IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 15, Issue 7 Ser. I (July 2022), PP 69-74
www.iosrjournals.org

Synthesis, Characterization and Biological Evaluation of Novel Tetrasubsituted Imidazole Compounds

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ABSTRACT
A novel set of tetrasubstituted imidazole derivatives were crafted through a multicomponent one-pot synthesis scheme. This procedure incorporated a cyclocondensation reaction involving benzil, aromatic primary amines, aldehydes, and ammonium acetate within glacial acetic acid. To characterize the resultant compounds, various methods were employed including determination of melting point, color assessment, conductivity evaluation, CHN analysis, along with FT-IR and UV-Visible spectroscopy. The progression of the reaction was monitored using Thin Layer Chromatography (TLC) at regular time intervals. To assess the biological efficacy of these compounds, they were tested against multiple bacterial strains. All three imidazole derivatives exhibited varying degrees of notable antibacterial activity against the tested bacterial strains.
Key points*: Imidazole Compound, Benzil Derivative, Formation Process, Analysis and Identification*

I. INTRODUCTION

The discovery of imidazole in the 1840s sparked a surge of investigation and development in imidazole-related compounds due to its multifaceted uses in areas such as pharmaceuticals, agriculture, ligands, catalysts, and synthetic acceptors, among others. These compounds are prominently positioned within the medicinal drug sector due to the critical role they play in treating various health conditions. Imidazole derivatives continually undergo exploration for their medicinal potential, thanks to the unique structural features of the imidazole ring, allowing for interactions with numerous enzymes and receptors in living organisms.

The imidazole ring is a key component of several important biological molecules, such as DNA, histamine, vitamin B12, and hemoglobin. Its use in the design and synthesis of biologically active molecules is also extensive since it is considered an isostere of triazole, oxazole, pyrazole, thiazole, tetrazole, amides, and more.

Research literature has unveiled imidazole-based compounds' potential in various applications like anticancer, antihypertensive, antihistaminic, antineurophatic, antihemolytic, cytotoxic, antimycobacterial, antioxidant, and antimicrobial activities, to name a few. Several imidazole-based compounds with medical applications have been reported, like dacarbazine, zoledronic acid, and azathioprine as anticancer, oxiconazole, and miconazole as antifungal, secnidazole and benznidazole as antiparasitic, cimetidine and dexmedetomidine as antihistaminic, losartan and olmesartan as antihypertensive, dexmedetomidine, and fipamezole as antineuropathic.

Imidazole synthesis can be achieved through various approaches, with multicomponent one-pot synthesis being a preferred method due to its convenience, efficiency, cost-effectiveness, and high yield. This method is particularly useful in synthesizing tetrasubstituted imidazole compounds, a class of imidazole with significant medicinal and biochemical processes. They exhibit potential applications as analgesic, anti-inflammatory, fungicidal, antibacterial, and antitumor activities. Thus, the synthesis of tetrasubstituted imidazole has gained worldwide attention in recent years.

In our current research, we have synthesized three tetrasubstituted imidazole compounds (K1, K2, and K3) using a cyclocondensation reaction of 1,2-diketone (benzil), aldehydes, primary amines, and ammonium acetate in glacial acetic acid as a catalyst. These novel compounds were initially analyzed by physical methods like solubility, melting point, conductivity, and thin layer chromatography (TLC) before being evaluated with spectroscopic techniques like UV-Visible and FT-IR. To the best of our knowledge, the synthesis and computational study of these compounds (K1, K2, K3) have not been reported previously.

II. Materials and Methods

For this study, all chemicals were of laboratory-grade quality, acquired from Sigma Aldrich. No additional refinement was conducted on these chemicals before use. Solvents were also procured from chemical

suppliers and were distilled before use. Reaction progression was tested using thin layer chromatography, and the chromatographic plates were irradiated in U.V and then assessed in iodine vapors. An EL III CHNOS elemental analyzer (Elementar, Hanau, Germany) was used to check the percentage composition of the newly synthesized compounds.

Instrumentation

A magnetic stirrer hot plate was used for heating and stirring the reaction materials, with stirring range 50 to 1200rpm and a heating range of 60-200°C. Materials were weighed using the AX200 model from Shimadzu, Japan. The melting point of the synthesized compounds was identified using a Gallenkamp melting point apparatus. Agilent technology (Cary-620) FTIR spectrophotometer was utilized to verify the presence of the desired functional groups in the synthesized compounds. A Shimadzu UV-240 spectrophotometer was employed to acquire the UV/Visible spectrum of the synthesized compounds. Conductivity of the compounds was measured using an SDT-600 conductivity meter. Elemental composition analysis was conducted using an EL III CHNOS elemental analyzer (Elementar, Hanau, Germany).

General Method for the Synthesis of Compounds; K1, K2, and K3

The tetrasubstituted imidazole derivatives (K1, K2, and K3) were produced following a previously reported protocol with minor modifications. The synthesis of 5-methyl-2-(2-methyl-4,5-diphenyl-1H-imidazol-1-yl)phenol (K1) was initiated by dissolving benzil (1.05 g, 0.005 mmol) and acetaldehyde (0.22 g, 0.005 mmol) in glacial acetic acid at room temperature. Subsequently, p-cresolamine (0.62 g, 0.005 mmol) and ammonium acetate (0.38 g, 0.005mmol) were added to the reaction mixture. The mixture was then refluxed at 110°C for 12 hours, with TLC used to monitor the reaction progress. After 12 hours, the mixture's volume was reduced by half through heating, then allowed to evaporate slowly in a beaker. K1 crystals were obtained within a week. These crystals were purified by washing with ethyl acetate followed by ethyl alcohol. The yield of the desired compound was around 75%, with a melting point of 182-184°C. Elemental analysis for $C_{23}H_{20}N_2O$ (340.41) yielded: Calculated: C, 81.15; H, 5.92; N, 8.23; O, 4.70%; Obtained: C, 81.05; H, 5.89; N, 8.13; O, 4.65%.

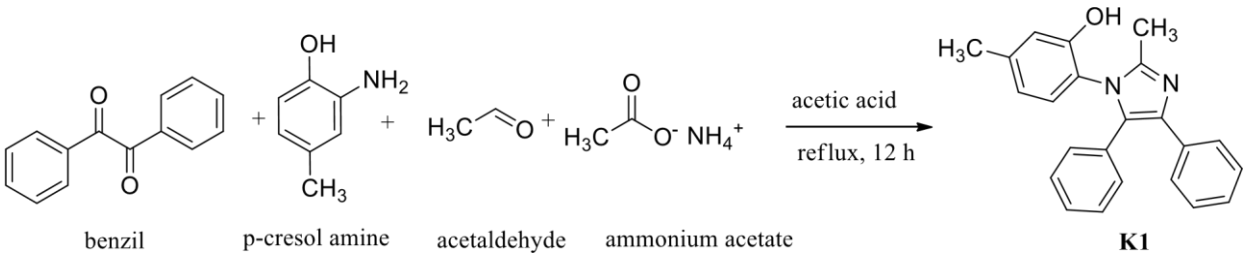

Figure1: synthesis of 5-methyl-2-(2-methyl-4,5-diphenyl-1H-imidazol-1-yl)phenol (K1)

The process to synthesize 2-[2-(furan-2-yl)-4,5-diphenyl-1H-imidazol-1-yl]-5-methylphenol (K2) started with the dissolution of benzil (1.05 g, 0.005 mmol) and furfural (0.36ml, 0.005 mmol) in glacial acetic acid under room temperature conditions. This was followed by the addition of p-cresolamine (0.62 g, 0.005 mmol) and ammonium acetate (0.38 g, 0.005mmol) into the reaction blend. The concoction was then subjected to reflux at a temperature of 110 °C for a duration of 12 hours. Throughout this period, TLC was employed to track the progression of the reaction. Following the 12-hour mark, the mixture's volume was halved through heating. To encourage slow evaporation, the mixture was transferred to a beaker. Within a week, K2 crystals were produced. To purify these crystals, they were first cleaned with ethyl acetate, then with ethyl alcohol. The synthesized compound yield was approximately 81%, with a melting point range of 195-197°C. For the compound with molecular formula $C_{26}H_20N_2O_2$ (392.44), the elemental analysis showed: Theoretical: C, 79.57; H, 5.14; N, 7.14; O, 8.15%; Experimental: C, 79.51; H, 5.11; N, 7.08; O, 8.03%.

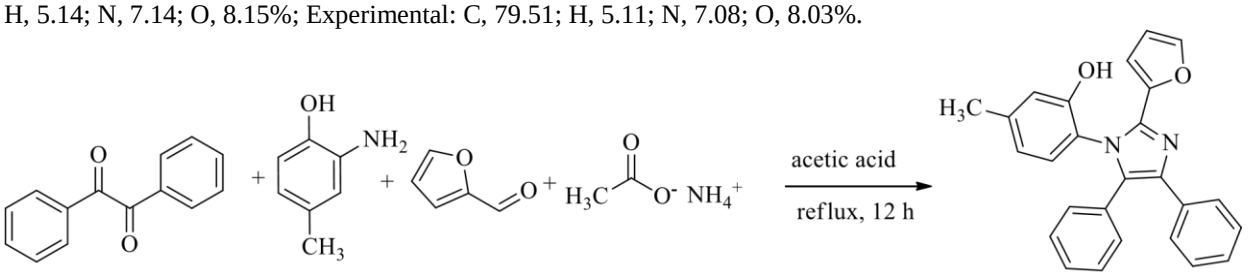

Figure2: Synthesis of 2-[2-(furan-2-yl)-4,5-diphenyl-1H-imidazol-1-yl]-5- methylphenol (**K2**)

Synthesis of *5-methyl-2-(2, 4, 5-triphenyl-1H-imidazole-1-yl) phenol* (K3)

The process to synthesize 2-[2-(furan-2-yl)-4,5-diphenyl-1H-imidazol-1-yl]-5-methylphenol (K2) started with the dissolution of benzil (1.05 g, 0.005 mmol) and furfural (0.36ml, 0.005 mmol) in glacial acetic acid under room temperature conditions. This was followed by the addition of p-cresolamine (0.62 g, 0.005 mmol) and ammonium acetate (0.38 g, 0.005mmol) into the reaction blend. The concoction was then subjected to reflux at a temperature of 110 °C for a duration of 12 hours. Throughout this period, TLC was employed to track the progression of the reaction. Following the 12-hour mark, the mixture's volume was halved through heating. To encourage slow evaporation, the mixture was transferred to a beaker. Within a week, K2 crystals were produced. To purify these crystals, they were first cleaned with ethyl acetate, then with ethyl alcohol. The synthesized compound yield was approximately 81%, with a melting point range of 195-197°C. For the compound with molecular formula C26H20N2O2 (392.44), the elemental analysis showed:
Theoretical: C, 79.57; H, 5.14; N, 7.14; O, 8.15%; Experimental: C, 79.51; H, 5.11; N, 7.08; O, 8.03%.

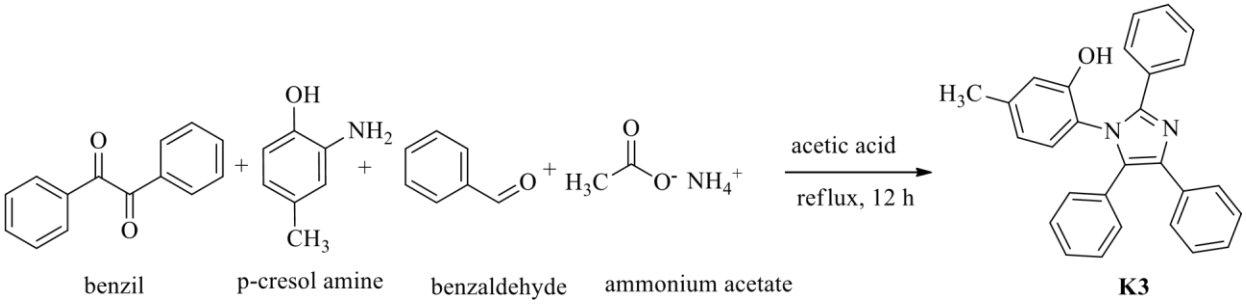

Figure3: Synthesis of 5-methyl-2-(2, 4, 5-triphenyl-1H-imidazole-1-yl) phenol (K3)

III. Results and Discussion

Physical characteristics

Color/Melting Point/Physical Appearance/yield: The newly prepared tetrasubsituted imidazole derivatives (K1, K2 and K3) inert against climate and humidity at room temperature.. They exist in crystalline form and have color differentiates. Color, physical appearance and melting points of synthesized compounds are shown in table 1.

Table 1: Melting point, color, physical appearance and yield of synthesized compounds (K1, K2 and K3)

No	Codes	Color	Melting point (°C)	Physical appearance	Yield
1	K1	blackish brown	182-184 °C	Crystalline	75 %
2					
	K2	brown	195-197 °C	Crystalline	81 %
3					
	K3	yellow	206-208 °C	Crystalline	70 %

Conductance Values:

The conductivity of synthesized compounds was determined at room temperature. About 1 M solution of synthesized compounds was prepared using DMSO as a solvent to check their conductance. The conductance values of synthesized compounds were low as they are organic compounds of covalent nature and non-electrolyte having conductivity range from 12 to 15 $\Omega^{-1}cm^2mol^{-1}$ as shown in table 2.

Table 2: Conductance values of synthesized compounds (K1, K2 and K3)

No	Codes	Ω-1cm-2mol-1
1	K1	15
2	K2	13
3	K3	12

UV-Visible study

Theλmax of all synthesized compounds was determined experimentally in the solvent phase. The experimentally determined values are tabulated in table 3. It was observed that compound K3 has highest λmax while K1 possess lowest one.

Table 3: λmax of synthesized compounds

No	Codes	λmax (nm)
1	K1	265
2	K2	316
3	K3	361

IR Spectra of synthesized compounds (K1, K2 and K3)

Agilent technology (Cary-620) FTRIR spectrophotometer was used to obtain and interpret IR Spectra of newly synthesized tetrasubsituted imidazole derivatives (K1, K2 and K3)

IR Spectra of 5-*methyl-2-(2-methyl-4,5-diphenyl-1H-imidazol-1-yl)phenol* (K1)

The infrared (IR) spectroscopy data of compound K1, as depicted in Table 4, reveals a new peak at 1650 cm^{-1}, possibly suggesting the formation of the C=N bond. The emergence of this peak indicates the probable creation of an imino bond (C=N) during the cyclocondensation of benzil, primary amine, aldehyde, and ammonium acetate. The peak corresponding to the C-N bond was found at 1440 cm^{-1}, supporting the synthesis of the target compound. An OH stretching frequency is responsible for the peak at 3345 cm^{-1}, while new peaks at 3050 cm^{-1} and 2912 cm^{-1} can be ascribed to sp^2(C-H) and sp^3(C-H) stretching frequencies respectively. The disappearance of the peak at 1680 cm-1 suggests the absence of benzil, but other group-related peaks remained largely unaltered.

For compound K2, a subset of its IR values is listed in Table 4. The data demonstrates the presence of a peak at 1658 $cm^{-1,}$ likely due to the azomethine (−C=N−) linkage. The C-N peak occurs at 1419 cm^{-1}, and the formation of the C=N bond, as indicated by the corresponding peak, suggests the successful condensation of benzil, p-cresolamine, and furfural. The peak at 1575 cm^{-1} can be attributed to the (C=C) bond in the aromatic imidazole ring, while the OH stretching frequency is responsible for the band appearing at 3315 cm^{-1}. A peak corresponding to C-O stretching frequency was also noted at 1325 cm^{-1}. All remaining peaks stayed consistent.

The IR spectra of compound K3, with selected values provided in Table 4, shows a peak at 1656 cm^{-1} that points towards the formation of the C=N bond within the imidazole ring. This suggests that the desired imino bond may have been formed through the condensation of benzil, p-cresolamine, and benzaldehyde. The presence of the aromatic imidazole ring can be inferred from the peak at 1522 cm^{-1}, attributed to the C=C bond. Other notable peaks include those at 3065cm^{-1} due to sp^2(C-H) and 1449 cm^{-1} likely due to C-N stretching frequency. Peaks corresponding to OH and sp3(C-H) stretching frequencies, appearing at 3305 cm^{-1} and 2908 cm^{-1} respectively, further support the synthesis of the target compound. The absence of a peak at 1680 cm^{-1}, originally attributed to benzil carbonyl, indicates the complete condensation of benzil with other reactants. No significant changes were observed for all other peaks.

Table 4: Some selected IR peaks of synthesized compounds (K1, K2 and K3)

No	Codes	C=N (cm^{-1})	C-N (cm^{-1})	OH (cm^{-1})	C-O (cm^{-1})
1	K1	1650	1440	3345	NA
2	K2	1658	1449	3315	1325
3	K3	1656	1419	3305	NA

Biological study

Antibacterial Activity (*in-vitro*)

The synthesized compounds K1, K2, and K3 underwent biological testing for activity against Escherichia coli (a Gram-negative bacterium) and Bacillus subtilis and Staphylococcus aureus (both Gram-positive bacteria), based on methodologies drawn from established scientific literature. The performance of these compounds was gauged relative to a well-known antibiotic, Ciprofloxacin. It was observed that these synthesized compounds demonstrated substantial antibacterial activity, with compound K2 exhibiting the highest effectiveness against certain bacterial strains. In contrast, compound K1 displayed the least activity. Both compounds K2 and K3 showed promising results, with inhibition zones larger than 20mm against all tested bacterial species. Compound K2 stood out as the most potent, displaying the highest activity (23mm inhibition zone) against Bacillus subtilis among all three synthesized compounds.

Table 5: Exhibition of antibacterial study of synthesized compounds

Bacterial strains	K1	K2	K3	Ciprofloxacin
		GRAM	POSITIVE	
Staphylococcus aureus (mm)	19	21	20	27
Bacillus subtilis (mm)	18	23	20	29
		GRAM	NEGATIVE	
Escherichia coli (mm)	16	22	21	27

IV. Conclusion

In this study, we created three new tetrasubstituted imidazole derivatives through a one-step, multicomponent synthesis process. The structural attributes of these compounds were confirmed using various spectroscopic methods such as Fourier Transform Infrared (FT-IR) and UV-Visible spectroscopy. These chemically engineered compounds were tested for their antimicrobial properties against several bacterial species including Staphylococcus aureus, Bacillus subtilis, and Escherichia coli. The results indicated that these synthetically produced compounds displayed appreciable antibacterial activity.

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