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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF 1,2,4,5-TETRA SUBSTITUTED IMIDAZOLE

Rizwan Ahmed Khan[*1], **Misbah Irshad**[2]

[*1]*MS Scholar of Chemistry, University of Education, Lahore, Pakistan*
[2]*Assistant Professor, University of Education, Lahore, Pakistan*

[*1]rizwanahmedkhan1001@gmail.com

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Corresponding Author: *
Rizwan Ahmed Khan

Abstract

This study includes the synthesis, characterization and bioscreening of tetra-substituted imidazole derivatives. This is based on the formation of benzil by the oxidation of benzoin with concentrated nitric acid. The synthesized benzil was used for the preparation of main product tetra tetra-substituted imidazole and its analogues by reacting benzil with ammonium acetate, aniline and different substituted aldehydes in the presence of solvent ethanol, maintaining reflux temperature. Varieties of tetra-substituted imidazole were obtained. Structures of these compounds were confirmed by IR and EIMS. All prepared compounds were screened for antimicrobial activity and they showed influential results such as 2-(2,4-dimethoxyphenyl)-1,4,5-triphenyl-1H-imidazole,2-(2,5-dimethoxyphenyl)-1,4,5-triphenyl-1H-imidazole,1,4,5-triphenyl-2-(2,4,5-trimethoxyphenyl)-1H-imidazole and 1,2,4,5-tetraphenyl-1H-imidazole show potency against microbes.

INTRODUCTION

Imidazole ($C_3N_2H_4$) is a five-membered aromatic heterocyclic compound containing two non-adjacent nitrogen atoms, classifying it as a diazole. It is colorless to white, produces a mildly alkaline solution in water, and exists in two tautomeric forms due to the mobility of hydrogen between nitrogen atoms (Harold et al., 2015; Weber et al., 1887). The term "imidazole" was introduced by Arthur Rudolf Hantzsch in 1887, although Heinrich Debus first synthesized it in 1858 using glyoxal, formaldehyde, and ammonia (Debus et al., 1858; Rosemeyer et al., 2004). Its amphoteric nature (pKa = 7 and 14.9) makes it reactive toward both nucleophilic and electrophilic attack, while its polarity (dipole moment = 3.67 D) ensures stability under acidic, basic, oxidative, and reductive conditions (Finar, 2009).

The imidazole ring is a structural component of many biologically important molecules including histidine, histamine, vitamin B12, biotin, purines, and nucleic acids (Grimmett et al., 1997; Brown et al., 1998). It is also present in natural products such as alkaloids and in many pharmaceutical agents like antifungals, antibiotics, sedatives (Midazolam), and proton pump inhibitors (Karitzky et al., 1998; Gilchrist et al., 1985). Imidazole derivatives exhibit diverse pharmacological activities, including antimicrobial, anticryptococcal, nitric oxide synthase inhibition, anti-inflammatory, antidiabetic, and antihypertensive properties (Kubicki et al., 2004; Munk et al., 1997). Furthermore, its derivatives function as kinase inhibitors, particularly against p38 MAP kinases involved in immunological and inflammatory disorders (Derstine et al., 1996, 1997).

Synthetic approaches for imidazole derivatives include multi-component condensation reactions involving diketones, aldehydes, and ammonium acetate, facilitated by catalysts such as silica sulfuric acid, microwaves, or heteropoly acids (Singh et al., 2008; Corey et al., 1999). Their structural versatility allows incorporation into 1,3,4-oxadiazoles and related heterocycles, which demonstrate antimicrobial, insecticidal, anti-tumor, antiviral, and anti-inflammatory activities (Bilodeau et al., 1998; Kitbunnadaj et al., 2003; De Esch et al., 2016). Beyond pharmaceuticals, imidazole is widely applied industrially, for example as a corrosion inhibitor for transition metals like copper, highlighting its dual biomedical and industrial significance (Bhatnagar et al., 2011).

1.1 Application

Imidazole and its derivatives exhibit a broad pharmacological spectrum, including analgesic, anti-inflammatory, antiviral, antifungal, anticancer, antihelmintic, antineoplastic, and cardiovascular activities (Sharma et al., 2005; Fizames et al., 1987; Huong et al., 1999; Mamalis et al., 1987; Wyssvl, 1985; Kiyota, 1988). Nitroimidazoles such as metronidazole, misonidazole, and clotrimazole are widely used as antibacterial, antifungal, and anticancer agents, while imidazolines such as priscol and privine serve as vasoconstrictors and vasodilators. These derivatives are also used as catalysts, dyestuffs, and polymerizing agents, extending their scope beyond medicine (Dutta et al., 2010).

Imidazoles demonstrate anthelmintic and anti-inflammatory potential, especially against nematodes, cestodes, and trematodes, with efficacy influenced by host metabolism, drug bioavailability, and parasite biology (Benoist et al., 1996; Dutta et al., 2010). Anti-inflammatory effects are seen in various substituted imidazoles, benzimidazoles, and imidazolones, which act by inhibiting edema, prostaglandin synthesis, and leukotriene pathways (Shanker et al., 1994; Barthwal et al., 1987; Hingorani et al., 1990). Structure–activity relationship studies indicate that hydroxyl, alkyl, and heteroaryl substitutions enhance potency (Sinha, 1985; Sawhney, 1990).

In oncology, imidazole-based derivatives are important as CDK inhibitors, farnesyltransferase inhibitors (FTIs), and multidrug resistance (MDR) modulators, showing strong antiproliferative activity against diverse cancer cell lines (Tyagi et al., 2008; Karoutchi et al., 2008; McGregor et al., 2008; Gollapudi et al., 1997; Devanarayan et al., 2002). Indole–imidazole hybrids and pyrido-imidazole analogs demonstrate exceptional cytotoxicity against MDR phenotypes. Moreover, imidazole derivatives like clotrimazole and ketoconazole revolutionized antifungal therapy, while environmental studies show imidazole stability, amphoteric reactivity, and potential release into wastewater (Trivedi et al., 1990). Collectively, imidazoles are promising multi-target scaffolds with applications spanning medicine, industry, and environmental sciences.

1.2 Objectives of the Study

- Develop efficient laboratory routes for imidazole and analogues via diverse synthetic pathways; synthesize novel, functionally substituted imidazolic molecules; and verify identity and purity using TLC and melting points, followed by comprehensive spectroscopic characterization (UV/Vis, IR, MS).
- Assess the synthesized compounds as metal-ion sensors and evaluate their biological potential—particularly antimicrobial activity—using appropriate microbiological assays.

2. Research Methodology

2.1 Materials and Research Setting

Analytical grade reagents and solvents (Alfa-Aesar, Sigma Aldrich, Merck) were used throughout the study. Experimental work was primarily conducted at the University of Education, Lahore, with additional support from Government College University (GCU) Lahore, HEJ Research Institute Karachi, University of Agriculture Faisalabad, University of Sargodha, and LUMS Lahore.

Materials Matrix

Category	Items/Examples
Apparatus	Beakers, condensers, conical funnels, magnetic stirrers, TLC tanks/cards, RBFs, separating funnels, hot plates, thermometers, pipettes, microsyringes, vials, capillary tubes, petri dishes
Chemicals	Benzoin, concentrated nitric acid, ammonium acetate, aniline, benzaldehyde & substituted benzaldehydes (2,4-dimethoxy, 2,5-dimethoxy, 2,4,5-trimethoxy, 3,4,5-trimethoxy, 4-acetoxy-3-methoxy), ethanol, methanol, dichloromethane, acetic acid, n-hexane, $FeCl_3$, NaOH, KOH, $NaHCO_3$
Instruments	UV lamp, digital balance, magnetic hot plate, IR spectrometer (Schimadzu), EIMS spectrometer

Synthesis Procedures

Preparation of Benzil:

Benzoin was oxidized with concentrated nitric acid under reflux for 15 minutes. After TLC confirmation, the precipitate was recrystallized in ethanol to obtain pure benzil.

General Imidazole Synthesis:

Equimolar benzil, aldehydes, amines, and ammonium acetate were refluxed in ethanol with $FeCl_3$ catalyst. Reaction progress was monitored by TLC, and products were isolated via dichloromethane extraction, recrystallized, and characterized.

2.2 Characterization

Purity was checked by TLC and melting points. Structural characterization employed UV/Vis spectroscopy, IR spectroscopy, and EIMS to confirm the functional groups and molecular structure of synthesized compounds.

2.3 Biological Screening

Antioxidant activity of imidazole analogues was tested using:

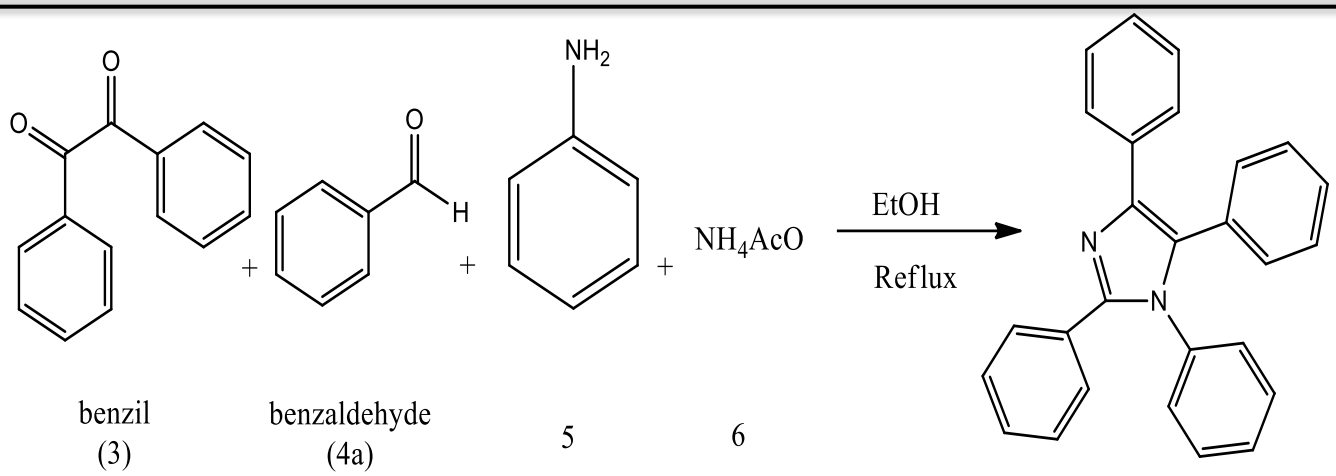
DPPH Radical Scavenging Assay (Zhang et al., 2012): Compounds at varying concentrations (0.25–1.5 mg/ml) were tested against DPPH solution; absorbance measured at 700 nm and scavenging % calculated.

Ferric Reducing Power Assay (Oyaizu method): Compounds (0.1 mg/ml) in phosphate buffer were incubated with potassium ferricyanide and trichloroacetic acid, followed by $FeCl_3$ addition. Absorbance was measured at 700 nm, and reducing power % was determined.

3. Results

3.1 Synthesis of 1,2,4,5-Tetraphenyl-1H-Imidazole

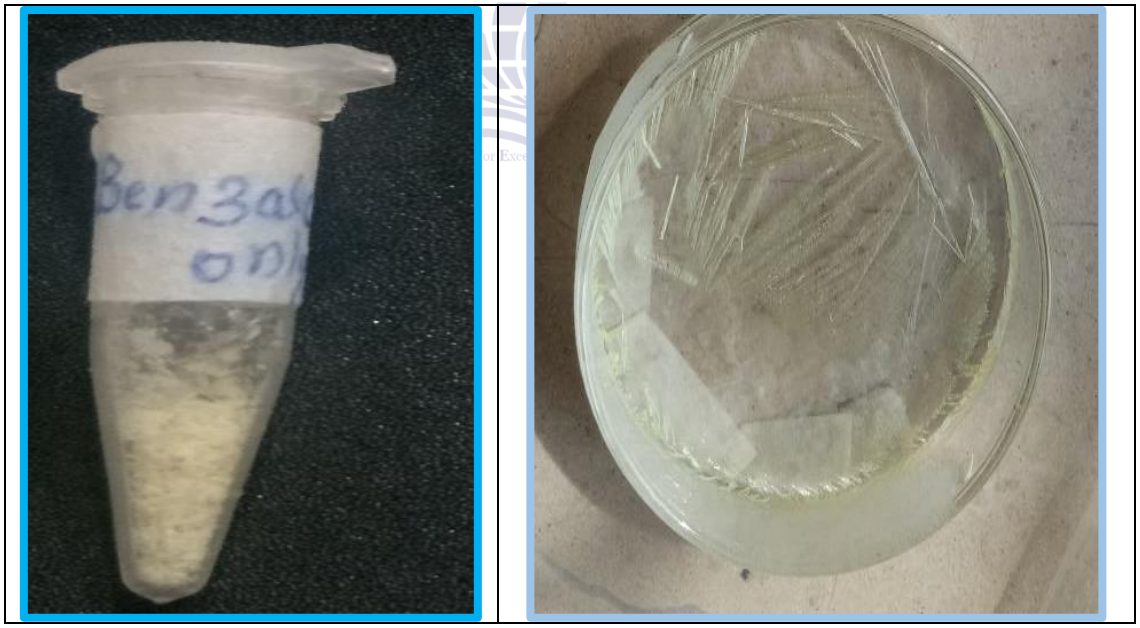
Benzaldehyde 0.065gm (1mmol) was taken in round bottom flask with benzil 0.19gm (1mmol), aniline 0.057ml (1mmol), ammonium acetate 0.24gm (1mmol) and the mixture solution was stirred in ethanol with iron chloride as catalyst. The mixture was refluxed for 180 minutes and the performance of the reaction was examined with the help of thin layer chromatography. The product was separated with the help of separating funnel with dichloromethane. The pale-yellow crystals of the compound obtained and characterized. The catalyst also separated by magnet or simple filtration for further use.

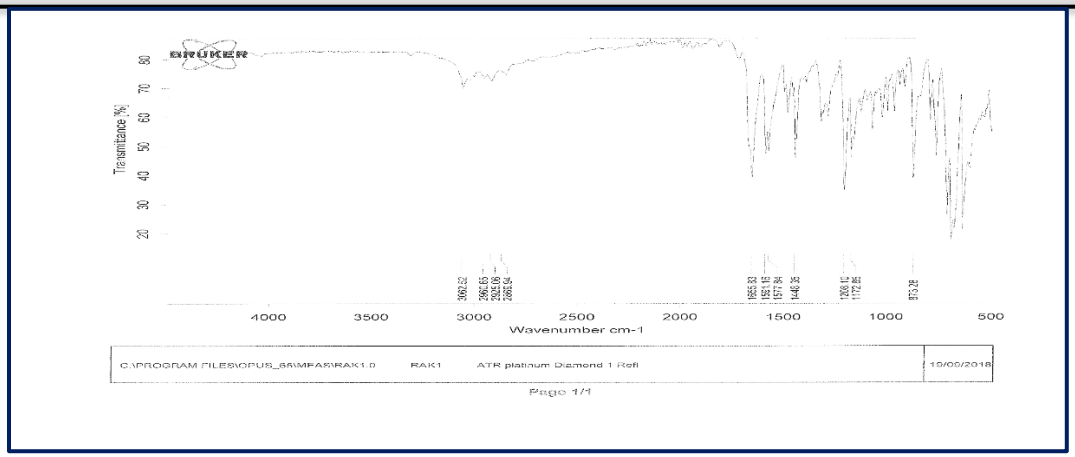


1,2,4,5-tetraphenyl-1H-imidazole (7A)

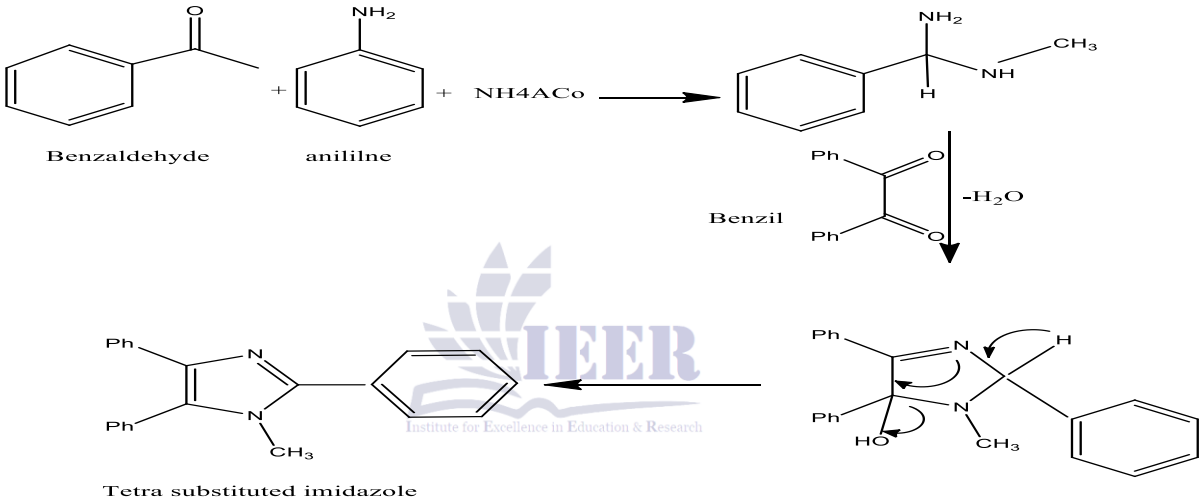
3.2 Characterization of 1,2,4,5-Tetraphenyl-1H-Imidazole

Chemical Formula: C27H20N2
Molecular Weight: 372.46
Uv/Vis: crescent peak, UV active
IR : 1650 stretching,3300 N-H stretching, 1360 (C-N stretching





Mechanism



3.2.1 Reagents and Concentration

Reagents	molar mass [g/mol]	wt/vol taken	n [mmol]
Benzoin	212.24	6.0g	28.3
acetic acid	60.05	30Ml	Excess
nitric acid	63.01	45mL	Excess

3.2.2 Description

Synthesized benzil was separated in the form of fine crystals and stored in voils for further reaction with the different derivatives of benzaldehyde to get the main product that is tetra substituted imidazole. Results of synthesized benzil is as follow;

In IR spectrum absorption peaks at 1637 owing to C=C stretching (aliphatic) and at 1685 owing to C=O (α-β unsaturtion, aliphatic) gave inidcaton for the formation of benzil. The molecular weight of benzil 210 was confirmed by mass fragmentation pattern from EIMS data. The parent peak at m/z 214 indicate the synthesis of benzil.

4. Conclusion

The successful synthesis of 1,2,4,5-tetraphenyl-1H-imidazole ($C_{27}H_{20}N_2$, MW = 372.46) was confirmed through UV/Vis activity, IR peaks corresponding to N–H (3300 cm^{-1}), C=N (1650 cm^{-1}), and C–N (1360 cm^{-1}) stretching, and by mass spectrometry data. The intermediate benzil was also successfully synthesized from benzoin, validated by IR absorption bands at 1637 cm^{-1} (C=C) and 1685 cm^{-1} (C=O) along with a parent peak at m/z 214 in EIMS, confirming its molecular weight. These results establish the effective preparation and characterization of tetra-substituted imidazole derivatives through the adopted synthetic route.

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