RESEARCH PAPER

Design, Synthesis And Evaluation Of Some Novel Tyrosyl-Trna Synthetase Inhibitors As Potent Anti-Microbial Agents

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Abstract:
This study focuses on the design, synthesis, and biological evaluation of novel benzimidazole derivatives as potential antimicrobial agents. The increasing challenge of antimicrobial resistance necessitates the development of new therapeutic molecules with improved efficacy and safety profiles. Benzimidazole, a biologically significant heterocyclic scaffold, was selected due to its wide range of pharmacological activities. A series of 1,2-disubstituted benzimidazole derivatives were synthesized through a multi-step chemical process involving o-phenylenediamine and substituted phenyl acetyl chloride. The synthesized compounds were characterized using spectroscopic techniques such as FTIR, ^{1}H NMR, and mass spectrometry, confirming their structural integrity. Molecular docking studies were performed using Schrodinger software against tyrosyl-tRNA synthetase (PDB ID: 1JIJ) to evaluate binding affinity. The compounds demonstrated significant interactions with the target enzyme, supporting their potential biological activity. Antimicrobial screening revealed notable antibacterial and antifungal activity against selected strains, with compounds 5b, 5j, and 3e showing the highest efficacy. The results indicate that these derivatives possess promising antimicrobial properties comparable to standard drugs. Overall, this research highlights the potential of benzimidazole derivatives as effective candidates for future antimicrobial drug development

Keywords
Benzimidazole, Antibacterial, Antifungal, Docking Analysis, o-phenylene diamine.
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Introduction:

Antimicrobial agents play a vital role in modern medicine, having saved countless lives through effective treatment of various microbial infections. However, the growing concern of antimicrobial resistance has limited the use of existing drugs due to toxicity risks and pharmacokinetic challenges. To combat this issue, researchers are focusing on developing novel antimicrobial compounds that can overcome resistance and provide better therapeutic outcomes. The design of new antimicrobial agents is crucial to addressing the challenges posed by resistant microorganisms and ensuring effective treatment options for patients[1,2].

Heterocyclic aromatic compounds are known for their diverse biological activities, showcasing a broad spectrum of pharmacological effects, including cancer treatment, antimicrobial therapy, and antifungal interventions. Additionally, they exhibit antioxidant, anti-inflammatory and enzyme-inhibiting properties, making them valuable for further research and development [3].

Heterocyclic compounds are a class of organic molecules that contain at least one non-carbon atom, such as nitrogen, oxygen, or sulfur, within their ring structure. These compounds are significant in pharmacology, with many active drugs already on the market [4]. Heterocycles can exist independently or be fused with other rings, including carbocycles or other heterocycles. In this research, benzimidazole, a benzo-fused five-membered nitrogen-containing heterocycle, was chosen for study [5]. Notably, benzimidazole's biological relevance was first explored in the mid-20th century, with its purine-like structure hypothesized to contribute to its activity. As an aromatic heterocyclic compound, benzimidazole serves as a crucial pharmacophore in medicinal chemistry, making it a valuable structure for drug development [6-8].

Material and Methods

The study utilized chemicals and solvents sourced from commercial suppliers. Key analytical techniques included thin-layer chromatography (TLC) on silica gel plates, with UV light used for spot visualization. Melting points were determined using the Thiele's tube method. Spectroscopic analysis involved FTIR spectroscopy (SHIMADZU) for IR spectra and a Bruker 400 MHz spectrometer for ^{1}H and ^{13}C NMR spectra with DMSO as the solvent and TMS as the reference standard.

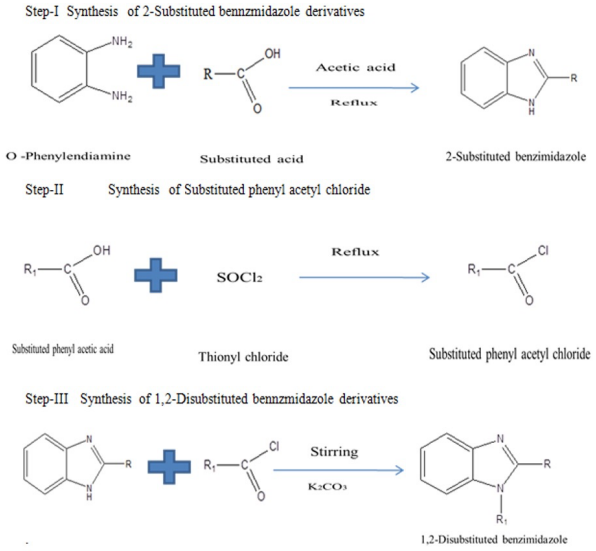
Molecular Docking Analysis

Molecular docking studies were conducted using the Schrodinger software (version 12.8) to assess binding interactions with target proteins. The 3D crystal structure

of tyrosyl-tRNA synthetase (PDB ID: 1JIJ) was retrieved from the RCSB Protein Data Bank for docking analysis.

Chemistry

The synthesis of 1, 2-disubstituted benzimidazole derivatives (5a, 5b, 5d, 5f, 5g, 5i, 5j, 3b and 3e) involves a multi-step process. Initially, o-phenylenediamine undergoes cycloaddition with a one-carbon donor source, facilitated by oxidative couples like H_2O_2/HCl or H_2O_2/$Fe(NO_3)_3$, yielding 2-substituted benzimidazole intermediates. These intermediates are then reacted with substituted phenyl acetyl chloride in the presence of potassium carbonate to form the target benzimidazole derivatives. The synthesized compounds were characterized using techniques such as IR, ^{1}H NMR, and mass spectrometry with TLC monitoring reaction progress. Their antimicrobial properties, including antibacterial and antifungal activities were evaluated and molecular docking simulations supported structural confirmation and assessment of biological potential. This study highlights the therapeutic potential of benzimidazole derivatives in antimicrobial applications [9].



Scheme 1. Methodology for synthesizing benzimidazole derivatives.

Common synthetic routes to 1, 2-disubstituted benzimidazole derivatives [10]

Procedure for Preparation of 2-substituted benzimidazole compounds

A mixture of o-phenylenediamine and substituted acids in acetic acid was heated under reflux at 70-80°C for 2-3 hours. TLC monitoring, using a 7:3 n-hexane-ethyl acetate mobile phase, tracked reaction progress. Upon completion, the mixture was cooled treated with ammonium hydroxide to adjust the pH and the resulting precipitate was filtered, dried and purified by methanol recrystallization.

Procedure for Preparation of substituted phenyl acetyl chloride

Substituted phenyl acetic acid was treated with thionyl chloride (8-10 ml) in a round-bottom flask and refluxed for 3-4 hours. Excess thionyl chloride was removed by evaporation and the resulting product was used directly in the subsequent synthesis step.

Preparation of 1, 2-disubstituted benzimidazole compounds

The 2-substituted benzimidazole from step 1 was dissolved in DMF and potassium carbonate was added. After 15 minutes of stirring, substituted phenyl acetyl chloride (from step 2) was introduced and the reaction mixture was stirred for 5 hours to overnight. Ice was added, and the mixture was cooled to induce precipitation. The solid was filtered, washed with cold water, dried, and purified by methanol recrystallization

Structural Confirmation through Spectral Analysis

3b. Prepration of 2-(2-bromophenyl)-1-(2-ethyl-1*H*benzo[d]imidazole-1-yl) ethanone

FTIR (KBr) ν (cm-1): 1112 (C-N str.), 2845 (C-H str.), 1449 (-CH_2), 1382 (-CH_3), 1665 (C=O str), 1588 (C=C str.), 672 (C-Br str), 3231 (C-OH str).1H NMR (400 MHz, DMSO, δ ppm): 7.1-7.7 ppm (m, 8H, Ar-H), 3.86 (s, 2H, -CH_2), 2.58 (t, 3H, -OH), 2.94 (q, 2H, -CH_2). MS (ESI) m/z [% rel. abundance]: 342.6 [M+], 344.3 [M+2].

3e. Synthesisof1-(2-ethyl-1H-benzo[d]imidazole-1-yl)-2-phenylethanone

FTIR (KBr) ν (cm-1): 1187 (C-N str.), 1403 (-CH_2), 1333 (-CH_3), 1637 (C=O str.), 2730 (C-H str.), 1497 (C=C str).1H NMR (400 MHz, δ ppm): 7.1-7.7 ppm (m, 9H, Ar-H), 2.6 (t, 3H, -CH_3), 3.9 (s, 2H, -CH_2), 2.9,(d,-2H, CH2). MS (ESI) m/z [% rel. abundance]: 263.8 [M+].

5a. Preparation of 2-(4-bromophenyl)-1-(2-ethyl-1H-benzo[d]imidazol-1-yl)ethanone Involved a multi-step chemical reaction

FTIR (KBr) ν (cm-1): 1120 (C-N str.), 1344 (-CH_2), 1648 (C=O str.), 1445 (N=O str.).1H NMR (400 MHz, δ ppm): 7.1-7.5 ppm (m, 8H, Ar-H), 2.48 (t, 3H, -OH), 3.1 (q, 2H, -CH_2). MS (ESI) m/z [% rel. abundance]: 298.5 [M+].

5b. Preparation of (2-ethyl-1H-benzo[d]imidazol-1-yl)(4-fluorophenyl) methanone involved a multi-step chemical reaction.

FTIR (KBr) ν (cm-1): 1687 (C=O stretching), 1352 (-CH_3), 698 (C-F stretching).1H NMR (400 MHz, DMSO, δ ppm): 7.1-7.6 ppm (m, 8H, Ar-H), 3.1 (s, 2H, -CH_2), 2.56 (t, 3H, -CH_3). MS (ESI) m/z [% rel. abundance]: 295.2 [M+].

5d. Preparationof2-(4-fluorophenyl)-1-(2-(4-nitrophenyl)-1H-benzo[d]imidazol- 1- yl) ethanone involved a multi-step chemical reaction.

FTIR (KBr) ν (cm-1): 1120 (C-N str.), 1645 (C=O str.), 1149 (-CH_2), 1352 (-CH_3), 1445 (C-N str.), 698 (C-F str.).1H NMR (400 MHz, δ ppm): 7-8.4 ppm (m, 12H, Ar-H), 3.45 (s, 2H, -CH_2). MS (ESI) m/z [% rel. abundance]: 375.3.

5f. Preparation of 1-(2-ethyl-1H-benzo[d]imidazol-1-yl)-2-(2-fluorophenyl) ethanone Involved a multi-step chemical reaction

FTIR (KBr) ν (cm-1): 1488 (-CH_2), 1325 (-CH_3), 1628 (C=O str.), 730 (C-Br str.) .1H NMR (400 MHz, δ ppm): 7.2-7.9 ppm (m, 8H, Ar-H), 3.6 (s, 2H, -CH_2), 3.7 (t, 3H, -OH), 3.3 (t, 2H, -CH_3). MS (ESI) m/z [% rel. abundance]: 382.2.

5g. Preparation of 1-(2-ethyl-1H-benzo[d]imidazol-1-yl)-2-(4-fluorophenyl) ethanone involved a multi-step chemical reaction

FTIR (KBr) ν (cm-1): 701 (C-F str.), 1752 (C=O str.), 1469 (-CH_2), 1349 (-CH_3).1H NMR (400 MHz, δ ppm): 7-7.5 ppm (m, 8H, Ar-H), 3.6 (s, 2H, -CH_2), 3.6 (q, 3H, -CH_3), 2.9 (t, 2H, -CH_2), MS (ESI) m/z [% rel. abundance]: 282.5 [M+].

5i. Preparation of 2-(4-chlorophenyl)-1-(2-ethyl-1H-benzo[d]imidazol-1-yl) ethanone Involved a multi-step chemical reaction

FTIR (KBr) ν (cm-1): 1145 (C-N str.), 1641 (C=O str.), 1345 (-CH_2), 1485 (-CH_3), 740 (C-Cl str.).1H NMR (400 MHz, δ ppm): 7.1-7.6 ppm (m, 8H, Ar-H), 3.46 (s, 2H, -CH_2), 3.40 (q, 2H, -CH_2), 2.87 (t, 3H, -CH_3). MS (ESI) m/z [% rel. abundance]: 298.5 [M+], 298.2 [M+1].

5j.Preparation of 2-(2-fluorophenyl)-1-(2-(2-nitrophenyl)-1H-benzo[d]imidazol-1-yl) ethanone

FTIR (KBr) ν (cm-1): 1535 (Aromatic C=N str.), 1643 (C=O str.), 1442 (N=O str.), 678 (C-F str.), 1352 (-CH_3). 1H NMR (400 MHz, DMSO, δ ppm): 6.7-8.1 ppm (m, 12H, Ar-H), 3.67 (s, 2H, -CH_2).MS (ESI) m/z [% rel. abundance]: 374.9 [M+].

Biological assessment of antibacterial and antifungal properties

The synthesized compounds were tested for their antibacterial and antifungal activity against Gram-positive and Gram-negative bacteria, as well as a fungal strain, using the agar diffusion method. Plates were incubated at 37°C for 24 hours. Compounds were tested at 50 µg/ml and 100 µg/ml against S.A, E.Coli and Aspergillus niger. Streptomycin, tetracycline and miconazole were used as standards. Compounds 5b, 5j and 3e showed the strongest activity against the tested strains (see Table 1 and Figure 1).

Table 1. Evaluation of the synthesized compounds for their antibacterial and antifungal potential through a comparative approach.

Organism	S. Aureus ATCC (Gram Positive)		E.Coli ATCC (Gram Negative)		Aspergillus Niger	
Synthesized Molecules	Concentration					
	50µg/ml	100µg/ml	50µg/ml	100µg/ml	50µg/ml	100µg/ml
3b	22	20	22	20	18	20
3e	26	24	26	24	20	22
5a	22	20	22	20	16	18
5b	26	24	26	24	20	21
5d	26	24	24	22	18	16
5f	22	20	22	20	16	14
5g	20	22	20	18	20	16
5i	24	22	22	20	18	18
5j	28	28	26	24	22	21
Streptomycin	28	32	-	-	-	-
Tetracycline	-	-	27	30	-	-
Miconazole	-	-	-	-	22	26

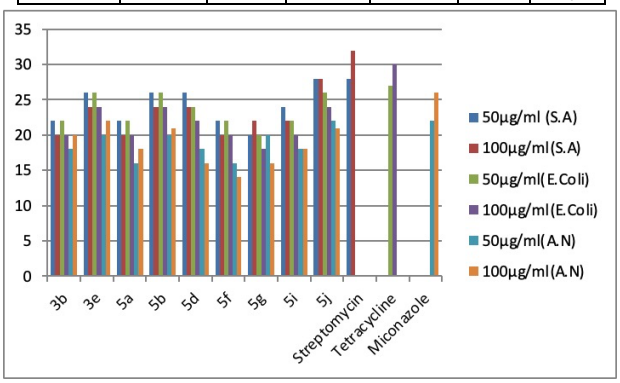


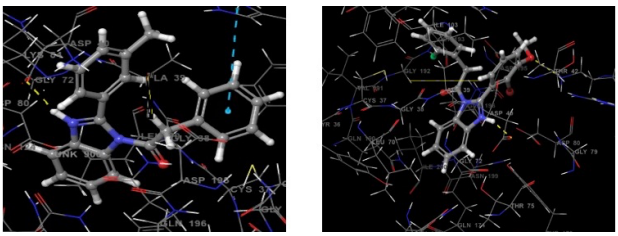
Figure 1 Assessment and comparison of the antibacterial and antifungal effects of the prepared compounds

Evaluation of Molecular Docking

Molecular docking studies were conducted to examine the interactions between the synthesized compounds and the selected target proteins. The docking outcomes are summarized in Table 2. Among the evaluated compounds 3b, 3e, 5a, 5b, 5d, 5f, 5g, 5i, and 5j exhibited notable binding affinity toward the enzyme tyrosyl-tRNA synthetase (PDB ID: 1JIJ).

Table 2 Molecular docking analysis of the tested compounds with tyrosyl-tRNA synthetase (PDB ID: 1JIJ)

Compound name.	Docking Score
3b	-9.031
3e	-10.142
5a	-10.331
5b	-10.092
5d	-9.231
5f	-9.278
5g	-9.045
5i	-9.048
5j	-10.031



5j

3e

Fig 2: Interaction of compound 5j and 3e with TyrRS (1JIJ)

Findings and Interpretation

The synthesized compounds 3b, 3e, 5a, 5b, 5d, 5f, 5g, 5i, and 5j were evaluated for their antimicrobial potential, including both antibacterial and antifungal activities. These derivatives were characterized using physical properties, chromatographic methods and spectroscopic techniques such as IR, ^{1}H NMR and mass spectrometry. The structural identity of the benzimidazole derivatives was confirmed through the combined interpretation of infrared, proton nuclear magnetic resonance and mass spectrometry data.

In the IR spectra, characteristic absorption bands were observed at 1643 cm^{-1} corresponding to C=O stretching, 1442 cm^{-1} attributed to N=O stretching, 1535 cm^{-1} indicating aromatic C=N stretching and 678 cm^{-1} assigned to C–F stretching vibrations. The ^{1}H NMR spectra displayed a broad multiplet in the region of δ 6.7–8.1 ppm, which was attributed to aromatic protons (Ar–H). Furthermore, mass spectral analysis of all synthesized derivatives showed the presence of characteristic molecular ion peaks [M+1] and [M+2] confirming their molecular weights.

The antibacterial activity of the compounds was tested against both Gram-positive and Gram-negative bacterial strains using the agar diffusion cup plate method. The inoculated plates were incubated at 37 °C for 24 hours. The compounds were tested at concentrations of 50 µg/ml and 100 µg/ml against *Staphylococcus aureus* and *Escherichia coli*. Tetracycline and Streptomycin were used as standard reference antibiotics for comparison. As presented in Table 1, the compounds 3b, 3e, 5a, 5b, 5d, 5f, 5g, 5i, and 5j exhibited notable inhibitory activity against both *S. aureus* and *E. coli*.

For antifungal evaluation the agar diffusion cup plate technique was also applied. The synthesized compounds at 50 µg/ml and 100 µg/ml concentrations were screened against the fungal strain *Aspergillus niger*, with Miconazole used as the reference antifungal agent. According to the results summarized in Table 1 all the tested compounds demonstrated considerable antifungal activity against *A. niger*.

Summary

Several benzimidazole derivatives were successfully synthesized, and their chemical structures were confirmed using various spectroscopic techniques, including IR, ^{1}H NMR and mass spectrometry. The synthesized compounds were further evaluated for their antimicrobial properties, and the results indicated appreciable activity in comparison with the standard reference drug. In addition, molecular docking studies were performed to better understand the possible interactions and mechanisms responsible for their observed biological activity.

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