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Synthesis and antimicrobial evaluation of thiadiazole-conjugated imidazole derivatives

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Abstract

The emergence of multidrug-resistant (MDR) pathogens has created an urgent need for novel antimicrobial agents with improved efficacy. In this study, a series of thiadiazole-conjugated imidazole derivatives (5a–5e) were synthesized and evaluated for antibacterial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). The synthetic route involved sequential preparation of substituted imidazoles, followed by conjugation with thiadiazole moieties. Structural confirmation was achieved through FTIR, ^1H NMR, and mass spectrometry analyses. Antibacterial activity was assessed using disc diffusion and broth dilution methods. Among the synthesized compounds, derivative 5e (bearing a methoxy substituent) exhibited the most promising activity, with inhibition zones up to 26 mm and IC_{50} values of 67.83 µg/mL (E. coli) and 63.56 µg/mL (S. aureus). Structure–activity relationship (SAR) analysis revealed that electron-donating substituents enhanced antibacterial potency, whereas electron-withdrawing groups diminished activity. Although less potent than ciprofloxacin, compound 5e represents a promising scaffold for further optimization toward next-generation antimicrobial agents.

Keywords: Thiadiazole, antimicrobial, imidazole, structure activity relationship, zone of inhibition

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Introduction

The global rise of healthcare-associated infections has emerged as a critical public health challenge, contributing significantly to morbidity and mortality.[1] A major factor exacerbating this crisis is the alarming increase in antibiotic-resistant bacteria, particularly multidrug-resistant (MDR) pathogens, which severely limit the effectiveness of conventional antimicrobial therapies. The rapid evolution of microbial resistance against widely used antibiotics has rendered many clinical treatments inadequate, creating an urgent demand for novel anti-infective agents capable of overcoming these resistance mechanisms.[1]

Despite the pressing need, the pace of discovery of new antibacterial molecules has slowed considerably in recent decades. Most advances in antibiotic research have focused on structural modifications of existing drug classes rather than the development of entirely new scaffolds. This limited innovation has left clinicians with few effective options, as resistance inevitably develops against every known antibiotic. Consequently, the search for new chemical entities with potent antimicrobial activity remains a priority in medicinal chemistry.

Heterocyclic compounds, particularly imidazole and thiadiazole derivatives, have attracted considerable attention due to their diverse biological activities, including antimicrobial, antifungal, and anticancer properties.[2-4] Imidazole moieties are well known for their ability to interact with biological targets through hydrogen bonding and π–π stacking, while thiadiazole rings contribute additional pharmacophoric features such as electron-rich heteroatoms and enhanced lipophilicity.[5] The conjugation of these two heterocycles is therefore hypothesized to yield novel scaffolds with improved antibacterial potency.

In this context, the present study was undertaken to synthesize a series of thiadiazole-conjugated imidazole derivatives and evaluate their antimicrobial activity against representative Gram-positive and Gram-negative bacteria. By exploring the structure–activity relationship (SAR) of these conjugates, the work aims to identify promising scaffolds that may serve as leads for the development of next-generation antibacterial agents

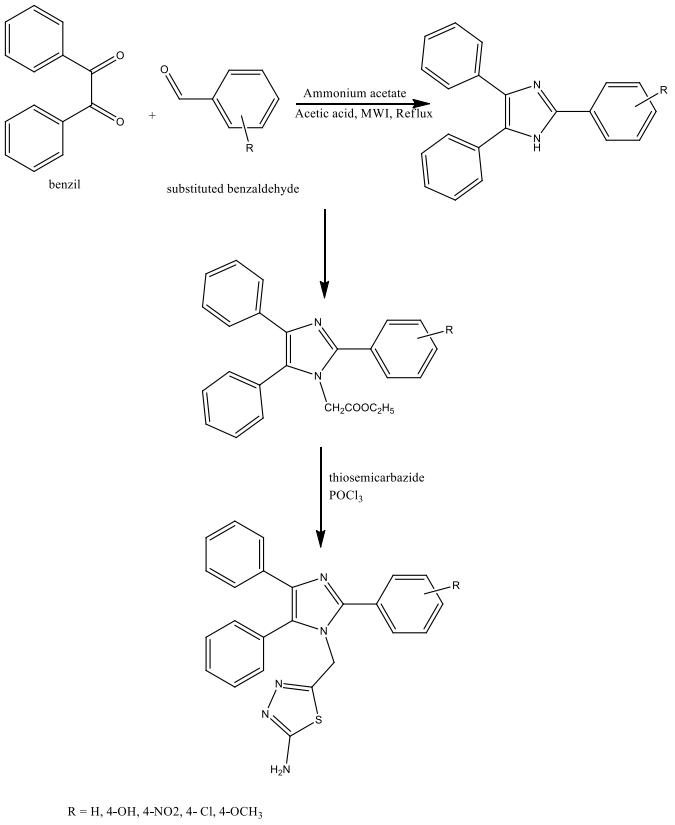
Material and Methods

Materials

Benzil was procured from Sulab; Ammonium acetate was purchased from Rankem, Aromatic aldehydes, glacial acetic acid and benzyl chloride were obtained from SD Fine Chemicals and Oxford Fine Chemicals. All other reagents and chemicals used for the synthesis were of synthetic grade and procured from Oxford Fine Chemicals. All the reagents and chemical were used as

obtained without any further purification. All glassware was of borosilicate glass and cleaned using chromic acid cleaning mixture (conc. H_2SO_4 + sodium dichromate) before use.

The synthesis of desired compounds was achieved using Scheme 1.



Scheme 1.

General method of Synthesis of substituted-2,4,5-triphenyl-1H-imidazole, 3

In to a clean dry round bottom flask, benzil (0.01 mol), ammonium acetate (0.05 mol), corresponding aromatic aldehyde (0.01 mol) and 25 ml of acetic acid were mixed. The mixture was refluxed for 1 h on a heating mantle. The reaction mixture was cooled and poured in to the 50 ml of water contained in a beaker. The precipitate that separated out was filtered and dried. The product was recrystallized from ethanol to obtain the 2,4,5-triphenyl-1H-imidazole. The reaction was monitored by TLC using (Methanol-dichloromethane, 1:1) as the solvent system.[6]

General method for synthesis of N-substituted imidazole, 4a-e

Anhydrous K_2CO_3 (0.006 mol) was added to a solution of the appropriate phenyl imidazole (0.003 mol) and ethylchloroacetate (0.003 mol) dissolved in anhydrous DMF (10 mL) in a round bottom flask and refluxed for 1-2 h. The mixture was poured onto crushed ice to precipitate the solid product. The product was filtered at pump using Buchner funnel.[7]

General method for synthesis of thiadiazole-imidazole conjugates, 5a-e

The synthesis of thiadiazole-imidazole conjugates was carried out by modification of the reported method.[8] A mixture of compound **4a-e** (0.01 mol), thiosemicarbazide (0.91 g ; 0.01 mol) and (5

mL) phosphorus oxychloride was refluxed for 8 h. The cold reaction mixture was poured on crushed ice and neutralized by adding sodium hydroxide solution. The resulting solid was filtered and recrystallized from chloroform to give a white-crystals of thiadiazole-conjugated derivatives, **5a-e**.

Antibacterial Activity

The synthesized Schiff's bases were dissolved in DMSO to obtain the solutions of 25, 50, 75 & 100 μg/mL. These solutions were used as the test samples. Lyophylized cultures of *Escherichia coli* (MTCC 40), and *Staphylococcus aureus* (MTCC 3160) were revived in nutrient broth for study of antibacterial action .

Determination of zone of inhibition

About 3 mm thick pre-poured nutrient agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. The antimicrobial action was screened using disc diffusion method.[9] Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200μL of the Schiff's bases (25, 50, 75 & 100 μg/mL) were placed in each hole. The plates were incubated for 24h at 37 ± 0.1°C to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters.

Determination of minimum inhibitory concentration

The broth dilution technique was used to determine the minimum inhibitory concentration of the synthesized compounds. The final inoculum size (of bacterial culture) was maintained to 10^5 CFU/mL. A set of tubes containing only the inoculated broth was used as the growth control, and one containing only the broth was used to ensure the sterility of the medium. A set of six tubes was labelled as 1 to 6 and to each tube was added 3 mL of nutrient broth. To the first tube was added 300μL of 1mg/mL of the test sample. The contents were mixed by swirling between hands and 300μL of content from the 1st tube was transferred to 2nd tube. The process was repeated from 2nd to 3rd tube up to the 6th tube. From the 6th tube, 300μL of content was withdrawn and discarded. To each of these tubes was added 200μL of the bacterial inoculum. All the tubes were incubated at 37°C for 24-48 h to allow for growth of micro-organism. After incubation, the optical density of the content from each tube was observed at 600 nm using UV-Visible spectrophotometer. The concentration that led to half of the optical density (50%) of the growth control tube was observed for each sample and standard (norfloxacin).[10]

Results and Discussion

Chemistry

Synthesis of the desired compounds was carried out by utilizing the scheme depicted in previous chapter. The scheme was optimized by varying the molar concentrations of the reactants and the

reaction time in order to achieve maximum yield for the compounds. The optimized conditions comprised of 1:5:1 ratio of benzyl-ammonium acetate and aldehyde.

The R_f value obtained from TLC, melting point and percent yield of the synthesized compounds is depicted in Table 1.

Table 1. R_f value, melting point and percent yield of 5a-e

Compound Code	Color	R_f Value	Melting Point	% Yield
5a	White	0.53	248-250°C	68
5b	Pale Yello	0.62	236-238°C	61
5c	White	0.47	251-253°C	70
5d	Pale Yellow	0.71	239-241°C	73
5e	Pale Yellow	0.77	244-246°C	67

The results of solubility studies reveal the all the compounds were insoluble in water and soluble in chloroform, methanol and DMSO.

The spectral studies (NMR, Mass and IR) were conducted to confirm the structure of the synthesized compounds. The spectra were obtained for the samples and the interpretation of each spectrum was carried out to ascertain the formation of desired bonds and incorporation of the functional groups.

The FTIR spectra of all the compounds exhibited stretching and bending vibrations for C-H, C=N, C-N, C-S, and N-H whereas C-Cl stretching peaks was prominent in the corresponding compounds. The peak for OH stretching was evident in compound 5c. The proton NMR spectra yielded shifts for aromatic protons as well as the CH_2 proton of the benzyl group. The mass spectra revealed molecular ion peaks and isotopic peaks corresponding to the molecular mass of the compounds.

5-((2,4,5-triphenyl-1H-imidazol-1-yl)methyl)-1,3,4-thiadiazol-2-amine, 5a

IR (cm^{-1}): 3027.29 (C-H stretching), 1706.09 (C=N stretching), 1603.19 (C=C stretching), 1475.38 (C-H bend), 1316.64 (C-N stretching); ^{1}HNMR (δ ppm): 7.28-7.69 (CH, aromatic), 6.07 (NH_2), 5.06 (CH_2); Mass (m/z): 409.14 (M^+ peak)

5-((2-(4-nitrophenyl)-4,5-diphenyl-1H-imidazol-1-yl)methyl)-1,3,4-thiadiazol-2-amine, 5b

IR (cm^{-1}): 3036.76 (C-H stretching), 1699.89 (C=N stretching), 1603.53 (C=C stretching), 1521.66 (N-O stretching), 1480.66 (C-H bend), 1318.44 (C-N stretching); ^{1}HNMR (δ ppm): 7.28-7.61 (CH, aromatic), 8.00/8.24 (CH, adj to NO_2), 6.02 (NH_2), 5.06 (CH_2); Mass (m/z): 454.12 (M^+ peak)

4-(1-((5-amino-1,3,4-thiadiazol-2-yl)methyl)-4,5-diphenyl-1H-imidazol-2-yl)phenol, 5c

IR (cm^{-1}): 3413.55 (O-H stretching), 3028.61 (C-H stretching), 1713.59 (C=N stretching), 1586.89 (C=C stretching), 1481.18 (C-H bend), 1323.49 (C-N stretching), 1079.31 (C-O stretching); ^{1}HNMR (δ ppm): 8.49 (OH), 7.28-7.68 (CH, aromatic), 6.92 (CH, adjacent to OH), 6.07 (NH_2), 5.02 (OH, CH_2); Mass (m/z): 425.13 (M^+ peak)

5-((2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazol-1-yl)methyl)-1,3,4-thiadiazol-2-amine, 5d

IR (cm^{-1}): 3024.20 (C-H stretching), 1682.94 (C=N stretching), 1575.12 (C=C stretching), 1462.53 (C-H bend), 1321.95 (C-N stretching), 802.86 (C-Cl stretching); ^{1}HNMR (δ ppm): 7.28-7.85 (CH, aromatic), 6.07 (NH_2), 5.02 (CH_2); Mass (m/z): 443.10 (M^+ peak)

5-((2-(4-methoxyphenyl)-4,5-diphenyl-1H-imidazol-1-yl)methyl)-1,3,4-thiadiazol-2-amine, 5e

IR (cm^{-1}): 3026.05 (C-H stretching), 1680.43 (C=N stretching), 1589.72 (C=C stretching), 1484.45 (C-H bend), 1313.34 (C-N stretching), 682.60 (C-O bending); ^{1}HNMR (δ ppm): 7.28-7.78 (CH, aromatic), 6.07 (NH_2), 5.06 (CH_2), 3.80 (CH_3); Mass (m/z): 439.15 (M^+ peak)

Antibacterial Action

The antibacterial activity of the synthesized thiadiazole-imidazole conjugates was determined measuring the zone of inhibition in the agar plate. Four concentrations of the synthesized compounds were tested for antibacterial action against ciprofloxacin as the standard drug for antibacterial action. The zone of inhibition of the test compounds is presented in table 2.

Table 2. Antibacterial activity of synthesized compounds

Compound Code	Zone of Inhibition (mm)*							
	E. coli				*S. aureus*			
	25µg	50µg	75µg	100µg	25µg	50µg	750µg	100µg
5a	10	10	13	14	10	10	10	13
5b	10	10	14	15	10	10	12	13
5c	10	10	17	18	10	10	14	15
5d	10	10	15	17	10	10	15	17
5e	10	10	**20**	**26**	10	10	18	**20**
Ciprofloxacin	**24**	**-**	**-**	**-**	**25**	**-**	**-**	**-**

* Below 12 mm – poor activity; 13-18 mm – moderate activity & above 18 mm – good activity

The MIC value of the test compounds was determined using broth dilution method by measuring the optical density of the broth solution incubated with diluted drug samples. The concentration that resulted in 50% optical density in comparison to the growth tube was taken as MIC of the test sample (Table 3).

Table 3. Calculated IC_{50} values of the test samples

Test sample	IC_{50} (µg/mL)	
	E. coli	***S. aureus***
5a	79.08	108.27
5b	148.13	162.31
5c	141.19	147.25
5d	142.38	170.44
5e	67.83	63.56
Ciprofloxacin	0.91	0.02

As visible from the results, the IC_{50} value for 5e was the lowest against both the pathogen (67.83 µg/mL for *E. coli* and 63.56 µg/mL for *S. aureus*). Also form the results it was very evident that attachment of electron donating groups in the molecule increased the IC_{50} (reduced antimicrobial potency) as seen in compounds 5b, 5c and 5d. In contrast non substituted compound, 5a was having a higher antibacterial activity in comparison to the electron donor substitution. NO_2 strongly withdraws electron density, destabilizing interactions with bacterial targets and possibly reducing lipophilicity, leading to poor cell penetration. OH can donate electrons, but also forms hydrogen bonds that may interfere with optimal binding or reduce permeability across bacterial membranes. Chlorine increases lipophilicity but its inductive withdrawal may reduce binding affinity. The balance doesn't favor antibacterial potency for the compound. Methoxy group donates electron density and enhances resonance stabilization, possibly improving binding to bacterial enzymes. It also increases lipophilicity moderately, aiding cell penetration. This dual effect likely explains its superior activity.

The SAR suggests that electron-donating substituents improve antibacterial potency, while electron-withdrawing groups diminish it. Compound 5e (OCH_3) is the most promising scaffold for further development, though it is still far less potent than ciprofloxacin. Future modifications could explore additional EDGs or lipophilic substituents to enhance activity further.

Conclusion

The present work successfully synthesized and characterized a novel series of thiadiazole-conjugated imidazole derivatives. Antibacterial evaluation demonstrated that these compounds possess moderate to good activity against both Gram-positive and Gram-negative bacteria, with compound 5e emerging as the most active candidate. The SAR analysis highlighted the importance of electron-donating substituents in enhancing antimicrobial potency, while electron-withdrawing groups reduced effectiveness. Although the activity of these derivatives remains lower than that of ciprofloxacin, the findings suggest that thiadiazole–imidazole conjugates, particularly methoxy-substituted analogs, can serve as valuable scaffolds for further structural modifications. Future studies should focus on optimizing lipophilicity, substituent positioning, and pharmacokinetic properties to improve antibacterial efficacy and clinical relevance.

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