

In-silico Designing, Synthesis, SAR and Microbiological Evaluation of Novel Amide Derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic Acid

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ABSTRACT

Aim/Background: Due to this increasing problem of antibiotic resistance, the number of different antibiotics available is dwindling and there are only a handful of new antibiotics in the drug development pipeline. Therefore, there is an urgent need for the development of new antimicrobial drugs. In the present study, we have synthesized and investigated antimicrobial activities of novel derivatives with molecular docking studies. **Methods:** In this article, amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid were synthesized by focusing the *aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase enzymes* and inhibition by docking study leads to antimicrobial action. Studies were carried out on a designed amide library of derivatives with the help of Schrodinger's maestro package and AutoDock Vina 4.2 of molecular docking software against crystal structure of enzymes (PDB ID: 1wny.PDBQT and 1jil.PDBQT). Best 23 focused amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid were selected based upon their dock score for synthesis and further investigated for *in vitro* antimicrobial. **Results:** *In vitro* results revealed that a large number of synthesized compounds were found to possess excellent antimicrobial. Among the library, best compound B-1, B-7 and B-23 possessing excellent dock score by AutoDock Vina/Schrodinger's maestro against 1wny.PDBQT:1jil.PDBQT is -10.2:-10.1/ -5.26:-5.456, -10.3:-10.0/-5.318:-5.231, -10.0:-10.5/-5.609:-5.876 and as compared to standard drugs dock score -10.2:-10.6/-5.847:-5.895, -9.8:-9.9/ -6.323:-6.13. Derivatives B-1, B-7 and B-23 also exhibited good MIC as compared to standard by tube dilution method. **Conclusion:** *In-silico* studies played an important role in designing the potent ligands against *aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase enzymes* as well as explained the binding pattern of designed and synthesized ligand within active pocket and good antimicrobial compounds can be used for *in vivo* studies for future.

Key words: Benzimidazole, Molecular Docking, 1wny.PDBQT, 1jil.PDBQT, Antimicrobial Activity.

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INTRODUCTION

At present lot of drugs are available for healing the microbial infection but most of them is becoming fruitless due to antimicrobial resistance by the microorganisms. So, there is a great need for the discovery of

new antimicrobial agents to overcome the antimicrobial resistance and side effects.¹ The *aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase enzymes* have been a focus of recent research for antibacterial drug discovery. These enzymes play crucial



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roles in protein biosynthesis by catalyzing the synthesis aminoacylation of transfer RNA (tRNA) by their cognate amino acid. Once these enzymes are inhibited, protein biosynthesis is halted, which in turn results in the attenuation of bacterial growth under both *in vitro* and infectious conditions.^{2,3} Docking softwares are very useful for the screening of thousands of molecules affinity towards a particular disease or drug target.⁴

The pharmacology and chemistry of benzimidazole have been meaningful to medicinal chemistry because possessed various pharmacological activities i.e. antimicrobial,⁵ antioxidant,⁵ anticancer,⁶ anti-inflammatory,⁷ analgesic,⁷ antidiabetic,⁸ anticonvulsant,⁸ anti-asthmatic,⁹ antiviral,¹⁰ antihypertensive,¹¹ antiproliferative,¹² antitubercular¹³ with many more.¹⁴ Benzimidazole moiety have been showing talented/flourishing activity in the treatment of several diseases and due to that reason benzimidazoles achieved much thoughtfulness as momentous pharmacophore and called as privileged structure in medicinal chemistry.¹⁵ By looking review contains the different pharmacological activities of benzimidazole and its derivatives, focused amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid were taken for lead development.

In last decade, the virtual screening approach for studying the docking of small molecules with protein structure is a powerful tool for lead design and has become the most active and useful technology now being used in drug discovery processing. It is an attempt to remove the element of luck from the drug designs, because pharmacological activity is dependent upon drug receptor binding which was analyzed by molecular docking.¹⁶ In the present study, we designed the focused amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid were tested for docking against *aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase* enzymes have been a focus of recent research for bacterial inhibitory activity and were used to study the binding modes and binding affinities to the 1wny.PDBQT (*Isoleucyl-tRNA synthetase* editing domain, source: *Thermus thermophilus*) (Figure 1 and 2) and 1jil.PDBQT (*tyrosyl-tRNA synthetase* editing domain, source: *Staphylococcus aureus*) (Figure 3 and 4), by using software AutoDock Vina 4.2¹⁷ and Schrodinger Glide.^{18,19} The final screening of the focused amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid is calculated. Molecular docking helps in studying the interaction between the receptor/protein with ligand by identifying the suitable active sites in protein and by obtaining the best geometry of ligand-receptor com-

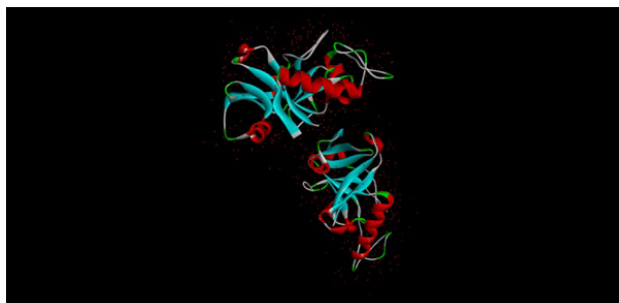


Figure 1: 1wny.PDBQT used for molecular docking (source: www.rcsb.org)

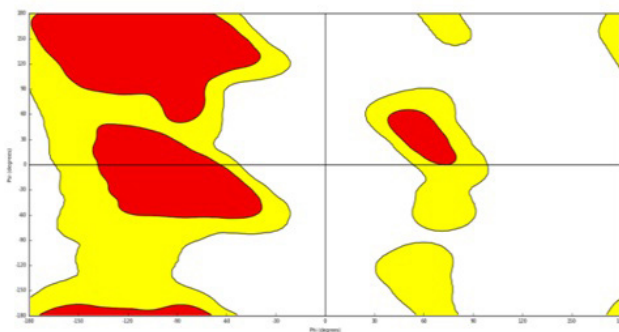


Figure 2: Ramchandran Plot used for optimization of targeted protein 1wny.PDBQT



Figure 3: 1jil.PDBQT used for molecular docking (source: www.rcsb.org)

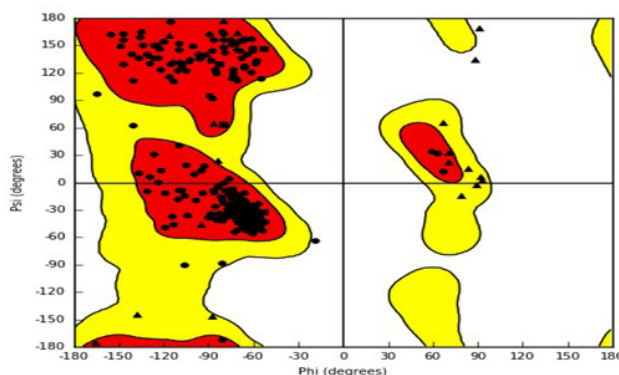


Figure 4: Ramchandran Plot used for optimization of targeted protein 1jil.PDBQT

plex and by calculating the energy of interaction for different ligands for design the new lead compounds for research.²⁰

MATERIAL AND METHODS

All the chemicals used in synthesis and antimicrobial activity were purchased from Himedia Laboratories and TM Media, Titan Biotech Ltd., Delhi. Cefadroxil, Ofloxacin and Fluconazole were obtained from CDH, Mumbai. All strains were acquired from MTCC, Chandigarh. Chemical reactions were maintained under observation by TLC on silica gel plate; spotting was examined in iodine and UV chambers, in mobile media Methanol: Ethyl Acetate: Hexane (1:4:5). Sonar melting point apparatus in open capillary tubes were used for the detection of melting points. ¹H NMR and ¹³C NMR spectra were confirmed in DMSO and deuterated CDCl₃ on Bruker Avance II 400 NMR spectrometer at a frequency of 400 MHz and 100 MHz downfield to tetramethyl silane standard. Coupling constants (J) and chemical shifts were reported in Hertz (Hz) and δ (parts per million). Waters Micromass Q-ToF Micro instrument was used for Mass spectrum were recorded. MIC were completed in BOD incubator (Haryana Scientific and Engg. Corporation Ltd., Rohtak) and Laminar Air Flow biosafety cabinet (Hicon, New Delhi) respectively.

Synthesis of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid outlined in Scheme 1 (Figure 5).^{21,22} Structural part of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid was in confirmed by Mass spectra,²³ ¹H NMR and ¹³CNMR²⁴ and elemental analysis which were found in full agreement with their structures. Detection of reaction completion was done by single spot TLC under UV lamp.²⁵

For the synthesis

1 mole of PEG 5000, 1.2 mole of 4-fluoro 3-nitro benzoic acid, 1.2 mole of DCC and a pinch of DMAP is dissolved in DCM and stirred for two days at room temperature. To the precipitated product add 1.1 mole of 3-nitrobenzenamine and kept for stirring for one day which is precipitated by ether. Recovered product is subjected to reduction by adding the mixture of 1 mole of NH₄Cl and 1 mole of Zn, in methanol and stirred for 3 hrs, centrifuge the reaction mixture and filter to remove Zn. Then 20 to 30 ml of DCM was added and again centrifuged to remove NH₄Cl. The product was treated with 1.2 moles 4-MBA, 1.2 moles of DCC, pinch of DMAP dissolved in the solvent DCM then stirred for one day. Product obtained was treated with TFA:EDC

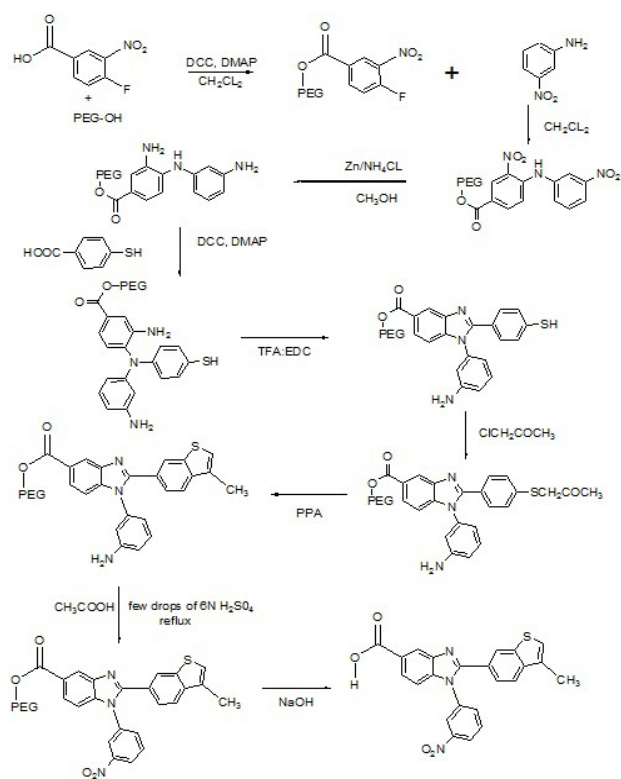


Figure 5: Scheme 1: Synthetic route for 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid.

in 1:10 ratio and stirred for one day. Product obtained was treated with 1.3 moles of chloroacetone and stirred for one day. Then product was treated with 1.4 moles of PPA and stirred for one day. Product obtained was treated with 1.2 moles tetra-n-propyl ammonium bromate in the 20 ml of glacial acetic acid and 5ml of 6N sulfuric acid and refluxed for 6 hrs and product was filtered with the help of water. Polymer was cleaved by treating the product obtained with 0.5g of sodium hydroxide by dissolving in methanol with stirring to get final product of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid.

NOTE: After each step product was recovered by precipitating with diethyl ether. Total 100 amide derivatives were designed using Chemdraw and docking studies were carried out, out of which best 23 compounds were selected based on docking score.

Amide library of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic outlined in Scheme 2 (Figure 6).²⁶ 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid (0.27 mmol, 1.0 equiv) was taken in the respective RBF. To each RBF, corresponding aromatic amines (1.2 equiv) (B-1 to B-23) (Table 2) was added followed by DMF (2 ml) and TEA (54 mg, 0.54

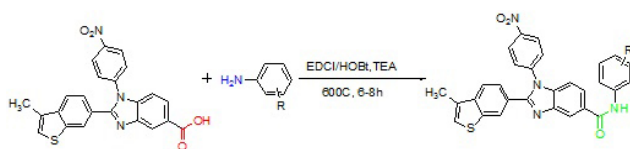


Figure 6: Scheme 2: Synthesis route for different amides of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid

mmol, 2.0 equiv), HOBt (0.54mmol, 2equiv) and EDCI (0.54mmol, 2 equiv). RBF were kept in magnetic stirrer with hot plate for 6-8 hr for heating at 60°C with stirring. After the reaction was over (TLC monitoring), 5-7 ml of an aq. Saturated solution of sodium hydrogen carbonate was added into each of the reaction tubes. The quenched reaction mixtures were either filtered by using filter paper. For liquid-liquid extraction, water (8-10 ml) was added into each of the reaction mixture and was extracted with organic solvent (8-10 ml). The combined organic layer was washed with water, separated using whatmann filter paper, dried over anhydrous sodium sulphate and concentrated using Rotary evaporator. Samples were purified by column chromatography (using an optimized mixture of Ethyl acetate:Hexane as an eluent).

Antimicrobial activity: Minimum inhibitory concentrations (MIC)

The tube dilution method was used for the determination of antimicrobial potential of the synthesized derivatives against *S. aureus* MTCC 3160, *B. subtilis* MTCC 441, *E. coli* MTCC 45, *T. thermophilus* MTCC 1494, *C. albicans* MTCC 183 and *A. niger* MTCC 282 strains. Dilutions of test and standard compounds were prepared in double strength nutrient broth I.P. (bacteria) or Sabouraud dextrose broth I.P. (fungi).^{27,28} The slants of *S. aureus* MTCC 3160, *B. subtilis* MTCC 441, *E. coli* MTCC 45 and *T. thermophilus* MTCC 1494, were incubated at the 30-35°C for 24h. The slants of *C. albicans* were incubated at 20-25°C for 48 hr whereas; the slants of *A. niger* were incubated at 20-25°C for 5 days. After the incubation period sterilized 0.9% NaCl solution was used to harvest the bacterial and fungal cultures from agar slant through proper shaking and then the suspensions of microorganisms were diluted with the sterile 0.9% NaCl solution to cfu count was adjusted by adjusting the density of microorganism suspension to that of 0.5 McFarland standards by adding distilled water. The number of cfu was determined by dilution pour-plate method.²⁹ The samples were incubated at 37°C for 24 hr (bacteria), at 25°C for 7 days (*A. niger*), and at 37°C for 48 hr (*C. albicans*) and the results were recorded.

In silico Molecular docking studies: Docking performed by Schrodinger Glide

The Schrodinger, Inc. (New York, USA) software was utilized to carry out the computational calculations and docking studies which was completed in the Laboratory for Enzyme Inhibition Studies, Department of Pharmaceutical Sciences and Natural Products, Central University of Punjab, Bathinda, INDIA. Software ensures that protein and ligands corrected form for docking, the receptor-grid files were generated by grid-receptor generation program Glide. Grid-based Ligand Docking uses a hierarchical sequence of filters to produce possible conformations of the ligand in the active-site region of the protein receptor. At this stage, crude score values and geometric filters were prepared out unlikely binding modes.³⁰ Docking Performed by Autodock Vina 4.2: By using prepared PDB ID grid performed molecular docking with. mol2-Tripos files by using Command prompt and after the collection of docking score then check the interaction/hydrogen bond distance between molecules with PDB ID grid with the help of Autodock Vina 4.2. Protein preparation: X-ray protein structure co-ordinates for *aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase* were downloaded from Protein Data Bank and were prepared with the help of the protein preparation wizard of Schrödinger (Prepwiz). PDB ID: 1wny having resolution: 1.6 Å and PDB ID: 1jil having resolution: 2.2 Å (selection criteria used was lowest resolution and availability) was utilized for the study. All the waters molecules (except those coordinated to metals) were removed except water present between the ligand and protein. The energy-restrained of the protein structure target site optimization of targeted protein *aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase* were made using OPLS-2005 as force field. Ligand preparation: The three-dimensional structural library was prepared using the Chemdraw software and then save as. mol2-Tripos file. The 3-D structures of the selected ligands were preceded for energy minimization using the LigPrep tool from Schrodinger to correct the coordinates, ionization, stereochemistry and tautomeric structure to gain the appropriate conformation through the addition or removal of hydrogen bonds. The ligands prepared after energy minimization were used for molecular docking studies.

RESULTS AND DISCUSSION

Spectral Data: B: 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid: MS: 430.0861 (M^{+}). ¹H NMR: δ 13.02 (s, 1H), 8.51 (s, 1H), 8.33 (s, 1H), 8.22 – 8.13 (m, 2H), 8.10 (d, $J = 9.4$

Hz, 1H), 8.05 (d, $J = 7.6$ Hz, 1H), 7.94 (d, $J = 9.6$ Hz, 2H), 7.85 (d, $J = 7.8$ Hz, 1H), 7.63 (t, $J = 7.4$ Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 167.55, 154.00, 147.11, 143.15, 139.67, 139.53, 137.58, 137.23, 130.89, 130.73, 130.02, 127.94, 127.29, 127.19, 126.69, 124.50, 124.32, 122.43, 121.87, 121.71, 121.32, 108.40, 14.17.

B-1: 2-(3-methylbenzo[b]thiophen-6-yl)-N-(naphthalen-1-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 555.1491 (M^+). ^1H NMR: δ 10.39 (s, 1H), 8.52 (s, 1H), 8.47 (s, 1H), 8.18 (d, $J = 14.5$ Hz, 3H), 8.06 (dd, $J = 15.0, 8.6$ Hz, 2H), 7.96 (d, $J = 9.4$ Hz, 1H), 7.89 – 7.82 (m, 2H), 7.69 (dt, $J = 15.2, 7.4$ Hz, 3H), 7.54 (t, $J = 7.4$ Hz, 2H), 7.29 (s, 2H), 7.24 (s, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 164.94, 154.00, 147.11, 143.01, 139.67, 139.53, 137.45, 137.23, 136.02, 134.44, 130.89, 130.73, 130.44, 130.02, 127.94, 127.29, 127.08, 126.93, 126.22, 125.80, 125.54, 125.12, 124.50, 124.41, 124.32, 123.86, 122.43, 121.87, 121.32, 119.86, 115.87, 109.11, 14.17.

B-2: N-(2,3-difluorophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 541.1101 (M^+). ^1H NMR: δ 10.94 (s, 1H), 8.49 (s, 1H), 8.44 (s, 1H), 8.18 (t, $J = 9.1$ Hz, 3H), 8.03 (q, $J = 8.7, 7.6$ Hz, 3H), 7.87 (d, $J = 8.6$ Hz, 2H), 7.71 (t, $J = 7.4$ Hz, 1H), 7.24 (s, 1H), 7.15 (q, $J = 7.4$ Hz, 1H), 6.97 (q, $J = 7.3$ Hz, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 165.61, 153.79, 144.47, 142.99, 142.22, 139.67, 139.53, 135.81, 130.89, 130.72, 130.51, 127.29, 125.19, 124.79, 124.50, 124.32, 123.86, 123.24, 123.16, 122.43, 119.86, 118.95, 118.87, 114.49, 114.29, 109.13, 15.14, 15.09, 14.17.

B-3: N-(2,4-dimethylphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 534.1636 (M^+). ^1H NMR: δ 9.35 (s, 1H), 8.34 – 8.26 (m, 2H), 8.19 (dt, $J = 7.2, 1.6$ Hz, 1H), 8.14 (d, $J = 1.7$ Hz, 1H), 8.06 (d, $J = 7.5$ Hz, 1H), 7.92 – 7.67 (m, 5H), 7.24 (t, $J = 0.7$ Hz, 1H), 7.19 (d, $J = 7.3$ Hz, 1H), 7.04 – 6.96 (m, 2H), 2.41 (s, 3H), 2.20 (d, $J = 5.4$ Hz, 6H). ^{13}C NMR: δ 164.94, 153.79, 144.47, 142.99, 142.22, 139.67, 139.53, 137.45, 135.81, 134.44, 130.89, 130.72, 130.44, 127.29, 127.08, 126.93, 126.22, 125.80, 125.54, 125.19, 125.12, 124.79, 124.50, 124.41, 124.32, 123.86, 122.43, 119.86, 115.87, 109.13, 14.17.

B-4: N-(2,4-dinitrophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 595.099 (M^+). ^1H NMR: δ 9.76 (s, 1H), 8.51 (s, 1H), 8.44 (s, 1H), 8.25 (s, 1H), 8.19 (d, $J = 8.2$ Hz, 1H), 8.08 (d, $J = 9.2$ Hz, 1H), 7.97 (t, $J = 7.6$ Hz, 2H), 7.89 (d, $J = 9.3$ Hz, 1H), 7.84 (s, 1H), 7.73 (d, $J = 7.7$ Hz, 1H), 7.64 (t, $J = 7.1$ Hz, 1H), 7.50 (d, $J = 7.7$ Hz, 1H), 7.24 (s, 1H), 7.08 (d, $J = 7.7$ Hz, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 164.42, 154.00, 147.11, 143.01, 140.32, 139.67, 139.53, 137.23, 136.92, 136.02, 135.25, 130.89,

130.73, 130.44, 130.14, 130.02, 127.94, 127.29, 124.50, 124.32, 123.86, 122.70, 122.43, 121.87, 121.32, 119.86, 119.72, 109.11, 14.17.

B-5: N-(2,5-dichlorophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 574.0447 (M^+). ^1H NMR: δ 9.76 (s, 1H), 8.51 (s, 1H), 8.44 (s, 1H), 8.25 (s, 1H), 8.19 (d, $J = 8.2$ Hz, 1H), 8.08 (d, $J = 9.2$ Hz, 1H), 7.97 (t, $J = 7.6$ Hz, 2H), 7.89 (d, $J = 9.3$ Hz, 1H), 7.84 (s, 1H), 7.73 (d, $J = 7.7$ Hz, 1H), 7.64 (t, $J = 7.1$ Hz, 1H), 7.50 (d, $J = 7.7$ Hz, 1H), 7.24 (s, 1H), 7.08 (d, $J = 7.7$ Hz, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 164.82, 154.00, 147.11, 143.01, 139.67, 139.53, 137.23, 136.02, 135.86, 131.84, 130.89, 130.80, 130.73, 130.44, 130.02, 127.94, 127.37, 127.29, 126.64, 125.18, 124.50, 124.32, 123.86, 122.43, 121.87, 121.32, 119.86, 109.11, 14.17.

B-6: N-(2,5-difluorophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 541.1101 (M^+). ^1H NMR: δ 10.72 (s, 1H), 8.51 (s, 1H), 8.44 (s, 1H), 8.25 (s, 1H), 8.19 (d, $J = 8.2$ Hz, 1H), 8.08 (d, $J = 9.3$ Hz, 1H), 7.96 (d, $J = 7.2$ Hz, 3H), 7.88 (d, $J = 9.4$ Hz, 1H), 7.73 (d, $J = 7.8$ Hz, 1H), 7.65 (d, $J = 7.3$ Hz, 1H), 7.30 (t, $J = 7.5$ Hz, 1H), 7.24 (s, 1H), 6.96 (d, $J = 8.6$ Hz, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 164.42, 155.26, 144.34, 143.96, 141.98, 140.32, 139.67, 139.53, 138.26, 136.92, 136.33, 135.25, 131.30, 130.89, 130.45, 130.15, 128.40, 127.71, 127.29, 124.49, 124.32, 123.81, 122.70, 122.42, 122.08, 119.78, 119.72, 109.57, 14.17.

B-7: N-mesityl-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 547.1759 (M^+). ^1H NMR: δ 9.48 (s, 1H), 8.34 – 8.26 (m, 2H), 8.19 (dt, $J = 7.2, 1.6$ Hz, 1H), 8.14 (d, $J = 1.7$ Hz, 1H), 8.10 – 8.03 (m, 1H), 7.92 – 7.67 (m, 5H), 7.24 (t, $J = 0.7$ Hz, 1H), 6.88 (s, 2H), 2.41 (s, 3H), 2.27 (s, 3H), 2.09 (s, 6H). ^{13}C NMR: δ 164.84, 155.26, 149.94, 144.42, 144.34, 143.96, 141.98, 139.67, 139.53, 138.26, 136.33, 131.30, 130.89, 130.45, 128.40, 127.92, 127.71, 127.29, 124.49, 124.32, 123.81, 122.42, 122.08, 119.78, 119.73, 115.38, 112.31, 109.57, 56.01, 34.53, 31.27, 14.17.

B-8: N-(2,6-difluorophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 541.1101 (M^+). ^1H NMR: δ 9.58 (s, 1H), 8.34 – 8.26 (m, 2H), 8.19 (dt, $J = 7.2, 1.6$ Hz, 1H), 8.14 (d, $J = 1.7$ Hz, 1H), 8.06 (d, $J = 7.4$ Hz, 1H), 7.92 – 7.67 (m, 5H), 7.26 – 7.15 (m, 2H), 6.97 (td, $J = 7.5, 1.0$ Hz, 2H), 2.41 (s, 3H). ^{13}C NMR: δ 167.04, 155.26, 144.34, 143.96, 141.98, 139.67, 139.53, 138.26, 136.33, 134.96, 133.66, 132.52, 131.30, 130.89, 130.50, 129.15, 128.40, 127.71, 127.29, 124.49, 124.32, 123.81, 122.42, 122.08, 119.78, 109.57, 20.74, 17.51, 14.17.

B-9: N-(2-bromo-4-methylphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 598.0497 (M^+). 1H NMR: δ 9.76 (s, 1H), 8.52 (s, 1H), 8.39 (s, 1H), 8.19 (d, J = 7.6 Hz, 2H), 8.08 (d, J = 9.4 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.96 (d, J = 5.9 Hz, 1H), 7.90 (d, J = 9.3 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.64 (t, J = 7.3 Hz, 1H), 7.46 (s, 1H), 7.25 (d, J = 9.2 Hz, 2H), 7.12 (d, J = 7.3 Hz, 1H), 2.41 (s, 3H), 2.28 (s, 3H). ^{13}C NMR: δ 164.83, 154.00, 147.11, 143.01, 139.67, 139.53, 137.23, 136.02, 135.73, 135.40, 132.61, 130.89, 130.73, 130.44, 130.02, 128.64, 127.94, 127.29, 124.50, 124.32, 123.86, 122.43, 122.26, 121.87, 121.32, 119.86, 114.48, 109.11, 20.65, 14.17.

B-10: N-(2-bromo-5-nitrophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 629.0192 (M^+). 1H NMR: δ 10.68 (s, 1H), 8.92 (s, 1H), 8.49 (s, 1H), 8.44 (s, 1H), 8.18 (s, 3H), 8.03 (d, J = 8.0 Hz, 2H), 7.96 (d, J = 9.3 Hz, 1H), 7.89 (d, J = 8.5 Hz, 1H), 7.84 (d, J = 8.8 Hz, 2H), 7.71 (t, J = 7.3 Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 164.77, 154.00, 147.11, 146.82, 143.01, 139.67, 139.53, 137.60, 137.23, 136.02, 135.00, 130.89, 130.73, 130.44, 130.02, 127.94, 127.29, 124.50, 124.32, 123.86, 122.43, 121.87, 121.32, 119.86, 119.02, 118.16, 115.87, 109.11, 14.17.

B-11: N-(2-chloro-4-methylphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 554.0993 (M^+). 1H NMR: δ 10.02 (s, 1H), 8.51 (s, 1H), 8.44 (s, 1H), 8.25 (s, 1H), 8.19 (d, J = 8.2 Hz, 1H), 8.08 (d, J = 9.3 Hz, 1H), 7.94 (dt, J = 29.4, 8.8 Hz, 4H), 7.73 (d, J = 7.8 Hz, 1H), 7.64 (t, J = 7.3 Hz, 1H), 7.47 (s, 1H), 7.24 (s, 1H), 7.08 (d, J = 7.7 Hz, 1H), 2.41 (s, 3H), 2.28 (s, 3H). ^{13}C NMR: δ 164.88, 154.00, 147.11, 143.01, 139.67, 139.53, 137.23, 136.41, 136.02, 134.07, 130.89, 130.73, 130.44, 130.02, 129.78, 128.15, 127.94, 127.29, 124.50, 124.32, 124.08, 123.86, 122.98, 122.43, 121.87, 121.32, 119.86, 109.11, 20.35, 14.17.

B-12: N-(2-chloro-4-nitrophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 585.0688 (M^+). 1H NMR: δ 10.68 (s, 1H), 8.52 (s, 1H), 8.44 (s, 1H), 8.34 (s, 1H), 8.26 (d, J = 8.5 Hz, 1H), 8.19 (d, J = 8.8 Hz, 2H), 8.11 (dd, J = 18.1, 8.9 Hz, 2H), 8.03 (d, J = 7.7 Hz, 1H), 7.97 (d, J = 5.8 Hz, 1H), 7.89 (d, J = 9.2 Hz, 1H), 7.83 (d, J = 7.7 Hz, 1H), 7.64 (t, J = 7.4 Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 164.88, 154.00, 147.11, 143.01, 140.00, 139.85, 139.67, 139.53, 137.23, 136.02, 130.89, 130.73, 130.44, 130.02, 127.94, 127.29, 125.90, 124.63, 124.50, 124.32, 123.94, 123.86, 122.43, 121.87, 121.64, 121.32, 119.86, 109.11, 14.17.

B-13: N-(2-chlorophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 540.0837 (M^+). 1H NMR: δ 9.71 (s, 1H), 8.34 – 8.26 (m, 2H), 8.19 (dt, J = 7.2, 1.6 Hz, 1H), 8.14 (d, J = 1.7 Hz, 1H), 8.06 (d, J = 7.4 Hz, 1H), 7.92 – 7.67 (m, 6H), 7.54 (dd, J = 7.5, 1.6 Hz, 1H), 7.38 (td, J = 7.4, 1.5 Hz, 1H), 7.24 (t, J = 0.7 Hz, 1H), 6.97 (td, J = 7.4, 1.5 Hz, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 164.88, 154.00, 147.11, 143.01, 139.67, 139.53, 137.23, 136.02, 135.67, 130.89, 130.73, 130.44, 130.02, 129.37, 128.01, 127.94, 127.29, 127.28, 125.85, 124.50, 124.32, 123.86, 122.92, 122.43, 121.87, 121.32, 119.86, 109.11, 14.17.

B-14: 2-(3-methylbenzo[b]thiophen-6-yl)-N-(2-nitrophenyl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 550.1140 (M^+). 1H NMR: δ 11.19 (s, 1H), 8.44 (dd, J = 7.5, 1.5 Hz, 1H), 8.35 – 8.26 (m, 3H), 8.19 (dt, J = 7.2, 1.6 Hz, 1H), 8.14 (d, J = 1.7 Hz, 1H), 8.10 – 8.03 (m, 1H), 7.92 – 7.65 (m, 6H), 7.27 – 7.18 (m, 2H), 2.41 (s, 3H). ^{13}C NMR: δ 164.42, 154.00, 147.11, 143.01, 139.67, 139.53, 137.23, 136.02, 135.80, 134.88, 130.89, 130.73, 130.44, 130.02, 127.94, 127.87, 127.29, 125.76, 124.50, 124.32, 123.86, 122.80, 122.43, 121.87, 121.32, 119.86, 118.88, 109.11, 14.17.

B-15: 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-N-o-tolyl-1H-benzo[d]imidazole-5-carboxamide: MS: 519.1446 (M^+). 1H NMR: δ 9.45 (s, 1H), 8.52 (s, 1H), 8.41 (s, 1H), 8.24 (s, 1H), 8.19 (d, J = 8.2 Hz, 1H), 8.06 (d, J = 9.3 Hz, 1H), 7.98 (d, J = 8.6 Hz, 1H), 7.93 (d, J = 10.9 Hz, 2H), 7.74 (d, J = 7.8 Hz, 1H), 7.64 (t, J = 7.2 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.36 (t, J = 7.7 Hz, 1H), 7.22 (d, J = 12.1 Hz, 3H), 2.41 (s, 3H), 2.20 (s, 3H). ^{13}C NMR: δ 164.84, 153.79, 149.94, 144.47, 144.42, 142.99, 142.22, 139.67, 139.53, 135.81, 130.89, 130.72, 130.44, 127.92, 127.29, 125.19, 124.79, 124.50, 124.32, 123.86, 122.43, 119.86, 119.73, 115.38, 112.31, 109.13, 56.01, 34.53, 31.27, 14.17.

B-16: 2-(3-methylbenzo[b]thiophen-6-yl)-N,1-bis(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 550.1140 (M^+). 1H NMR: δ 10.14 (s, 1H), 8.67 (s, 1H), 8.52 (s, 1H), 8.41 (s, 1H), 8.19 (d, J = 9.2 Hz, 2H), 8.12 (d, J = 8.5 Hz, 2H), 8.04 (q, J = 10.0, 8.9 Hz, 3H), 7.93 (d, J = 9.4 Hz, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.70 (t, J = 7.3 Hz, 1H), 7.62 (t, J = 8.2 Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 165.61, 154.00, 148.71, 147.11, 143.01, 139.67, 139.53, 138.56, 137.23, 136.02, 131.13, 130.89, 130.73, 130.51, 130.02, 127.94, 127.29, 126.44, 124.50, 124.32, 123.86, 122.43, 121.87, 121.32, 119.86, 118.21, 114.96, 109.11, 14.17.

B-17: 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-N-(3-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 573.12 (M^+). 1H NMR: δ 9.39 (s, 1H), 8.52 (s, 1H), 8.42 (s, 1H), 8.24

(s, 1H), 8.19 (d, $J = 8.2$ Hz, 1H), 8.06 (d, $J = 9.2$ Hz, 2H), 7.95 (dt, $J = 21.0, 8.7$ Hz, 4H), 7.74 (d, $J = 7.7$ Hz, 1H), 7.64 (t, $J = 7.3$ Hz, 1H), 7.48 (t, $J = 8.1$ Hz, 1H), 7.37 (d, $J = 8.1$ Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 165.00, 155.26, 144.34, 143.96, 141.98, 139.67, 139.53, 138.26, 136.33, 135.34, 132.70, 131.30, 130.89, 130.45, 129.02, 128.40, 127.71, 127.29, 127.16, 124.49, 124.45, 124.32, 123.81, 122.42, 122.08, 120.55, 119.78, 109.57, 17.80, 14.17.

B-18: N-(4-bromo-2-methylphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 598.05 (M^+). ^1H NMR: δ 9.58 (s, 1H), 8.34 – 8.26 (m, 2H), 8.19 (dt, $J = 7.2, 1.6$ Hz, 1H), 8.14 (d, $J = 1.7$ Hz, 1H), 8.10 – 8.03 (m, 1H), 7.92 – 7.67 (m, 5H), 7.41 (d, $J = 1.4$ Hz, 1H), 7.29 (dd, $J = 7.5, 1.5$ Hz, 1H), 7.24 (t, $J = 0.7$ Hz, 1H), 6.85 (d, $J = 7.5$ Hz, 1H), 2.41 (s, 3H), 2.19 (s, 3H). ^{13}C NMR: δ 165.00, 154.00, 147.11, 143.01, 139.67, 139.53, 137.23, 136.02, 135.12, 132.71, 130.89, 130.84, 130.73, 130.44, 130.02, 129.90, 127.94, 127.29, 124.50, 124.32, 123.86, 123.49, 122.43, 121.87, 121.32, 119.86, 116.93, 109.11, 17.98, 14.17.

B-19: N-(4-fluoro-3-methylphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 537.14 (M^+). ^1H NMR: δ 9.46 (s, 1H), 8.52 (s, 1H), 8.43 (s, 1H), 8.19 (d, $J = 10.1$ Hz, 2H), 8.08 (d, $J = 9.3$ Hz, 1H), 7.99 (dd, $J = 17.4, 6.9$ Hz, 2H), 7.87 (d, $J = 9.3$ Hz, 1H), 7.79 (d, $J = 7.8$ Hz, 1H), 7.74 – 7.68 (m, 1H), 7.64 (t, $J = 7.0$ Hz, 1H), 7.22 (d, $J = 13.2$ Hz, 2H), 7.14 (d, $J = 8.0$ Hz, 1H), 2.41 (s, 3H), 2.25 (s, 3H). ^{13}C NMR: δ 164.84, 153.79, 149.94, 144.47, 144.42, 142.99, 142.22, 139.67, 139.53, 135.81, 130.89, 130.72, 130.44, 127.92, 127.29, 125.19, 124.79, 124.50, 124.32, 123.86, 122.43, 119.86, 119.73, 115.38, 112.31, 109.13, 56.01, 34.53, 31.27, 14.17.

B-20: 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-N-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 550.11 (M^+). ^1H NMR: δ 8.52 (s, 1H), 8.42 (s, 1H), 8.36 (s, 1H), 8.30 (d, $J = 8.6$ Hz, 2H), 8.19 (s, 2H), 8.09 (d, $J = 9.3$ Hz, 1H), 8.02 (d, $J = 8.5$ Hz, 3H), 7.96 (d, $J = 6.0$ Hz, 1H), 7.87 (d, $J = 9.4$ Hz, 1H), 7.80 (d, $J = 7.9$ Hz, 1H), 7.64 (t, $J = 7.3$ Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 164.42, 155.26, 144.34, 143.96, 141.98, 139.67, 139.53, 138.26, 136.33, 136.21, 135.12, 133.51, 131.30, 130.89, 130.45, 128.40, 128.35, 127.71, 127.29, 125.42, 124.49, 124.32, 123.84, 123.81, 122.42, 122.08, 119.78, 109.57, 14.17.

B-21: N-(5-tert-butyl-2-methoxyphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 591.20 (M^+). ^1H NMR: δ 9.94 (s, 1H), 8.52 (s, 1H), 8.43 (s, 1H), 8.25 (s, 1H), 8.19 (d, $J = 8.2$ Hz, 1H), 8.06 (d, $J = 9.2$ Hz, 1H),

7.96 (dd, $J = 18.2, 10.2$ Hz, 4H), 7.74 (d, $J = 7.7$ Hz, 1H), 7.64 (t, $J = 7.3$ Hz, 1H), 7.24 (s, 1H), 7.14 (d, $J = 7.0$ Hz, 1H), 6.91 (d, $J = 7.3$ Hz, 1H), 3.83 (s, 3H), 2.41 (s, 3H), 1.31 (s, 9H). ^{13}C NMR: δ 164.86, 164.74, 160.90, 160.87, 153.84, 153.79, 151.33, 144.47, 142.99, 142.22, 139.67, 139.53, 135.81, 130.89, 130.72, 130.44, 128.48, 128.28, 127.29, 125.19, 124.79, 124.50, 124.32, 123.86, 122.43, 119.86, 116.21, 116.01, 110.22, 110.14, 109.13, 108.54, 108.46, 14.17.

B-22: N-benzyl-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 519.14 (M^+). ^1H NMR: δ 8.51 (s, 1H), 8.44 (s, 1H), 8.24 (s, 1H), 8.19 (d, $J = 8.1$ Hz, 1H), 8.15 (d, $J = 9.1$ Hz, 1H), 8.06 (d, $J = 9.1$ Hz, 1H), 7.94 (dt, $J = 23.5, 8.8$ Hz, 3H), 7.73 (d, $J = 7.6$ Hz, 1H), 7.63 (t, $J = 7.4$ Hz, 1H), 7.32 (s, 5H), 7.24 (s, 1H), 4.65 (d, $J = 8.9$ Hz, 2H), 2.41 (s, 3H). ^{13}C NMR: δ 165.00, 154.00, 147.11, 143.01, 139.67, 139.53, 137.29, 137.23, 136.02, 133.73, 130.89, 130.73, 130.44, 130.02, 128.58, 127.94, 127.55, 127.29, 124.50, 124.32, 123.86, 122.69, 122.43, 121.87, 121.32, 119.86, 109.11, 20.85, 18.40, 14.17.

B-23: N-(4-chloro-2-nitrophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 519.14 (M^+). ^1H NMR: δ 11.25 (s, 1H), 8.47 (d, $J = 7.6$ Hz, 2H), 8.22 (dd, $J = 16.6, 9.2$ Hz, 4H), 8.14 (s, 1H), 8.06 (d, $J = 8.5$ Hz, 2H), 7.97 (d, $J = 9.4$ Hz, 1H), 7.89 (d, $J = 7.9$ Hz, 1H), 7.72 (t, $J = 7.3$ Hz, 1H), 7.67 (d, $J = 7.6$ Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 164.42, 154.00, 147.11, 143.01, 139.67, 139.53, 137.23, 136.21, 136.02, 135.12, 133.51, 130.89, 130.73, 130.44, 130.02, 128.35, 127.94, 127.29, 125.42, 124.50, 124.32, 123.86, 123.84, 122.43, 121.87, 121.32, 119.86, 109.11, 14.17. Table 1

Antimicrobial activity: Minimum inhibitory concentrations (MIC)

The synthesized derivatives were evaluated for their *in vitro* antibacterial activity against *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli*, *T. thermophilus* and antifungal activity against *A. niger* and *C. albicans*. Antimicrobial activity of the synthesized compounds was completed by the tube dilution method. From the recorded pMIC values, it was observed that compound compound **B-1**, **B-7** and **B-23** was found to be most active against all the strains having pMIC **B-3**: 2.53, 2.53, 2.53, 2.23, 2.53, 2.23 & **B-7**: 2.54, 2.54, 2.54, 2.54, 2.54, 2.54 and **B-23**: 2.28, 2.28, 2.28, 2.28, 2.58, 2.58 $\mu\text{M}/\text{ml}$ value. Compound **B-14** was found to be least antibacterial active and Compound **B-20** was found to be least antifungal active in the series. In general, the results of MIC studies exposed that the synthesized compounds have bacteriocidal and fungicidal potential as compared to standard Cefadroxil,

Table 1: Physical and Analytical data of title compounds.

Sr. No.	Compounds	Molecular Formula	Molecular Weight	Melting Points (°C)	% Yields	R _f
B	2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid	C ₂₃ H ₁₆ N ₃ O ₄ S	429.45	110-112	91	0.49
Different Anilines (R)						
B-1	naphthalen-1-amine	C ₃₃ H ₂₂ N ₄ O ₃ S	554.62	94-96	78.62	0.61
B-2	2,3-difluoroaniline	C ₂₉ H ₁₈ F ₂ N ₄ O ₃ S	540.54	215-217	78.25	0.72
B-3	2,4-dimethylaniline	C ₃₁ H ₂₄ N ₄ O ₃ S	532.61	155-157	87.64	0.85
B-4	2,4-dinitroaniline	C ₂₉ H ₁₈ N ₆ O ₇ S	594.55	207-209	68.9	0.56
B-5	2,5-dichloroaniline	C ₂₉ H ₁₈ Cl ₂ N ₄ O ₃ S	573.45	86-88	75.63	0.39
B-6	2,5-difluoroaniline	C ₂₉ H ₁₈ F ₂ N ₄ O ₃ S	540.54	73-75	86.7	0.82
A-7	2,4,6-trimethylaniline	C ₃₂ H ₂₆ N ₄ O ₃ S	546.64	158-160	92.85	0.87
B-8	2,6-difluoroaniline	C ₂₉ H ₁₈ F ₂ N ₄ O ₃ S	540.54	97-99	77.5	0.44
B-9	2-bromo-4-methylaniline	C ₃₀ H ₂₁ BrN ₄ O ₃ S	597.48	75-77	83.9	0.6
B-10	2-bromo-5-nitroaniline	C ₂₉ H ₁₈ BrN ₅ O ₅ S	628.45	102-104	82.4	0.86
B-11	2-chloro-4-methylaniline	C ₃₀ H ₂₁ ClN ₄ O ₃ S	553.03	93-95	69.54	0.7
B-12	2-chloro-4-nitroaniline	C ₂₉ H ₁₈ ClN ₅ O ₅ S	584	87-87	79.8	0.54
B-13	2-chloroaniline	C ₂₉ H ₁₉ ClN ₄ O ₃ S	539	57-59	74.25	0.63
B-14	2-nitroaniline	C ₂₉ H ₁₉ N ₅ O ₅ S	549.56	119-121	75.15	0.73
B-15	o-toluidine	C ₃₀ H ₂₂ N ₄ O ₃ S	518.59	150-152	91.22	0.84
B-16	3-nitroaniline	C ₂₉ H ₁₉ N ₅ O ₅ S	549.56	75-77	67.5	0.67
B-17	3-(trifluoromethyl)aniline	C ₃₀ H ₁₉ F ₃ N ₄ O ₃ S	572.56	72-74	61.72	0.64
B-18	4-bromo-2-methylaniline	C ₃₀ H ₂₁ BrN ₄ O ₃ S	597.48	95-97	77.8	0.69
B-19	4-fluoro-3-methylaniline	C ₃₀ H ₂₁ FN ₄ O ₃ S	536.58	103-105	77.5	0.51
B-20	4-nitroaniline	C ₂₉ H ₁₉ N ₅ O ₅ S	549.56	111-113	66.5	0.55
B-21	5-tert-butyl-2-methoxyaniline	C ₃₄ H ₃₀ N ₄ O ₄ S	590.69	82-84	88	0.5
B-22	Phenylmethanamine	C ₃₀ H ₂₂ N ₄ O ₃ S	518.59	151-153	80.5	0.88
B-23	4-chloro-2-nitroaniline	C ₂₉ H ₁₈ ClN ₅ O ₅ S	585.07	201-203	89.4	0.65

Ofloxacin and Fluconazole. Here, Figure 7 graphically represent the pMIC, Table 2 represent the pMIC values of compounds with comparison to standards.

Molecular Docking research

Newly designed molecules/ligands were studied for their molecular docking behavior with the help of Schrodinger's Maestro package docking software and AutoDock Vina 4.2, Table 3. Molecules were docked was in to binding modes and binding affinities to the 1wny. PDBQT and 1jil. PDBQT. Induced fit docking (IFD) method was utilized for the same. The predicted binding pattern revealed that synthesized ligand binds within catalytic cavity firmly via. hydrogen bond formation,

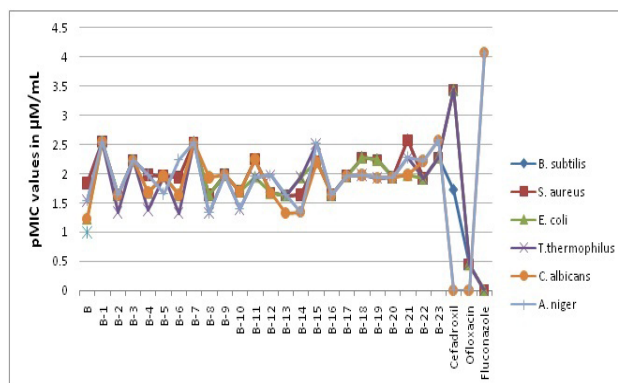


Figure 7: Antimicrobial activity (pMIC in µM/mL) of synthesized derivatives against different microorganisms.

Table 2: pMIC values ($\mu\text{M/mL}$) of synthesized derivatives against different standard microbial strains.

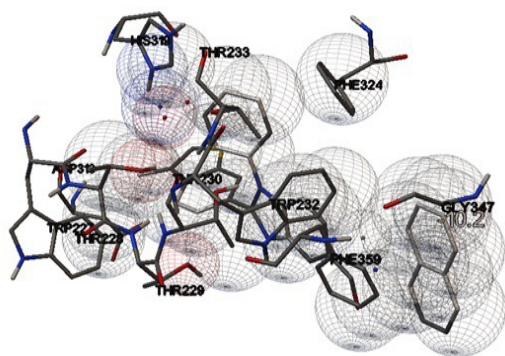
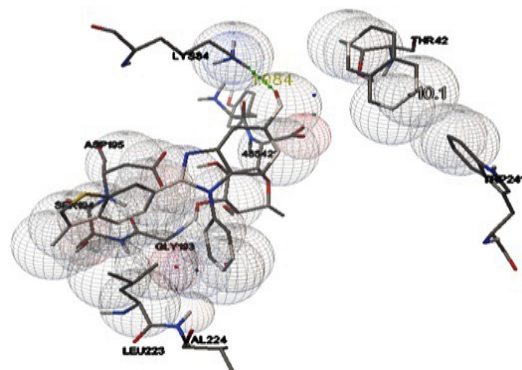
Compound	pMIC values in $\mu\text{M/ml}$					
	Bacterial Strains				Fungal Strains	
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>T. thermophilus</i>	<i>C. albicans</i>	<i>A. niger</i>
B	1.84	1.84	1.23	1.54	1.23	1.54
B-1	2.55	2.55	2.55	2.55	2.55	2.55
B-2	1.64	1.64	1.64	1.34	1.64	1.64
B-3	2.23	2.23	2.23	2.23	2.23	2.23
B-4	1.98	1.98	1.68	1.38	1.68	1.98
B-5	1.96	1.96	1.96	1.96	1.96	1.66
B-6	1.94	1.94	1.64	1.33	1.64	2.24
B-7	2.54	2.54	2.54	2.54	2.54	2.54
B-8	1.64	1.64	1.64	1.33	1.94	1.33
B-9	1.98	1.98	1.98	1.98	1.98	1.98
B-10	1.70	1.70	1.70	1.40	1.70	1.40
B-11	2.25	2.25	1.95	1.95	2.25	1.95
B-12	1.67	1.67	1.67	1.97	1.67	1.97
B-13	1.63	1.63	1.63	1.63	1.33	1.63
B-14	1.64	1.64	1.94	1.94	1.34	1.34
B-15	2.22	2.22	2.22	2.52	2.20	2.52
B-16	1.64	1.64	1.64	1.64	1.64	1.64
B-17	1.96	1.96	1.96	1.96	1.96	1.96
B-18	2.28	2.28	2.28	1.98	1.98	1.98
B-19	2.24	2.24	2.24	1.93	1.93	1.93
B-20	1.94	1.94	1.94	1.94	1.94	1.94
B-21	2.58	2.58	1.98	2.28	1.98	2.28
B-22	1.92	1.92	1.92	1.92	2.22	2.22
B-23	2.28	2.28	2.28	2.28	2.58	2.58
Cefadroxil	1.72	3.44	3.44	3.44	0	0
Ofloxacin	0.44	0.44	0.44	0.44	0	0
Fluconazole	0	0	0	0	4.08	4.08

pi-pi stacking and hydrophobic interaction. Molecular docking parameters and interaction pattern of ligands within the pocket. In the binding model the most active compounds are B-1, B-7 and B-23. In compound B-1, 1WNY PDB, Visual inspection of the pose of Umbrageous in to the inhibitor binding cavity revealed that ligand is inserted in to “aromatic case” framed by THR 229, THR 228, TRP 227, ASP 310, HIS 319, THR 223, THR 230, TRP 232, PHE 324, GLY 347 and the FAD aromatic ring of the protein (Figure 8) and 1JIL PDB, Visual inspection of the pose of Umbrageous in to the inhibitor binding cavity revealed that ligand is inserted in to “aromatic case” framed by LYS 84, THR 42, TRP 241, GLY 193, AL 224, LEU 223, SER 194, ASP 195 and the FAD aromatic ring of the protein. The binding is further stabilized by π - π stacking and the oxygen of NO_2 of the N-aryl part of compound forms a hydrogen

bond interaction with the OH of LYS 84 having value 1.984 (Figure 9). In compound B-7, 1WNY PDB, Visual inspection of the pose of umbrageous in to the inhibitor binding cavity revealed that ligand is inserted in to “aromatic case” framed by THR 229, THR 228, VAL 318, TRP 232, PHE 324, VAL 343, PHE 359, GLY 347 and the FAD aromatic ring of the protein (Figure 10) and 1JIL PDB, Visual inspection of the pose of umbrageous in to the inhibitor binding cavity revealed that ligand is inserted in to “aromatic case” framed by GLN 17, TRP 197, SER 194, ASP 153, ASP 195, GLY 83, LY 884, GLY 49, LYS 231, LYS 234 and the FAD aromatic ring of the protein (Figure 11). In compound B-23, 1WNY PDB, Visual inspection of the pose of umbrageous in to the inhibitor binding cavity revealed that ligand is inserted in to “aromatic case” framed by THR 2229, THR 223, TRP 227, GLU 312, GLN 311, SER 310, VAL 318,

Table 3: Docking Score of Synthesized Derivatives.

Sr No.	Docking Score by Autodock Vina 4.2.		Docking Score by Schrodinger Glide			
	1wny	1jil	1wny		1jil	
			Docking Score	glide gscore	Docking Score	glide gscore
B	-9.9	-8	-4.12	-4.12	-3.03	-3.03
B-1	-10.2	-10.1	-5.26	-5.26	-5.456	-5.456
B-2	-9.9	-10.1	-4.84	-4.84	-4.957	-4.957
B-3	-9.9	-9.9	-24.342	-4.342	-4.56	-4.56
B-4	-8.8	-9.6	-4.502	-4.502	-4.864	-4.864
B-5	-9.7	-9.9	-4.586	-4.586	-4.957	-4.957
B-6	-9.1	-10.0	-4.9	-4.9	-5.314	-5.314
B-7	-10.3	-10.0	-5.318	-5.318	-5.231	-5.231
B-8	-9.4	-9.9	-4.459	-4.459	-4.796	-4.796
B-9	-10.3	-10.0	-5.318	-5.318	-5.231	-5.231
B-10	-9.4	-9.9	-4.459	-4.459	-4.796	-4.796
B-11	-10.0	-9.9	-4.425	-4.425	-4.896	-4.896
B-12	-9.6	-10.0	-4.952	-4.952	-5.03	-5.03
B-13	-9.7	-9.8	-4.783	-4.783	-4.964	-4.964
B-14	-9.7	-10.4	-4.976	-4.976	-5.03	-5.03
B-15	-9.6	-9.9	-4.37	-4.37	-4.658	-4.658
B-16	-9.4	-9.5	-4.729	-4.729	-4.967	-4.967
B-17	-9.6	-10.1	-4.596	-4.596	-4.88	-4.88
B-18	-9.2	-9.8	-4.53	-4.53	-4.867	-4.867
B-19	-9.4	-9.9	-4.776	-4.776	-4.997	-4.997
B-20	-9.4	-9.7	-4.714	-4.714	-4.968	-4.968
B-21	-9.3	-9.8	-4.778	-4.778	-4.951	-4.951
B-22	-10.0	-10.1	-4.969	-4.969	-5.186	-5.186
B-23	-10.0	-10.5	-5.609	-5.609	-5.876	-5.876
Cefadroxil	-10.2	-10.6	-5.847	-5.847	-5.895	-5.895
Ofloxacin	-9.8	-9.9	-6.325	-6.325	-6.131	-6.131

**Figure 8: Docking Result of B-1 with PDB 1WN Y****Figure 9: Docking Result of B-1 with PDB 1JIL**

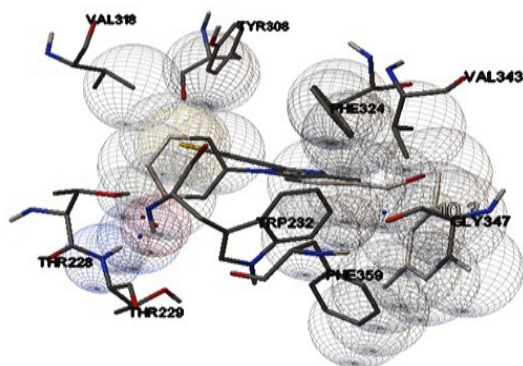


Figure 10: Docking Result of B-7 with PDB 1WNY

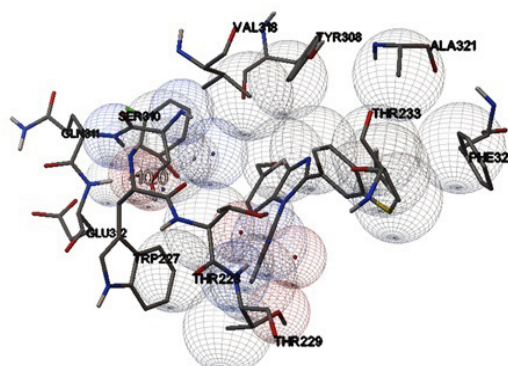


Figure 12: Docking Result of B-23 with PDB 1WNY

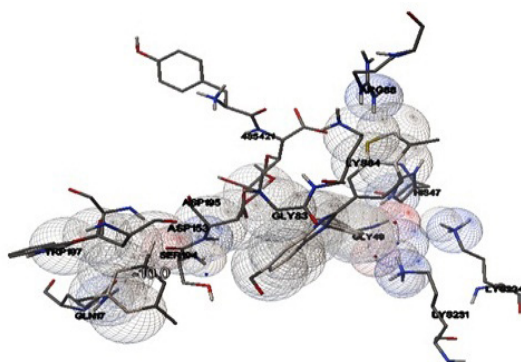


Figure 11: Docking Result of B-7 with PDB 1JIL

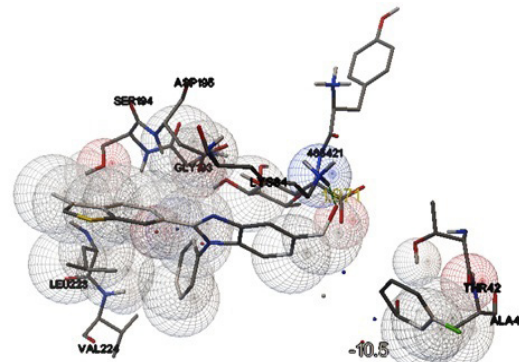


Figure 13: Docking Result of B-23 with PDB 1JIL

TYR 308, ALA 321, THR 233, PHE 324 and the FAD aromatic ring of the protein (Figure 12) and 1JIL PDB, Visual inspection of the pose of umbrageous in to the inhibitor binding cavity revealed that ligand is inserted in to “aromatic case” framed by VAL 224, LEU 223, SER 194, ASP 195, GLY 193, LYS 84, THR 42, ALA 43 and the FAD aromatic ring of the protein. The binding is further stabilized by π - π stacking and the oxygen of NO_2 of the N-aryl part of compound forms a hydrogen bond interaction with the OH of LYS 84 having value 1.971 (Figure 13). Docking studies revealed that hydrogen bond interactions fix the ligands firmly and tightly in the active site.

SAR (structure-activity relationship) studies (Figure 14)

Amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid of good biological activity as compared with compound B.

Substitution with aromatic amine having electron donating groups at different position shows most active among the series.

Substitution of naphthalene ring shows best antimicrobial activity among the series i.e. compound B-1.

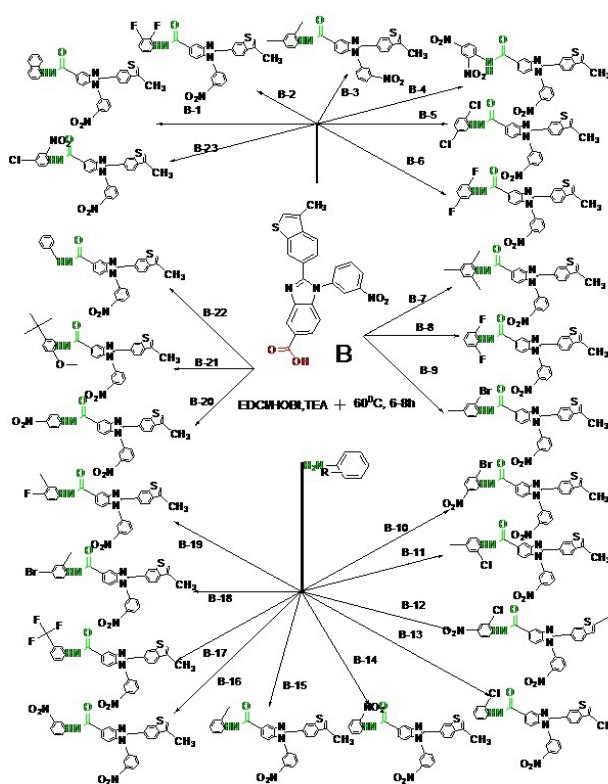


Figure 14: Scheme 2: Amide derivatives of 1-(3-nitrophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1H-benzo[d]imidazole-5-carboxylic acid

Methyl group (electron donating group) at 2,4,6 position shows the second best antimicrobial activity among the series i.e. compound B-7.

Substitution of methyl group at 2,4 shows the best antibacterial activity but decreases antifungal activity i.e. compound B-3.

Substitution of Nitro at 2 and chloro at 4 shows the best antifungal activity but decreases antibacterial activity i.e. compound B-23.

Substitution of di-floro at 2,3 and 2,5 and 2,6 shows the weak antimicrobial activity among the series i.e. compound B-2, B-6, B-8.

Substitution of bromo at 2 decrease the activity e.g. compound B-9, B-10 but Substitution of bromo at 4 and electron donating group at position 2 shows good antibacterial activity i.e. compound B-18.

Substitution of one electron withdrawing group and one electron donating group at different position of aromatic ring shows moderate activity among the series i.e. compound B-9, B-11, B-18, B-19.

Substitution with aromatic amine having electron withdrawing groups at different position leads to decrease in activity i.e. compound B-4, B-5, B-10 and B-12.

Antibacterial activity decreases when aniline is replaced by phenylmethanamine shows moderate antifungal activity i.e. compound B-22.

Substitution of one electron withdrawing group at different position of aromatic ring shows weak activity i.e. compound B-13, B-14, B-16 and B-20.

CONCLUSION

In vitro results revealed that a large number of synthesized compounds were found to possess excellent antimicrobial. Among the library, best compound B-1, B-7 and B-23 possessing excellent dock score by AutoDock Vina/Schrodinger's maestro against 1wny.PDBQT:1jil. PDBQT is -10.2:-10.1/ -5.26:-5.456, -10.3:-10.0/-5.318:-5.231, -10.0:-10.5/-5.609:-5.876 and as compared to standard drugs dock score -10.2:-10.6/-5.847:-5.895, -9.8:-9.9/ -6.323:-6.131. Derivatives B-1, B-7 and B-23 also exhibited good MIC as compared to standard by tube dilution method. *In-silico* studies played an important role in designing the potent ligands against *aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase* enzymes as well as explained the binding pattern of designed and synthesized ligand within active pocket and good antimicrobial compounds can be used for *in vivo* studies for future. *In-silico* studies and *in vitro* results comply with each other.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATIONS

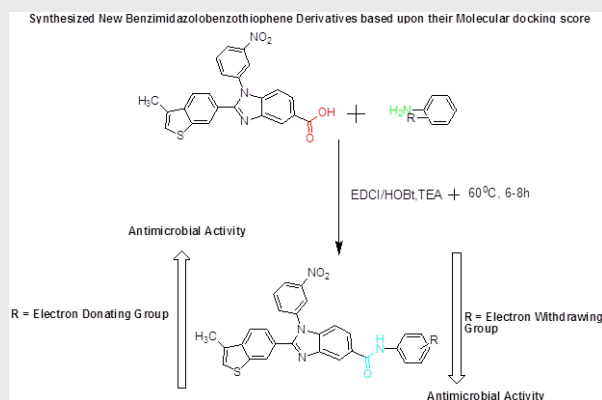
¹³C NMR: Carbon 13 Nuclear magnetic resonance; **¹H NMR:** Proton Nuclear magnetic resonance; **4-MBA:** 4-Mercapto Benzoic acid; **BOD:** Biological oxygen demand; **CFU:** Colony forming unit; **DCC:** Di Cyclohexyl carbodimide; **DCM:** Dichloro methane; **DMAP:** 4-dimethyl amino pyridine; **DMF:** Dimethylsulfoxide; **DMSO:** Dimethyl sulfoxide; **EDC:** Ethylene dichloride; **EDCI:** 1-Ethyl 3-(3-dimethylaminopropyl) carbodiimide; **HOBt:** 1-Hydroxybenzotriazole; **MIC:** Minimum inhibitory concentrations; **MS:** Mass Spectra; **MTCC:** Microbial Type Culture Collection; **MW:** Molecular weight; **OPLS:** Optimized Potential for Liquid Simulations; **PDB ID:** Protein Data Bank Identification; **PEG-5000:** Poly Ethylene Glycol 5000; **PPA:** Polyphosphoric acid; **RBF:** Round Bottom Flask; **RT:** Room temperature; **TEA:** Triethylamine; **TFA:** Trifluoro acetic acid; **TLC:** Thin layer chromatography; **UV:** Ultra violet.

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PICTORIAL ABSTRACT



SUMMARY

There has been a continual battle between humans and the multitude of microorganisms that cause infection and disease. Infectious diseases are responsible for great number of deaths in the world population. The careless use and overconsumption of antibiotics has led to the emergence of antibiotic-resistant strains. Therefore, there is an urgent need for the development of new antimicrobial drugs. In the present study, we have synthesized and investigated antimicrobial activities of novel derivatives with molecular docking studies. In this article, amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid were synthesized by focusing the *aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase enzymes* and inhibition by docking study leads to antimicrobial action. Studies were carried out on a designed amide library of derivatives with the help of Schrodinger's maestro package and AutoDock Vina 4.2 of molecular docking software against crystal structure of enzymes (PDB ID: 1wny.PDBQT and 1jil.PDBQT). Best 23 focused amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(3-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid were synthesized based upon their dock score and further investigated for *in vitro* antimicrobial. *In-silico* studies and *in vitro* results comply with each other.

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