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A Study of Pyrazole Derivative Synthesis and Characterization

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Abstract – Pyrazoles and their variously substituted derivatives are important biological agents and substantial amount of study activity has been directed towards this class. In particular, they are used as antitumor, antibacterial and antifungal, antiviral, antiparasitic, antitubercular, insecticidal, anti-inflammatory, anti-diabetic, anesthetic and analgesic agents. A four new 1,3,5-trisubstituted pyrazole derivatives (2a-d) were synthesized from the reaction of corresponding 1,3-diaryl-2-propene-1-one compounds (1a-d) with phenyl hydrazine in glacial acetic acid. The pyrazole derivatives were divided for their antimicrobial activities in contradiction of several bacterial species and candida albicans.

Keywords – Pyrazoles, Pyrazole Derivatives, Antimicrobial Activities

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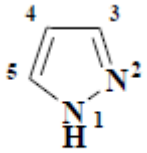
INTRODUCTION

Pyrazoles are five-membered two-nitrogen containing heterocyclic compound and they have the formula C3H4N2 and are colorless liquid. Pyrazoles are aromatic compounds and have different tautomeric structures. Unsubstituted pyrazole may be presented in two tautomeric forms and for the pyrazole derivatives that have two carbon atoms neighboring the nitrogen atoms on the ring have dissimilar substituent, five tautomeric structures are possible. When substitutions are introduced on the first, third and fourth position of this ring, it is highly activated and readily reacts to give various moieties.



Structure of Pyrazole

Synthesis of 1,3,5-trisubstituted pyrazoles (2a-2d): These compounds were synthesized according to modified procedure described in the reference [10]. To the mixture of equivalent chalcone 1a-1d (1 mmol) in 20 mL of glacial acetic, phenyl hydrazine (1 mmol) was added & reaction mixture was refluxed immediate. Later completion of reaction as monitored by TLC using ethyl acetate: hexane system (5:5 and 3:7), the reaction mixture was cooled and poured into ice water. The precipitate washed with cold water and recrystallized from ethanol.

N-(4-(1-phenyl-5-(thiophen-2-yl)-1H-pyrazol-3-yl)-phenyl)-acetamide (2a): Brown powder, yield 60%, M.P. 90°C to 93°C; IR (cm-1): 3275 (NH), 3103 (aromatic C-H), 2964 (aliphatic C-H), 1670 (C=O), 1651 (C=N), 1593 (C=C). 1H-NMR (300 MHz, DMSO-d6) δ (ppm): 2.06 (s, 3H, CH3), 6.60-8.15 (m, 13 H, 12 Ar-H, pyrazol-H), 10.05 (s, 1H, NH). GCMS (NCI) m/e: 359 M+ for C21H17N3OS.

4-(1-Phenyl-5-thiophen-2-yl-1H-pyrazol-3-yl)-phenol (2b): Brown powder, yield 82%, M.P. 62°C to 64°C; IR (cm-1): 3150 broad peak (OH), 3034 (aromatic C-H), 2950 (aliphatic C-H), 1641(C=N), 1597 (C=C). 1H-NMR (300 MHz, DMSO-d6) δ (ppm): 6.75-8.10 (m, 13 H, 12 Ar-H, pyrazol-H), 11.50 (s, 1H, OH). GCMS (NCI) m/e: 318 M+ for C19H14N2OS.

N-(4-(5-(3,4-dimethoxyphenyl)-1-phenyl-1H-pyrazol-3-yl)-phenyl)-acetamide (2c): Brown powder, yield 85%, M.P. 68-71°C; IR (cm-1): 3404 (NH), 3050 (aromatic C-H), 2929 (aliphatic C-H), 1672 broad peak (C=O, C=N), 1593 (C=C). 1H-NMR (300MHz, DMSO-d6) δ (ppm): 2.06 (s, 3H, CH3), 3.75 (s, 3H, OCH3), 3.81 (s, 3H, OCH3), 6.80-8.15 (m, 13H, 12 Ar-H, pyrazol-H), 10.15 (s, 1H, NH). GCMS (NCI) m/e: 413 M+ for C25H23N3O3.

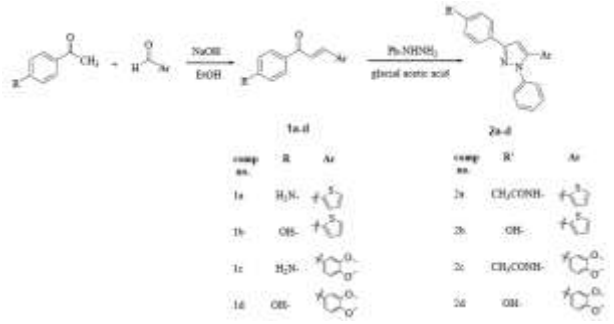
4-(5-(3,4-dimethoxyphenyl)-1-phenyl-1H-pyrazol-3-yl)-phenol (2d): Brown powder, yield 34%, M.P 88°C to 91°C; IR (cm-1): 3281 broad peak (OH), 3037 (aromatic C-H), 2931(aliphatic C-H), 1651(C=N), 1597 (C=C). 1H-NMR (300 MHz, DMSO-d6) δ (ppm): 3.70 (s, 3H, OCH3), 3.75 (s, 3H, OCH3), 6.75-7.65 (m, 12H, Ar-H), 8.59 (s, 1H, pyrazol-H), 9.80 (s, 1H, OH). GCMS (NCI) m/e: 372 M+ for C23H20N2O3.

Antimicrobial study

The 1,3,5-trisubstituted pyrazole derivatives (2a-2c) were screened for their antimicrobial activity in contradiction of *Escherichia coli*, *Pseudomonas aeruginosa* (gram positive), *Staphylococcus aureus*, *Bacillus subtilis* (gram positive) as well as *candida albicans* using the well diffusion method. Dimethyl sulfoxide (DMSO) was used as a control and the test was achieved at 10 mg/mL and 100 mg/mL concentration using DMSO solvent. Each experiment was run in triplicate and the average reading was recorded.

Synthesis

Chalcone derivatives (1a-d) were obtained by the reaction of methyl ketones with substituted aldehydes in ethanol in the presence of sodium hydroxide (Claisen-schmidt method) Scheme 1.



SCHEME 1. Synthesis of 1,3,5-trisubstituted pyrazole derivatives (2a-2d).

The 1,3-diaryl-2-propene-1-one derivatives (1a-1d) were characterized using IR, 1H-NMR and GCMS technique. The IR spectrum of (E)-1-(4-aminophenyl)-3-(thiophen-2-yl)-prop-2-en-1-one (1a) derivative shows absorption at 3427 and 3313 cm-1 related to -NH2 and 1631 cm-1 which is due to the stretching frequency of C=O group, while the aliphatic CH=CH and C=C stretching frequencies appeared at 1595 cm-1 and 1575 cm-1 respectively.

The 1H-NMR of 1a showed a singlet signal at ð 6.12 due to NH2 protones, doublet signals at ð 6.61 related to two aromatic protons, while the other aromatic protons and the COCH=CH protons appears as a multiplet signal at 7.15 ppm to 7.86 ppm. The GCMS peak at 229 further confirms the molecular ion M+ of compound. 1,3,5-Trisubstituted pyrazole derivatives (2a- 2d) were synthesized by refluxed of (1,3-disubstituted)-prop-2-ene-1-one (1a-d) and phenyl hydrazine mixture in glacial acetic acid as concise in SCHEME 1. The synthesized derivatives were categorized after recrystallization from suitable solvent by recording their IR, 1H-NMR and GCMS spectra. The IR spectrum of compound 2a showed absorption at 3275 referring to stretching frequency of N-H and 1670 cm-1 related to amide carbonyl (C=O), while absorptions of C=N and C=C groups appear at 1651 cm-1 and 1593 cm-1, respectively. The 1H-NMR of pyrazole derivative 2a showed singlet appeared at ð 2.06 due to methyl protons of amide group (COCH3) and multiplet signals at ð 6.60-8.15 related to aromatic protons as well as pyrazole proton. The NH amide proton appears as a singlet at 10.05 ppm. The molecular ion (359) M+ of pyrazole derivatives (2a) strongly confirmed by GCMS spectrum. Physical properties, spectral information, and mass analysis of all the synthesized compounds are illustrated in the experimental part.

Antimicrobial study

This research depicted the In Vitro assay of the 1,3,5-trisubstituted pyrazole derivatives (2a-c) against several microbial species (TABLE). In Vitro assay accomplished by two concentrations of synthesized derivatives 10 and 100 mg/mL. All the synthesized derivatives 2a-2d, especially compound 2b exhibited promising activities against *candida albicans* at 10 and 100 mg/ mL concentrations. The promising discovered pyrazole derivative 2d shows manifest antimicrobial activity against all bacterial species as well as *candida albicans* as described by TABLE .

TABLE . Antimicrobial activity of 1,3,5-trisubstitutedpyrazole derivatives against versatile microbial species.

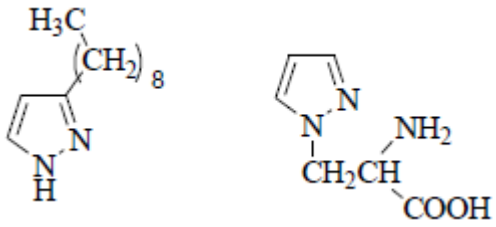
Pyrazole derivatives	Inhibition zone (mm) at 10 and 100 mg/mL				
	Gram Negative		Gram Positive		Fungi
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*
2a	-	-	-	-	13, (21)*
2b	-	-	15, (15)*	15, (17)*	16, (21)*
2c	-	-	-	-, (14)*	13, (17)*
2d	-, (14)*	19, (20)*	-, (11)*	10, (12)*	12, (18)*

(-): No inhibition observed *: Inhibition zone in mm at 100 mg/mL

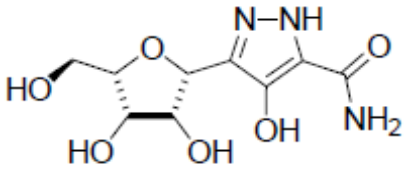
LITERATURE REVIEW

Pyrazole and its derivatives have been well known in pharmaceutical chemistry because of their wide spectrum of biological activities. In the following paragraphs carrying the literature review, the importance of pyrazole and its varied therapeutic uses have been explained. Naturally occurring pyrazoles were isolated after 1950's.

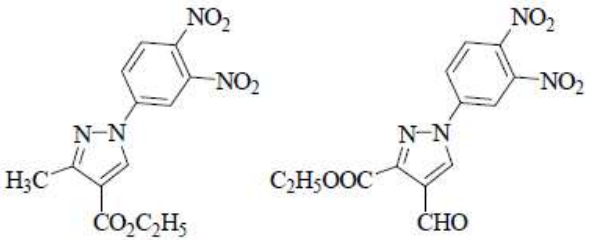
The first natural pyrazole, 3-*n*-nonylpyrazole, was obtained from a plant of the *piperaceae* family namely *Houttuynia Cordata.*This was obtained from tropical Asia and it showed antimicrobial activity. The other natural pyrazole derivative, *levo*-β-(1-pyrazolyl) alanine, was isolated from watermelon seeds (*Citrullus Vulgaris*) by Japanese researchers.



An azole class of C-nucleoside, 4-hydro-3-β-D-ribofuranosylpyrazole-5- carboxmide or pyrazofuran was isolated by Buchanan *et al.* (1981) from the fermentation broth of *Streptomyces candidus.* This antibiotic has antiviral activity and also showed very good cytotoxicity.

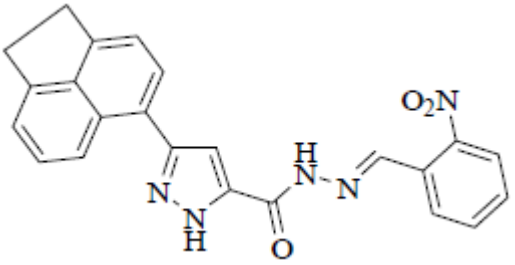


Sridhar *et al.* (2004) prepared a new 1*H*-pyrazole derivatives for their antimicrobial activities. Antibacterial activity tested against *Escherichia coli*, *Pseudomonas aeuroginosa*, *Enterobacter facecalis* and *Staphylococcus aureus.* Antifungal activity tested against *Alernarnia alternate*, *Bipolaris oryzae Curuvularia lunata*, *Fusaricom oxysperum* and *Rhizochonia solani.* All compounds showed significant activity. Among all, compounds showed good antibacterial and antifungal activity against tested microorganisms.

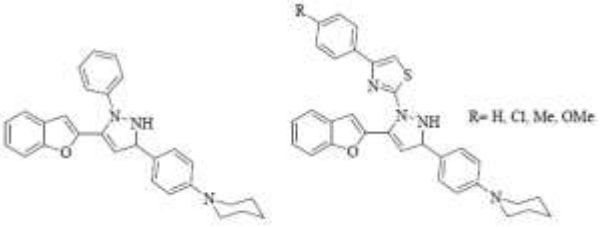


Three tripod pyrazole derivatives have been organized by Bouabdallah *et al.* (2006). These derivatives were tested for their anticancer studies on two cell line murin mastocytoma (P815) and human laryngeal carcinome (Hep). Compound showed promising cytotoxicity with IC50 17.82 against P815 and IC50 3.25 against Hep cell lines. More importantly, cytotoxicity of compound against Hep cell lines is potent than standard Adriamycin.

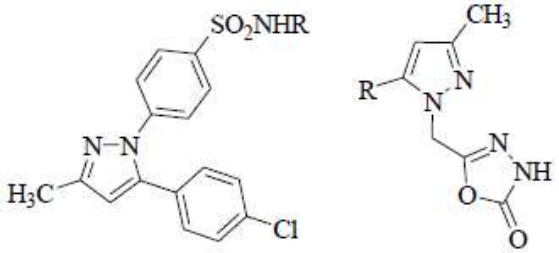
Shih *et al.* (2010) reported pyrazole based influenza agent as well as antiviral activity. Compound showed both activities in sub-micromolar level and this moiety becomes a good influenza and antiviral agent in the future.



Hamad et al (2012) synthesized novel pyrazole-1-carbothioamide and pyrazole piperidine derivatives. Target compounds were appraised for *in vitro* antibacterial and anti-inflammatory activity. Compounds showed more than 70 % inhibition and these compounds may be promising anti-inflammatory agents in the future.

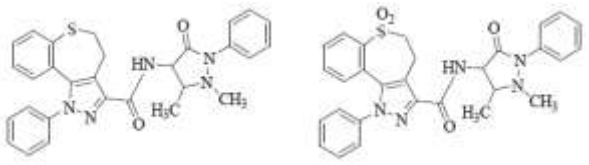


A novel series of pyrazole bearing benzenesulfinamide (**S-1.29**) and 1,3,4- oxadiazole (**S-1.30**) derivatives were synthesized by El-Moghazy *et al.* (2012). All target compounds were tested for *in vivo* anti-inflammatory activity by acute carrageenan-induced paw edema method. The active compounds (61.12-62.67 % inhibition of edema) were tested for ulcerogenic liability in rats and proved to be safer than standard Indomethacin.

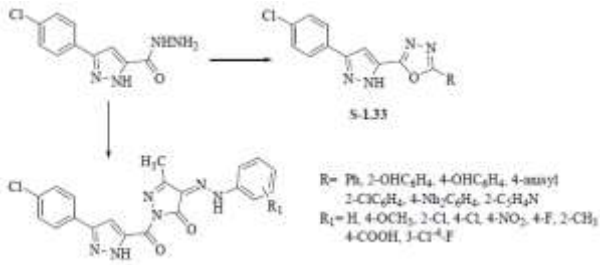


R= H, thiazolyl, Ph, Me, 4-chlorophenyl

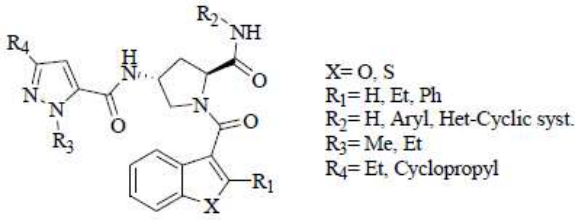
Palanisamy and Kumaresan (2013) developed 4,5-dihydro-1*H*- [1]benzothiepino[5,4-*c*]pyrazole derivatives and tested for antimicrobial, antitubercular and antitumoral activity. Compounds (**S-1.31** and **S-1.32**) showed significant activity against *M. tuberculosis* (8.2 and 7.8 µM) and also showed highest antitumor activity of IC50 value 18 and 12 µM respectively.



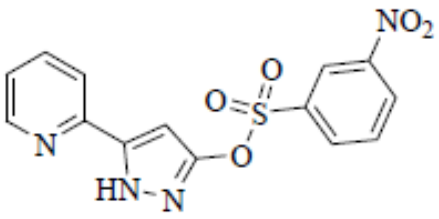
Horrocks *et al.* (2013) developed 1,3,4-oxadiazole (**S-1.33**) and 5- pyrazolinones (**S-1.34**) linked pyrazole as core moiety and tested for their *in vitro* antitubercular and antifungal activity. All the compounds showed excellent antitubercular activity against *M. tuberculosis* H37Rv strain. The substitutions (-Cl and -NO2) on oxadiazole increased the biological potency and hydroxyl substitution on phenyl ring showed poor antifungal activity.



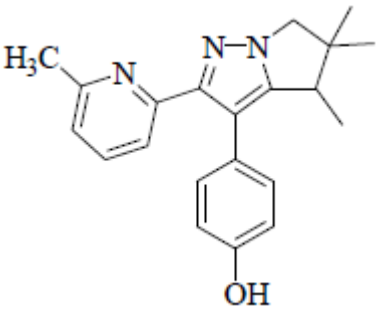
Benzofuran pyrrolidine pyrazole derivatives were synthesized & proved as highly potent antitubercular agents by Kamsri *et al.* (2015). These derivatives are potent InhA inhibitors with IC50 values at nanomolar levels and led to the conclusion that core structure of these compounds are the key portion for binding in the InhA inhibitor.



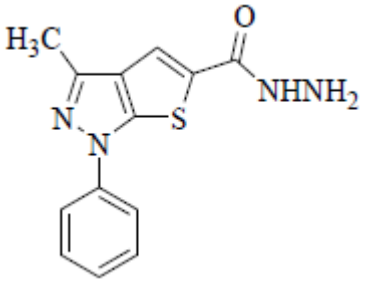
Pyrazole based pyridine-sulfonate derivatives were designed & synthesized for anti-HBV activity by Chuang *et al.* (2016). Structure activity relationship were established in HepG 2 2.2.15 cells. They found inhibition of HBV gene expression & viral DNA replication. Among all the compounds, compound showed potent inhibitory activity with IC50 value of 9.19 μM and higher selectivity index value of 35.46.



Eva *et al.* (2017) synthesized a series of 2,3,4-substituted 5,5-dimethyl-5,6- dihydro-4H-pyrrolo[1,2-b]pyrazoles (DPPs) and evaluated for their ALK5 inhibition activity. The peak potent compounds showed submicromolar IC50 values for ALK5. In cells, the compounds caused dose-dependent dephosphorylation of SMAD2, a well-established substrate of ALK5. In accumulation, the compounds blocked translocation of SMAD2/3 to nuclei of cells stimulated with TGFβ & protein continued mostly in cytoplasm, extra endorsing their molecular target. Therefore, novel DPP derivatives proved to be active as ALK5 inhibitors.

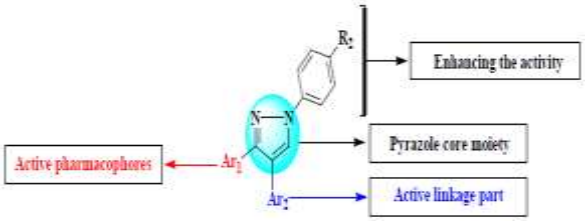


Novel pyrazole containing hydrazone derivatives were synthesized by Pravin *et al.* (2017) and tested for their *in-vitro* antimicrobial activity. Some of the compounds were more active against tested bacterial strain *V. cholera.*



IMPORTANT STRUCTURAL FEATURES OF PYRAZOLE DERIVATIVES

On the basis of literature review, the structure activity association of compounds with the structure represented in Figure indicated that the core pyrazole moiety is responsible for the activity of the compound. The structure activity relationship study was based on (i) a pyrazole ring, (ii) a linkage moiety (iii) hydrophobic moiety. Auxiliary of the pyrazole ring by either a naphthyl or aphenyl ring resulted in the loss of activity. The activity of the compounds is also affected by the distance between the hydrophobic moiety and the basic nitrogen.



Structural features of pyrazole derivatives

CONCLUSION

Four new 1,3,5-trisubsituted pyrazole derivatives were synthesized &characterized using IR, 1H-NMR

and GCMS Technique. The pyrazole compounds (2a-d) were divided in contradiction of several bacterial species as well as against *candida albicans*. Compound 2d exhibited promising antimicrobial activity against all species. In particular, they are used as antitumor, antibacterial and antifungal, antiviral, antiparasitic, antitubercular, insecticidal, anti-inflammatory, anti-diabetic, anesthetic and analgesic agents.

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