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Evaluation Of New Pyrazole Derivatives For Their Biological Activity: Structure-Activity Relationship

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Abstract: A series of new 3-Aryl-4-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole-5-carbonitriles (1) were screened *in vitro* for their antibacterial and antifungal activities against four different organisms. The minimal inhibitory concentration (MIC's) was determined against each organism. The compounds were tested for their antioxidant activity and reducing power ability. The effect of substitution on the activity, and the possible structure activity relationship mechanism of the compounds for their antioxidant activity are presented.
Key words: Pyrazoles, antibacterial, antifungal, antioxidant, DPPH, reducing power.

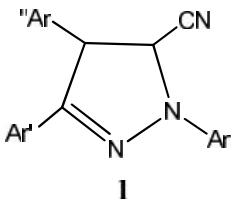
INTRODUCTION

The pyrazoles constitutes an interesting class of organic compounds with diverse chemical and biological application. They are known to possess variety of biological activities such as analgesic, anti-inflammatory, protein kinase C inhibitor[1]. A series of pyrazoles have known to exhibit significant fungicidal activities against *Corynespora cassiicola*[2], antitubercular, antimicrobial activity[3], anticancer[4], analgesic, anti-inflammatory[5] and antibacterial activity[6]. Many pyrazole derivatives possess remarkable antiepileptic and antimicrobial[7], potent and selective inhibitors of tissue-nonspecific alkaline phosphatase[8], antiamoebic[9], and antiandrogenic activities[10].

The pyrazole nucleus is a ubiquitous feature of pharmacological interest and has been proven to be a fertile source of medicinal agents. Some of these compounds have also exhibited antidiabetic[11], anaesthetic[12] properties. *N*-phenyl pyrazoles synthesized from 4-(2-bromoacetyl)-5-methyl-1-phenylcarbamoyl-1*H*-pyrazole as versatile synthons exhibited a potent antimicrobial activity[13]. Rai et al[14-15] reported the synthesis of pyrazoles, pyrazolones, bis-heterocycles bearing pyrazoline and imidazole moieties and their antimicrobial and antioxidant activity. This paper describes *in vitro* screening results of the title compounds for their antimicrobial activity, minimum inhibitory concentrations, antioxidant activity and reducing power.

EXPERIMENTAL

In view of the enormous biological potency associated with pyrazole derivatives, a series of new 3-Aryl-4-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole-5-carbonitriles (1a-h) were selected in the present work for the study of their biological activities. The antimicrobial activity of the synthesized compounds (1a-h) was done by paper disc diffusion method[16].



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a) Ar = C_6H_5, Ar' = 4-FC_6H_4, Ar'' = 4-$H_3COC_6H_4$;
b) Ar = C_6H_5, Ar' = 4-ClC_6H_4, Ar'' = 4-$H_3COC_6H_4$;
c) Ar = C_6H_5, Ar' = 4-BrC_6H_4, Ar'' = 4-$H_3COC_6H_4$;
d) Ar = C_6H_5, Ar' = 4-CNC_6H_4, Ar'' = 4-$H_3COC_6H_4$;
e) Ar = C_6H_5, Ar' = C_6H_5, Ar'' = 4-$H_3COC_6H_4$;
f) Ar = C_6H_5, Ar' = 4-$H_3COC_6H_4$, Ar'' = 4-$H_3COC_6H_4$;
g) Ar = C_6H_5, Ar' = 3,4-$(OCH_3)_2C_6H_3$, Ar'' = 4-$H_3COC_6H_4$;
h) Ar = C_6H_5, Ar' = 4-Furan-2-yl, Ar'' = 4-$H_3COC_6H_4$.

ANTIBACTERIAL ACTIVITY

The representative compounds (1a-h) were tested at a concentration (50 µg/mL) in methanol on the nutrient agar media against Gram-negative bacteria species *Escherichia coli, Salmonella typhimurium*, Gram-positive bacteria species *Bacillus substilis*, and *Staphylococus aureus.* The antibiotic streptomycin was used as standard drug against bacteria. The paper discs inoculated with bacteria were incubated for 24 hrs at 37^0C. After incubation, the zone of inhibition produced by the test compounds was measured. The screening tests were performed in triplicate and the results were taken as a mean of three determinations.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contain logarithmic serially two-fold diluted amount of test compound and controls were inoculated with approximately 5 x 10^5 c.f.u of actively dividing bacteria cells. The cultures were incubated for 24 hrs at 37^0C and the growth was monitored visually and spectrophotometrically. The lowest concentration required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). All the experiments were carried out in triplicate and the results were taken as a mean of three determinations.

ANTIFUNGAL ACTIVITY

The test compounds (1a-h) were evaluated for their antifungal activity against the fungi species *Aspergillus niger, Aspergillus flavus, C. albicans*, and *Fusarium oxysporium* strains at a concentration of 25 µg/mL in DMF in the potato dextrose agar media. The antibiotic Griseofulvin was used as standard drug against fungi. The paper discs inoculated with fungi were incubated for 72 hrs at 37^0C. After incubation, the zone of inhibition produced by the test compounds was measured in mm. The screening tests were performed in triplicate and the results were taken as a mean of three determinations.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The cultures were incubated for 72 hrs at 37^0C and the growth was monitored visually and spectrophotometrically. All the experiments were carried out in triplicate and the results were taken as a mean of three determinations.

ANTIOXIDANT ACTIVITY

The antioxidant activity of the compounds (1a-h) was assessed by evaluating their DPPH radical scavenging ability[17]. Butylated hydroxyl toluene (BHT) was used as an antioxidant.. Samples dissolved in methanol (0-50 µg/mL for samples 1a-h; 0-5 µg/mL for BHT) in 200 µL aliquot was mixed with 100 mM tris-HCl buffer (800 µL, pH 7.4) and then added 1 mL of 500 µM DPPH in ethanol (final concentration of 250 µM). The mixture was shaken vigorously and left to stand for 20 min at room temperature in dark. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. All the experiments were carried out in triplicates at different concentrations (10-50 µg/mL) and the results are expressed as mean of the three determinations.

REDUCING POWER

The reducing power ability of samples 1a-h was determined by a known method[18]. The samples 1a-h (0-50 µg/mL) was mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6 and 1% potassium ferricyanide. The mixture was incubated at 50^oC for 20 min. Then an equal volume of 10% trichloroacetic acid was added to the mixture and then centrifuged at 5000 rpm for 10 min. the upper layer of solution was mixed with distilled water and 0.1% ferric chloride at a ratio of 1:1:2 and the absorbance were measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. All the experiments were carried out in triplicates and the results are expressed as mean of three determinations.

RESULTS AND DISCUSSION

The results of antibacterial activity of the test compounds shows that; the compounds 1a-c showed remarkable activity against *E.coli, S.typhimurium* and *B. substilis* and moderate against *S. aureus*,

which is attributed to the presence of fluoro, chloro, bromo substituents at C_3-substituted benzene ring. The compounds 1d and 1h showed lesser activity against all the bacterium, this might be expected due to the presence of strong electron withdrawing -CN substituent on the aromatic ring and the 2-furanoyl substituent to the pyrazole nucleus respectively. The compounds 1e-g showed higher activity against *E.coli* and *S.typhimurium*, moderate against *B. substilis* and weak against *S. aureus*, this was expected to the presence of electron donating $–OCH_3$ groups on the aromatic ring. It was interesting to notice that, with increase in the number of $–OCH_3$ groups on the ring; enhanced their activity from 1e to 1g. The results that the presence of electron donating groups such as $–OCH_3$ on the substituted benzene ring and fluoro, chloro and bromo substituents C_5-substituted benzene ring were better antibacterial agents (Fig 1). The results indicate that the compounds 1a-c may be used as control measures against different bacteria. The results of MIC's determined reveal that some of these test compounds can act as good antibacterial agents at very lower concentrations (Fig 2).

The results of antimicrobial activity of the compounds show that all possess promising antifungal activity against *A. niger* and *A. flavus*; moderate or weak activity against *C. albicans* and *F. oxysporium* The compound 1d exhibited very weak inhibition against all the organisms tested, it might be expected due to the presence of electron with drawing –CN group on the aromatic ring. From the results of the study, it was observed that the presence of halogen substituents and electron donating substituents enhances the activity against many fungi organisms (Fig 3). The results of MIC's determined reveal that some of these test compounds can act as good antibacterial agents at very lower concentrations (Fig 4).

The free radical scavenging ability of samples 1a-h was evaluated by DPPH scavenging model system using the equation-1. All the synthesized compounds showed promising free radical scavenging ability, but of lesser activity when compared with the standard antioxidant. At the initial concentrations of (10-20 µg/mL), not much significant variations in the free radical scavenging ability of samples 1a-h was observed. However, when the concentration was increased (30-50 µg/mL) all showed a promising radical scavenging ability. The compounds 1a-d showed radical scavenging ability up to 60%; the compounds 1e,f,h showed up to 45% and the compound 1g showed 32% with reference to the standard antioxidant. From the results, it was observed that, the presence of strong electron withdrawing substituents enhanced the antioxidant property of the test compounds; the electron donating substituents retards the antioxidant property of the test compounds (Table 1). The EC_{50} values in µg/mL were determined for the antioxidant activity of the test samples measured at different concentrations (Fig 5). The experimental results indicate the potential electron donating ability of synthesized compounds.

$$\text{DPPH Scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad \text{Equation 1}$$

The samples 1a-h was evaluated for their reducing power ability to reduce ferric chloride and potassium ferricyanide complex. It was observed that at the initial concentrations of (10-20 µg/mL), there was no significant variations in the activity. However, when the concentration was increased (30-50 µg/mL), all showed remarkable reducing power. The compounds 1a-d showed higher reducing power and 1e-h showed moderate reducing power. The increased absorbance at 700 nm indicated the presence of reducing power of the synthesized compounds (Fig 6).

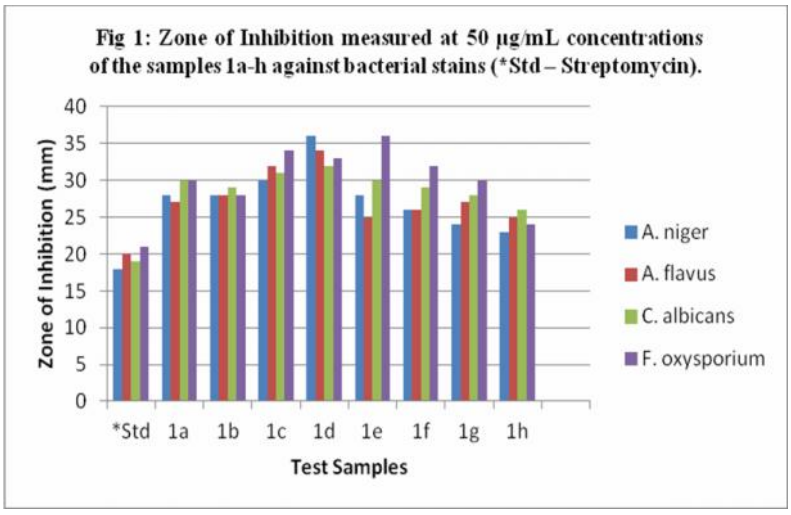


Fig 2: Minimal inhibitory concentrations in µg/mL of test samples 1a-h measured against bacterial stains (*Std – Streptomycin).

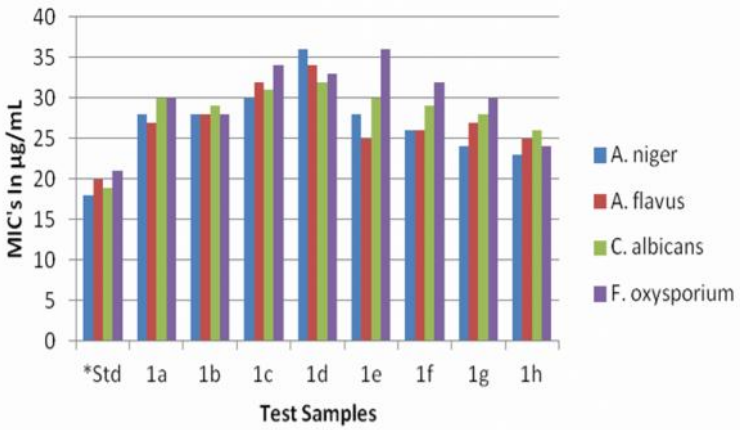


Fig 3: Zone of Inhibition measured at 50 µg/mL concentrations of the samples 1a-h against fungal stains (*Std – Griseofulvin).

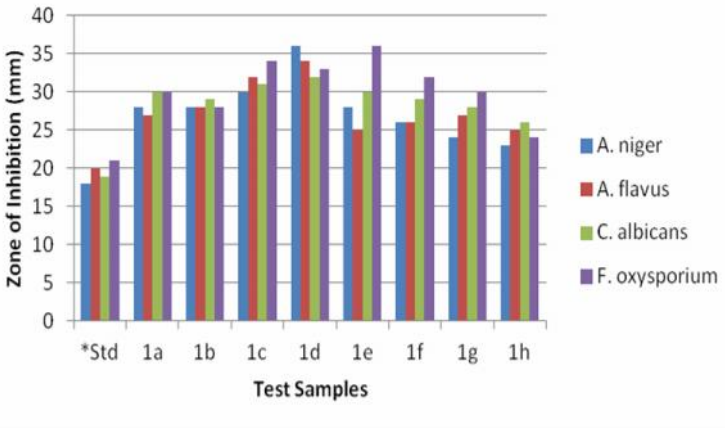


Fig 4: Minimal inhibitory concentrations in µg/mL of test samples 1a-h measured against fungal stains (*Std – Griseofulvin).

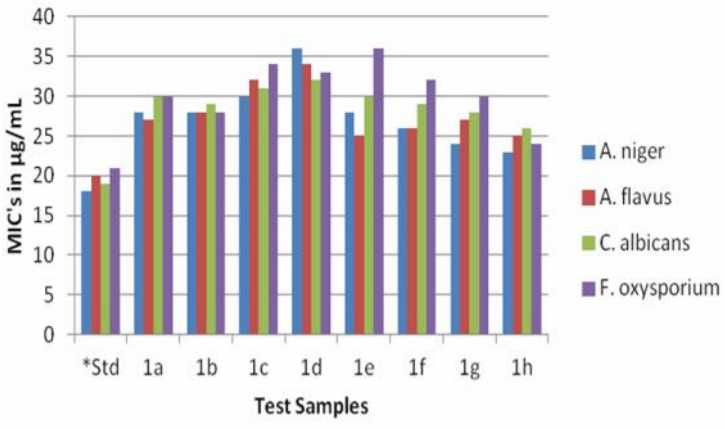
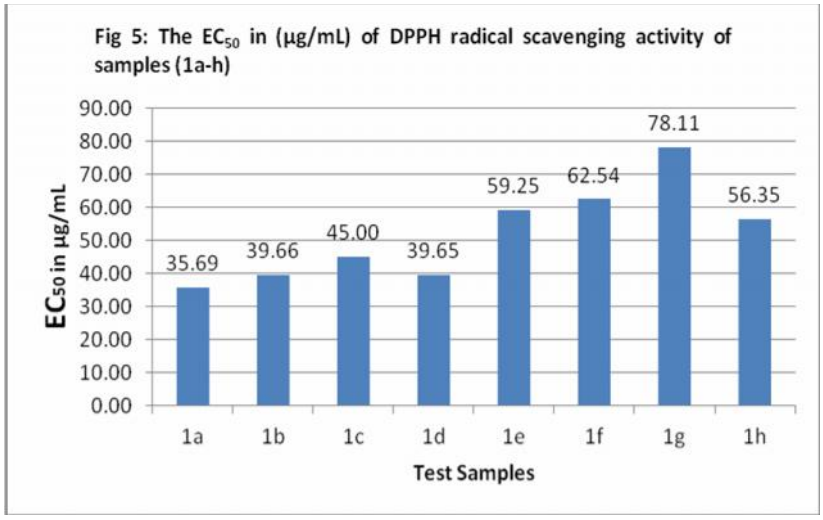
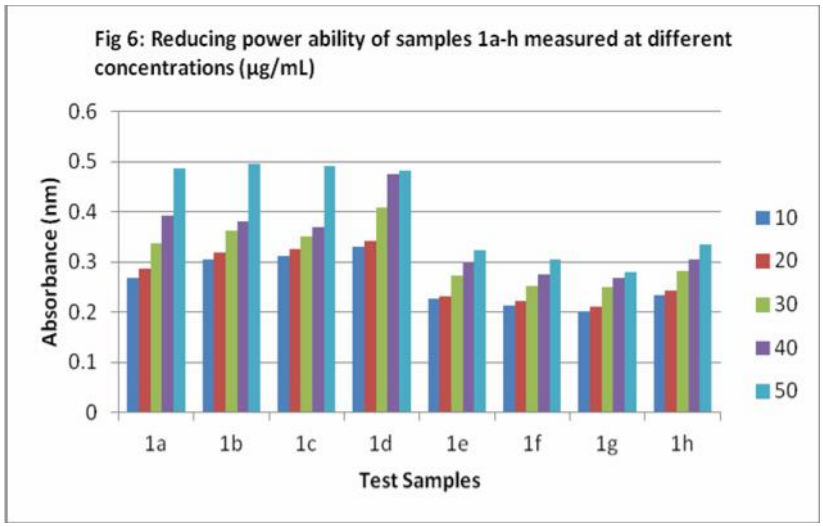


Table 1: Percentage of Radical Scavenging activity of samples 1a-h relative to the standard antioxidant BHT.

Samples	Concentration (µg/mL)				
	10	20	30	40	50
1a	10.46	13.30	28.81	38.52	58.73
1b	13.12	16.36	29.42	40.23	59.92
1c	11.22	14.96	27.61	36.96	52.67
1d	14.76	16.23	31.90	45.66	60.17
1e	11.16	13.22	26.90	34.61	42.18
1f	09.32	11.22	22.26	30.22	38.96
1g	8.12	10.36	19.80	26.75	31.80
1h	12.12	14.36	28.80	35.75	44.80

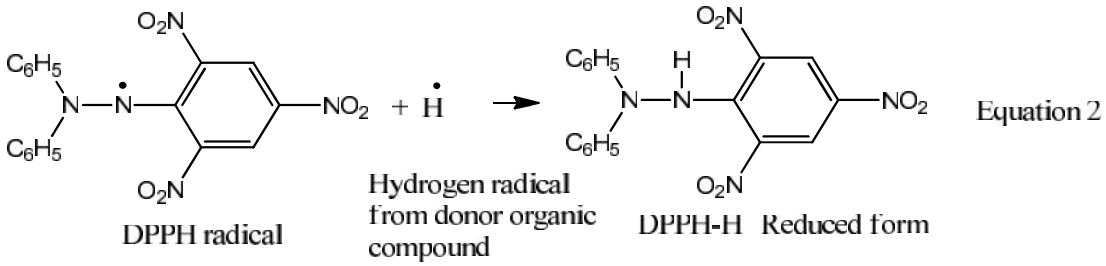
*Values are expressed as mean of the three determinations (n=3)





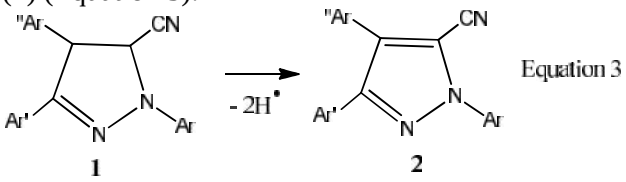
Structure-Activity relationship of antioxidant activity

2,2-Diphenyl-1-1-picrylhydrazyl is a stable organic nitrogen radical that acts as a scavenger for other radicals. It was characterized by a typical deep purple color and has a maximum absorbance in the range of 515-520 nm. DPPH radical scavenging test evaluates *in vitro* antioxidant capacity. In presence of hydrogen/electron donor or free radical scavenging antioxidants, the absorption intensity is decreased and the radical solution is decolorized to pale yellow color according to the number of electrons captured (Equation 2).



Equation 2

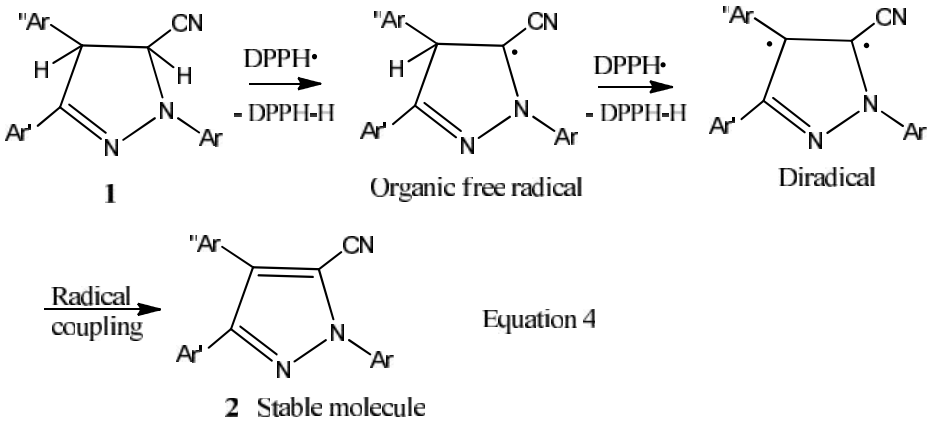
The relatively unstable non-aromatic 3-Aryl-4-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole-5-carbonitriles (**1**) on catalytic dehydrogenation, produces a stable aromatic 3-Aryl-4-(4-methoxyphenyl)-1-phenyl-pyrazole-5-carbonitriles (**2**) (Equation 3).



1 **2**

Equation 3

The instability of the 3-Aryl-4-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole-5-carbonitriles (1) was expected to be the driving force for their antioxidant activity. From the experimental results, the stiochiometry of the reaction was found to be 1:2 for test compounds: DPPH free radical. This suggests that each molecule (1) has a tendency to donate two hydrogen atom and two electrons to the acceptor molecules. In the presence of hydrogen donor organic compound (1) the DPPH free radical abstract the hydrogen atom bonded to C_5-atom along with one of its bonded electron to give organic free radical and it becomes reduced (DPPH-H). The second molecule of DPPH free radical abstracts the hydrogen atom of C_4-atom with one of its bonded electron to give organic diradical and it becomes reduced (DPPH-H). The organic diradical expected to undergo intramolecular coupling to form stable organic compound (2) (Equation 4).



Equation 4

On the basis of this speculation, the C_4 and/or C_5 positions of the pyrazole ring may be the active site responsible for antioxidant activity of the screened pyrazole derivatives.

CONCLUSION

The potency in their biological activity of the new compounds validates the significance of this study. The study revealed that the most of the compounds tested showed moderate to good activity. However, the effect of compounds on the host cell and their mode of action remain to be studied.

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