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Suprita
Department of Chemistry
CCSHAU Hisar, Haryana, India

Pardeep Loura
Department of Chemistry
CCSHAU Hisar, Haryana, India

Suman
Department of Chemistry
CCSHAU Hisar, Haryana, India

Susheel Gulati
Department of Chemistry
CCSHAU Hisar, Haryana, India

Rajvir Singh
Department of Chemistry
CCSHAU Hisar, Haryana, India

Corresponding Author:
Suprita
Department of Chemistry
CCSHAU Hisar, Haryana, India

Grinding: A novel method for synthesis of potential antifungal pyrazoles

Suprita, Pardeep Loura, Suman, Susheel Gulati and Rajvir Singh

Abstract
A benign, rapid and solvent free synthesis of pyrazoles have been done by grinding techniques by using substituted benzaldehyde, malanonitrile and 2,4-dinitrophenyl hydrazine and comparison has been done with conventional method in which solvent is used. Special features of grinding techniques involve ecofriendly, non hazardous, excellent yield and short time reaction. The synthesized compounds have been screened for their antifungal activity against *Asperillus niger* and *Rhizoctonia solani.* These synthesized compounds were characterized by IR, ^{1}HNMR and melting point.

Keywords: Pyrazole, solvent free, grinding, synthesis and antifungal activity

1. Introduction
Pyrazoles consist of 5-membered cyclic ring structure which contains 3-carbon and 2-nitrogen atoms in adjacent position. Pyrazoles belongs to the series of simple aromatic ring of the heterocyclic compounds, which have a large scale of pharmacological effect due to its alkaloid nature. 1-pyrazolyl-alanine, was the first natural pyrazole isolated from the seeds of watermelon in 1959. Pyrazole and its derivatives have a broad spectrum of application in agrochemicals and pharmaceutical industry. Synthesized the derivatives of pyrazole and evaluated its antidepressant and anticonvulsant activities [1]. These heterocyclic compounds have a unique position among heterocyclic compounds due to its biological activities such as antibacterial, hypoglycaemic, sedative-hypotonic [2] anticonvulsant and antiviral [3]. These compounds proceeds a good example of antifungal and antibacterial activities against fungal and bacterial strains respectively [4]. The newer techniques of pyrazole synthesis by using ecofriendly one pot multicomponent, solvent free, solid support, microwave [5] and ultrasound synthetic methods are more useful than that of the conventional methods [6].
The potential derivatives of these special biologically active moities represent one of the most important classes of heterocyclic compounds in pharmacological field due to their potent activities covering a wide range of application as potential antimicrobial, antiviral and herbicide [7]. The structure, synthesis and biological activities of pyrazole derivatives have long been focus of the research interests in the field of agriculture and medicine. Different pyrazole derivatives have shown nematicidal, herbicidal and insecticidal properties [8]. Novel pyrazole derivatives are screened for their antifungal activity against *Asperillus niger* [9]. The results revealed that the growth of microbial strain got affected by the synthesized compounds. Pyrazoles are also known for its antibacterial, anti HIV, anticancer, analgesic and hypoglycaemic activities [10]. Pyrazole derivatives have a long history of application in agrochemicals and pharmaceutical industry as herbicides and other active pharmaceuticals respectively. Thus, pyrazoles represent an important role in heterocyclic chemistry and occupy a prime place in organic chemistry.

2. Experimental
2.1 Method A (Conventional)
A mixture of substituted benzaldehyde, malanonitrile and 2,4-dinitrophenyl hydrazine in ethanol was taken in a flask. The reaction mixture was refluxed at 75°C for the 3 hours. Progress of the reaction was monitored with the help of thin layer chromatography. After completion of the reaction, the reaction mixture was cooled and then recrystallized from ethylacetate to afford the final compound.

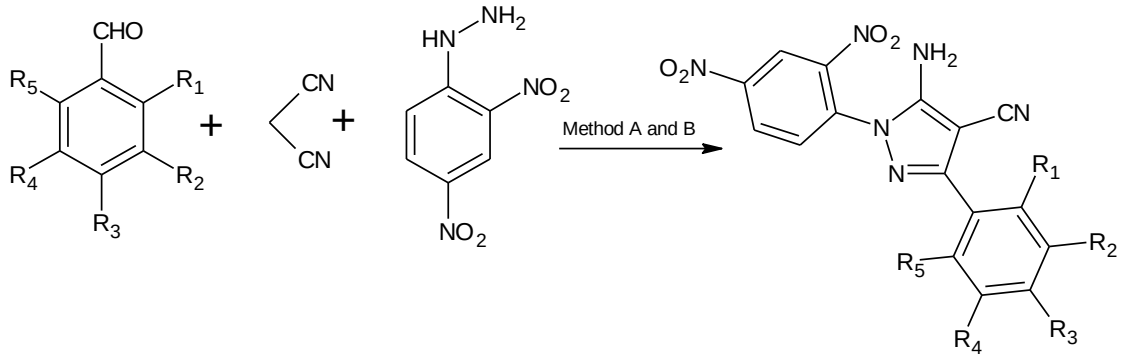
2.2 Method B (Grinding)
A mixture of substituted benzaldehyde, malanonitrile and 2,4-dinitrophenyl hydrazine were ground for 12 minutes in a mortar with pestle and left overnight at room temperature. Completion of the reaction was monitored by thin layer chromatography.

It was poured over crushed ice and solid that separated out with the help of filter paper and washed with ice cold water for 5-6 times. After that, it was crystallized from ethylacetate to afford the compound.

3. Results

3.1 Synthesis of substituted Pyrazoles (8-12)

2-hydroxy benzaldehyde (1), 4-chloro benzaldehyde (2), 4-methyl benzaldehyde (3), 2,4-dimethoxy benzaldehyde (4), 2,3-dimethoxy benzaldehyde (5), malononitrile (6) and 2,4-dinitro phenyl hydrazine (7) in equimolar ratio were taken in a flask. With constant stirring (in presence of solvent)/grinding (without solvent), it gives 5-amino-1-(2,4-dinitrophenyl)-3-(2-hydroxy-phenyl)-1H-pyrazole-4-carbonitrile (8), 5-amino-1-(2,4-dinitrophenyl)-3-(4-chloro-phenyl)-1H-pyrazole-4-carbonitrile (9), 5-amino -1- (2,4-dinitrophenyl) -3- (methyl-phenyl) -1H-pyrazole -4- carbonitrile (10) 5-amino -1- (2,4-dinitrophenyl)-3-(2,4-dimethoxy-phenyl)-1H-pyrazole-4-carbonitrile (11), 5-amino -1- (2,4-dinitrophenyl)-3-(2,3-dimethoxy-phenyl)-1H-pyrazole-4-carbonitrile (12) in good yields (scheme 1). Physical data of 5-amino-1-(2,4-dinitrophenyl)-3-(2-hydroxy/ methoxy/ chloro/ 3-hydroxy/ methoxy -phenyl) -1H-pyrazole -4-carbonitrile (8-12) have been given in table 2 and 3.



1-5, 6 7 8-12
Reagents and Reaction Conditions
Method A: Conventional method (ethanol)
Method B: Grinding at room temp.

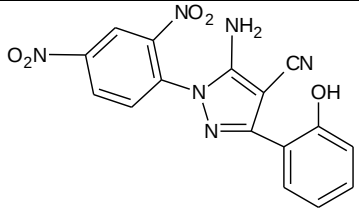
Table 1: Various functional group of pyrazole derivatives

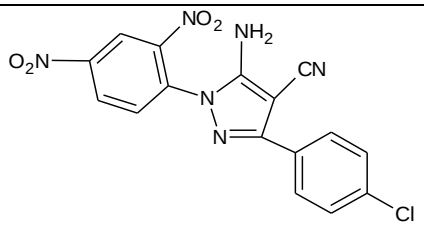
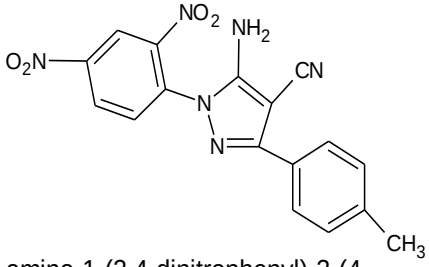
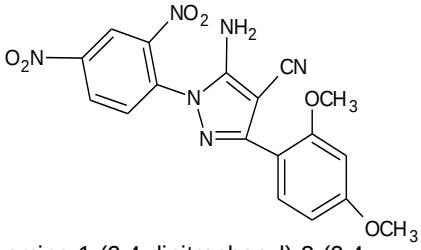
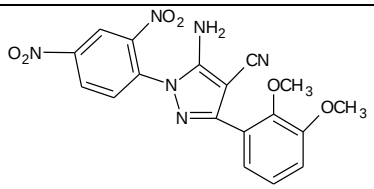
Compound Number	R_1	R_2	R_3	R_4	R_5
1,8	OH	H	H	H	H
2,9	H	H	Cl	H	H
3,10	H	H	CH_3	H	H
4,11	OCH_3	H	OCH_3	H	H
5,12	OCH_3	OCH_3	H	H	H

Table 2: A comparative analysis of physical data of substituted pyrazoles (8-12) between conventional and grinding method

Compound Number	Conventional Method		Grinding Method	
	Time (Hours)	Yield (%)	Time (minute)	Yield (%)
8	3	77	12, left overnight	82
9	3	79	10	81
10	3.5	78	8	82
11	4	81	10	85
12	3.5	83	11	89

Table 3: Physical data of synthesized Pyrazoles (8-12)

Compound Number	Molecular formula	Structure	Melting point (°C)	
			Conventional method	Grinding method
8	$C_{16}H_{10}N_6O_5$	 5-amino-1-(2,4-dinitrophenyl)-3-(2-hydroxy-phenyl)-1H-pyrazole-4-carbonitrile	232-234	233-235

9	$C_{16}H_9ClN_6O_4$	 5-amino-1-(2,4-dinitrophenyl)-3-(4-chloro-phenyl)-1H-pyrazole-4-carbonitrile	178-80	179-181
10	$C_{17}H_{12}N_6O_4$	 5-amino-1-(2,4-dinitrophenyl)-3-(4 methyl-phenyl)-1H-pyrazole-4-carbonitrile	183-84	182-184
11	$C_{18}H_{14}N_6O_6$	 5-amino-1-(2,4-dinitrophenyl)-3-(2,4 dimethoxy-phenyl)-1H-pyrazole-4-carbonitrile	229-30	229-231
12	$C_{18}H_{14}N_6O_6$	 5-amino-1-(2,4-dinitrophenyl)-3-(2,3dimethoxy-phenyl)-1H-pyrazole-4-carbonitrile	209-10	208-210

3.2 Spectral data of some selected compounds:

5-amino-1-(2,4-dinitrophenyl)-3-(2-hydroxyphenyl)-1H-pyrazole-4-carbonitrile (8): yellow solid. mp: 232-234 ºC; ^{1}H NMR (400 Hz, $CDCl_3$): 11.01 (s, 1H, OH); 10.01 (s, 2H, NH_2); 6.85-6.88 (s, 1H); 7.60-8.86 (d, 2H); 7.41-7.63 (d, 4H); IR (KBr) cm^{-1}:3356 (OH), 3267 (NH), 3105 (C=CH), 1614 (C=C, aromatic), 2223(CN), 1331 (NO_2)

5-amino-1-(2,4-dinitrophenyl)-3-(4-chlorophenyl)-1H-pyrazole-4-carbonitrile (9): pale yellow solid. mp: 178-180 °C; ^{1}H NMR (400 Hz, $CDCl_3$): 8.91 (s, 2H, NH_2); 7.41-7.52 (s, 1H); 7.65-8.23 (d, 2H); 7.23-7.75 (d, 4H); IR(KBr)cm^{-1}: 3286 (NH), 3092 (C=CH), 2227 (CN), 1328 (NO_2)

5-amino-1-(2,4-dinitrophenyl)-3-(4-methylphenyl)-1H-pyrazole-4-carbonitrile (10) pale yellow solid. mp: 182-184ºC; ^{1}H NMR (400 Hz, $CDCl_3$): 8.87 (s, 2H, NH_2); 7.27 (s, 1H); 7.42-8.64 (d, 2H); 7.40-7.69 (d, 4H), 2.31-2.50 (s, 3H, CH_3); IR (KBr) cm^{-1}: 3286 (NH_2), 3090 (C=CH), 2224 (CN), 1611 (C=C, aromatic), 1327 (NO_2)

4. Bioevaluation

The synthesized compounds have been screened for their antifungal activity against *Rhizoctonia solani* and *Aspergillus niger* fungi by Poisoned Food Techniques (Tuite., 1968) [11] at 50, 100, 150 and 200µg/ml concentrations. The degree of inhibition of growth was calculated from the mean differences between treatments and the control or percentage of latter by using the formula mentioned below. The data are presented in table 4.1.

$$\% \text{ inhibition} = \frac{C-T}{C} \times 100$$

Where

C = mycelial growth in control dish

T = mycelial growth in treated dish

Table 4: Antifungal activity of substituted pyrazoles (8-12)

Compound no.	% age growth inhibition								*Rhizoctonia solani*	*Aspergillus niger*
	Fungi									
	Rhizoctonia solani (Conc.) µg/ml				*Aspergillus niger* (Conc.) µg/ml					
	50	100	150	200	50	100	150	200	EC_{50} µg/ml	EC_{50} µg/ml
8	a	A	29	52	42.00	50.25	56.58	65.00	195.66	98.49

9	37.14	45.71	58.57	68.57	35.00	46.25	55.58	69.00	116.68	120.1
10	38.57	45.71	55.71	62.85	a	a	a	a	121.45	A
11	41.42	44.28	52.85	58.57	40.26	46.14	57.00	68.54	133.38	117.78
12	a	A	25.75	48.48	43.00	49.25	59.21	70.65	a	106

a: no growth inhibition

5. Discussion

The given scheme (1) was proceeds by given methods and comparison of both methods showed that grinding method is very safe, environmentally benign and economic method which enlighten the era of green synthesis of heterocyclic compounds. These synthesized compounds represents a great examples of antifungal compounds.

As outlined in figure 1, compound 5-amino-1-(2,4-dinitrophenyl)-3-(4-chloro-phenyl)-1H-pyrazole-4-carbonitrile (9) was found most active as compared to other compounds in this series against the tested fungi *Rhizoctonia solani*. This compound has shown percentage growth inhibition 37.14, 45.71, 58.57 and 68.57% at 50, 100, 150 and 200µg/ml concentrations respectively with 116.68µg/ml EC_{50} value.

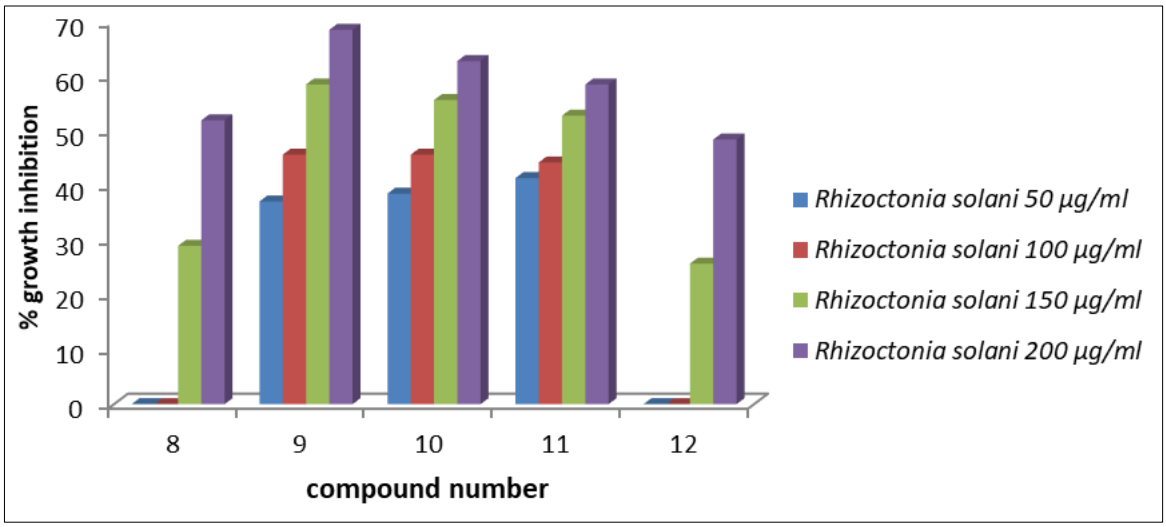


Fig 1: Antifungal activity of substituted Pyrazoles (8-12) against *Rhizoctonia solani*

The antifungal activity data delineated in figure 2 showed that compound 5-amino-1-(2,4-dinitrophenyl)-3-(2-hydroxyphenyl)-1H-pyrazole-4-carbonitrile (8) has shown the percentage growth inhibition 42.00, 50.25, 56.58 and 65% against the tested fungi *Aspergillus niger* at 50, 100, 150 and 200µg/ml concentrations. This compound has been found most active as compared to other compounds in this series with 98.49 µg/ml EC_{50} value.

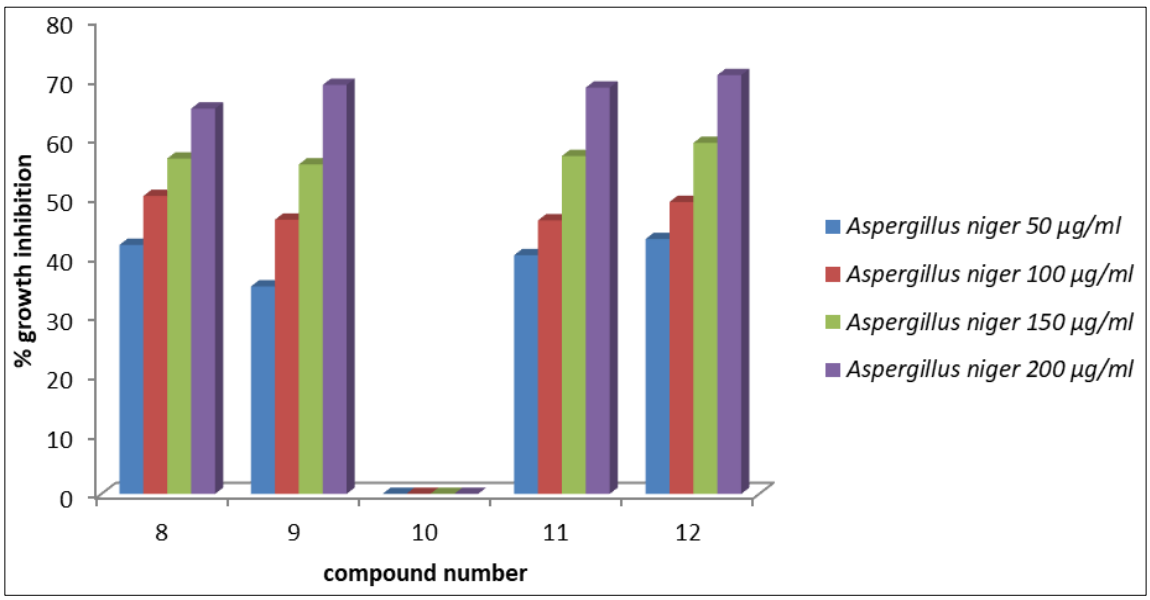


Fig 2: Antifungal activity of substituted pyrazoles (8-12) against *Aspergillus niger*

6. Conclusion

We have explained an ecofriendly method for the synthesis of pyrazoles. Grinding technique has been used for the synthesis of pyrazoles which is ecofriendly in nature, solvent free, easy to handle and give good yields as compared to conventional method. These compounds have been screened for their antifungal activity against *Aspergillus niger* and *Rhizoctonia solani* fungi.

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8. References

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