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Novel 5-functionalized-pyrazoles: Synthesis, characterization and pharmacological screening

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ABSTRACT

In the present study a series of *O*-substituted pyrazoles **7**(**a**–**f**) and *N*-substituted pyrazoles **9**(**a**–**f**) were synthesized via phase-transfer catalyzed reaction of ethyl 5-(bromomethyl)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate **5** with various oxygen and nitrogen containing compounds in presence of tetrabutylammonium bromide (TBAB) in THF. The compound **5** was obtained by the efficient bromination with *N*-bromosuccinimide (NBS) in presence of a catalytic amount of azoiso-bis-butyro nitrile (AIBN) in refluxing CCl_4. The synthesized compounds were evaluated for their in vitro antimicrobial and antidiabetic activity and were compared with standard drugs. Among the synthesized compounds, compound **9b** emerged as an excellent antimicrobial and antidiabetic agent. Newly synthesized compounds were characterized by analytical and spectral (IR, ^{1}H NMR, ^{13}C NMR and LC–MS) methods.

Due to the increased rate of microbial infections[1] and resistance to antimicrobial agents,[2] identification of novel structure leads that may be of use in designing new, potent and broad spectrum antimicrobial agents remains a major challenge for medicinal chemistry researchers. Thus, intense efforts in antimicrobial drug discovery is still needed to develop more promising, economical and effective drugs for use in the clinical arena.[3]

DM (Diabetes mellitus) is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose levels with disturbances in carbohydrate, fat and protein metabolism resulting from absolute or relative lack of insulin secretion.[4] Diabetes, being one of the most common global diseases, affects approximately 200 million individuals worldwide and approximately 300 million people worldwide are at risk of diabetes.[5] The management of the blood glucose level is a critical strategy in the control of diabetes complications. It is widely accepted that the most challenging goal in the management of patients with diabetes mellitus is to maintain blood glucose levels as close to normal as possible.

The inhibition of enzymes involved in the digestion of carbohydrates can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet by delaying the process of carbohydrate hydrolysis and absorption. The control of postprandial hyperglycemia is an important strategy in the management of diabetes mellitus, especially type II diabetes and reducing chronic complications associated with the disease. Therefore, such enzyme inhibitors can be useful in the treatment of type II diabetes.[6]

Pyrazole is a five membered ring system with two nitrogen atom represents an important class of compounds not only for their theoretical interest but also for anti-inflammatory, analgesic, antitumor, anti hypertensive, antipyretic, sedatives, antibacterial and antidiabetic activities.[7,8] In fact, some of the pyrazole derivatives like Celecoxib, Viagra, Fipronil etc., are now widely used in the market as therapeutic agents.[9–13]

In the light of these facts and in continuation of our interest in the synthesis of heterocycles containing a multi-structure for biological activity[14] we thought of synthesizing a new class of 5-functionalized-pyrazole, to see the additive effect of these rings towards the in vitro antimicrobial and antidiabetic activity, which is the current passion being accomplished in most of the drug discoveries.[15,16]

The synthesis of title compounds ethyl 5-(*O*-alkyl-substituted)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate derivatives **7**(**a**–**f**) and 5-(*N*-alkyl-substituted)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate derivatives **9**(**a**–**f**) were prepared as shown in Scheme 1 and

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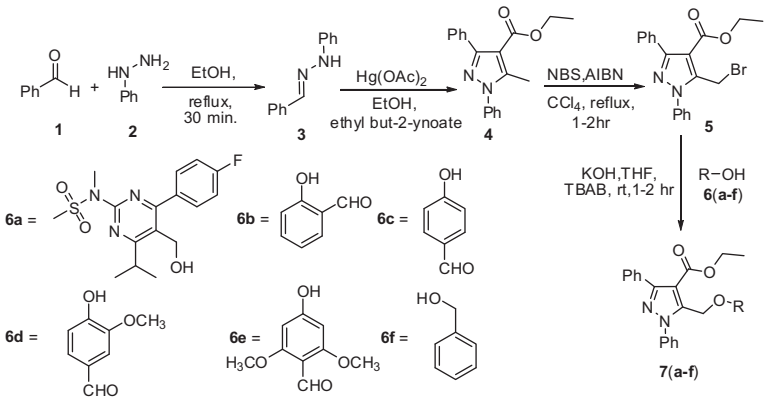
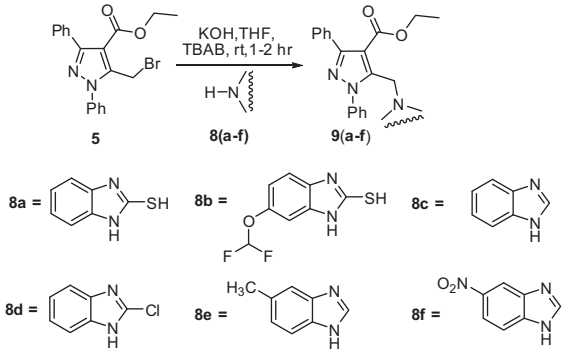


Scheme 1. Synthesis of *O*-alkylated pyrazoles **7**(**a**–**f**).



Scheme 2. Synthesis of *N*-alkylated pyrazoles **9**(**a**–**f**).

Scheme 2, respectively. The crucial compound ethyl-5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylate **4** was synthesized regioselectively,[17] which involves mild one pot reaction of benzaldehyde and phenyl hydrazine with ethyl but-2-ynoate in presence of $Hg(OAc)_2$ and EtOH as the solvent.

In the next step bromination of methyl group attached to the 5th position of the pyrazole **4** was achieved by using *N*-bromosuccinimide (NBS) as a brominating agent[18] in the presence of a catalytic amount of a free-radical initiator such as AIBN in CCl_4 under reflux. This procedure works well giving good yield of corresponding brominated pyrazole **5**.

In order to evaluate biological activity of different oxygen and nitrogen containing pyrazole derivatives, in the last step we have carried out *O*-alkylation and *N*-alkylation of brominated pyrazole **5** with various hydroxyl bearing compounds **6**(**a**–**f**) and secondary amines **8**(**a**–**f**) in presence of tetrabutyl ammonium bromide (TBAB) and KOH in THF as the solvent to yield ethyl 5-(*O*/*N*-substituted)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate derivatives **7**(**a**–**f**) and **9**(**a**–**f**). The *N*-(6-(4-fluorophenyl)-5-(hydroxymethyl)-4-isopropyl-4,5-dihydropyrimidin-2-yl)-*N*-methylmethanesulfonamide, a component of *Rosuvastatin* which is used as dislypidemia (**6a**) was a gift from my Professor, compound **8a** and **8b** were synthesized from the existing literature[19,20] The remaining hydroxyl bearing compounds **6**(**b**–**f**) and amines **8**(**c**–**f**) were obtained from commercial suppliers and used without further purification. Alkylation was carried out by using Phase transfer catalysis (PTC).[21] PTC is a relatively new method for the promotion[22,23] of two-phase reactions. Afresh synthesized compounds were characterized by analytical and spectral methods.

The structural assignments to newly synthesized compounds **7**(**a**–**f**) and **9**(**a**–**f**) were based on their elemental analysis and spectral (IR, ^1H NMR, ^{13}C NMR and Mass) data. The ^1H NMR spectra of the compound **5a** showed disappearance of peaks at δ 1.15 (3H, s) $-CH_3$ protons and appearance of singlet due to CH_2Br of ethyl 5-(bromomethyl)-1,3-diphenyl-1*H*-pyrazole-4-carboxylate at δ 4.83 confirms the formation of product. Similarly the appearance of a new singlet peak at δ 4.16 due to $-CH_2-O-$ and δ 4.15 due to $-CH_2-N<$ confirms the formation of novel ethyl 5-Substituted-1,3-diphenyl-1*H*-pyrazole-4-carboxylate **7a** and **9a**. In ^{13}C NMR spectra, absence of peak at δ 11.5 ($-CH_3$) and 20.2 (CH_2Br) of **4** and **5** and appearance of peak at 65.9 and 47.9 due to $-CH_2-O-$ and $-CH_2-N<$ substantiated the formation of compounds **7a** and **9a**.

The newly synthesized compounds **7**(**a**–**f**) and **9**(**a**–**f**) were screened in vitro for their antibacterial activity against four bacterial species, viz.: *Bacillus cereus* (NCIM, 2016; MTCC 8372), *Staphylococcus aureus* (NCIM, 2079; MTCC 96), (gram-positive bacteria), *Escherichia coli* (NCIM, 2065; MTCC 724), *Klebsiella pneumonia* (NCIM, 2957; MTCC 3384), (gram-negative bacteria) and antifungal activity against two fungal species, viz.: *Aspergillus flavus* (MTCC 873), *Aspergillus niger* (MTCC 281), by disc diffusion[24] and microdilution method.[25] The antibiotic *Tetracycline* and *Nystatin* were used as positive reference to determine the sensitivity of each microbial species tested. The smallest amount of synthesized compounds or standard (*Tetracycline*) antibiotic was required to inhibit the visible growth of a test microorganism (MIC) and the lowest concentration of an antibiotic required to kill a particular bacterium/fungi (MBC/MFC). The results are compiled in Tables 1 and 2. In all the determinations tests were performed in six replicate and the results were taken as a mean of at least three determinations.

All compounds exhibit good to potent in vitro antimicrobial activity against Gram-positive and Gram-negative stains. The results revealed that, compounds **7a**, **7e**, **9a**, **9b**, **9d** and **9f** exhibit excellent antibacterial activity. The compound **7a** was active against gram positive while the compound **7e** showed better activity against gram negative strain. Among the compounds **9**(**a**–**f**), compound **9b** emerged as a promising broad spectrum anti-bacterial agent, while the fungal strains were inhibited by the compounds **7a**, **9a**, **9d** and **9e**. The compound **9a** which has thiol group shows better activity than **9d** which contain chloro group. The compound **9e** containing electron donating $-CH_3$ group was less active against bacterial strains but possess good antifungal activity while the compound **9f** containing electron withdrawing $-NO_2$ group was less active against both the fungal strains. Compare to the *O*-substituted-pyrazole derivatives, the *N*-substituted pyrazolyl-benzimidazole derivatives are very potent and this was attributed to the presence of benzimidazole ring.