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SYNTHEISIS OF HYBRID BENZIMIDAZOLE AND PYRAZOLE DERIVATIVES AND THEIR PHARMACOLOGICAL EVALUTION

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ABSTRACT

Prominent biological activities of benzimidazole and pyrazole compounds include antibacterial, antiviral, antitumor, analgesic, anti-inflammatory, anti-Alzheimer's, anti-ulcer, and antidiabetic effects. Hence Pyrazole substituted Benzimidazole derivatives were synthesised and evaluated for their antimicrobial, antifungal and antitubercular activity. Of the two synthesised compounds, bromo substituted derivatives has better antifungal activity when compared with chloro substituted derivatives. Both the compounds showed mild to moderate anti-tubercular activity against H37RV strain of Mycobacterium tuberculosis using Pyrazinamide, Ciprofloxacin and Streptomycin as standard.

Keywords: Benzimidazole, Pyrazole, Anti-microbial, Anti-fungal, Anti-tuberculosis

1. INTRODUCTION

Pharmacophore hybridization is a cutting-edge technique in medicinal chemistry that has been gaining traction for its ability to create novel bioactive compounds. By merging two or more molecules with distinct bioactive structural characteristics, researchers can produce new products with enhanced potency. This method has shown significant promise in developing compounds with a wide range of therapeutic applications **[1, 2]**.

A preferred structural motif in this approach is the benzimidazole nucleus which is notable for its versatility in medical applications, leading to the creation of a diverse array of medications. This

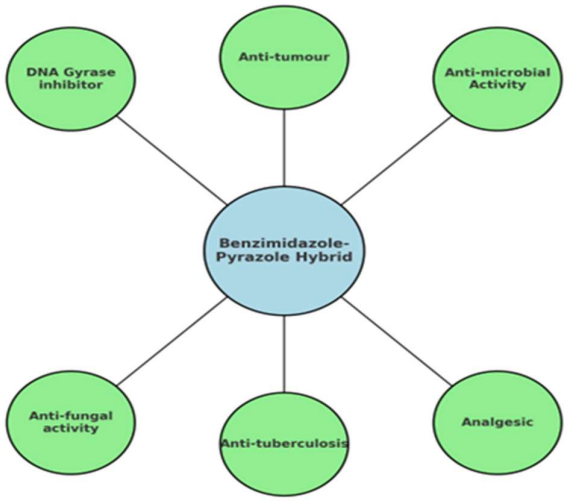
heterocyclic aromatic compound consists of a fused imidazole and benzene ring, incorporating two nitrogen atoms within its ring structure. This unique configuration grants benzimidazole its distinct properties and biological activities, making it a valuable scaffold in drug development **[3]**.

Among the azole family, pyrazole stands out as one of the most extensively studied compounds. Pyrazole and its analogues are recognized for their potential as scaffolds in medicinal chemistry, offering a variety of biological activities. Compounds incorporating pyrazole have demonstrated antiviral, antifungal, antioxidant, anticancer, and antidepressant properties. These attributes have drawn significant attention from researchers aiming to harness these qualities for therapeutic purposes **[4]**.

Combining benzimidazole and pyrazole through hybridization has yielded compounds with remarkable antimicrobial properties, including antiviral and potential anti-COVID-19 activities. These hybrid molecules have shown efficacy as topoisomerase IV and DNA-Gyrase inhibitors, conferring antibacterial and antifungal effects. Additionally, benzimidazole-pyrazole hybrids exhibit analgesic, anticancer, and antiulcer activities, further broadening their therapeutic potential. Their potent antioxidant properties also make them effective in combating oxidative stress, adding another layer of therapeutic benefit **[5, 6]**.

Our current research focuses on the synthesis of hybrid benzimidazole and pyrazole compounds and the evaluation of their biological activities. The medical community has shown substantial interest in benzimidazole compounds due to their extensive range of therapeutic applications. These applications span across anticancer, antibacterial, anthelmintic, antihistaminic, proton pump inhibitory, anti-inflammatory, and antihypertensive properties **[7, 8]**.

In conclusion, the wide-ranging therapeutic applications of benzimidazole fused pyrazole derivatives in treating serious diseases, role in combating parasitic infections, ease of synthesis, and fundamental presence in essential nutrients make this compound a subject of significant interest and importance in the medical community. The ongoing research into benzimidazole and pyrazole hybrids holds promise for developing new and more effective treatments for a variety of medical conditions, further solidifying their role in advancing healthcare **[7, 9–11]**.



2. MATERIALS AND METHODS

2.1 Synthesis of Acetophenone Phenylhydrazone

20 ml of glacial acetic acid and 4.12g of precisely measured acetophenone was carefully mixed together in a reaction jar. 5.495g of phenyl hydrazine dissolved in a solution made of 10 ml of glacial acetic acid and 10 ml of water and this was added to the acetophenone mixture gently. Under careful monitoring, the reaction mixture was allowed to cool in ice with regular shaking until hydrazones precipitate out. The product was filtered, washed with dilute acetic acid and water to yield hydrazone as colorless crystals. The product was recrystallized using ethanol **[7, 12]**.

2.2 Synthesis of Pyrazole substituted Carbaldehyde

A precise quantity of dimethyl formamide [DMF] 64.77 ml was first chilled to the proper temperature in order to reduce the emission of fumes. The chilled DMF was gently

added to 3.62ml of phosphorus oxy chloride and subsequently 3g of hydrazine was added. The reaction mixture that resulted was subsequently kept at a temperature of between 70 and 80°C under reflux conditions for a duration of six to seven hours with frequent stirring during the process. Continuous stirring was necessary to provide a homogeneous reaction and avoid any localized overheating **[13]**.

The reaction mixture was progressively added to a huge volume of ice-cold water when the reflux phase was over. In order to return the mixture to room temperature, this step helped to quench the reaction and started the cooling process. The mixture was carefully neutralized by adding a saturated sodium bicarbonate solution after it had had enough time to cool. In order to guarantee the safe handling of the resulting

combination, this neutralizing step was essential.

After neutralization, the collected solid was carefully washed with cold water to get rid of any contaminants or leftover reactants. The solid product was dried to remove any last traces of moisture after washing and was recrystallized using ethanol **[7, 10]**.

2.3 Synthesis of Benzimidazole

O-phenylenediamine 0.100g and substituted carbaldehyde 0.125g were combined with the aid of ammonium chloride 0.15g dissolved in 4ml of ethanol as catalyst. To guarantee complete mixing of the reactants and effective reaction progression, the reaction was continually stirred on refluxing while maintaining at a temperature of 80°C during the process. Thin-layer chromatography [TLC] was used to track the reaction's development and assess its completion using 1:2 ethyl acetate and n-hexane. After the completion of the reaction, the mixture was cautiously transferred into cold-water, and the obtained precipitate was gathered using filtration. The precipitate was washed with water to assure purity and get rid of any soluble contaminants and dried. Recrystallizing the dried product from ethanol was the last purification stage **[7, 13, 14]**.

3. PHARMACOLOGICAL EVALUATION

3.1 Antitubercular Activity

The antitubercular activity is evaluated using the H37 RV strain of Mycobacterium tuberculosis. using Pyrazinamide, Ciprofloxacin and Streptomycin as standard. The synthesised drugs are evaluated for activity at various concentrations ranging from 100 to 0.2µg/ml **[13, 15–17]**.

3.2 Antimicrobial Activity

Agar well plate assays for antimicrobial susceptibility testing were carried out in-line with the standard method to assess if the compounds had any appreciable antibacterial activities. For test compounds, each derivative of [0.05g] was dissolved in 10 ml DMSO, from the above solution 50mg/ml concentration is prepared and further drawn to 500ug/ml concentration. Each compound was loaded from the working stock solution onto 6-mm diameter sterile. They were allowed to dry. Each plate comprises of two impregments one for the test compound and other for the control [tetracycline 5ug/ml] to compare the activity with that of the standard. The plates were incubated at 37 °C for 24 h. At the end of incubation, the plates were examined for zones of inhibition. Diameters of inhibition zones were measured [in mm] and recorded. The in vitro antimicrobial activity was performed using the disc diffusion method with different strains of bacteria such as *E.coli* and *S. aureus* strains. The anti-bacterial activity was expressed as the

minimum inhibitory concentration in µg/ml **[16–19]**.

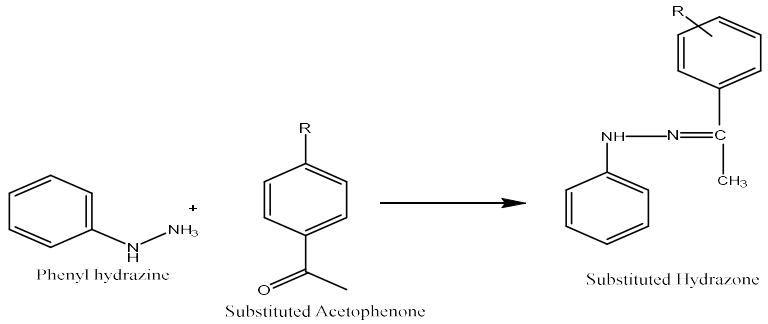
3.3 Anti-Fungal Activity

Antifungal activity was determined by Agar disc diffusion method on sabouraud dextrose agar [SDA] medium as per the procedure specified in Balouiri M 2016. Amphotericin B is taken as positive control. Sample and positive control of 20µl each were added in sterile discs and placed in SDA plates. The plates were incubated at 280C for 24 hours. Then antifungal activity was determined by measuring the diameter of zone of inhibition **[4, 5, 20]**.

4. RESULTS AND DISCUSSION

Two pyrazoles substituted benzimidazole derivatives compounds A and B were synthesised with good yield of 80% and 78% respectively.

STEP 1



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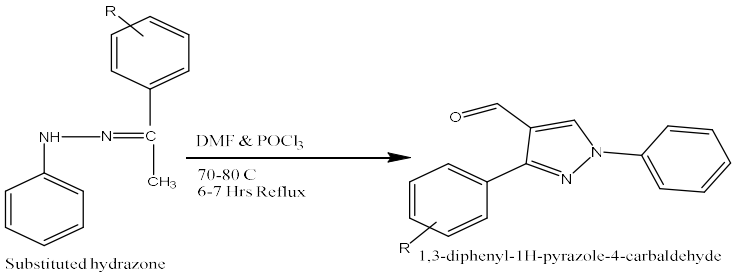
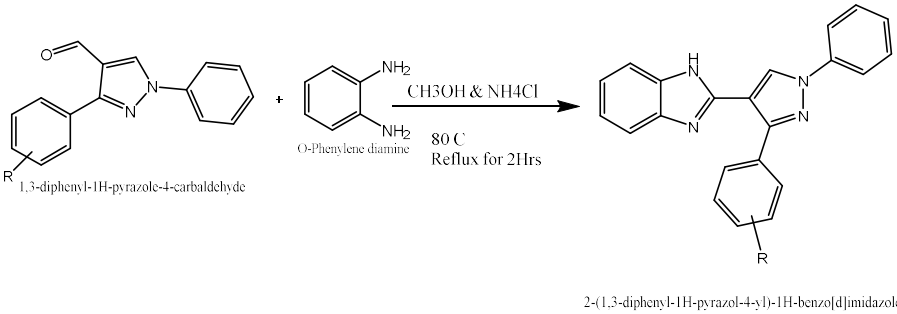
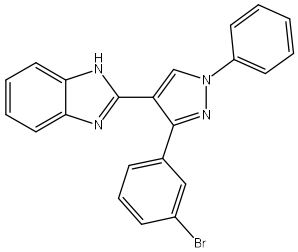


STEP 3



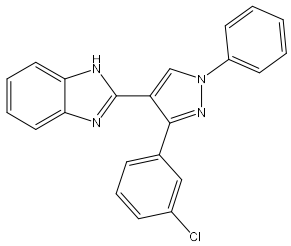
Compound A is 3-bromo substituted derivative and compound B is 3-chloro substituted derivative. Compound A is crystalline powder with a light-yellow colour with molecular weight of 444 and melting point of 115 to 116°C, and the

compound is soluble in methanol and chloroform. Compound A on IR interpretation proves C=N [imine] around 1600-1680 cm^{-1}, N-N [azides] around 2100-2300 cm^{-1}, often appearing as a broad peak, C-Br [bromomethyl] around 500-600 cm^{-1}, N-H [amine] around 3200-3500 cm^{-1} appearing as a broad peak due to hydrogen bonding.



On the other hand, compound B is crystalline and yellow in colour with molecular weight of 503, which indicates that its molecular structure is relatively bigger. Because the chemical is soluble in both methanol and chloroform, it can be used in a variety of organic solvent applications. It also has a melting point of 110 to 112°C, which is important information regarding its thermal stability and possible uses in heating operations.

IR interpretation shows C=N [imine] around 1600-1680 cm^{-1}, N-N [azides] around 2100-2300 cm^{-1}, C-Cl [halogen] around 600-800 cm^{-1}, and N-H [amine] around 3200-3500 cm^{-1}.



4.1 Evaluation of Anti-Microbial activity using Agar Well Plate Method

By evaluating the zone of inhibition against Escherichia coli [E. coli] for the two compounds using tetracycline as standard which showed zone of inhibition of 27mm against *E.coli* and 22mm against *S. aureus* , Compound A and B showed zone of inhibition of 25 and 24mm respectively. Similarly for the zone of inhibition against *S. aureus* Compound A and B showed zone of inhibition of 20 and 19mm respectively.

4.2 Evaluation of Antifungal activity

The zone of inhibition for Aspergillus niger and Candida albicans for the two compounds were evaluated taking antibiotic Amphotericin-B as the standard. The study observed the antifungal efficacy of compound A against *Aspergillus niger* and *Candida albicans* through their zones of inhibition of 11 and 7mm at concentration of 500µg/ml, whereas that of compound B was found to be 9 and 8mm respectively at concentration of 500µg/ml indicating that compound A has better antifungal activity when compared with compound B.

4.3 Evaluation of Antitubercular activity

Antitubercular activity was performed using MABA and the drug concentration was tested at 100 to 0.2µg/ml. Bromoacetophenone demonstrates antitubercular sensitivity at higher concentrations. Specifically, it is effective at inhibiting the growth of tuberculosis bacteria at concentrations of 100 µg/ml and 50 µg/ml. However, as the concentration decreases, bromoacetophenone loses its effectiveness. It shows resistance to antitubercular activity at the lower concentrations. On the other hand, chloroacetophenone also displays antitubercular sensitivity, but at a slightly broader range of concentrations compared to bromoacetophenone. Chloroacetophenone is effective at 100 µg/ml, 50 µg/ml, and 25 µg/ml. Nevertheless, similar to bromoacetophenone, its efficacy diminishes with lower concentrations. In summary, both bromoacetophenone and chloroacetophenone exhibit antitubercular properties at higher concentrations but become ineffective at lower concentrations, with chloroacetophenone maintaining sensitivity over a slightly wider concentration range compared to bromoacetophenone.

5. CONCLUSION

Hybrid benzimidazole and pyrazole compounds with chloro and bromo substitution were synthesised and evaluated for anti-tubercular, antifungal and antimicrobial activities. The investigation's findings show that these hybrids could be useful bridges to create biological agents with greater potency.

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