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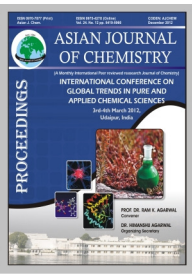

In vitro Antimicrobial Studies of Some Pyrazolones and Their SAR Studies†

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In vitro antimicrobial *viz.*, antifungal studies of some selective pyrazolones on the certified pure cultures of some specified pathogens were carried out. Compounds were established on the basis of analytical methods and advanced spectroscopic techniques. Theoretical semi-empirical studies of these compounds were also carried out. The SAR studies involving lab activities and properties obtained on semi-empirical studies were also done. Two compounds *viz.*, 4-amino antipyrine (**C4**), 3-methyl-4-nitroso-5-pyrazolone (**C5**) recorded significant antimicrobial activity against *Alternaria solani*.

Key Words: *Alernaria solani*, Antifungal activity, Pyrazolones, SAR study.

INTRODUCTION

A plant disease is a condition in which a plant exhibits some malfunction or abnormality in its growth or development. The frequency of acquiring bacterial, viral or fungal infectious diseases on plants increasing each year[1]. The battle against the infectious diseases has become a never ending process. Several synthetic[2] as well as natural compounds were screened against these microbial diseases by several workers. *Alternaria solani* is a fungal pathogen, producing a disease in *Solanum lycopersicum* (tomato)[3,4] and *Solanum tuberosum* (potato)[5] called early blight disease[6]. Pyrazole derivatives have been reported to show a broad spectrum of biological activity including antibacterial[7-12], antifungal[13-16] and antiinflammatory[17,18]. Due to its wide range of biological activity pyrazoles have received a considerable interest in the field of drug discovery. In this view medicinal chemists are also trying out their best to speed up the drug discovery process for finding the lead molecule by using different drug design techniques which includes quantitative structural activity relationship (QSAR), molecular modeling and combinatorial chemistry was practiced to reduce the time of synthesis. With the use of all these techniques the time required for the primary screening of the drug molecule to find a lead molecule is largely decreased. In this view, we planned to synthesize some novel pyrazolone derivatives and to find their SAR activity. Moisture[19] and temperature are probably the most important factor affecting plant. The key to controlling parasitic diseases is to involve the application of a protective chemical on plant[20,21]. In the present study, five pyrazolone derivatives were screened *in vitro* against *Alternaria solani*.

EXPERIMENTAL

The pathogens used in the study *viz.*; *Alternaria solani* was procure from ITCC division of plant pathology, Indian Agriculture Research Institute, New Delhi. Fresh inoculation of fungi was done by taking a loopful from IARI culture tube and streaking was done on potato dextrose media agar plates and slants and incubated at 22-24 ºC for *Alternaria solani* for revival and sub culturing purpose.

Synthetic compounds: All the five derivatives of pyrazolones, (**C1-C5**) (Table-1) were synthesized in the laboratory and their structures were confirmed by standard analytical techniques. The structures and some physical properties of pyrazolone compounds are given in Table-1.

Preparation of petri plates and media: A well known media potato dextrose agar (PDA) and potato dextrose broth (PDB) were autoclaved at 121 ºC for 15 lbs pressure for 15 min. Media were cooled and then plates were prepared by dispensing 15-20 mL medium per plate. Plates were kept in the same position for 0.5 h to solidify media and kept inverted in the incubator at 28 ºC overnight for sterility checking.

Preparation of antimicrobial discs: Whatmann 1 filter paper discs of 6 mm. Diameters were punched out from barge sheet and were autoclaved at 121 ºC, at 15 lbs for 15 min.

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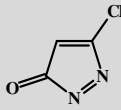
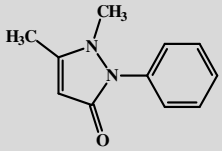
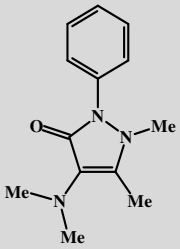
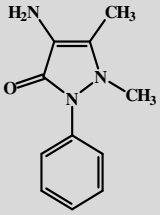
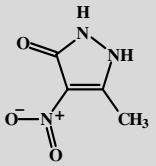
TABLE-1
STRUCTURE AND PHYSICO-CHEMICAL PROPERTIES OF PYRAZOLONES EMPLOYED IN THE STUDY

Code (name of compd.)	m.w. (m.p., ºC)	Structure
C-1 (3-Methyl-5-pyrazolone)	72 (217-223)	
C-2 (Antipyrine)	188.23 (109-113)	
C-3 (4-(Dimethyl amino)antipyrine)	231.29 (96-98)	
C-4 (4-Amino antipyrine)	203.244 (105-110)	
C-5 (3-Methyl-4-nitroso-5-pyrazolone)	143.103 (154-156)	

Preparation of serial dilutions of test compounds: Each compound was serially diluted (5 mg/mL → 0.5 mg/mL) in DMF. Each dilution of each compound was tested against fungal pathogen.

Inoculation and incubation: 100 µL of test organism was used to inoculate sterile potato dextrose agar plate by spread plate swab method. Whatmann paper discs were dispensed on glass plates and each was loaded with 5 µL volume of pre designated dilution of **C1→C5**. The discs were left air dried in the laminar air flow and were then carefully transferred to inoculated plates at pre designated positions. The plates were then incubated at 28 ºC for 48 h.

RESULTS AND DISCUSSION

Antifungal activity of pyrazolone compound: A total of five pyrazolones *i.e.*, 3-methyl-5-pyrazolone (**C1**), antipyrine (**C2**), 4-(dimethyl amino)antipyrine (**C3**), 4-amino antipyrine (**C4**), 3-methyl-4-nitroso-5-pyrazolone (**C5**) were tested for antifungal activity against *Alternaria solani*. Of the five compounds tested 4-amino antipyrine (**C4**), was found highly effective at the concentrations of (3 mg/mL to 5 mg/mL), while 3-methyl-4-nitroso-5-pyrazolone (**C5**) was effective at the concentration *i.e.* 2.5 to 5 mg/mL and 4-(dimethyl amino)-antipyrine (**C3**) was moderate while the rest *viz.*, 3-methyl-5-pyrazolone (**C1**), antipyrine (**C2**), were not effective against *Alternaria solani* (Table-2)

Analytical studies of compounds: All the compounds were investigated by melting point, CHN analysis, IR and mass spectral studies. The preliminary investigation of the compounds *viz.*, m.p./b.p. determination were carried out in Research Laboratory, Department of Chemistry of Govt. Kamala Raja Girls Post-Graduate Autonomous College Gwalior (M.P.). CHN analyses of these compounds were carried out on Elemental analyzer Elemental Vario EL III and the results obtained are included in the Table-1.

Mass spectral studies of the compounds: Mass spectral studies of the compounds were recorded on mass spectrometers Jeol SX-102 (FAB) at SAIF CDRI Lucknow. Mass spectral studies of the compounds help in establishing the compounds by means of its fragmentation studies. Apart from it studies of parent ion peak in mass spectra of any compound help in the establishment of its molecular weight. Mass spectral studies of the compounds chosen for this study show that the parent ion peaks in the spectra of these compounds appear at the m/e values where these are expected to come.

Infrared spectral studies: Infrared studies of the compounds were recorded on Perkin-Elmer infrared spectrophotometer in the range 4000 to 50 cm^{-1}, at SAIF CDRI Lucknow. Infrared absorption studies of pyrazolones have been assigned by a comparison of these spectra with those of pyrazole, five membered ring systems the mono-substituted benzene ring system and with those already reported. The strong band has been assigned to the ring stretching of 5 membered ring in pyrazolone compounds. Five membered ring hetero atomic compounds are found to have two strong bands near 1590-1560 and 1450-1430 cm^{-1} which are considered to be characteristic of five membered ring.

Computational studies of compounds: The most common and popular computational (Semi-empirical) methods used today in the field of Chemi-informatics are MNDO, ZINDO, MNDO/3 AM1 and PM3 *etc*. In this paper AM1, PM3, MNDO and ZINDO calculations for the compounds under study are being reported. For this study, Hyperchem 8.0 software package were used to calculate the structure activity relationship (SAR) related parameters such as heat of formation (HF), zero point energy (ZPE), dipole moment (DM), hydration energy (HE), refractivity (RF), polarizability (PZ), surface area (approx) (SAA), surface area (Grid), (SAG) and volume (VOL). All these calculations were carried out. The lab reported antimicrobial data have been converted into log values for SAR analysis (p MIC). All the computed parameters/descriptors were taken as dependent variable. Step wise regression analysis method was used to develop the SAR equation statistical parameters such as correlation coefficient (CC), standard error (SD) and Fischer test (F-test) were considered to select best SAR Model. When set of parameters/descriptors were subjected to stepwise linear regression analysis in order develop the SAR equations with different values of CC, SD and F-test. In present study, following significant SAR equation were obtained.

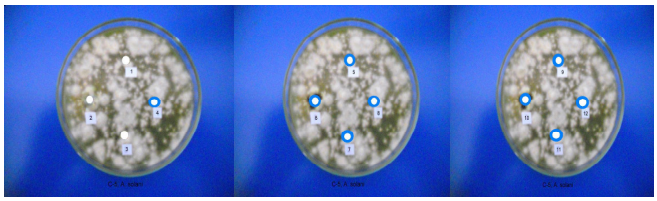
p (MIC) = (2.8600241) log P -4.8046472
N = 5, SD = 0.55217383, CC=0.8333267, F-test = 0.00110292

TABLE-2
ANTIFUNGAL ACTIVITIES OF PYRAZOLONES AGAINST *Alternaria solani*

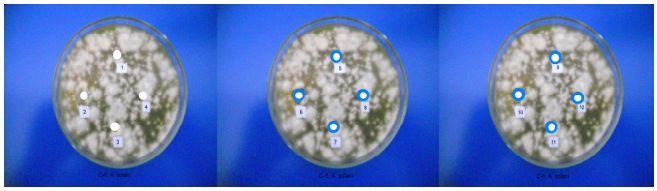
Conc.	1 mL DMF	0.5 mg	1.0 mg	1.5 mg	2.0 mg	2.5 mg	3.0 mg	3.5 mg	4.0 mg	4.5 mg	5.0 mg
Compd.											
C-1	0	-	-	-	-	-	-	-	-	-	-
C-2	0	-	-	-	-	-	-	-	-	-	-
C-3	0	-	-	-	-	1	1	1	1.5	1.5	1.5
C-4	0	-	-	1.5	1.5	1.5	**2**	**2**	**2**	**2**	**2**
C-5	0	-	-	-	1.5	**2**	**2**	**2**	**2**	**2.5**	**2.5**

Note: The figures given the zone of inhibition (ZOI) in mm

p (MIC) = (1.168857038) DM +5.058315699
N=5, SD =1.414586701, CC = 0.156484578, F-test = 1.60576E-06



Antifungal activity of 4-amino antipyrine (**C4**) against *Alternaria solani*



Antifungal activity of 3-methyl-4-nitroso-5-pyrazolone (**C5**) against *Alternaria solani*

Conclusion

The present study involves synthetic compounds to check their antifungal property with their different concentrations (5→0.5 mg/mL) in DMF against fungal phytopathogen *Alternaria solani*. This is the first study evaluating pyrazolone compounds showing their antifungal activity. The significant MIC of two compounds (*viz.* C4 and C5) has been recorded as 2.5 mg/mL of DMF. The present paper also discusses structure activity relationship along with some significant SAR equations.

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