with ethylacetoacetate in presence of sodium nitrite and sodium acetate which yielded 1-ethyl-2-(substituted phenyl thioureido)-hydrazono-3-oxobutyrate (II). Compounds (II) reacted with phenyl hydrazine and 2,4-dinitrophenyl hydrazine to give the title compounds (III &IV) respectively. All the newly synthesized compounds were characterized on the basis of IR, ^{1}H NMR spectra and elemental analysis data. These compounds have been screened for their antifungal activities.

Introduction
The synthesis of pyrazole and its analogues has been a subject of consistent interest because of the wide range of applications for such heterocycles in the field of pharmaceutical and agrochemical industries. The pyrazole nucleus has been reported to possess a wide range of biological properties such as antifungal, anti-bacterial,[1,2] analgesic, anti-inflammatory,[3,4] antiviral, anticonvulsant,[5] anticancer,[6,7] antihelmintic,[8] antioxidant,[9] antimicrobial,[10-12] herbicidal and cytotoxic agent.[13]
Pyrazolones show anti-inflammatory,[14] anti-bacterial,[15] antipyretic,[16] anti-microbial,[17] and anticancer[18] activities. In continuation of our work on heterocyclic compounds,[19-23] we have synthesized some new 2-pyrazolin-5-ones.

Experimental
Purity of all the newly synthesized compounds was checked on silica gel G plates using iodine vapour as the detecting agent. Melting points were determined in open capillary tubes using Gallen Kamp melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu-8400 FT-IR spectrophotometer in KBr pellets. ^{1}H NMR spectra (chemical shift in δ ppm) were recorded at 89.99 MHz using Bruker Avance II 400 spectrometer (300 MHz) using $CDCl_3$ as a solvent. Chemical shifts being expressed in δ ppm downfield from TMS as an internal standard.



R= 2-NO_2, 3-NO_2, 2,5-diCl, 3-Cl, 4-NO_2, 2-CH_3. R^1= -C_6H_5, 2,4-di$NO_2C_6H_4$.

Scheme 1

Ethyl-2-(substituted phenyl thioureido) hydrazono-3-oxobutyrate (II)

Substituted-phenylthiourea (0.01 mol) was dissolved in a mixture of HCl (8 ml) and water (6 ml) then cooled to 0°C in an ice bath and a cold aqueous solution of $NaNO_2$ (0.02 mol) was added. The diazonium salt was filtered directly into a cold solution of aceto acetic ester (0.01 mol) and CH_3COONa (0.1 mol) in ethanol (50 ml) and the resulting solid was washed with water and then crystallized from ethanol to give compound **II**.

1-Phenyl-4-(substitutedphenyl thioureido) hydrazono-3-methyl-2-pyrazolin-5-ones (III)

To compound **II** (0.002 mol) dissolved in glacial acetic acid (20 ml) a solution of phenyl hydrazine (0.002 mol) in glacial acetic acid was added and the mixture was refluxed for 4 hrs and then cooled and allowed to stand overnight. The resulting solid was dried and then crystallized from ethanol to give the title compound **III**.

1-(2,4-dinitrophenyl)-4(substitutedphenyl thioureido)hydrazono-3-methyl-2-pyrazo lin-5-ones (IV)

To compound **II** (0.002 mol) dissolved in glacial acetic acid (20 ml) solution of 2,4-dinitrophenylhydrazine (0.002 mol) in glacial acetic acid was added and the mixture was refluxed for 4 hrs and then cooled and allowed to stand overnight. The resulting solid was dried and then crystallized from ethanol to give the title compound **IV**.

Result and Discussion

Formation of compound **II** were confirmed by IR spectra in which these compounds show presence of peaks at 1690 and 1620 cm–1 (>C=O) of ester and acetyl group respectively. Peaks at 3240, 1570, 1060 and 1345 cm^{-1}show the presence of >NH, C=N, >CS and $–NO_2$ groups respectively. ^{1}H NMR showed peak at δ 5.3 ppm for >NHCSNH<, δ 4.3 ppm for $–\mathbf{CH_2}CH_3$, 1.7 for $>CH_2\mathbf{CH_3}$, δ 13 ppm for NHN=C, H bonded, δ 2.27 ppm for $>COCH_3$, δ 7.0-7.5 ppm for aromatic protons.

Compound **III** show disappearance of peak of ester due to cyclization with phenylhydrazine. IR spectrum shows C=O at 1680 cm^{-1} and 1315 cm^{-1} $–NO_2$. ^{1}H NMR shows peak at δ 2.2 ppm for $=C–CH_3$, δ 6.7-7.0 for aromatic proton (9H), δ 10.3 ppm for (–N=C) δ, 5.9 ppm for >NHCS.

Compound **IV** show disappearance of peak due to ester and cyclization occurs by the reaction of 2,4-dinitrophenyl hydrazine. In IR spectrum >C=O appeared at 1700 cm^{-1} for cyclic ketone and 1345cm^{-1} $–NO_2$. ^{1}H NMR shows peak at δ 2.02 ppm for >CH3, δ 7.2-7.3 ppm for aromatic protons (8H) and δ 8.9 ppm for (–N=C), δ 8.2 ppm for >NHCS.

Table 1: Physical and analytical data of the compounds

Compounds	M. P (^{o}C)	Yield %	Mol. Formula	Elemental Analysis			
				N %		S%	
				Found	Calc.	Found	Calc.
IIa	81	67	$C_{13}H_{14}N_4O_5S$	16.53	16.56	9.43	9.46
IIb	128	70	$C_{13}H_{14}N_4O_5S$	16.55	16.56	9.47	9.46
IIc	122	64	$C_{13}H_{14}N_4O_5S$	16.52	16.56	9.42	9.46
IId	94	72	$C_{13}H_{13}N_3O_3Cl_2S$	11.57	11.60	8.82	8.84
IIe	>300	65	$C_{13}H_{14}N_3O_3ClS$	12.78	12.82	9.73	9.77
IIf	>300	62	$C_{14}H_{17}N_3O_3S$	13.64	13.68	10.40	10.42
IIIa	96	68	$C_{17}H_{14}N_6O_3S$	21.96	21.99	8.34	8.37
IIIb	116	60	$C_{17}H_{14}N_6O_3S$	21.97	21.99	8.38	8.37
IIIc	108	54	$C_{17}H_{14}N_6O_3S$	21.95	21.99	8.34	8.37
IIId	98	59	$C_{17}H_{13}N_5OCl_2S$	17.22	17.24	7.85	7.88
IIIe	>300	70	$C_{17}H_{14}N_5OClS$	18.40	18.43	8.58	8.61
IIIf	>300	62	$C_{18}H_{17}N_5OS$	19.91	19.94	9.10	9.11
Iva	92	59	$C_{17}H_{12}N_8O_7S$	23.70	23.72	6.76	6.78
IVb	142	65	$C_{17}H_{12}N_8O_7S$	23.74	23.72	6.79	6.78
IVc	120	52	$C_{17}H_{12}N_8O_7S$	23.69	23.72	6.75	6.78
IVd	148	70	$C_{17}H_{11}N_7O_5Cl_2S$	19.72	19.75	6.42	6.45
IVe	>300	68	$C_{17}H_{12}N_7O_5ClS$	21.25	21.28	6.91	6.94
IVf	>300	65	$C_{18}H_{15}N_7O_5S$	22.20	22.22	7.23	7.25

Biological activity

Microorganisms Used

Clinical laboratory bacterial isolates of fungal isolates viz. *Fusarium Oxysporium* and *Penicillium funicolsum* were collected from the stock cultures of Microbiology Laboratory, SMS Medical College, Jaipur, India.

Preparation of Extract

The samples were prepared by 10mg/ml in DMSO and kept on a rotary shaker for 24 hrs. and stored at 4ºC in airtight bottles.

Determination of antifungal assay

Anti fungal activity of the experimental plant was investigated by agar well diffusion method[24] (Bonjar *et al*, 2005). Fungus colonies were subcultured onto Sabouraud's dextrose agar, SDA (Merck, Germany) and respectively incubated at 37°C for 24hrs and 25°C for 2-5 days. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 106 cells/mL. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube 0.1 mL of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37°C. After incubation of 24 hrs bioactivities were determined by measuring the diameter of inhibition zone in mm. All experiments were made in triplicate and means were calculated.

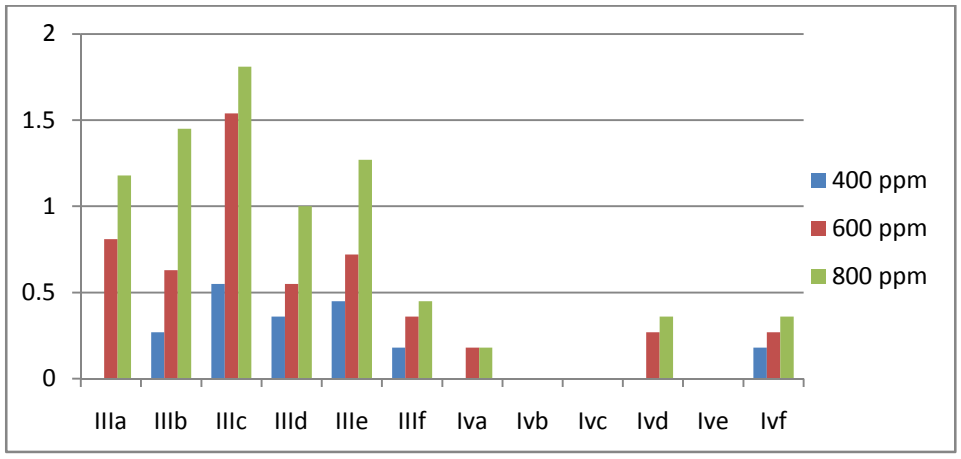
$$\textbf{Activity Index} = \frac{\text{Sample of compound}}{\text{activity of control condition}}$$

Table II Fungicial Screening Data of Compounds III a-f and IV a-f

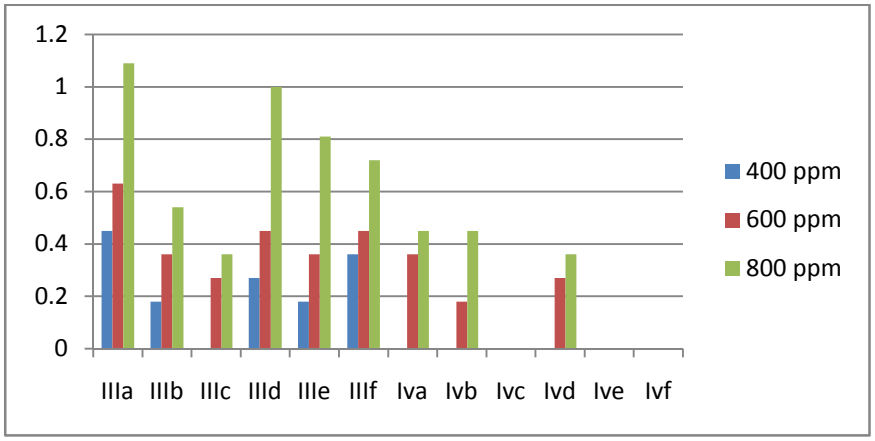
Activity Index of compounds III a-f and IV a-f						
Compounds	Penicillium funicolsum Conc. (ppm)			Fusarium Oxysporium Conc. (ppm)		
	400	600	800	400	600	800
IIIa	---	0.81	1.18	0.45	0.63	1.09
IIIb	0.27	0.63	1.45	0.18	0.36	0.54
IIIc	0.55	1.54	1.81	---	0.27	0.36
IIId	0.36	0.55	1.00	0.27	0.45	1.00
IIIe	0.45	0.72	1.27	0.18	0.27	0.36
IIIf	0.18	0.36	0.45	0.36	0.45	0.72
IVa	---	0.18	0.18	---	0.36	0.45
IVb	---	---	---	---	0.18	0.45
IVc	---	---	---	---	---	---
IVd	---	0.27	0.36	---	0.27	0.36
IVe	---	---	---	---	---	---
IVf	0.18	0.27	0.36	---	---	---

Activity Index of compounds IVb in Penicillium funicolsum

Activity Index of compounds IVb in Fusarium Oxysporium



Activity Index of compounds III a-f and IV a-f in Penicillium funicolsum



Activity Index of compounds III a-f and IV a-f in Fusarium Oxysporium

Antifungal activity

Same compounds were tests for antifungal activity against *Penicillium funicolsum* and *Fusarium Oxysporium* at a concentrations of 400, 600 and 800 ppm. respectively (Table 2).

The standard drug used in the present study was "Ketokenazole" for evaluating antifungal activity which showed 1 µg/mL against *P. funicolsum* and *F. Oxysporium.*

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