RESEARCH PAPER

SYNTHESIS, CHARACTERISATION, ANTIMICROBIAL ACTIVITY, AND MOLECULAR DOCKING OF NOVEL BENZOTHIAZOLE DERIVATIVES

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ABSTRACT

In important class of heterocyclic compounds, benzothiazole derivatives constitute with diverse pharmacological applications. Aim of the study was designed to synthesize, characterize and evaluate the antimicrobial activity as well as molecular docking of novel benzothiazole derivatives. The compounds were characterized by physical and spectral analysis such as FT-IR, 1H-NMR, 13C-NMR and mass spectrometry. The antimicrobial potential of the derivatives was evaluated against bacterial and fungal pathogenic strains by MIC (minimum inhibitory concentration) assays. Results of the study show that all the synthesized derivatives possess significant antibacterial and antifungal activities comparable to standard drug. Furthermore, molecular docking study against bacterial enzyme/protein targets active pockets of lanosterol 14α-demethylase to elucidate binding modes. The synthesized molecules RKN4, RKN7 and RKN8 showed good binding affinity in comparison with the standard, itraconazole. The binding affinity of RKN8 was -10.7 Kcal/mol and showed the best binding affinity. All the tested compounds exhibited significantly high inhibition at 12.5-200 µg/mL in antibacterial and antifungal testing. The analogues RKN4 and RKN8 exhibited better activity contrary to all the fungal as well as bacterial strains. The better activity is accredited to the occurrence of 2,4-dichloro group attached to phenyl residues at position 2 of quinazoline-4-one in the derivatives. Other analogues exhibited modest activity related to standard levofloxacin, fluconazole and amphotericin-B against all the fungal and bacterial strains. Thus, RKN4 and RKN8 are acting as lead compounds as antibacterial and antifungal agents and this demonstrates that structural modification of the benzothiazole scaffold can lead to promising antimicrobial candidates.

KEYWORDS: Benzothiazole, lanosterol-14α-demethylase, antibacterial activity, antifungal activity.

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.INTRODUCTION

The increasing incidence of multidrug resistance (MDR) among pathogenic microorganisms represents a serious global health threat, demanding the urgent development of novel antimicrobial agents. Traditional antibiotics are becoming less effective against resistant bacterial and fungal strains, necessitating the exploration of new heterocyclic frameworks with improved potency and selectivity. Importantly, subtle modifications on the benzothiazole ring, such as substitution at the 2- or 6-position with electron-donating or electron-withdrawing groups, can drastically influence biological activity, providing opportunities for structure–activity relationship (SAR) exploration.

Benzothiazole nucleus has attracted remarkable attention as a privileged pharmacophore because of its rigid bicyclic aromatic system, resonance stabilization, and favourable lipophilicity. These structural features enable benzothiazole derivatives to exhibit versatile binding interactions with biological macromolecules, making them promising scaffolds in drug discovery and development[1]. Indeed, numerous benzothiazole-based compounds are reported in the literature with significant antimicrobial, antifungal, anticancer, antitubercular, anti-oxidant, enzyme inhibitory, and anticonvulsant activities. Some clinically used drugs such as riluzole (anti-ALS agent), zopolrestat (antidiabetic), methabenzthiazuron (urea-based herbicide), TMCB (antifungal), frentizole (antiviral) and ethoxzolamide (carbonic anhydrase inhibitor) also contain the benzothiazole core highlighting its pharmacological importance. From a synthetic standpoint, benzothiazoles can be conveniently prepared by the condensation of substituted anilines with ammonium thiocyanate, often followed by oxidative cyclization. This route offers a straightforward and versatile approach to introduce structural diversity by varying substituents on the aniline precursor. The resulting derivatives can then be purified and structurally characterized using

spectroscopic and analytical methods, to confirm the successful construction of heterocyclic system[2].

In addition to experimental evaluation, molecular docking studies have emerged as powerful computational tools in modern drug design. Docking not only predicts the binding affinity of small molecules toward biological targets but also provides insights into their orientation, key hydrogen-bond interactions, hydrophobic contacts, and potential mechanisms of action. For benzothiazole derivatives, docking against microbial enzymes such as lanosterol-14α-demethylase (CYP51), DNA gyrase, dihydrofolate reductase, enoyl-ACP reductase, or fungal cytochrome P450 enzymes can help rationalize observed antimicrobial activities and guide further optimization. Such integrated approaches bridge experimental microbiological data with computational predictions, offering a more complete understanding of molecular mechanisms.

In this framework, the present study was undertaken with objectives to synthesize and characterize a new series of benzothiazole derivatives, followed by to evaluate their antimicrobial potential and computational docking studies. To complement biological screening, docking simulations were executed to investigate the binding connections and the most active combinations with key microbial enzyme targets. This combined synthetic, biological, and computational study is expected to provide valuable insights into the structural features of benzothiazole derivatives that govern their antimicrobial activity, thereby contributing to the design of more potent and selective therapeutic agents[3].

MATERIALS AND METHODS

Chemistry

A series of benzothiazole derivatives was successfully designed and synthesized through a multi-step synthetic pathway starting from appropriately substituted aromatic precursors as shown in reaction scheme **Figure 1**. The synthetic route involved key steps such as cyclization, acylation, and nucleophilic substitution, yielding the desired target compounds in moderate to good yields.[9] Purity and identity of all prepared derivatives were checked by thin layer chromatography (TLC) and further characterized by spectral practices, including mass spectrometry, ^{1}H-NMR, ^{13}C-NMR and IR[4].

All chemicals including reagents as well as solvents used without further purification in this were procured from commercial suppliers and of analytical grade. The silica gel plates (Merck, 60 F254) were used to monitor the development of reactions, routinely by TLC with visualization of spots achieved under iodine vapours. The final benzothiazole derivatives were purified by recrystallization. Melting points of the synthesized compounds were determined using the open capillary method and are reported without correction[5].

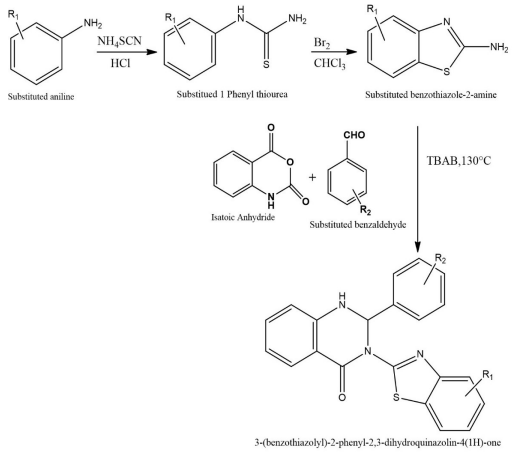

Figure 1. Reaction scheme.

General Method of Preparation of Substituted 2-Aminobenzothiazole

Synthesis of substituted 1-phenylthiourea from aniline

To aniline derivatives (1 eq.), conc. HCl (1 eq.) was mixed slowly and mixture was heated for approximately 30 min. A freshly prepared saturated mixture of ammonium thiocyanate in water (1.5 eq.) was slowly poured in above mixture. The concentrate was heated till the mixture becomes turbid. This muddled solution was then transferred in ice water. Subsequent precipitate was then filtered as well as re-crystallized with 80% ethanol[6].

Synthesis of substituted benzothiazol-2-amine from substituted 1-phenylthiourea

Substituted phenylthiourea in chloroform was bromated by means of 5% Br_2 mixture (1 eq.) in trichloromethane until the yellow-orange colour appeared. Slurry was reserved immediate for whole night. Precipitate gotten was washed and filtered with trichloromethane till the colour disappeared. The hydrobromide as ppt, was mixed and basified with rectified spirit and ammonia water solution. Precipitate was splashed with water and recrystallized by means of mixture dichloromethane:ethanol (2:1).

Synthesis of substituted 3-(benzothiazolyl)-2-phenyl-2,3-dihydroquinazolin-4(1H)-one from substituted benzothiazol-2-amine

A combination of isatoic anhydride (1.1 eq), substituted benzaldehyde (1.1 eq), and 2-aminobenzothiazole (1 eq) was successively added to a stoppered flat-bottomed flask holding a bead which was magnetic and the TBAB, ionic liquid and then, was boiled at approximately 130°C in a previously heated oil bath for not less than 30 minutes. After that, the reaction complex was mixed with ice water as required (2×100 ml), then splashed

with water and the solid remainder was crystallized with the help of ethanol[7].

Molecular Docking Study of Synthesized Compounds

Molecular docking study was performed against the target, Lanosterol 14α-demethylase (CYP51) using AutoDock Vina 1.5.7 tools to gain insight into the binding interactions of the synthesized benzothiazole compounds at the target using itraconazole as standard. The three-dimensional crystal structure of the receptor protein (PDB ID: 4LXJ) was obtained from Protein Data Bank (PDB). Preceding to docking, the protein assembly was pre-processed by deleting all crystallographic water particles. Also, heteroatoms/co-crystallized ligands were deleted. Hydrogens atoms were added by selecting "Polar Only". Also, Kollman charges were added to protein. Then receptor was saved in write PDBQT in format 4LXJ.pdbqt. After that ligand was pre-processed by minimizing MM2 minimize in ChemDraw and saved as. mol/.sdf which was further converted to .pdb format in Open Babel. Then in AutoDock Tools, Ligand was in input section was opened. Gasteiger charges were added, set detect root and set torsions to by choosing "All Active". In Output section file was saved as PDBQT format. The above steps were followed by all the derivatives and standard drug. After that, Grid box setup was done by identifying binding site. Then AutoDock Vina was run command line was given. Lastly analysis and validation were done to see 2D as well as 3D interaction diagram with the help of Discovery Studio[8,9,10].

Evaluation of Antimicrobial Activities of Synthesized Compounds

Antibacterial activity of Synthesized Compounds

Determination of MIC (minimum inhibitory concentration) of synthesized benzothiazole compounds was done using 2-fold serial dilution in 96-well plate against bacterial strains Gram+ve (*S. aureus* ATCC 29213) and Gram-ve (*E. coli* ATCC 25922, *K. pneumoniae* BAA 1705, *A. baumannii* BAA 1605 and *P. aeruginosa* ATCC 27853) by broth microdilution (Clinical Laboratory Stantards Institute, CLSI M07-A11) method. Sterile water was added approximately 200 µL to outer wells to prevent evaporation. Media was prepared by dispensing 100 µL cation-adjusted Mueller Hinton Broth (MHB) into B3–G11. Then, drug dilution was done by adding 100 µL test compound at 256 µg/mL to col 3. Serial dilution was done through 2-fold across 3→11. Discard 100 µL from column 11. Final range was maintained between 128 → 0.125 µg/mL after inoculum. Inoculum was prepared and adjusted with bacteria to 0.5 McFarland, diluted it in ratio of 1:100 in MHB. It was added with 100 µL/well. Finally, it was maintained to 5×10^5 CFU/mL, 200 µL/well. Control was prepared such as B2 column which gives growth control (no drug) while G12 was for sterility control (no bacteria). Levofloxacin was used as standard. Plates were incubated at 35±2°C and for 16–20 h for all the bacterial strains. MIC (lowest concentration with no visible growth) was checked through visualization by adding 20 µL resazurin for 1–2 h. Blue colour indicated no growth[11,12,13,14].

Antifungal activity of Synthesized Compounds

Determination of MIC (minimum inhibitory concentration) of synthesized benzothiazole compounds was done using 2-fold serial dilution in 96-well plate against fungal strains (Candida albicans ATCC 90028, Candida parapsilosis ATCC 22019, and Candida tropicalis ATCC 750) by broth microdilution (Clinical Laboratory Stantards Institute, CLSI M27-A4) method. Sterile water was added approximately 200 µL to outer wells to prevent evaporation. Media was prepared by dispensing 100 µL RPMI 1640 with L-glutamine, buffer with 0.165M MOPS (pH 7), filter and sterilize into B3–G11. Then, drug dilution was done by adding 100 µL test compound at 128 µg/mL to B3-G3. Serial dilution was done through 2-fold across 3→11. Discard 100 µL from column 11. Final range was maintained between 64 → 0.06 µg/mL after inoculum. Inoculum was prepared and adjusted with bacteria to 0.5 McFarland, diluted it in ratio of 1:1000 in RPMI-MOPS. It was added with 100 µL/well. Finally, it was maintained to 0.5-2.5×10^3 CFU/mL, 200 µL/well. Control was prepared such as B2 column which gives growth control (no drug) while G12 was for sterility control (no bacteria). Amphotericin-B and fluconazole were used as standard. Plates were incubated at 35±2°C and for 24 h for *C. albicans* while 48 h for *C. parapsilosis*. MIC (lowest concentration with no visible growth) was checked through visualization or OD530. MIC for fluconazole means showing ≥50% inhibition vs control while MIC for amphotericin-B means showing ≥90% inhibition vs control[11,15,16].

RESULTS AND DISCUSSION

In the present investigation, eight benzothiazole derivatives were synthesized. The intermediates required for the preparation of these derivatives were obtained following previously reported procedures. The final derivatives (RKN1-RKN8) were synthesized via condensation reactions. Thin-layer chromatography (TLC) analysis of each compound, using n-hexane: ethyl acetate as the mobile phase, yielded a single, well-defined spot without tailing, confirming their purity. All synthesized benzothiazole compounds were further characterized and confirmed by their physical, spectral, and analytical data.

Spectral Analysis of Synthesized Compounds

3-(6-Chlorobenzothiazol-2-yl)-2-(2-hydroxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (RKN-1)

Yield: 85%; MP: 137-139°C; Solubility: DMSO, $CDCl_3$; R_f value (ethyl acetate/n-hexane::0.5:1): 0.55; FTIR (*v*, ATR, cm^{-1}): 1253.52 (C=N), 1646.43 (C=O), 3350.62 (N-H); ^1H-NMR δ (ppm, 500 MHz, $CDCl_3$) = 3.848 (3H, *s*, -OCH_3), 5.193-5.201 (1*H*, *s*, N-H), 6.784-6.800 (1*H*, *d*, C-H), 6.896-6.926 (1*H*, *t*, Ar-H), 6.984-7.006 (1*H*, *dd*, Ar-H), 7.143-7.286 (5*H*, *m*, Ar-H), 7.346-7.379 (1*H*, *t*, Ar-H), 7.584-7.593 (1*H*, *d*, Ar-H), 7.623-7.640 (1*H*, *d*, Ar-H), 7.977-7.992 (1*H*, *d*, Ar-H); ^{13}C-NMR δ (ppm, 125 MHz, $CDCl_3$): 56.08 (-OCH_3), 68.37, 104.10, 115.44, 116.72, 120.88, 122.15, 127.82, 129.02, 129.53, 134.82, 135.43, 138.08, 142.58, 145.05, 156.29, 157.18 (C=O), 161.71(C = N); ESI-MS (*m/z*): 422 $[M+H]^+$.

3-(6-Methoxybenzothiazol-2-yl)-2-(p-tolyl)-2,3-dihydroquinazolin-4(1H)-one (RKN2)

Yield: 84%; MP: 117-119°C; Solubility: $CDCl_3$, R_f value (ethyl acetate/n-hexane::0.5:1): 0.60; FTIR (*v*, ATR, cm^{-1}): 1253.64 (N=H), 1646.13 (C=O), 3352.77 (N-H); ^{1}H-NMR δ (ppm, 500 MHz, DMSO): 2.200 (3*H*, *s*, -CH_3), 3.843 (3*H*, *s*, -OCH_3), 5.179-5.186 (1*H*, *d*, -NH) 6.739-6.755 (1*H*, *d*, C-H), 6.857-7.887 (1*H*, *t*, Ar-H), 6.970-6.995 (3*H*, *m*, Ar-H), 7.195-7.212 (2*H*, *d*, Ar-H), 7.278-7.282 (1*H*, *d*, Ar-H), 7.313-7.346 (1*H*, *t*, Ar-H), 7.576-7.584 (1*H*, *d*, Ar-H), 7.630-7.638 (1*H*, *d*, Ar-H), 7.976-7.991 (1*H*, *d*, Ar-H); ^{13}C-NMR δ (ppm, 125 MHz, $CDCl_3$): 21.22 (-CH_3), 56.07 (-OCH_3), 68.94 (-CH), 104.05, 115.30, 116.31, 116.46, 120.39, 122.15, 126.21, 129.46, 129.51, 134.86, 135.24, 136.58, 138.52, 142.72, 145.51, 156.41, 157.06 (C=O), 161.97 (C=N); ESI-MS (*m/z*): 402.1 $[M+H]^+$.

3-(6-Methoxybenzothiazol-2-yl)-2-(4-methoxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (RKN3)

Yield: 83%; MP: 118-120°C; Solubility: DMSO, $CDCl_3$; R_f value (ethyl acetate/n-hexane::0.5:1): 0.40; FTIR (*v*, ATR, cm^{-1}): 1252.21 (N=H), 1647.56 (C=O), 3352.98 (N-H); ^{1}H-NMR δ (ppm, 500 MHz, DMSO): 3.655 (3*H*, *s*, -OCH_3), 3.840 (3*H*, *s*, -OCH_3), 5.213-5.220 (1*H*, *d*, -NH) 6.683-6.701 (2*H*, *d*, Ar -H), 6.743-6.759 (1*H*, *d*, C-H), 6.859-6.889 (1*H*, *t*, Ar-H), 6.973-6.996 (1*H*, *dd*, Ar-H,), 7.230-7.7.278 (3*H*, *m*, Ar-H), 7.316-7.350 (1*H*, *t*, Ar-H), 7.563-7.571 (1*H*, *d*, Ar-H), 7.629-7.647 (1*H*, *d*, Ar-H) 7.978-7.994 (1*H*, *d*, Ar-H); ^{13}C-NMR δ (ppm, 125 MHz, $CDCl_3$): 55.37 (-OCH_3), 55.78 (-OCH_3), 68.72 (-CH), 104.04, 114.12, 114.98, 115.30, 116.31, 120.31, 122.12, 127.60, 129.41, 131.66, 134.58, 135.27, 142.69, 145.55, 156.40, 157.05, 159.79 (C=O), 161.97 (C=N); MS (ESI^+) m/z: 418.0 $[M+H]^+$.

2-(2,4-Dichlorophenyl)-3-(6-methoxybenzothiazol-2-yl)-2,3-dihydroquinazolin-4-(1H)-one (RKN4)

Yield: 88%; MP: 138-140°C; Solubility: DMSO; R_f value (ethyl acetate/*n*-hexane::0.5:1)- 0.50; FTIR (*v*, ATR, cm^{-1}): 1245.78 (C=N), 1650.72 (C=O), 3405.78 (N-H); ^{1}H-NMR δ (ppm, 500 MHz, DMSO): 3.790 (3*H*, *s*, -OCH_3), 6.832 (1*H*, *s*, C-H), 6.846-6.86 (1*H*, *d*, Ar-H), 6.989-7.011 (1*H*, *dd*, Ar-H), 7.037-7.054 (1*H*, *d*, Ar-H,), 7.261-7.281 (1*H*, *dd*, Ar-H), 7.369-7.400 (1*H*, *t*, Ar-H), 7.581-7.587 (1*H*, *d*, N-H), 7.593-7.598 (2*H*, *m*, Ar-H), 7.710-7.714 (1*H*, *s*, Ar-H), 7.882-7.896 (1*H*, *d*, Ar-H), 8.070-8.078 (1*H*, *s*, Ar-H); ^{13}C-NMR δ (ppm, 125 MHz, $CDCl_3$): 56.05 (-OCH_3), 67.21 (-CH), 103.96, 114.86, 115.40, 115.55, 120.33, 122.51, 127.57, 127.61, 129.33, 130.56, 132.92, 134.86, 135.02, 135.37, 135.59, 142.55, 144.87, 155.15, 157.20 (C=O), 162.22 (C=N); MS (ESI^+) *m/z*: 456.0 $[M^+]$, 456.9 $[M^+$+1], 457.9 $[M^+$+2], 458.9 $[M^+$+3].

2-(4-Chlorophenyl)-3-(6-methylbenzothiazol-2-yl)-2,3-dihydroquinazolin-4(1H)-one (RKN5)

Yield: 85%; MP: 136-138°C; Solubility: DMSO; R_f value (ethyl acetate/*n*-hexane::0.5:1): 0.60; FTIR (*v*, ATR, cm^{-1}): 1250.26 (C=N), 1640.41 (C=O), 3353.56 (N-H); ^{1}H-NMR δ (ppm, 500 MHz , $CDCl_3$): 2.475 (3*H*, *s*, -CH_3), 6.011 (1*H*, *s*, C-H), 6.884-6.930 (2*H*, *m*, Ar-H, NH), 7.159-7.173 (2*H*, *d*, Ar-H), 7.223-7.239 (1*H*, *d*, Ar-H), 7.321-7.385 (3*H*, *m*, Ar-H), 7.641-7.667 (3*H*, *d*, Ar-H), 7.980-7.995 (1*H*, *d*, Ar-H); ^{13}C-NMR δ (ppm, 125 MHz, $CDCl_3$): 21.45 (-CH_3), 68.04 (-CH), 115.86, 116.43, 119.98, 120.80, 120.95, 127.48, 127.75, 128.64, 129.15, 133.48, 134.03, 134.12, 135.15, 137.97, 145.51, 146.09, 157.29 (C=O), 161.85 (C=N); MS (ESI^+) *m/z*: 406 $[M^+]$, 407 $[M^+$+1], 408 $[M^+$+2], 409 $[M^+$+3].

3-(6-Methylbenzothiazol-2-yl)-2-(p-tolyl)-2,3-dihydroquinazolin-4(1H)-one (RKN6)

Yield: 81%; MP: 116-118°C; Solubility: DMSO; R_f value (ethyl acetate/*n*-hexane::0.5:1): 0.40; FTIR (*v*, ATR, cm^{-1}): 1244.41 (C=N), 1626.09 (C=O), 3344.63 (N-H); ^{1}H-NMR δ (ppm, 500 MHz, DMSO): 2.219 (3*H*, *s*, -CH_3), 2.464 (3*H*, *s*, -CH_3), 5.245-5.252 (1*H*, *d*, -NH), 6.766-6.782 (1*H*, *d*, C-H), 6.877-6.907 (1*H*, *t*, Ar-H), 6.995-7.011 (2*H*, *d*, Ar-H), 7.217-7.253 (3*H*, *m*, Ar-H,), 7.335-7.366 (1*H*, *t*, Ar-H), 7.629-7.658 (3*H*, *m*, Ar-H), 8.000-8.015 (1*H*, *d*, Ar-H); ^{13}C-NMR δ (ppm, 125 MHz, $CDCl_3$): 21.50 (-CH_3), 22.67 (-CH_3), 68.94 (-CH), 116.14, 116.29, 117.15, 120.19, 120.89, 120.96, 126.03, 127.43, 129.30, 129.88, 133.59, 133.93, 135.08, 136.36, 138.30, 145.35, 146.20, 157.39 (C=O), 161.88 (C=N); MS (ESI^+) *m/z*: 386.1 $[M^+]$, 387.1 $[M^+$+1], 388.1 $[M^+$+2], 389.1 $[M^+$+3].

2-(4-Methoxyphenyl)-3-(6-methylbenzothiazol-2-yl)-2,3-dihydroquinazolin-4(1H)-one (RKN7)

Yield: 82%; MP: 119-121°C; Solubility: DMSO; R_f value (ethyl acetate/*n*-hexane::0.5:1): 0.50; FTIR (*v*, ATR, cm^{-1}): 1250.26 (C=N), 1640.43 (C=O), 3353.56 (N-H); ^{1}H-NMR δ (ppm, 500 MHz, DMSO): 2.465 (3*H*, *s*, -CH_3), 3.678 (3*H*, *s*, -OCH_3),

5.239-5.247 (1*H*, *d*, -NH) 6.703-6.721 (2*H*, *dd*, C-H, Ar-H), 6.772-7.788 (1*H*, *d*, Ar-H), 6.883-6.913 (1*H*, *t*, Ar-H), 7.212-7.228 (1*H*, *d*, Ar-H), 7.254-7.279 (2*H*, *m*, Ar-H), 7.344-7.374 (1*H*, *t*, Ar- H), 7.628-7.670 (3*H*, *m*, Ar-H), 8.003-8.019 (1*H*, *d*, Ar-H); ^{13}C-NMR δ (ppm, 125 MHz, $CDCl_3$): 21.46 (-CH_3), 55.16 (-OCH_3), 68.49 (-CH), 113.91, 116.11, 116.24, 120.16, 120.84, 120.93, 127.40, 129.28, 131.41, 131.98, 133.54, 133.92, 135.07, 145.32, 146.16, 157.36, 159.58 (C=O), 161.80 (C=N); MS (ESI^+) *m/z*: 402.1 [M^+], 403.1 [M^++1], 404.0 [M^++2], 405.1 [M^++3].

2-(2,4-Dichlorophenyl)-3-(6-methylbenzothiazol-2-yl)-2,3-dihydroquinazolin-4(1H)-one (RKN8)

Yield: 86%; MP: 141-143°C; Solubility: DMSO; R_f value (ethyl acetate/*n*-hexane::0.5:1): 0.60; FTIR (*v*, ATR, cm^{-1}): 1239.86 (C=N), 1616.53 (C=O), 3403.46 (N-H); ^{1}H-NMR δ (ppm, 500 MHz, DMSO): 2.448 (3*H*, *s*, -CH_3), 5.570-5.575 (1*H*, *s*, N-H), 6.633-6.649 (1*H*, *d*, C-H), 6.882-6.912 (1*H*, *t*, Ar-H), 6.967-7.003 (2*H*, *m*, Ar-H), 7.181-7.197 (1*H*, *d*, Ar-H), 7.309-7.342 (1*H*, *t*, Ar-H), 7.421-7.424 (1*H*, *d*, Ar-H), 7.603-7.618 (2*H*, *m*, Ar-H), 7.749-7.756 (1*H*, *d*, Ar-H), 8.052-8.068 (1*H*, *d*, Ar-H); ^{13}C-NMR δ (ppm, 125 MHz, $CDCl_3$): 21.46 (-CH_3), 68.99 (-CH), 114.60, 115.32, 120.07, 120.84, 121.18, 127.30, 127.38, 127.48, 129.13, 130.31, 132.69, 133.52, 134.15, 134.80, 135.11, 135.38, 144.66, 145.98, 156.08 (C=O), 162.07 (C=N); MS (ESI^+) *m/z*: 440.0 [M^+], 440.9 [M^++1], 442.0 [M^++2], 443.0 [M^++3], 444.0 [M^++4].

Molecular Docking Study of Synthesized Compounds

The molecular docking results of the synthesized compounds are represented in **Table 1**. Their 3D and 2D interactions with lanosterol 14α-demethylase (CYP51) are displayed in **Figure 2**. RKN8 shows the best binding affinity against the target protein as compared to the other synthesized molecules viz, RKN1, RKN2, RKN3, RKN4, RKN5, RKN6 and RKN7. In the above table the standard drug Itraconazole shows the binding affinity of -9.8 kcal/mol. The synthesized molecules RKN4, RKN7 and RKN8 shows good binding affinity in comparison with the standard well known drug i.e. itraconazole. The binding affinity of RKN8 was -10.7 kcal/mol with binding sites of amino acid with ligand interactions THR507- Conventional H_2 bond and π-Sigma, PRO238- π-Alkyl, VAL242- π-Alkyl, LEU96- π-Sigma, ALA69- π-Alkyl, PHE243- π-Alkyl. The binding affinity of RKN7 was -10.6 kcal/mol with binding sites of amino acids with interaction MET509- π-Sulphur, PRO238- π-Alkyl, HIS381- π- π Stacked, LEU380- Alkyl, PHE241- π-Sulphur, π- π Stacked and Alkyl, PHE384- Alkyl and π- π Stacked, LEU95- π-Alkyl. The binding affinity of RKN4 was -10.5 kcal/mol with binding sites of amino acids THR507, PRO238, TYR72, VAL242, LEU96, PHE243[9].

Table 1. Docking scores and key interactions of synthesized benzothiazole compounds with the target lanosterol 14α-demethylase (CYP51).

Compound	Binding Energy (kcal/mol)	Residues Involved with Key Interactions
RKN1	–8.9	PRO238- Alkyl, MET509- π-Sulphur, LEU380- Alkyl, PHE241- π-Sulphur and π- π T Shaped, HIS381- π- π Stacked, SER382- Alkyl, LEU95-Alkyl, PHE384- π- π Stacked, LEU383- Alkyl
RKN2	–8.5	THR507- Conventional H-bond, PRO238- π-Alkyl, VAL242- π-Alkyl, LEU96- π-Sigma and Alkyl, ALA69- π -alkyl, VAL66- Alkyl
RKN3	–8.6	PRO238- π-Alkyl and Alkyl, ALA69- π- Alkyl, VAL70- π-Alkyl, HIS381- π-Alkyl, PHE384- π-Alkyl, THR507- C-H Bond
RKN4	–10.5	THR507- Conventional H-Bond, PRO238- π-Alkyl, TYR72- π-π T Shaped, VAL242- π-Sigma, LEU96- π-Akyl, PHE243- π- Alkyl
RKN5	–9.2	LEU95- Alkyl, PHE241- π-Sulphur, π- π T Shaped, π Stacked, LEU380- π-Alkyl, PRO238- Alkyl, PHE384- π- π Stacked, HIS381- π-π Stacked, MET509- π-Sulphur
RKN6	–9.3	TYR227- Alkyl, ALA226- π-Alkyl, ILE205- π sigma, Alkyl
RKN7	–10.6	MET509- π Sulphur, PRO238-

		π- Alkyl, HIS381- π-πStacked, LEU380- Alkyl, PHE241- π-Sulphur, π- π Stacked and Alkyl, PHE384- Alkyl and π- π Stacked, LEU95- π Alkyl
RKN8	–10.7	THR507- Conventional H bond and π Sigma, PRO238- π Alkyl, VAL242- π Alkyl, LEU96- π Sigma, ALA69- π Alkyl, PHE243- π Alkyl
Itraconazole (Standard)	–9.8	ILE471 Conventional H bond, Alkyl, LEU158 π- π Stacked, VAL311 Alkyl π, TYR140 Alkyl , SER382 π-π Stacked , LEU380 π-Alkyl, TYR126 Alkyl

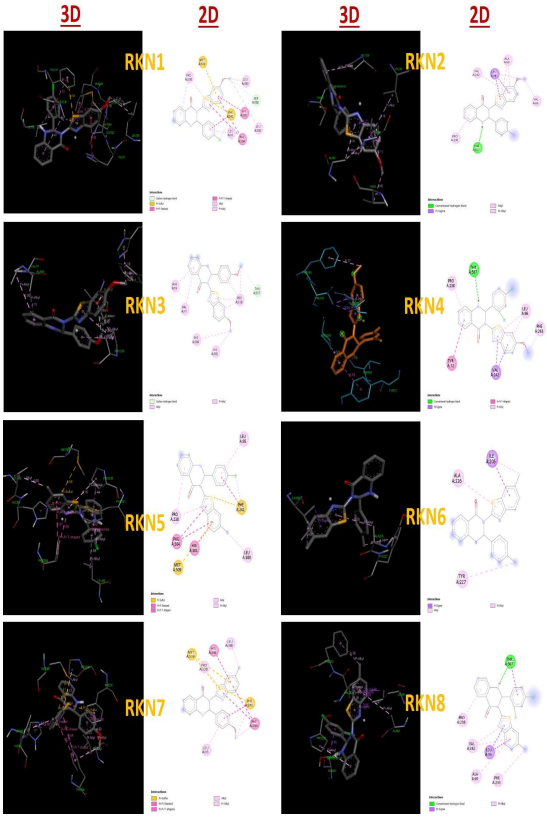

Figure 2. 3D and 2D interactions of the synthesized benzothiazole compounds with the target lanosterol 14α-demethylase.

Antimicrobial Activity of Synthesized Compounds

Antimicrobial activity of synthesized benzothiazole compounds are depicted in **table 2** and **table 3**. The examination of antibacterial and antifungal testing stats exposed that all the tested compounds exhibited significantly high inhibition at 12.5-200 µg/mL. The analogues RKN4 and RKN8 exhibited better activity contrary to all the fungal as well as bacterial strains. The better activity is accredited to the occurrence of pharmacological active 2,4-dichloro group that is attached to phenyl residues at position 2 of quinazoline-4-one. Other analogues exhibited modest activity related to standard levofloxacin, fluconazole and amphotericin-B against all the fungal and bacterial strains. Thus, RKN4 and RKN8 are acting as lead compounds as antibacterial agents due to having same MIC profile. RKN4 and RKN8 are also the lead compounds as antifungal agents due to both beating fluconazole on two fungal strains *Candida albicans* and *Candida tropicalis*. RKN4 is 2 times more potent than amphotericin-B against the fungal strain *Candida albicans*.

Table 2. Antibacterial activity of synthesized benzothiazole compounds as MIC value.

Sl. No.	**Compound(s)**	**Solubility**	**MIC value (µg/mL)**				
			E. coli	***S. aureus***	***K. pneumoniae***	***A. baumannii***	***P. aeruginosa***
1	RKN 1	DMSO	100	12.5	25	25	50
2	RKN 2	DMSO	100	50	50	50	25
3	RKN 3	DMSO	200	100	50	50	50
4	RKN 4	DMSO	25	25	12.5	12.5	12.5
5	RKN 5	DMSO	100	50	50	25	25
6	RKN 6	DMSO	150	50	100	50	50
7	RKN 7	DMSO	100	50	50	25	50
8	RKN 8	DMSO	25	25	12.5	12.5	12.5
9	Levofloxacin	DMSO	12.5	12.5	12.5	25	12.5

Table 3. Antifungal activity of synthesized benzothiazole compounds as MIC value.

Sl. No.	Compound(s)	MIC value (µg/mL)		
		Candida albicans	***Candida parapsilosis***	***Candida tropicalis***
1	RKN1	0.5	2	0.1
2	RKN2	2	8	4
3	RKN3	2	4	2
4	RKN4	0.125	1	0.25
5	RKN5	0.5	1	0.5
6	RKN6	2	4	2
7	RKN7	0.5	2	2
8	RKN8	0.25	0.5	0.25
9	Amphotericin-B	0.5	0.016	0.125
10	Fluconazole	0.25	2	1

The docking results showed a strong correlation with the observed *in vivo* antimicrobial activity, supporting the hypothesis that the receptor protein channel, lanosterol 14α-demethylase serves as a likely molecular target for the synthesized benzothiazole compounds. Compounds exhibiting higher binding affinities, such as RKN8 also demonstrated enhanced antimicrobial effects, indicating that effective receptor binding contributes to their pharmacological activity. The detailed docking poses and ligand-receptor interaction maps provide valuable structural insights that can guide future lead optimization and structure-based drug design efforts aimed at improving potency and selectivity[10].

CONCLUSION

The molecules RKN4, RKN7 and RKN8 possess good binding affinity in comparison with the standard, itraconazole against lanosterol 14α-demethylase. RKN8 showed the best binding affinity. All the synthesized derivatives possess significant antimicrobial activity comparable to the standard drug levofloxacin, fluconazole and amphotericin-B. The analogues RKN4 and RKN8 exhibited better activity contrary to all the fungal as well as bacterial strains. The better activity is accredited to the occurrence of 2,4-dichloro group attached to phenyl residues at position 2 of quinazoline-4-one in the derivatives. Thus, RKN4 and RKN8 may be the lead compounds as antimicrobial agents. These studies provide a strong foundation for further structural optimization and development of benzothiazole-based agents as potential candidates in antimicrobial drug discovery.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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