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Biological Evaluation, Synthesis and Design of Benzothiazole Derivatives for Antimicrobial Activity

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Abstract: ***Benzothiazoles are a crucial class of heterocyclic compounds known for their broad spectrum of biological activities, particularly their antimicrobial properties. This study investigates the synthesis, molecular docking, and biological evaluation of various benzothiazole derivatives for their potential antimicrobial activity. A series of benzothiazole derivatives were synthesized and characterized using spectroscopic methods. Molecular docking studies were performed to predict the binding affinities of these derivatives to target microbial enzymes, providing insights into their mechanisms of action. Biological evaluation involved testing the synthesized compounds for their antifungal and antibacterial activities against selected microbial strains. Compounds A1, A2, A4, A6, and A9 demonstrated significant antifungal activity against Aspergillus niger and Candida albicans (NCIM 3102), with Amphotericin-B used as the standard antifungal drug for comparison. Among these, compounds A1, A2, and A9 also exhibited promising antibacterial activity against Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 29737), with Ciprofloxacin serving as the standard antibacterial drug. The results indicate that specific structural modifications in the benzothiazole core can enhance antimicrobial efficacy, suggesting that these compounds have potential as therapeutic agents. Further studies, including in vivo testing and optimization of lead compounds, are warranted to fully explore their clinical potential.***

Keywords: Molecular docking, Benzothiazole, anti-bacterial, anti-fungal, heterocyclic compounds

1. Introduction

Benzothiazoles are a prominent class of heterocyclic compounds that contain a benzene ring fused to a thiazole ring. [1-3] They are notable for their broad spectrum of biological activities, which make them important scaffolds in medicinal chemistry and pharmaceutical research. This overview discusses the structure, synthesis, and diverse biological activities of benzothiazole derivatives, highlighting their significance in drug discovery and development. Benzothiazole is characterized by a fused ring system consisting of a benzene ring and a thiazole ring (a five-membered ring containing nitrogen and sulfur). The basic structure of benzothiazole allows for various substitutions on the benzene and thiazole rings, which can significantly alter their chemical and biological properties. These modifications enable the design of compounds with tailored activity for specific therapeutic targets. [4]

The synthesis of benzothiazole derivatives can be achieved through several methods. Some of the common synthetic routes include: Cyclization of 2-aminothiophenols with aldehydes or ketones is a widely used method for synthesizing benzothiazoles. This reaction typically involves the formation of a Schiff base intermediate, which cyclizes to form the benzothiazole ring system. Substitution reactions on preformed benzothiazole rings can introduce various functional groups, allowing for fine-tuning of biological activity. [5-7]

Biological Activities

Benzothiazole derivatives exhibit a wide range of biological activities, making them valuable in various therapeutic areas:

Antimicrobial Activity

Benzothiazoles have been extensively studied for their antimicrobial properties. They are effective against a variety of bacterial and fungal pathogens. 2-Substituted benzothiazoles showing potent activity against Staphylococcus aureus and Escherichia coli. [8]

Anticancer Activity

Certain benzothiazole derivatives have shown significant anticancer activity by targeting specific cellular pathways involved in cancer progression. 2-(4-Aminophenyl) benzothiazole has been investigated for its ability to inhibit tumor growth. [9]

Anti-inflammatory Activity

Benzothiazoles can also exhibit anti-inflammatory properties by modulating inflammatory pathways and cytokine production. Benzothiazole derivatives inhibiting COX-2 enzyme, which plays a key role in inflammation. [10] The versatility of benzothiazole derivatives in interacting with various biological targets makes them promising candidates for drug development. They serve as core structures in many drugs currently under clinical investigation or already in therapeutic use. The ability to modify the benzothiazole scaffold enables the development of compounds with optimized pharmacokinetic and pharmacodynamic properties. Benzothiazoles are an essential class of

heterocyclic compounds with diverse biological activities. Their structural flexibility and the ability to undergo various chemical modifications make them valuable in the design and development of new therapeutic agents. Continued research into benzothiazole derivatives holds promise for discovering new drugs to treat a range of diseases, including infections, cancer, and inflammatory conditions.

2. Experimental

Molecular Docking

The molecular docking studies was carried out using the Glide module incorporated into Schrodinger's molecular modeling software. All the molecular properties were calculated using the Qikprop module. The analysis of binding free energies corresponding with the docked complexes was carried out using the Prime MMGBSA module (Prime, 2017) installed on Linux based computer having 16 GB RAM. Molecular docking of Schiff's bases of 2-amino-benzthiazole derivatives with the target E. coli Dihydroorotase (PDB: 2Z2A) using the standard ligand 2-oxo-1,2,3,6-tetrahydropyrimidine-4,6-dicarboxylic acid (HDDP). [11]

Preparation of Receptor (E. coli Dihydroorotase)

Download the 3D structure of E. coli Dihydroorotase from the Protein Data Bank (PDB) with the accession code 2Z2A. Use molecular visualization software (e.g., PyMOL, Chimera, VMD) to remove any water molecules, cofactors, and other heteroatoms that are not part of the protein structure. Save the protein structure in an appropriate file format (e.g., PDB, PDBQT).

Preparation of Ligands (Schiff's Bases of 2-Amino-Benzthiazole Derivatives and HDDP)

Generate 3D structures of Schiff's bases of 2-amino-benzthiazole derivatives using a molecular modeling software or draw them using a molecular sketching tool (e.g., ChemDraw, MarvinSketch). Ensure that the structures are in a suitable format for molecular docking (e.g., SDF, MOL2, PDB). For the standard ligand HDDP, obtain its 3D structure from a chemical database or draw it using a molecular sketching tool.

Protein and Ligand Preparation

Prepare the receptor and ligand structures for docking using a molecular modeling software (e.g., AutoDockTools, Open Babel). Assign Gasteiger charges to the receptor and ligand atoms. Add polar hydrogen atoms to the receptor and ligand structures. Save the prepared receptor and ligand structures in the appropriate, file format required by the docking software.

Define the Binding Site

Identify the binding site on the receptor where the ligands are expected to bind. This can be based on experimental data, known active sites, or predicted binding pockets using computational tools (e.g., CASTp, SiteMap). Define a grid box or search space around the binding site to guide the docking software during the search for ligand binding modes.

Docking Setup

Choose a suitable molecular docking software (e.g., AutoDock, AutoDock Vina, Glide) for performing the docking calculations. Set up the docking parameters, including the search algorithm, scoring function, and number of docking poses to generate. Specify any constraints or preferences for ligand flexibility during the docking process.

Docking Calculation

Run the docking simulation using the prepared receptor and ligand structures and the defined docking parameters. Allow the software to explore different orientations and conformations of the ligand within the binding site of the receptor. Perform multiple docking runs or increase the sampling if necessary to ensure comprehensive exploration of the conformational space. [12]

Analysis of Docking Results

Analyze the docking results to identify potential binding modes and interactions between the ligands and the receptor. Evaluate the docking poses based on scoring functions provided by the docking software. Visualize the docked complexes using molecular visualization software to inspect the binding interactions, such as hydrogen bonds, hydrophobic contacts, and π-π stacking.

Validation and Refinement

Validate the docking results using experimental data if available, such as binding affinity measurements or structural studies. Refine the docking parameters or perform additional simulations to improve the accuracy of the predictions. Consider performing molecular dynamics simulations or other computational analyses to further characterize the stability and dynamics of the ligand-receptor complexes.

Synthesis of Compound

Scheme for Benzothiazole Derivatives

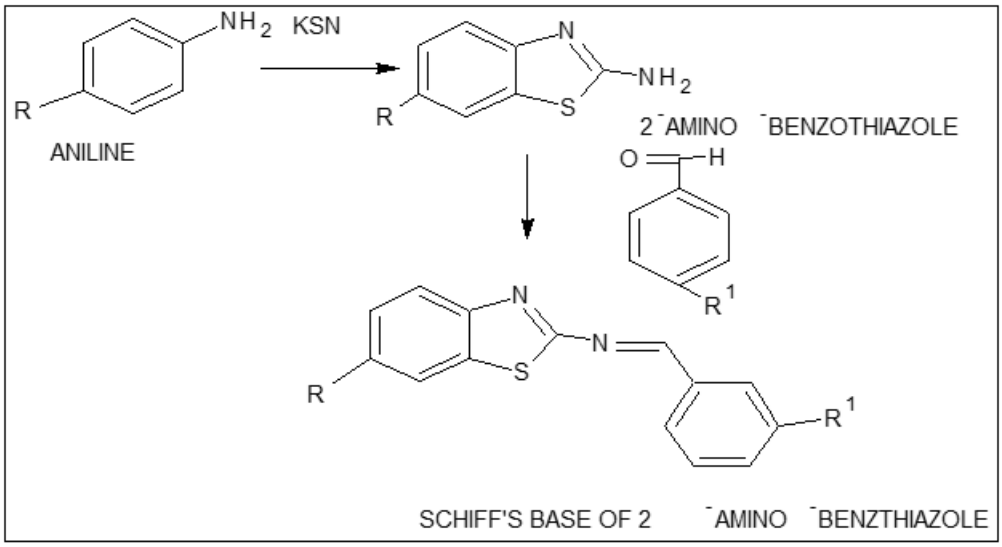


Figure 1: Scheme for Benzothiazole Derivatives

Table 1: Derivatives of Benzothiazole

Sr. No.	Label	R	R_1
1	A1	-ORTHO- CHLORO	Ortho-Hydroxy
2	A2	-ORTHO- CHLORO	Anisaldehyde
3	A3	-ORTHO- CHLORO	Ortho-Chloro
4	A4	-PARA-CHLORO	Ortho-Hydroxy
5	A5	-PARA-CHLORO	Anisaldehyde
6	A6	-PARA-CHLORO	Ortho-Chloro
7	A7	-META- CHLORO	Ortho-Hydroxy
8	A8	-META- CHLORO	Anisaldehyde
9	A9	-META- CHLORO	Ortho-Chloro

General procedure for synthesis of Scheme

Step 1

To synthesize 2- substituted amino benzothiazole using potassium thiocyanate; begin by mixing aniline (1 mole) and potassium thiocyanate (1 mole) in a suitable reaction vessel. Then heat the mixture under reflux with the addition of a hydrochloric acid until the reaction is complete, as indicated by the appearance of a characteristic color change. Then dissolve the resulting 2-mercaptobenzothiazole in ethanol and add an excess of sodium hydroxide to the solution. Reflux the mixture until the conversion to 2-amino-benzothiazole is achieved. After completion, cool the reaction mixture, neutralize it with dilute hydrochloric acid, and extract the product using an ethanol. Purify the crude product through recrystallization method, and confirm the identity and purity of 2-amino-benzothiazole using spectroscopic techniques such as NMR, IR spectrometry. Safety precautions should be strictly followed throughout the procedure, conducted in a well-ventilated laboratory, and optimized based on specific reaction conditions and reagents used.

Step 2

To synthesize the Schiff's base of 2-amino-benzothiazole and benzaldehyde, begin by adding 2-amino-benzothiazole (1 equivalent) and benzaldehyde (1 equivalent) to a suitable reaction vessel. Stir the mixture at room temperature in the presence of an ethanol until the Schiff's base is formed, typically evidenced by the appearance of a characteristic color change. To enhance the reaction efficiency, used acetic acid, can be employed. Once the reaction is complete, precipitate the Schiff's base by adding a diethyl ether to the reaction mixture. Collect the precipitate through filtration, wash it with the solvent, and allow it to air-dry. Purify the Schiff's base further, if necessary, using technique recrystallization. Confirm the identity of the synthesized compound through spectroscopic methods such as NMR, IR spectrometry. Adherence to safety protocols, such as proper ventilation and protective measures, is crucial during the synthesis, and the procedure may be optimized based on specific reaction conditions and desired product characteristics.

Physiochemical Characteracterization

Melting point determination

Melting point is a valuable criterion for the purity of the organic compound. The melting points were determined by open capillary method using digital melting point apparatus.

Solubility determination

The solubility of synthesized compounds was tested in different polar, semi polar, and non-polar solvent.

TLC analysis (R_f value)

Thin Layer Chromatography is an important technique, which provides information regarding progress of reaction and determines the purity of compounds. R_F Value is the characteristic for each compound and calculated through TLC analysis by using the equation given below:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Spectroscopic Characterization

Structural interpretation of Schiff's bases of 2-Amino-Benzothiazole Derivatives

Interpreting the structural features of Schiff's bases of 2-amino-benzthiazole derivatives using spectroscopic techniques such as Infrared Spectroscopy (IR), Nuclear Magnetic Resonance Spectroscopy (NMR)can provide valuable insights into their chemical composition and bonding patterns:

Infrared Spectroscopy (IR)
IR spectroscopy provides information about the functional groups present in a molecule based on the absorption of infrared radiation by specific chemical bonds. In Schiff's bases of 2-amino-benzthiazole derivatives, characteristic IR absorption bands may include:

The C=N stretching vibration: Typically appears in the range of 1600-1670 cm^{-1}, indicating the presence of the Schiff base functional group.

Aromatic C-H stretching vibrations: Appears as sharp peaks in the region of 3000-3100 cm^{-1} for the aromatic rings of benzothiazole derivatives. C-S stretching vibration: Observed around 600-700 cm^{-1}, confirming the presence of the thiazole ring. The absence of absorption bands corresponding to carbonyl groups (around 1700 cm^{-1}) would indicate successful formation of the Schiff base, as the carbonyl group of the aldehyde or ketone is involved in the condensation reaction.

^{1}HNMR Spectra
All the synthesized compounds were characterized by ^{1}HNMR spectral analysis. The results of NMR spectra resembles to molecular structure.

Microbiological Screening [13-14]

Anti-Bacterial Activity
Nutrient agar plates were prepared by pouring 15-20 mL of the medium into each sterilized Petri dish and were allowed to set at room temperature. The cell suspension was standardized to the density of 530 nm using spectrophotometer and was inoculated over the surface of agar medium using sterile cotton swab. The cups were scooped in each plate using a sterile cork borer of 6 mm diameter. Then the solutions of test compounds (0.10 mL) were added in cups by using micropipettes and these plates were incubated at 37°C for 48 hrs. The zone of inhibition was measured in mm for each organism.

Anti-Fungal Activity
Sabouraud-Dextrose agar plates were prepared by pouring 15-20 mL of the medium into each sterilized Petri dish and were allowed to set at room temperature. The cell suspension was standardized to a density of 530 nm using a spectrophotometer and was inoculated over the surface of medium using a sterile cotton swab. Three cups were scooped in each plate using a sterile cork borer of 6mm diameter, standard and test solution. The solution of each test compound (0.10 mL/0.15 mL) was added in the cups by using micropipettes and these plates were subsequently incubated at 37°C for 48 hrs. The zone of inhibition was measured in mm for each organism.

3. Results and Discussion

Molecular docking score of compounds A1-A9

Table 2: Molecular docking score of Compounds A1-A9

Comp.code	Binding Affinity	rmsd/ub	rmsd/lb
A1	-6.1	17.184	15.148
A2	-6.2	21.497	19.448
A3	-6.5	15.823	14.021
A4	-6.8	15.078	13.503
A5	-6.2	7.365	3.401
A6	-6.8	15.544	13.387
A7	-6.6	25.731	24.7
A8	-6.2	24.798	23.365
A9	-6.4	14.914	13.986

Melting Point, Molecular Formula, Percentage Yields and Rf Values Synthesized Derivatives of Scheme I:

Table 3: Analytical data of compounds

Comp	Mol. Formula	Mol. Wt.	M.P ^{0}C	Rf Value	Yield %
A_1	$C_{14}H_9N_2ClOS$	288.75	224-226	0.46	67
A_2	C15H11ClN2S	286.77	250-252	0.65	78
A_3	$C_{14}H_9N_2Cl2OS$	307.19	218-221	0.53	67
A_4	$C_{14}H_9N_2ClOS$	288.75	191-193	0.52	66
A_5	C15H11ClN2S	286.77	228-229	0.48	75
A_6	$C_{14}H_9N_2Cl2OS$	307.19	212-214	0.48	61
A_7	$C_{14}H_9N_2ClOS$	288.75	132-134	0.51	63
A_8	C15H11ClN2S	286.77	135-138	0.51	44
A_9	$C_{14}H_9N_2Cl2OS$	307.19	131-133	0.56	64

Spectral Analysis of Synthesized Derivatives
Results of ^{1}H NMR Spectra of Compound of Scheme

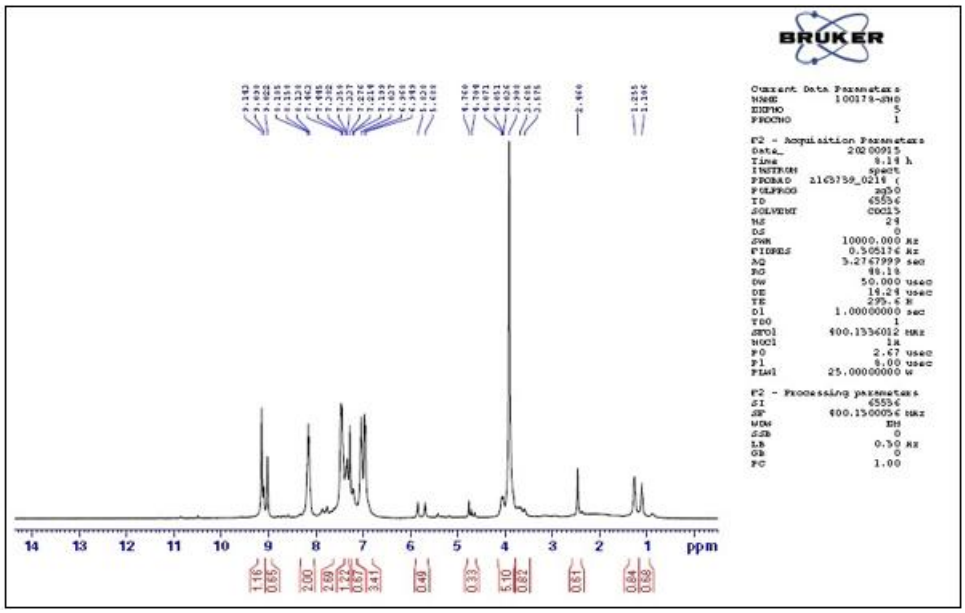
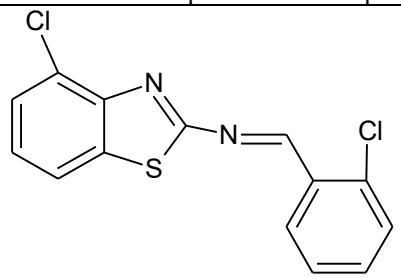


Figure 2: 1^{H}-NMR spectra of Compound A3

Table 4: ^{1}H NMR Interpretation of compound A3



Chemical Shift (ppm)	Coupling Constant (J,Hz)	Peak (Multiplicity)	Nature of proton and Assignment
6.54-8.45	Various	Multiplet	7H of -C6H5
4.76	N/A	Singlet	1H of (-CH=N)

Results of FTIR Spectral analysis of compounds from Scheme I:

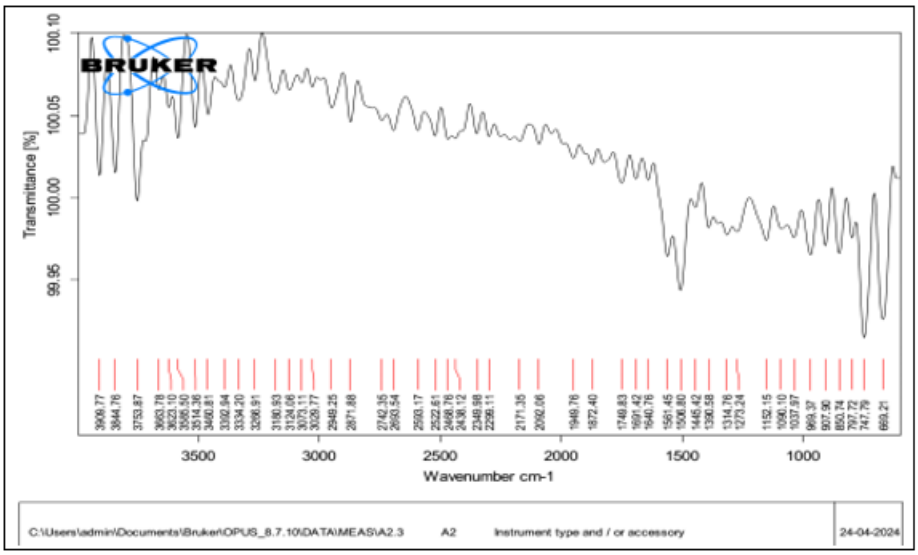
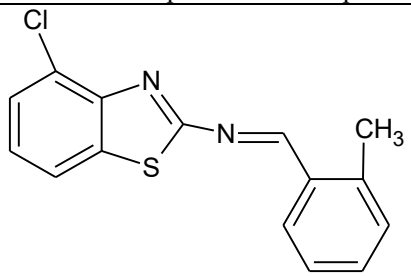


Figure 3: FTIR Spectra of Compound A2

Table 5: FTIR Interpretation of compound A2



Wavenumber (cm^{-1})	Assignment
3029.77	Aromatic -CH stretching.
2817.88	-CH2 stretching (methyl group)
1506.80	-C=N stretching (carbonitrile)
869.37	-C-Cl stretching (chloro group)
669.21	-C-S-C stretching. (thio group)

Biological Activity

Results of *In Vitro* Antibacterial and Antifungal activity of compounds

Table 6: Anti-bacterial and Anti-fungal activity of compounds

Compd.	*Zone of inhibition at 200µcg/mL (in mm.)*			
	E. coli	*S. aureus*	*A. niger*	*C. albicans*
A_1	19	21	20	19
A_2	21	21	23	21
A_3	17	18	19	17
A_4	14	13	24	20
A_5	18	20	15	18
A_6	16	18	21	21
A_7	15	17	14	19
A_8	19	12	17	15
A_9	21	23	20	23
Ciprofloxacin	25	26		-
Amphotericin-B	-	-	26	25

Compounds **A_1, A_2, A_4, A_6, A_9** have shown promising **antifungal** activity against *A.niger, C. albicans* (NCIM 3102). **Amphotericin-B** was used as standard drug. Compounds **A_1, A_2, A_9** have shown promising **antibacterial** activity against *E.coli* (ATCC25922)**),** *S. aureus* (ATCC 29737). Ciprofloxacin was used as std.drug. The integration of molecular docking, synthesis, and biological evaluation has led to the identification of benzothiazole derivatives with significant antimicrobial activity. The molecular docking studies provided insights into the binding affinities and interaction mechanisms of the synthesized compounds with key microbial enzymes, such as DNA gyrase and dihydrofolate reductase. These computational predictions guided the design and synthesis of benzothiazole derivatives, ensuring that they possess the structural characteristics necessary for high antimicrobial efficacy.

Antifungal Activity

Compounds A1, A2, A4, A6, and A9 exhibited notable antifungal activity against Aspergillus niger and Candida albicans. The zones of inhibition produced by these compounds were comparable to those of Amphotericin-B, indicating their potential as effective antifungal agents. The presence of specific functional groups, such as electron-donating or withdrawing substituents, likely contributed to their enhanced activity.

Antibacterial Activity

Compounds A1, A2, and A9 demonstrated strong antibacterial properties against Escherichia coli and Staphylococcus aureus. The zones of inhibition for these compounds were comparable to those of Ciprofloxacin, a widely used antibacterial drug. The structure-activity relationship (SAR) analysis revealed that certain substitutions on the benzothiazole ring enhance the compounds' ability to interact with bacterial targets, thus improving their antibacterial potency.

The promising antimicrobial activity of these benzothiazole derivatives suggests their potential for development as therapeutic agents. The findings from this study highlight the importance of structural modifications in optimizing the biological activity of benzothiazole derivatives. Future research should focus on further optimization of these lead compounds to enhance their efficacy and minimize potential toxicity.

4. Conclusion

The integration of molecular docking, synthesis, and biological evaluation has successfully identified benzothiazole derivatives with significant antimicrobial activity. Compounds A1, A2, A4, A6, and A9 showed notable antifungal activity, while A1, A2, and A9 exhibited strong antibacterial properties. These findings underscore the potential of benzothiazole derivatives as effective antimicrobial agents. Further optimization and in vivo studies are essential to fully explore their clinical potential and to develop these compounds into viable therapeutic options.

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