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Evaluation of New Tetra Substituted Pyrazolines for Their Antimicrobial and Antioxidant Activity; Structure-Activity Relationship

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***Abstract**: A series of synthesized new 8-[5-aryl-4-octyl-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-octanoic acid ethyl esters **1** were evaluated in vitro for their antibacterial and antifungal activity against different organisms. The compounds were tested for their antioxidant activity and their reducing power ability. The structure-activity relationship of the compounds was described.*

***Key words**: Antibacterial, antifungal, antioxidant, pyrazolines, reducing power.*

I. Introduction

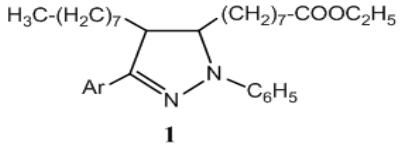
The five membered heterocycles pyrazoles and their derivatives have flourished with considerable intensity because of their synthetic and biological applications. The literature review shows that pyrazoles have known to exhibit enormous biological activity such as antibacterial, antifungal, anti-inflammatory, anticonvulsant, hypoglycemic and anticancer activities. The pyrazoles possess antipyretic, antitumour, tranquilizing and herbicidal activities. Pyrazole derivatives are reported to exhibit antioxidant [1] and antimicrobial [2-4]. A series of 3-substituted-5-hydroxy-5-trifluoro(chloro)methyl-*1H*-isonicotinoyl-4,5-dihydropyrazoles were synthesized and their in vitro antimicrobial activity was tested against INH susceptible mycobacterium tuberculosis H37Rv [5].

Human body requires tats to function properly. Fatty acids serve as the components of more complex membrane lipids and as major components of stored fat in the body. Chemically, fatty acids and their esters are used in the synthesis of much class of compounds. 4,4-Dimethyl-2-oxazolines of fatty acids are useful derivatives for mass spectrometric analysis. The utility of these adducts for structural investigations of natural and artificial fatty acids is now well established [6]. Recently Rai et al [7] reported the synthesis of 5-methyl-2-(5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carbonyl)2,4-dihydro-pyrazol-3-ones and their antimicrobial and antioxidant activity. They also demonstrated the synthesis and antimicrobial activity of bis-heterocycles bearing pyrazoline and imidazole moieties [8]. Pyrazolines obtained by the reaction of 4-acetyl thioanisole with aryl aldehydes through α,β-unsaturated ketones found to exhibit analgesic and anti-inflammatory activities [9].

In view of the varied biological potency associated with pyrazole and their derivatives, a series of synthesized new tetra substituted pyrazolines [10] were considered for studying their biological activities.

II. Materials And Methods

The chemicals/reagents used were purchased from sigma-aldrich chemicals (India) and Merck Chemicals (India). In view of the biological potency associated with pyrazole derivatives, in the present investigation; the synthesized new 8-[5-aryl-4-octyl-2-phenyl-3,4-dihydro-2*H*-pyrazol-3-yl]-octanoic acid ethyl esters **1** [10] were considered for studying their antifungal, antibacterial, antioxidant activities and their reducing power ability.



1

a) Ar = C_6H_5; b) Ar = 4-$OCH_3C_6H_4$;
c) Ar = 3,4-$(OCH_3)_2C_6H_3$; d) Ar = 4-$CH_3C_6H_4$;
e) Ar = 4-FC_6H_4; f) Ar = 4-ClC_6H_4;
g) Ar = 4-BrC_6H_4; h) Ar = 4-$NO_2C_6H_4$;
i) Ar = Furan-2-oyl.

ANTIMICROBIAL ACTIVITY: Antimicrobial activity of the synthesized compounds (3a-i) was done by paper disc diffusion method [11].

Antibacterial activity:

Gram-negative bacteria species such as *Escherichia coli, Salmonella typhimurium,* Gram-positive bacteria species such as *Bacillus substilis*, *Staphylococcus aureus* were used as antibacterial test strains. The representative compounds 1a-i was screened at the concentration (50 µg/ml) in methanol on the nutrient agar

media. The antibiotic chloromycetin was used as standard drug against bacteria. The paper discs inoculated with bacteria were incubated for 24 hrs at 37^0C. After the period of 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 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:

Four fungi species such as *Aspergillus niger, Aspergillus flavus, C. albicans, Fusarium oxysporium* were used as antimicrobial test strains. The screening of the representative compounds (1a-i) at the concentration (50 µg/ml) in DMF in the nutrient 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 the period of 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

DPPH radical scavenging assay:

The effect of the samples 1a-i in addition to the standard antioxidant butylated hydroxyl toluene (BHT) on DPPH radical was estimated according to the method of Lai et al [12-13]. Samples dissolved in methanol (0-50 µg/ml for samples 1a-i; 0-5 µg/mLfor 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 the dark. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The capability to scavenge DPPH radical was calculated using the following equation.

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

Statistical analysis: All the experiments were carried out in triplicates (n=3) and the results are expressed as mean of the three determinations.

Reducing power ability:

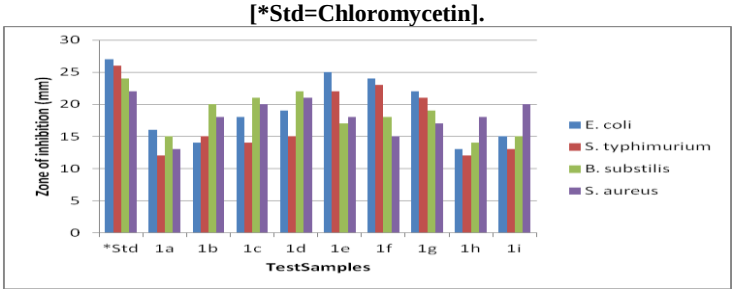
The reducing power of samples 1a-i was determined according to the method of Yen and Chen [14]. The samples 1a-i (0-50 µg/ml) was mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6 and 1% potassium ferricyanide. The mixture was incubated at 50^oC for 20 min. Then an equal volume of 10% trichloroacetic 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 (n=3) and the results are expressed as mean of the three determinations.

III. Results And Discussion

The results of antibacterial activity of the test samples 1a-i were given in (Fig 1). The investigation revealed that all these compounds showed moderate to good antibacterial activity against *Escherichia coli, Salmonella typhimurium, Bacillus substilis* and *Staphylococus aureus.* Compounds 1e, 1f, 1g, were active against the bacterium *Escherichia coli* and *Salmonella typhimuriu and moderate against Bacillus substilis* and *Staphylococus aureus.* This can be attributed to the presence of chloro, bromo and fluoro substituents on the C_5-substituted benzene ring. Compounds 1a, 1h and 1i were moderately active against all the bacteria. The compounds 1a and 1i were moderate to good active against *Staphylococus aureus* and *Bacillus substilis.* From the experimental results we found that the presence of electron donating groups such as $–OCH_3$ on the substituted benzene ring and chloro, bromo and fluoro substituents C_5-substituted benzene ring were better antibacterial agents than the other unsubstituted benzene ring or 2-furanoyl containing pyrazolines. The results thus obtained reveal that nature of substituents on the benzene ring may have a considerable impact particularly

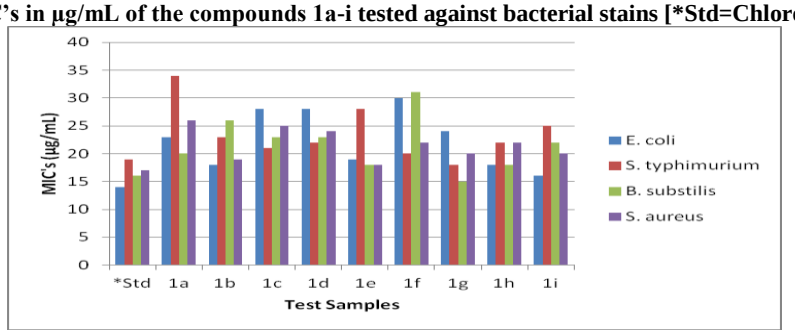
at para position. The results indicate that the compounds 1e, 1f and 1g may be used as control measures against different bacteria.

Fig 1: Zone of Inhibition (mm) at 50 µg/mL concentrations of the compounds 1a-i tested against bacterial stains [*Std=Chloromycetin].



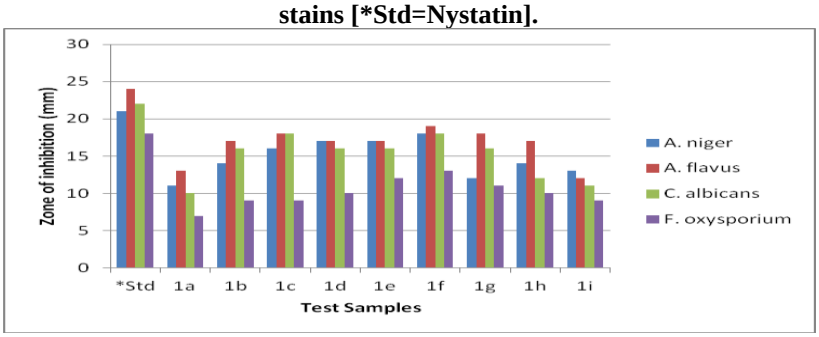
The MIC's results shows that, the concentration of the test samples 1a-f required to exhibit inhibition were not much deviated from the MIC's of the standard streptomycin (Fig 2), which shows these can be used as effective antibacterial agents against these organisms at lower concentrations.

Fig 2: MIC's in µg/mL of the compounds 1a-i tested against bacterial stains [*Std=Chloromycetin].



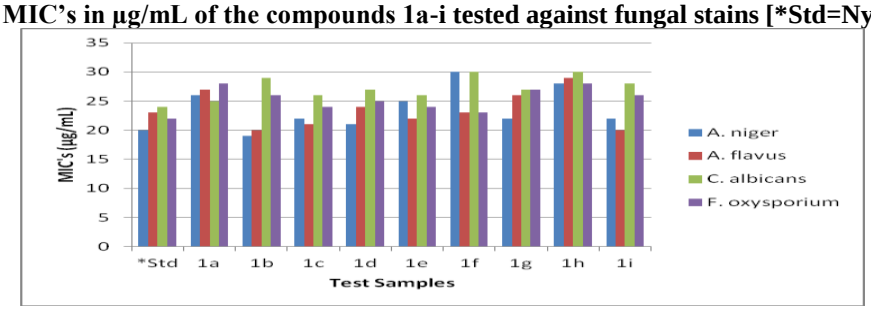
The results of antifungal activity of the test samples 1a-i were presented in (Fig 3). The investigation revealed that that all these compounds showed promising antifungal activity against *Aspergillus niger*, *Aspergillus flavus, C. albicans* and *Fusarium oxysporium. All the c*ompounds 1a-i were active against the fungi *Aspergillus niger* and *Aspergillus flavus* and moderately active against *C. albicans* and relatively less active against *Fusarium oxysporium.* The compounds containing chloro, bromo and fluoro para substituents on the C_5-benzene ring showed remarkable activity against all the organisms. Compounds 1a and 1i were moderately active against all the fungi. The compounds 1b, 1c and 1d were moderate to good active against *C. albicans.* From the experimental results we found that the presence of electron donating groups such as $-OCH_3$ on the C_5-benzene ring and chloro, bromo and fluoro substituents C_5-benzene ring were better antifungal agents than the other unsubstituted benzene ring or 2-furanoyl containing pyrazolines. The results thus obtained reveal that nature of substituents on the benzene ring may have a considerable impact particularly at para position. The results indicate that the compounds 1e, 1f, 1g and 1h may be used as control measures against different fungi.

Fig 3: Zone of Inhibition (mm) at 50 µg/mL concentrations of the compounds 1a-i tested against fungal stains [*Std=Nystatin].



The MIC's results shows that, the concentration of the test samples 1a-f required to exhibit inhibition were not much deviated from the MIC's of the standard streptomycin (Fig 4), which shows these can be used as effective antibacterial agents against these organisms at lower concentrations.

Fig 4: MIC's in µg/mL of the compounds 1a-i tested against fungal stains [*Std=Nystatin].



The results of antioxidant activity of the test samples 1a-i evaluated by DPPH radical scavenging were presented in (Fig 5). The free radical scavenging ability of samples 1a-i was evaluated by DPPH scavenging model system using the equation 1. All the samples showed free radical scavenging ability, but when compared with the standard antioxidant the samples tested showed 50% lesser activity. The results indicate that the compounds 1e, 1f, 1g and 1h possess potential electron donating ability. The IC_{50} values in µg/ml determined were given in (Fig 6).

Fig 5: Percentage of Radical Scavenging ability of the test samples 1a-i with reference to the standard BHT at different concentrations (g/ml)

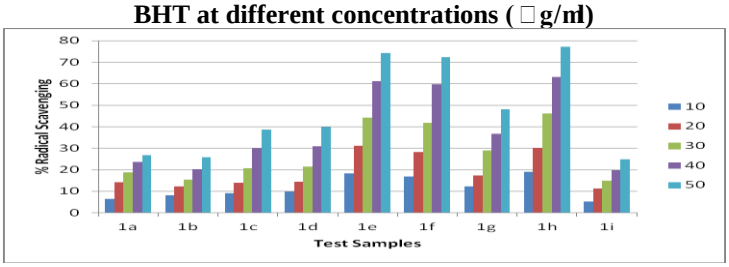
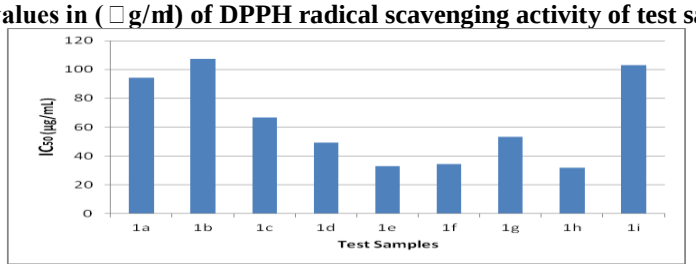
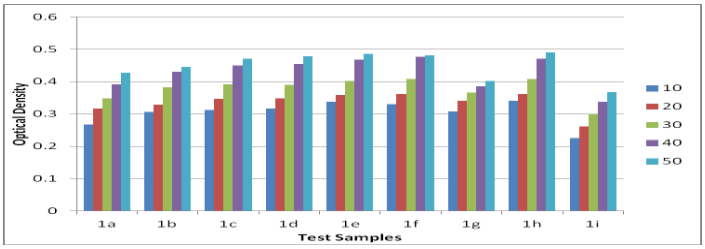


Fig 6: IC_{50} values in (g/ml) of DPPH radical scavenging activity of test samples 1a-i.

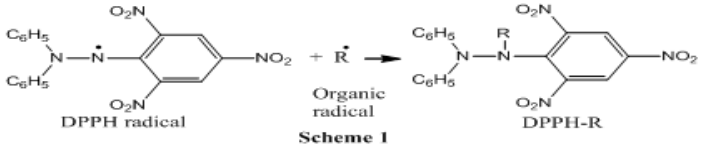


The reducing power of the samples 1a-i was evaluated for their ability to reduce ferric chloride and potassium ferricyanide complex. At the initial concentrations (10-20 µg/mL) there was no significant variations in the activity were observed. However, when the concentration was increased (30-50 µg/mL) 1f showed higher reducing power and 1a showed lower reducing power. The increased absorbance at 700 nm indicated the presence of reducing power of the synthesized compounds. The experimental results were presented in (Fig 7).

Fig 7: Reducing Power Ability Of Samples 1a-f Measured At 700nm As Absorbance At Different Concentrations.

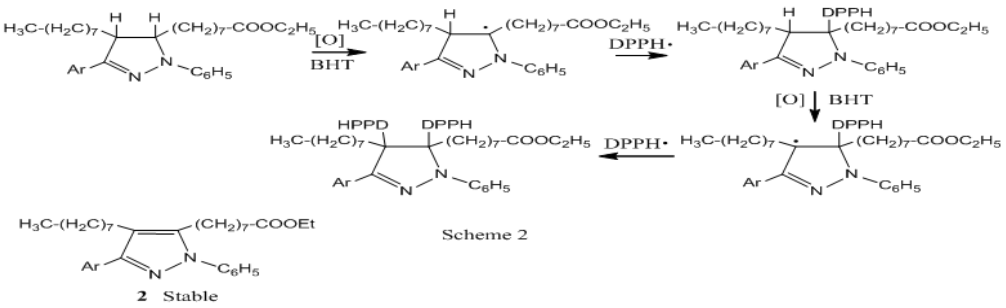


Structure-activity relationship of antioxidant activity: 2,2-Diphenyl-1-1-picrylhydrazyl is a stable organic nitrogen radical used as a scavenger for other radicals (Scheme 1). It was characterized by a typical deep purple color and has maximum absorbance in the range of 515-520 nm. DPPH radical scavenging test evaluates *in vitro* antioxidant capacity. In presence of hydrogen/electron donor (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.



Scheme 1

The new tetra substituted pyrazolines are relatively instable due to non aromatic pyrazolines ring (2). In order to become more stable aromatic pyrazole, pyrazolines are having a tendency to give off two protons and two electrons. The stability of the test compounds was expected to be the driving force for their antioxidant activity. The standard oxidant Butylated hydroxyl toluene (BHT) abstract the hydrogen atom bonded either to C_3 atom along with one of its bonded electron to give free radical of the title compounds, which was trapped in situ by the DPPH free radical. The second molecule of BHT abstracts the remaining hydrogen atom either of C_4 atom to form other organic free radical, which was scavenged by second molecule of DPPH radical (Scheme 2).



Scheme 2

IV. Conclusion

The divergence in the above biological activity of these compounds validates the significance of this study. The activity of the sample depends upon the nature of the substituents present on the pyrazoline ring. However, the effect of compounds on the host cell and their mode of action remain to be studied.

V. Acknowledgements

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References:

[1] E. Abdu Musad, R. Mohamed, B.A. Saeed, B.S. Vishwanath, K.M.L. Rai, "Synthesis and evaluation of antioxidant and antibacterial activities of new substituted bis(1,3,4-oxadiazoles), 3,5-bis(substituted) pyrazoles and isoxazoles", *Bioorg. and Med. Chem. Letters*, 21(12), 2011, 3536-3540.

[2] Vijay V. Dabholkar, Faisal Y. Ansari, "Synthesis and characterization of selected fused isoxazole and pyrazole derivatives and their antimicrobial activity", *J. Serb. Chem. Soc*., 74(11), 2009, 1219-1228.

[3] N. Satheesha Rai, Balakrishna Kalluraya, B. Lingappa, Shaliny Shenoy, Vedavathi G Puranic, "Convenient access to 1,3,4-trisubstituted pyrazoles carrying 5-nitrothiophene moiety via 1,3-dipolar cycloaddition of sydnones with acetylenic ketones and their antimicrobial evaluation", *Eur. J. of Med. Chem.*, 43, 2008, 1715-1720.

[4] Ahmad M Farag, Abdelrahman S Mayhoub, Saber E Barakat, Ashraf H Bayomi, "Synthesis of new N-phenylpyrazole derivatives with potent antimicrobial activity", *Bioorg. and Med. Chem.*, 16(8), 2008, 4569-4578.

[5] P.E. Almeida da Silva, D.F. Ramos, H.G. Bonacorso, A.I. de la Iglesia, Marli R. Oliveira, Tatiane Coelho, Jussara Mavarini, Hector R. Morbidoni, Nilo Zanatta, Marcos A.P. Martins, "Synthesis and in vitro antimycobacterial activity of 3-substituted 5-hydroxy-5-trifluoro[chloro]methyl-4-5-dihydro-1H-(isonicotinoyl) pyrazoles", *Int. J. of Antimicrobial Agents*, 32(2), 2008, 139-144.

[6] V. Spitzer, "Structure analysis of fatty acids by gas chromatography-low resolution electron impact mass spectrometry of their 4,4-dimethyloxazoline derivatives-a review", *Prog. Lipid Res*., 35, 1997, 387-408.

[7] K.B. Umesha, K.M.L. Rai, M.A. Harish Nayaka, "Antioxidant and Antimicrobial Activity of 5-methyl-2-(5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carbonyl)-2,4-dihydro-pyrazol-3-one", *Int. J. of Biomed. Sci.*, 5(4), 2009, 359-368.

[8] B. Jayashankara, K.M. Lokanatha Rai, "Synthesis and antimicrobial studies of new series of pyrazoline bearing bis heterocycles via 1,3-dipolar cycloaddition reactions", *E-Journal of Chemistry*, 5(2), 2008, 309-315.

[9] T. Karibasanagouda, Airody Vasudeva Adhikari, M. Girisha, "Synthesis of some new pyrazolines and isoxazoles carrying 4-methylthiophenyl moiety as potential analgesic and anti-inflammatory agents", *Indian J. of Chem.*, 48B, 2009, 430-437.

[10] M. Govindaraju, G. Vasantha Kumar, K. Ajay Kumar, "Synthesis of 8-(5-Aryl-4-octyl-2-phenyl-3,4-dihydro-2*H*-pyrazol-3-yl)-octanoic acid ethyl esters Via 1,3-Dipolar cycloaddition reaction", *IOSR J. of Applied Chem*,, 2012, (Communicated).

[11] B. El-Amraoui, J.-F. Biard, M.J. Uriz, S. Rifai, A. Fassouane, Antifungal and antibacterial activity of Porifera extracts from the Moroccan Atlantic coasts. *Journal of Medical Mycology.* 20, 2010, 70-74.

[12] L.S. Lai, S.T. Chou, W.W. Chao. Studies on the antioxidative activities of Hsian-tsao (Mesona procumbens Hemsl) leaf gum. *J. Agri. Food Chem*., 49, 2001, 963-968.

[13] K. Ajay Kumar, K.M. Lokanatha Rai, G. Vasanth Kumar, B.N. Mylarappa. A facile route for the synthesis of ethyl *N*-aryl-2,6-dioxo-piperid-3-ene-4-carboxylates and their biological activity. *Int. J. of Pharm. and Pharm. Sci.*, 4, Suppl 4, 2012, 564-568.

[14] Gow-ChinYen, Hui-Yin Chen. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.*, 43, 1995, 27-32.