Synthesis, *in-silico* Designing, SAR and Microbiological Evaluation of Novel Amide Derivatives of 1-(4-Nitrophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1H-benzo[d]imidazole-5-carboxylic Acid

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ABSTRACT

Background: Due to this increasing predicament of antibiotic confrontation, the lot of distinct antibiotics available is dwindling and there are only smatterings of new antibiotics in the drug development channel. Therefore, an intense necessitate for new antimicrobial drugs. In current study, we have synthesized the new derivatives with the help of molecular docking studies and investigated antimicrobial activities. **Methods:** By focusing the *enzymes i.e. aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase,* new amides of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid were synthesized and inhibition by docking study precedence to antimicrobial action. Studies were accomplished on a designed library of amide derivatives with the help of docking softwares i.e. AutoDock Vina 4.2 and Schrodinger's maestro package against crystal structure of enzymes (PDB ID: 1wny.PDBQT and1jil.PDBQT). Based upon their dock score, superlative 23 focused amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid were synthesized and further investigated for *in vitro* antimicrobial. **Results:** Among the library, superlative compounds C-1, C-10 and C-19 having tremendous dock score by AutoDock Vina/Schrodinger's maestro against 1wny.PDBQT:1jil.PDBQT-9.0:-9.9/ -5.144:-5.328,-9.2:-9.4/ -5.007:-5.369, -9.0:-9.6/ -5.184:-5.264 as compared to standard drug dock score -10.2:-10.6/-5.847:-5.895.C-1, C-10 and C-19 derivatives also exhibited good MIC against six representative micro-organisms as compared to standard by tube dilution method. **Conclusion:** *In-vitro* results revealed that a large number of synthesized compounds having good antimicrobial activity and dock score can be used for *in vivo* studies for future. *In-silico* studies and *in vitro* results comply with each other.

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

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INTRODUCTION

At present lot of drugs are accessible for curing the microbial infection but most of them is becoming abortive due to anti-microbial resistance by the micro-organisms. So there is an immense need for the discovery of novel antimicrobial agents to overcome the antimicrobial resistance and side effects.[1] The *tyrosyl-tRNA synthetase* and *aminoacyl-tRNA synthetase (AaRS)* enzymes have been a hub of latest research for drug discovery and play decisive roles in biosynthesis of protein by catalyzing the synthesis aminoacylation of transfer RNA (tRNA) by their cognate amino acid. Once enzymes are inhibited, halted protein biosynthesis, which in turn outcome in the shrinking

of bacterial growth under both *in vitro* and contagious conditions.[2,3] Docking softwares are extremely valuable for the screening of abundance of molecules affinity towards a selective disease or drug target.[4]

The chemistry and pharmacology of benzimidazole have been purposeful to medicinal chemistry because benzimidazole derivatives possessed various pharmacological activities i.e. antimicrobial, antioxidant, anticancer, anti-inflammatory, analgesic, antidiabetic, anticonvulsant, anti-asthmatic, antiviral, antihypertensive, antiproliferative, antitubercular with many more.[5-14] Benzimidazole moiety have been showing talented/flourishing activity in the healing of numerous diseases and due to that reason benzimidazoles attained much contemplation as meaningful pharmacophore and called as honored structure in medicinal chemistry.[15] By examine review contains the various benzimidazoles and their pharmacological, focused amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid were taken for lead development depending upon their docking score. In last decade, the virtual screening approach for studying the docking of small moieties with protein structure is a authoritative tool for lead design and has become the most active and valuable technology now being used in drug discovery processing. It is an effort to remove the element of fortune from the drug designs, because pharmacological activity is reliant 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-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid were tested for docking to study the binding modes and binding affinities to the 1wny. PDBQT (*Isoleucyl-tRNA synthetase* editing domain, source: *Thermus thermophilus)* and 1jil.PDBQT(*tyrosyl-tRNA synthetase* editing domain, source: *Staphylococcus aureus*) in which *aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase* enzymeshave been a center of attention of recent research for bacterial inhibitory activity by using software AutoDock Vina 4.2 and Schrodinger Glide.[17-19] Both software's showed exceptional non-commercial researcher docking program that is broadly used around the world. The final screening of the focused amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acidis calculated. Molecular docking helps in studying the interaction between the receptor/protein with ligand by identifying the suitable active/binding sites in protein and by obtaining the finest geometry of ligand-receptor complex and calculating the energy of interaction between ligands and protein for design the

new lead compounds for research. GlideScore is a practical scoring function that estimates the ligand binding free energy, including force field i.e. electrostatic, van der Waals etc, assistance and terms rewarding or penalizing interactions known to influence ligand binding. It has been enhanced for prediction of docking accuracy, database enrichment and binding affinity.[20]

MATERIALS AND METHODS

All chemicals used in synthesis and antimicrobial activity were acquired from Himedia Laboratories and TM Media, Titan Biotech Ltd., Delhi. Cefadroxil, Ofloxacin and Fluconazole were gathered from CDH, Mumbai. All strains were seized from *MTCC*, Chandigarh. Chemical reactions were sustained under surveillance by TLC on silica gel plate; spotting was investigated in iodine and UV chambers, in mobile media Methanol: Ethyl Acetate: Hexane (0.5:3.5:6). For detection of melting points sonar melting point apparatus in open capillary tubes were used. ^{1}H NMR and ^{13}C NMR spectra were proved in DMSO and deuterated $CDCl_3$ 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 noted in Hertz (Hz) and d (parts per million). Mass spectrums were recorded by Waters Micromass Q-ToF Micro instrument. MIC were concluded in BOD incubator (Haryana Scientific and Engg. Corporation Ltd., Rohtak) and Laminar Air Flow biosafety cabinet (Hicon, New Delhi) respectively.

Synthesisof 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid outlined in Scheme 1 (Figure 1**)**.[21,22] Structural part of compounds was in confirmed by Mass spectra,[23] ^{1}H NMR and ^{13}CNMR[24] 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 – fluro 3 – nitro benzoic acid [2], 1.2 mole of DCC and a pinch of DMAP is dissolved in DCM and kept for stirring for two days at room temperature. To the precipitated product [3] add 1.1 mole of 4-nitrobenzenamine and then stirred for one day which is precipitated by ether. The product [4] was then reacted with 1.2 moles 4-MBA, 1.2 moles of DCC, pinch of DMAP dissolved in the solvent DCM then stirred for one day. Retrieved product [5] is subjected to reduction by adding the mixture of 1 mole of NH_4Cl and 1 mole of Zn, in methanol and stirred for 3 hrs, centrifuge the reaction mixture and filter to remove Zn. Then

| Sr. No.	Different Compounds	Molecular Formula	Molecular Weight	Melting Points (°C)	% Yields	R_f
	Table 1: Physical data of title compounds.					
C	1-(2,4-dinitrophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1H-benzo[d]imidazole-5-carboxylic acid	$C_{23}H_{15}N_3O_4S$	429.45	120-122	84.4	0.36
	Different Anilines (R)					
C-1	naphthalen-1-amine	$C_{33}H_{22}N_4O_3S$	554.62	102-103	80.12	0.59
C-2	2,3-difluoroaniline	$C_{29}H_{18}F_2N_4O_3S$	540.54	132-134	68.75	0.65
C-3	2,4-dimethylaniline	$C_{31}H_{24}N_4O_3S$	532.61	162-164	82.34	0.77
C-4	2,4-dinitroaniline	$C_{29}H_{18}N_6O_7S$	594.55	242-244	78	0.52
C-5	2,5-dichloroaniline	$C_{29}H_{18}Cl_2N_4O_3S$	573.45	92-94	85.65	0.44
C-6	2,5-difluoroaniline	$C_{29}H_{18}F_2N_4O_3S$	540.54	99-101	80.4	0.55
C-7	2,4,6-trimethylaniline	$C_{32}H_{26}N_4O_3S$	546.64	163-165	85.92	0.8
C-8	2,6-difluoroaniline	$C_{29}H_{18}F_2N_4O_3S$	540.54	187-189	75.75	0.6
C-9	2-bromo-4-methylaniline	$C_{30}H_{21}BrN_4O_3S$	597.48	173-179	81.9	0.7
C-10	2-bromo-5-nitroaniline	$C_{29}H_{18}BrN_5O_5S$	628.45	228-230	92.5	0.81
C-11	2-chloro-4-methylaniline	$C_{30}H_{21}ClN_4O_3S$	553.03	168-170	72.69	0.62
C-12	2-chloro-4-nitroaniline	$C_{29}H_{18}ClN_5O_5S$	584	167-169	83.54	0.5
C-13	2-chloroaniline	$C_{29}H_{19}ClN_4O_3S$	539	152-154	77.85	0.61
C-14	2-nitroaniline	$C_{29}H_{19}N_5O_5S$	549.56	124-126	70.16	0.71
C-15	o-toluidine	$C_{30}H_{22}N_4O_3S$	518.59	161-163	82.5	0.89
C-16	3-nitroaniline	$C_{29}H_{19}N_5O_5S$	549.56	183-185	75.6	0.79
C-17	3-(trifluoromethyl)aniline	$C_{30}H_{19}F_3N_4O_3S$	572.56	179-181	72.65	0.42
C-18	4-bromo-2-methylaniline	$C_{30}H_{21}BrN_4O_3S$	597.48	153-155	66.8	0.67
C-19	4-fluoro-3-methylaniline	$C_{30}H_{21}FN_4O_3S$	536.58	206-208	85.7	0.76
C-20	4-nitroaniline	$C_{29}H_{19}N_5O_5S$	549.56	276-278	76.5	0.51
C-21	5-tert-butyl-2-methoxyaniline	$C_{34}H_{30}N_4O_4S$	590.69	188-190	74.8	0.63
C-22	Phenylmethanamine	$C_{30}H_{22}N_4O_3S$	518.59	159-161	85.4	0.74
C-23	4-chloro-2-nitroaniline	$C_{29}H_{18}ClN_5O_5S$	585.07	209-211	80.9	0.69
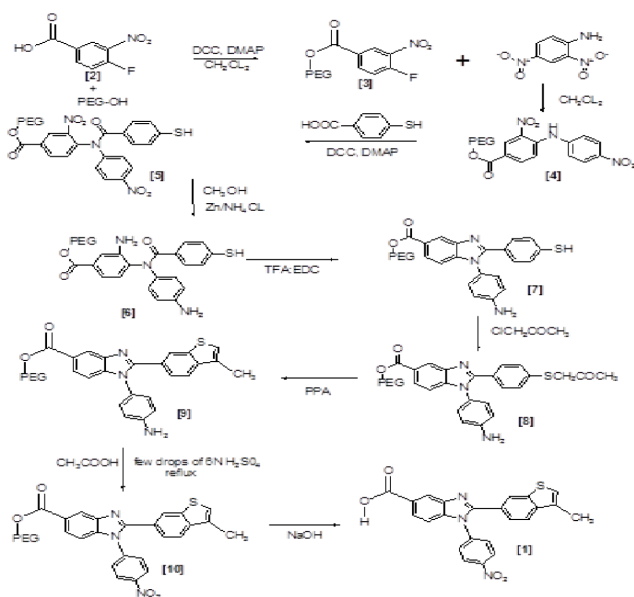
Figure 1: Scheme 1. Synthetic route for 1-(4-nitrophenyl)-2-(3-methylbenzzo[b]thiophen-6-yl)-1h-benzo[d]imidazole-5-carboxylic acid.

20 to 30 ml of DCM was added and again centrifuged to remove NH_4Cl. Product obtained [6] was treated with TFA:EDC in 1:10 ratio and then stirred for one day. Product obtained [7] was treated with 1.3moles of chloroacetone and stirred for one day. Then product [8] was reacted with 1.4 moles of PPA and stirred for one day. Product recovered [9] was treated with 1.2 moles tetra-n-propylammoniumbromate in the 20 ml of glacial acetic acid and 5ml of 6N sulfuric acid and refluxed for 6 hrs and product was filtered/washed with the help of water. Polymer was cleaved by treating the product recovered [10] 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-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid.

Note: After each step product was recovered by precipitating with diethyl ether.
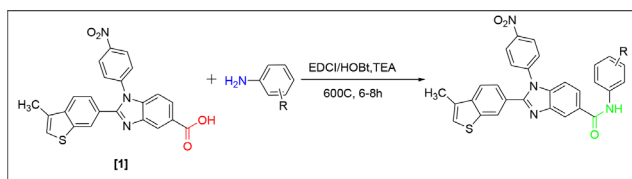
Figure 2: Scheme 2. Amide derivatives of 2-(3-methylbenzo[b] thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-car- boxylic acid.

Total 100 amide derivatives were designed using Chemdraw and docking studies were carried out, out of which superlative 23 compounds were selected based on docking score.

Amide library of 2-(3-methylbenzo[b]thiophen- 6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole- 5-carboxylic acid outlined in Scheme 2 (Figure 2):[26] 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)- 1H-benzo[d]imidazole-5-carboxylic acid(0.27 mmol, 1.0 equiv) [1] was taken in the respective RBF. To each RBF, corresponding aromatic amines (C-1 to C-23) (1.2 equiv) (Table 2) was added followed by DMF (2 ml) and TEA (54 mg, 0.54 mmol, 2.0 equiv), HOBt (0.54mmol, 2equiv) and EDCI (0.54mmol, 2equiv). RBF were reserved in magnetic stirrer with hot plate for 6 -8h for heating at 60°C with stirring. After the reaction was completed (TLC monitoring), 5-7 ml of an aqueous saturated solution of sodium hydrogen carbonate was added into each reaction RBF. The quenched reaction mixtures were either filtered by using filter paper, for liquid-liquid extraction, water (8-10 ml) was added into each reaction mixture and extracted using organic sol- vent (8-10 ml). The combined organic layer washed with water, separated/filtered using whatman filter paper, dried over anhydrous sodium sulphate and concentrated by using Rotary evaporator. Samples were purified by using column chromatography (using an optimized mix- ture of Ethyl acetate: Hexane as an eluent).

Antimicrobial activity: Minimum inhibitory concentrations (MIC):

The tube dilution method was used for the determination of microbial inhibitory potential of the synthesized derivatives against *E. coli MTCC* 45, *T. thermophilus MTCC* 1494, *S. aureus MTCC* 3160, *B. subtilis MTCC 441*, *C. albicans MTCC* 183 and *A. niger MTCC* 282 strains. Dilutions of standard and test compounds were processed in double strength nutrient broth I.P. (bac- teria) or Sabouraud dextrose broth I.P. (fungi).[27,28] The slants of *B. subtilis MTCC 441*, *S. aureus MTCC* 3160, *E. coli MTCC* 45 and *T. thermophilus MTCC* 1494, *were* incubated at the 30-35°C for 24 hr. 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 suitable agitation and then the suspensions of micro-organisms were diluted with the sterile 0.9 % NaCl solution to cfu count was adjusted by adjusting the density of micro-organism suspension to that of 0.5 McFarland standards by adding distilled water. The number of cfu was resolved 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 reported in terms of minimum inhibitory concentration (MIC).

In silico Molecular docking studies: Docking achieved by Schrodinger Glide

The Schrodinger, Inc. (New York, USA) soft- ware was exploited to study out the computa- tional calculations and docking studies. All the computational work was accomplished 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 amended form for docking, the receptor-grid files were engendered by grid-receptor generation program Glide. Grid-based Ligand Docking uses a stratified sequence of filters to fabricatepotential conformations of the ligand in the active site region of the protein receptor. At this stage, rudimentary score values and geometric filters were equipped out unlikely binding modes.[30]

Docking achieved by Autodock Vina 4.2: By using equipped PDB ID grid implemented molecular docking with .mol2-Tripos files by using Command prompt and after the compilation of docking score then confirm 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 equipped with the help of the protein preparation wizard of Schrödinger (Prepwiz) (67). PDB ID: 1wny having resolution: 1.6 A[0] and PDB ID: 1jil having resolution: 2.2 A[0] (selection criteria used was lowest resolution and availability) was exploited for the study. All the waters molecules (except those coordinated to metals) were separated except water present between the ligand and protein. The energy- controlled of the protein structure target site optimi-

Compounds	pMIC values in µM/ml					
	Bacterial Strains				Fungal Strains	
	B. subtilis	*S. aureus*	*E. coli*	*T. thermophilus*	*C. albicans*	*A. Niger*
C	1.84	1.84	1.84	2.14	1.84	1.84
C-1	2.55	2.55	2.25	2.25	2.25	2.25
C-2	1.64	1.64	1.64	1.33	1.64	1.33
C-3	1.63	1.63	1.63	1.93	1.32	1.32
C-4	1.68	1.68	1.68	1.98	1.68	1.98
C-5	2.26	2.26	2.26	2.26	2.26	1.96
C-6	2.24	2.24	2.24	2.24	1.94	1.94
C-7	1.64	1.64	1.94	1.64	1.34	1.34
C-8	1.64	1.64	1.64	1.64	1.33	1.33
C-9	1.98	1.98	1.98	1.98	1.98	1.67
C-10	2.61	2.61	2.61	2.61	2.61	2.61
C-11	1.95	1.95	1.95	1.65	1.95	1.65
C-12	1.67	1.67	1.67	1.67	1.67	1.67
C-13	1.94	1.94	1.94	1.63	1.63	1.63
C-14	1.94	1.94	1.94	1.64	1.94	1.64
C-15	1.68	1.68	1.68	1.68	1.68	1.68
C-16	1.94	1.94	1.94	1.64	1.64	1.64
C-17	1.96	1.96	1.96	1.96	1.96	1.96
C-18	2.28	2.28	2.28	1.98	1.98	1.98
C-19	2.54	2.54	2.54	2.54	1.93	1.93
C-20	1.64	1.64	1.64	1.34	1.04	1.04
C-21	1.67	1.67	1.67	1.37	1.67	1.67
C-22	1.68	1.68	1.38	1.38	1.02	1.02
C-23	2.27	2.27	2.27	2.27	2.27	2.27
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

Table 2: pMIC values (µM/mL) of synthesized derivatives against different standard microbial strains.

zation of targeted protein *aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase* were completed using OPLS-2005 as force field.

Ligand preparation: The three-dimensional structural library was generated using the Chem-draw software and then save as.mol2-Tripos. The 3-D structures of the selected ligands were antici-pated for energy minimization using the LigPrep tool from Schrodinger to correct the coordinates, ionization, stereochemistry and tautomeric structure to gain the suitable conformation through the addition or deletion of hydrogen bonds. The ligands prepared after energy minimization were recycled for molecular dock-ing studies.

RESULTS AND DISCUSSION

Physical and analytical data of synthesized derivatives are presented in Table 1.

Spectral Data: C: 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid:MS: 430.45 (M^{+1}). ^{1}H NMR: δ 13.02 (s, 1H), 8.50 (s, 1H), 8.28 (d, J = 7.8 Hz, 2H), 8.16 (s, 1H), 8.04 (d, J = 10.2 Hz, 3H), 7.88 (d, J = 7.9 Hz, 2H), 7.83 (d, J = 8.1 Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 167.55, 155.26, 144.34, 143.96, 142.17, 139.67, 139.53, 138.26, 138.07, 131.30, 130.89, 128.40, 127.71, 127.29, 127.21, 126.67, 124.49, 124.32, 122.42, 122.08, 121.69, 108.82, 14.17.

C-1: 2-(3-methylbenzo[b]thiophen-6-yl)-N-(naphthalen-1-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 555.14 (M^{+1}). ^{1}H NMR: δ 10.39 (s, 1H), 8.46 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.16 (s, 1H), 8.04 (d, *J* = 8.4 Hz, 2H), 7.92 (t, *J* = 10.8 Hz, 3H), 7.85 (t, *J* = 8.5 Hz, 3H), 7.68 (d, *J* = 7.4 Hz, 1H), 7.63 (d, *J* = 7.6 Hz, 1H), 7.54 (t, *J* = 7.2 Hz, 2H), 7.28 (t, *J* = 8.0 Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 166.76, 158.19, 158.11, 155.67, 155.59, 154.00, 147.11, 143.01, 139.67, 139.53, 137.23, 136.02, 130.89, 130.73, 130.48, 130.02, 128.10, 127.94, 127.29, 124.50, 124.32, 123.86, 122.43, 121.87, 121.32, 119.86, 118.96, 111.51, 111.48, 111.31, 111.28, 109.11, 14.17.

C-2: N-(2,3-difluorophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 541.11 (M^{+1}). ^{1}H NMR: δ 10.94 (s, 1H), 8.43 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.23 (s, 1H), 8.04 (d, *J* = 10.1 Hz, 1H), 7.98 (d, *J* = 9.1 Hz, 2H), 7.88 (t, *J* = 11.1 Hz, 3H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.24 (s, 1H), 7.15 (q, *J* = 7.6 Hz, 1H), 6.97 (q, *J* = 7.2 Hz, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 164.82, 155.26, 144.34, 143.96, 141.98, 139.67, 139.53, 138.26, 136.33, 135.86, 131.84, 131.30, 130.89, 130.80, 130.45, 128.40, 127.71, 127.37, 127.29, 126.64, 125.18, 124.49, 124.32, 123.81, 122.42, 122.08, 119.78, 109.57, 14.17.

C-3: N-(2,4-dimethylphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 533.16 (M^{+1}). ^{1}H NMR: δ 9.35 (s, 1H), 8.45 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.23 (s, 1H), 8.03 (d, *J* = 9.2 Hz, 1H), 7.98 (d, *J* = 7.5 Hz, 1H), 7.92 (t, *J* = 11.7 Hz, 3H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.24 (s, 1H), 7.04 (d, *J* = 6.8 Hz, 1H), 6.99 (d, *J* = 9.9 Hz, 2H), 2.40 (s, 3H), 2.20 (d, *J* = 5.7 Hz, 6H). ^{13}C NMR: δ 164.84, 154.00, 149.94, 147.11, 144.42, 143.01, 139.67, 139.53, 137.23, 136.02, 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, 119.73, 115.38, 112.31, 109.11, 56.01, 34.53, 31.27, 14.17.

C-4: N-(2,4-dinitrophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 595.10 (M^{+1}). ^{1}H NMR: δ 11.61 (s, 1H), 8.78 (s, 1H), 8.58 (d, *J* = 8.5 Hz, 1H), 8.47 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 2H), 8.24 (s, 1H), 8.12 – 8.01 (m, 2H), 7.99 (d, *J* = 6.0 Hz, 2H), 7.90 (d, *J* = 7.8 Hz, 2H), 7.73 (d, *J* = 7.9 Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ^{13}C NMR: δ 167.04, 154.00, 147.11, 143.01, 139.67, 139.53, 137.23, 136.02, 134.96, 133.66, 132.52, 130.89, 130.73, 130.48, 130.02, 129.15, 127.94, 127.29, 124.50, 124.32, 123.86, 122.43, 121.87, 121.32, 119.86, 109.11, 20.74, 17.51, 14.17.

C-5: N-(2,5-dichlorophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 574.04 (M^{+1}). ^{1}H NMR: δ 9.76 (s, 1H), 8.43 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 2H), 8.24 (s, 1H), 8.04 (d, *J* = 9.3 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 1H), 7.89 (d, *J* = 8.2 Hz, 3H), 7.84 (s, 1H), 7.72 (d, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.24 (s, 1H), 7.08 (d, *J* = 8.0 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.

C-6: N-(2,5-difluorophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 541.11 (M^{+1}). ^{1}H NMR: δ 10.72 (s, 1H), 8.44 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.24 (s, 1H), 8.04 (d, *J* = 9.2 Hz, 1H), 7.98 (d, *J* = 8.3 Hz, 2H), 7.89 (d, *J* = 8.6 Hz, 3H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.34 – 7.26 (m, 1H), 7.24 (s, 1H), 6.95 (t, *J* = 7.9 Hz, 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.

C-7: N-mesityl-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 547.18 (M^{+1}). ^{1}H NMR: δ 9.48 (s, 1H), 8.44 (s, 1H), 8.28 (d, *J* = 7.7 Hz, 2H), 8.23 (s, 1H), 8.04 (d, *J* = 9.2 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 3H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.24 (s, 1H), 6.88 (s, 2H), 2.41 (s, 3H), 2.27 (s, 3H), 2.08 (s, 6H). ^{13}C NMR: δ 164.74, 155.26, 144.34, 143.96, 141.98, 141.85, 139.67, 139.53, 138.26, 136.33, 131.30, 130.89, 130.45, 128.40, 128.05, 127.85, 127.71, 127.29, 124.85, 124.82, 124.49, 124.32, 123.81, 122.42, 122.08, 119.78, 117.62, 117.54, 110.70, 110.62, 109.57, 14.17.

C-8: N-(2,6-difluorophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 541.11 (M^{+1}). ^{1}H NMR: δ 9.58 (s, 1H), 8.45 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.24 (s, 1H), 8.04 (d, *J* = 9.3 Hz, 1H), 7.98 (d, *J* = 7.8 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 3H), 7.72 (d, *J* = 8.1 Hz, 1H), 7.22 (d, *J* = 13.9 Hz, 2H), 7.01 (d, *J* = 8.3 Hz, 2H), 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.

C-9: N-(2-bromo-4-methylphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 598.05 (M^{+1}). ^{1}H NMR: δ 9.76 (s, 1H), 8.44 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 2H), 8.24 (s, 1H), 8.04 (d, *J* = 9.3 Hz, 1H), 7.98 (d, *J* = 7.8 Hz, 1H), 7.91 (dd, *J* = 15.6, 7.4 Hz, 4H), 7.72 (d, *J* = 7.9 Hz, 1H),

7.45 (s, 1H), 7.24 (s, 1H), 7.17 (d, *J* = 7.4 Hz, 1H), 2.40 (s, 3H), 2.28 (s, 3H). ¹³C NMR: δ 164.83, 155.26, 144.34, 143.96, 141.98, 139.67, 139.53, 138.26, 136.33, 135.73, 135.40, 132.61, 131.30, 130.89, 130.45, 128.64, 128.40, 127.71, 127.29, 124.49, 124.32, 123.81, 122.42, 122.26, 122.08, 119.78, 114.48, 109.57, 20.65, 14.17.

C-10: N-(2-bromo-5-nitrophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 629.02 (^(M+1)). ¹H NMR: δ 10.67 (s, 1H), 8.92 (s, 1H), 8.44 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.16 (s, 1H), 8.04 (d, *J* = 8.2 Hz, 2H), 7.98 – 7.89 (m, 2H), 7.88 (d, *J* = 6.5 Hz, 2H), 7.84 (d, *J* = 9.7 Hz, 2H), 7.24 (s, 1H), 2.41 (s, 3H). ¹³C NMR: δ 164.77, 155.26, 146.82, 144.34, 143.96, 141.98, 139.67, 139.53, 138.26, 137.60, 136.33, 135.00, 131.30, 130.89, 130.45, 128.40, 127.71, 127.29, 124.49, 124.32, 123.81, 122.42, 122.08, 119.78, 119.02, 118.16, 115.87, 109.57, 14.17.

C-11: N-(2-chloro-4-methylphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 554.11 (M^+1). ¹H NMR: δ 10.01 (s, 1H), 8.43 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.24 (s, 1H), 8.04 (d, *J* = 9.1 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 1H), 7.97 – 7.88 (m, 2H), 7.87 (d, *J* = 9.9 Hz, 2H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.46 (s, 1H), 7.24 (s, 1H), 7.08 (d, *J* = 7.8 Hz, 1H), 2.40 (s, 3H), 2.28 (s, 3H). ¹³C NMR: δ 164.88, 155.26, 144.34, 143.96, 141.98, 139.67, 139.53, 138.26, 136.41, 136.33, 134.07, 131.30, 130.89, 130.45, 129.78, 128.40, 128.15, 127.71, 127.29, 124.49, 124.32, 124.08, 123.81, 122.98, 122.42, 122.08, 119.78, 109.57, 20.35, 14.17.

C-12: N-(2-chloro-4-nitrophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 585.07 (M^+1). ¹H NMR: δ 10.67 (s, 1H), 8.43 (s, 1H), 8.34 (s, 1H), 8.26 (dd, *J* = 16.8, 7.4 Hz, 4H), 8.13 (d, *J* = 8.6 Hz, 1H), 8.04 (d, *J* = 9.2 Hz, 1H), 7.98 (d, *J* = 7.8 Hz, 1H), 7.88 (t, *J* = 9.2 Hz, 3H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ¹³C NMR: δ 164.88, 155.26, 144.34, 143.96, 141.98, 140.00, 139.85, 139.67, 139.53, 138.26, 136.33, 131.30, 130.89, 130.45, 128.40, 127.71, 127.29, 125.90, 124.63, 124.49, 124.32, 123.94, 123.81, 122.42, 122.08, 121.64, 119.78, 109.57, 14.17.

C-13: N-(2-chlorophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 540.08 (M^+1). ¹H NMR: δ 10.13 (s, 1H), 8.43 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.15 (s, 1H), 8.04 (d, *J* = 8.8 Hz, 2H), 7.91 (s, 1H), 7.87 (d, *J* = 13.4 Hz, 3H), 7.78 (d, *J* = 8.1 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.38 (t, *J* = 7.8 Hz, 1H), 7.24 (s, 1H), 6.97 (t, *J* = 7.8 Hz, 1H), 2.41 (s, 3H). ¹³C NMR: δ 164.88, 155.26, 144.34, 143.96, 141.98, 139.67, 139.53, 138.26, 136.33, 135.67, 131.30, 130.89, 130.45, 129.37, 128.40, 128.01, 127.71, 127.29, 127.28, 125.85, 124.49, 124.32, 123.81, 122.92, 122.42, 122.08, 119.78, 109.57, 14.17.

C-14: 2-(3-methylbenzo[b]thiophen-6-yl)-N-(2-nitrophenyl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 550.11 (M^+1). ¹H NMR: δ 11.19 (s, 1H), 8.44 (d, *J* = 7.8 Hz, 2H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.15 (s, 1H), 8.04 (t, *J* = 8.4 Hz, 2H), 7.90 (d, *J* = 8.6 Hz, 4H), 7.84 (d, *J* = 7.6 Hz, 1H), 7.69 (t, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 11.2 Hz, 2H), 2.41 (s, 3H). ¹³C NMR: δ 164.42, 155.26, 144.34, 143.96, 141.98, 139.67, 139.53, 138.26, 136.33, 135.80, 134.88, 131.30, 130.89, 130.45, 128.40, 127.87, 127.71, 127.29, 125.76, 124.49, 124.32, 123.81, 122.80, 122.42, 122.08, 119.78, 118.88, 109.57, 14.17.

C-15: 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-N-o-tolyl-1H-benzo[d]imidazole-5-carboxamide: MS: 519.14 (M+1). ¹H NMR: δ 9.45 (s, 1H), 8.40 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.23 (s, 1H), 8.03 (d, *J* = 9.0 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 1H), 7.91 (t, *J* = 9.7 Hz, 3H), 7.73 (d, *J* = 7.9 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.36 (t, *J* = 7.7 Hz, 1H), 7.22 (d, *J* = 12.0 Hz, 3H), 2.40 (s, 3H), 2.20 (s, 3H). ¹³C 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.

C-16: 2-(3-methylbenzo[b]thiophen-6-yl)-N-(3-nitrophenyl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 550.11 (M^+1). ¹H NMR: δ 10.14 (s, 1H), 8.72 (s, 1H), 8.41 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.17 – 8.09 (m, 2H), 8.04 (d, *J* = 8.8 Hz, 3H), 7.91 (t, *J* = 10.2 Hz, 3H), 7.85 (d, *J* = 7.7 Hz, 1H), 7.62 (t, *J* = 8.3 Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ¹³C 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.

C-17: 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-N-(3-(trifluoromethyl)phenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 550.11 (M+1). ¹H NMR: δ 9.39 (s, 1H), 8.41 (s, 1H), 8.28 (d, *J* = 7.9 Hz, 2H), 8.18 (s, 1H), 8.07 – 7.98 (m, 3H), 7.88 (dd, *J* = 16.6, 8.5 Hz, 4H), 7.79 (d, *J* = 7.9 Hz, 1H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.24 (s, 1H), 2.41 (s, 3H). ¹³C 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.

C-18: N-(4-bromo-2-methylphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 598.05 (M^+1). ¹H NMR: δ 9.58 (s, 1H), 8.39 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 2H), 8.23

(s, 1H), 8.03 (d, *J* = 9.3 Hz, 1H), 7.98 (d, *J* = 7.8 Hz, 1H), 7.90 (d, *J* = 7.7 Hz, 3H), 7.73 (d, *J* = 7.7 Hz, 1H), 7.64 (d, *J* = 7.1 Hz, 1H), 7.45 (s, 1H), 7.29 (d, *J* = 6.9 Hz, 1H), 7.24 (s, 1H), 2.40 (s, 3H), 2.29 (s, 3H). ^{13}C NMR: δ 165.00, 155.26, 144.34, 143.96, 141.98, 139.67, 139.53, 138.26, 136.33, 135.12, 132.71, 131.30, 130.89, 130.84, 130.45, 129.90, 128.40, 127.71, 127.29, 124.49, 124.32, 123.81, 123.49, 122.42, 122.08, 119.78, 116.93, 109.57, 17.98, 14.17.

C-19: N-(4-fluoro-3-methylphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 537.14 (M+1). ^{1}H NMR: δ 9.46 (s, 1H), 8.41 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 2H), 8.23 (s, 1H), 8.04 (d, *J* = 9.3 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 1H), 7.88 (dd, *J* = 14.8, 8.6 Hz, 3H), 7.72 (d, *J* = 8.6 Hz, 2H), 7.24 (s, 1H), 7.19 (d, *J* = 6.4 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 2.41 (s, 3H), 2.25 (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.

C-20: 2-(3-methylbenzo[b]thiophen-6-yl)-N,1-bis(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 550.11 (M^{+1}). ^{1}H NMR: δ 8.41 (s, 1H), 8.36 (s, 1H), 8.29 (t, *J* = 6.5 Hz, 4H), 8.15 (s, 1H), 8.02 (d, *J* = 8.7 Hz, 4H), 7.93 – 7.84 (m, 3H), 7.84 (s, 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.

C-21: N-(5-tert-butyl-2-methoxyphenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 591.20 (M^{+1}). ^{1}H NMR: δ 9.94 (s, 1H), 8.42 (s, 1H), 8.28 (d, *J* = 7.7 Hz, 2H), 8.23 (s, 1H), 7.96 (ddd, *J* = 35.4, 17.6, 8.3 Hz, 6H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.24 (s, 1H), 7.14 (d, *J* = 7.0 Hz, 1H), 6.91 (d, *J* = 7.1 Hz, 1H), 3.83 (s, 3H), 2.41 (s, 3H), 1.31 (s, 9H). ^{13}C NMR: δ 164.86, 164.74, 153.79, 151.26, 151.06, 144.47, 144.37, 142.99, 142.22, 141.85, 139.67, 139.53, 135.81, 130.89, 130.72, 130.44, 128.05, 127.85, 127.29, 125.19, 124.85, 124.82, 124.79, 124.50, 124.32, 123.86, 122.43, 119.86, 117.62, 117.54, 110.70, 110.62, 109.13, 14.17.

C-22: N-benzyl-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 519.14 (M+1). ^{1}H NMR: δ 8.46 (s, 1H), 8.32 – 8.21 (m, 3H), 8.14 (t, *J* = 9.1 Hz, 1H), 8.00 (dd, *J* = 17.3, 8.6 Hz, 2H), 7.91 – 7.81 (m, 3H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.32 (s, 4H), 7.24 (s, 1H), 4.65 (d, *J* = 8.9 Hz, 2H), 2.41 (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.

C-23: N-(4-chloro-2-nitrophenyl)-2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxamide: MS: 519.14 (M^{+1}). ^{1}H NMR: δ 11.25 (s, 1H), 8.43 (s, 1H), 8.28 (d, *J* = 7.8 Hz, 2H), 8.22 (s, 1H), 8.05 (d, *J* = 11.9 Hz, 2H), 7.98 (d, *J* = 7.8 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 3H), 7.73 (d, *J* = 7.6 Hz, 1H), 7.63 (d, *J* = 7.4 Hz, 1H), 7.56 (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, 136.02, 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.

Antimicrobial activity

Minimum inhibitory concentrations (MIC)

The synthesized derivatives were evaluated for their *in vitro* antibacterial activity against *S. aureus, B. subtilis, E. coli, T. thermophilus* and antifungal activity against *C. albicans* and *A. niger*. Antimicrobial activity of the synthesized compounds was concluded by the tube dilution method. From the reported pMIC values, it was noticed that compound C-1, C-10 and C-19 was found to be most active against all the strains having pMIC C-1: 2.55, 2.55, 2.25, 2.25, 2.25, 2.25 and C-10: 2.61, 2.61, 2.61, 2.61, 2.61, 2.61 and C-19: 2.54, 2.54, 2.54, 2.54, 1.93, 1.93μM/ml value. Compound C-3 was found to be least antibacterial active and Compound C-22 was found tobe least antifungal active in the series. In general, the results of MIC studies exposed that the synthesized derivatives have bacteriocidal and fungicidal potential as comparted to standards Cefadroxil, Ofloxacin and Fluconazole. Here, Figure 3 graphically represent the pMIC, Table 2 represent the pMIC values of compounds with comparision to standards.
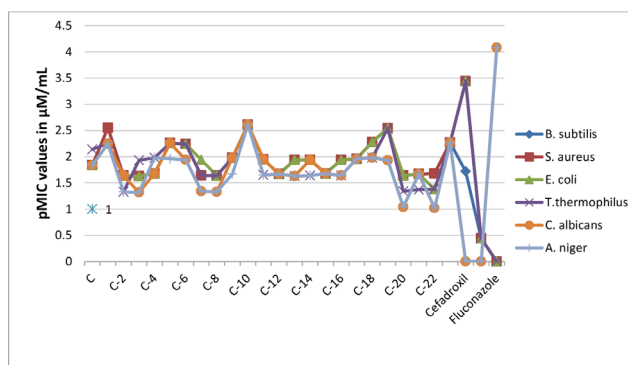
Figure 3: Antimicrobial activity (pMIC in µM/mL) of synthesized derivatives.

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
C-1	-9.0	-9.9	-5.144	-5.144	-5.328	-5.328
C-2	-9.0	-9.5	-4.867	-4.867	-4.981	-4.981
C-3	-8.8	-9.4	-4.2	-4.2	-4.658	-4.658
C-4	-8.5	-8.7	-4.526	-4.526	-4.861	-4.861
C-5	-8.8	-8.8	-4.299	-4.299	-4.637	-4.637
C-6	-9.0	-9.6	-4.683	-4.683	-4.997	-4.997
C-7	-9.0	-9.5	-4.354	-4.354	-4.698	-4.698
C-8	-8.5	-9.6	-4.762	-4.762	-4.891	-4.891
C-9	-8.8	-9.4	-4.596	-4.596	-4.965	-4.965
C-10	-9.2	-9.4	-5.007	-5.007	-5.369	-5.369
C-11	-8.7	-9.4	-4.563	-4.563	-4.968	-4.968
C-12	-8.8	-9.4	-4.231	-4.231	-4.885	-4.885
C-13	-8.5	-9.3	-4.532	-4.532	-4.986	-4.986
C-14	-9.2	-9.2	-4.883	-4.883	-5.007	-5.007
C-15	-8.6	-8.7	-4.632	-4.632	-4.862	-4.862
C-16	-8.6	-8.9	-4.611	-4.611	-4.882	-4.882
C-17	-9.1	-9.6	-4.74	-4.74	-4.967	-4.967
C-18	-9.1	-9.4	-4.499	-4.499	-4.986	-4.986
C-19	-9.0	-9.6	-5.184	-5.184	-5.264	-5.264
C-20	-9.1	-9.2	-4.477	-4.477	-4.821	-4.821
C-21	-8.7	-8.7	-4.583	-4.583	-4.565	-4.565
C-22	-9.0	-9.3	-4.093	-4.093	-4.284	-4.284
C-23	-9.3	-9.1	-4.268	-4.268	-4.199	-4.199
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

Table 3: Docking Score of Synthesized Derivatives.

Molecular Docking research: Newly designed and synthesized molecules/ligands along with standards were studied for their molecular docking behavior with the help of Schrodinger's Maestro package docking software and AutoDock Vina 4.2, Table 3. Ligands were docked was into 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 exposed that synthesized ligands bind within catalytic cavity firmly via Hydrogen bond formation, 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 C-1, C-10 and C-19.

In compound C-1, 1WNY PDB, Visual inspection of the pose of structure umbrageous into the inhibitor binding cavity revealed that ligand is inserted in to "aromatic case" framed by PHE 324, TRP 232, THR 230, THR 233, VAL 318, GLY 316, THR 315, TRP 227, ASP 313 and the FAD aromatic ring of the protein (Figure 4) and 1JIL PDB, Visual inspection of the pose of structure umbrageous into the inhibitor binding cavity revealed that ligand is inserted in to "aromatic case" framed by LYS 231, PHE 232, VAL 224, LEU 223, ASP 195, GLY 83, LY 384, ARG 88, GLY 49, ALA 43, HIS 47 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 ALA 43 having value 3.063 (Figure 5).

In compound C-10, 1WNY PDB, Visual inspection of the pose of structure umbrageous into the inhibitor binding cavity revealed that ligand is inserted in to "aromatic case" framed by PRO 294, TYR 301, LYS 212, PRO 288, PHE 302, GLU 288, TYR 335, LYS 299 and the FAD aromatic ring of the protein (Figure 6) and
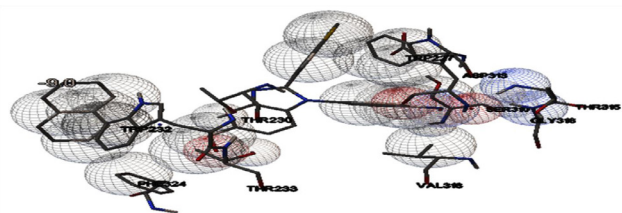
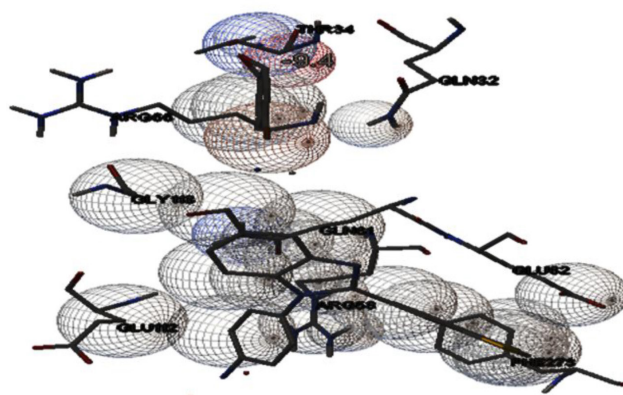**Figure 4: Docking Result of C-1 with PDB 1WNY.**
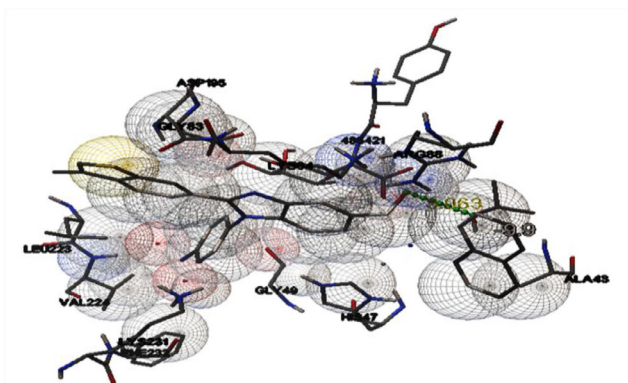

Figure 5: Docking Result of C-1with PDB 1JIL.

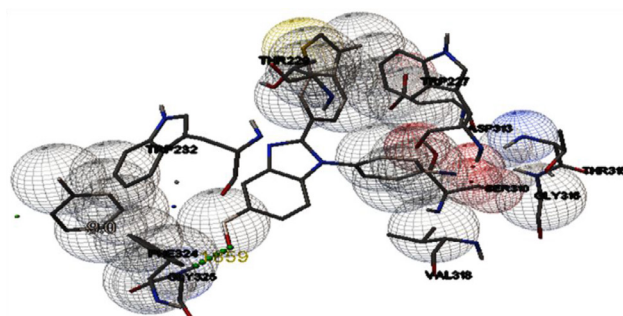**Figure 6: Docking Result of C-10 with PDB 1WNY.**

Figure 7: Docking Result of C-10 with PDB 1JIL.
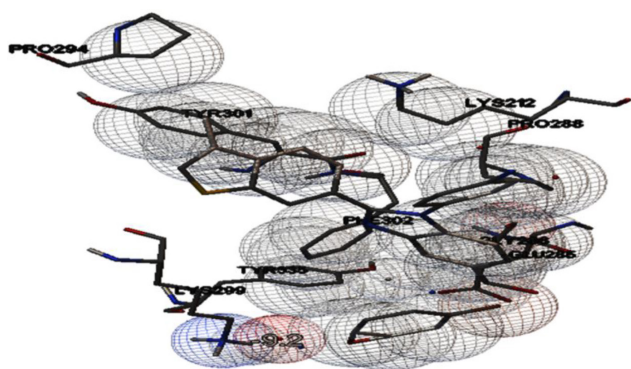

Figure 8: Docking Result of C-19 with PDB 1WNY.

1JIL PDB, Visual inspection of the pose of structure umbrageous into the inhibitor binding cavity revealed that ligand is inserted into "aromatic case" framed by THR 34, GLN 32, ARG 66, GLY 118, GLN 81, GLU 62, GLU 112, ARG 58, PHE 273 and the FAD aromatic ring of the protein (Figure 7).

In compound C-19, 1WNY PDB, Visual inspection of the pose of structure umbrageous into the inhibitor binding cavity revealed that ligand is inserted in to "aromatic case" framed by VAL 318, GLY 318, SER 310, THR 316, ASP 313, TRP 217, THR 229, TRF 232, PHE 324, GLY 325 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 GLY 325 having value 1.859 (Figure 8) and 1JIL PDB, Visual inspection of the pose of structure umbrageous into the inhibitor binding cavity revealed that ligand is inserted in to "aromatic case" framed by ALA 43, HIS 47, GLY 49, PHE 232, LYS 231, VAL 224, LEU 223, ASP 195, LYS 84, ARG 88 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 protein having value 3.082 (Figure 9). Docking studies revealed that hydrogen bond interactions fix the ligands firmly and tightly in the active site.

SAR (structure-activity relationship) studies

1. Amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid of good biological activity as compared with compound C.

2. Substiution of Bromo group (electron withdrawing group) at 2 and Chloro at 4 (electron withdrawing group) shows the best antimicrobial activity among the series i.e. compound C-10.

3. Substitution of naphthalene ring shows second best antimicrobial activity among the series i.e. compound C-1.
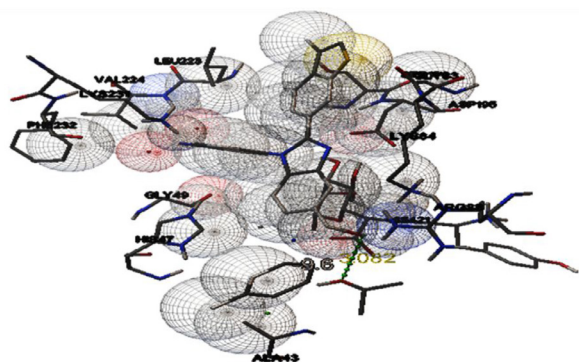
Figure 9: Docking Result of C-19 with PDB 1JIL.

4. Substiution of methyl group at 3 and Nitro at 4 shows the best antibacterial activity but decreases antifungal activity i.e. compound C-19.

5. Substiution of Nitro at 2(electron withdrawing group) and chloro(electron withdrawing group) at 4 shows the good antimicrobial activity i.e. compound C-23.

6. Di-nitro group (High electron withdrawing group) at 2,4 position shows poor antimicrobial activity among the series i.e. compound C-4.

7. Substiution of Chloro group at 2,5 shows the good antimicrobial activity i.e. compound C-5.

8. Substiution of di-floro at 2,3 and 2,6 shows the moderate antimicrobial activity i.e. compound C-2, C-8 but substiution at 2,5 shows good activity i.e. compound C-6.

9. substiution of bromo at 2 and electron donating group at position at 4, vice versa shows good antimicrobial activity i.e. compound C-18 and C-9.

10. Substiution 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 C-11, C-18.

11. Substiution of one electron withdrawing group at different position of aromatic ring shows moderate activity i.e. compound C-13, C-14, C-16.

12. Antifungal activity decreases when aniline is replaced by phenylmethanamine shows week among the series i.e. compound C-22.

Antibacterial activity decreases when methyl group substituted at position 2,4 shows week among the series i.e. compound C-3.

CONCLUSION

In-vitro outcome exposed that a vast number of synthesized compounds were found to havetremendous antimicrobial activity. Among the library, C-1, C-10 and C-19 possessing admirable dock score by both software: AutoDock Vina/Schrodinger's maestro against 1wny.PDBQT:1jil.PDBQT is -9.0:-9.9/ -5.144:-5.328,-9.2:-9.4/ -5.007:-5.369, -9.0:-9.6/ -5.184:-5.264 and as compared to standard drugs dock score -10.2:-10.6/-5.847:-5.895, -10.2:-10.6/-5.847:-5.895, -9.8:-9.9/ -6.323:-6.131.C-1, C-10 and C-19 derivatives also presented good MIC as compared to standard by tube dilution method. *In- silico* studies played an symbolic role in designing the potent ligands against *aminoacyl-tRNA synthetase (AaRS)* and *tyrosyl-tRNA synthetase* enzymes as well as interpreted/studyingthe binding pattern of designed/synthesized ligand within active pocket and excellent antimicrobial compounds can be used for *in vivo* studies for future. *In-silico* studies and *in vitro* results comply with each other.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

[13]**C NMR:** Carbon 13 Nuclear magnetic resonance; [1]**H 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:** Trifluro acetic acid; **TLC:** Thin layer chromatography; **UV:** Ultra violet.
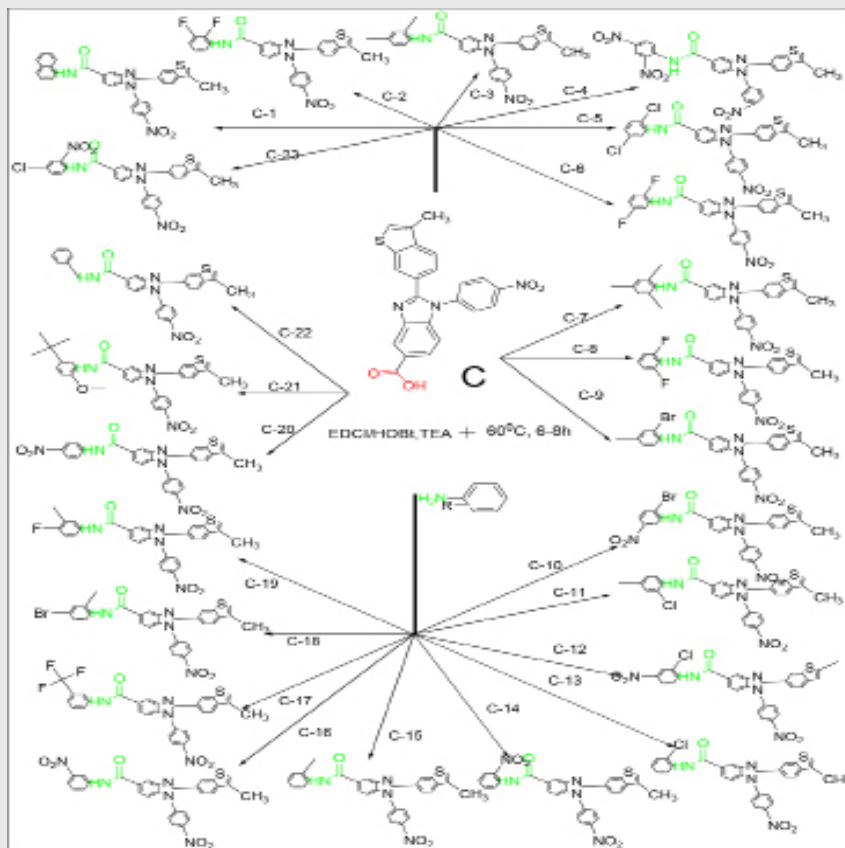
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SUMMARY

There has been a repetitive fight among humans and the multitude of micro-organisms that origin infection and disease. Infectious diseases are accountable for large figure of deaths in the world population. The casual use and overconsumption of antibiotics has led to the emergence of antibiotic-resistant strains. Therefore, there is a vital need for the growth of new antimicrobial drugs. In the study, we have synthesized and investigated antimicrobial activities of novel derivatives with molecular docking studies. In this, amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-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 precedence to antimicrobial action. Studies were accomplished on a designed library of amide derivatives with the help of docking softwares i.e. AutoDock Vina 4.2 and Schrodinger's maestro package against crystal structure of enzymes (PDB ID: 1wny.PDBQT and 1jil.PDBQT). Based upon their dock score, superlative 23 focused amide derivatives of 2-(3-methylbenzo[b]thiophen-6-yl)-1-(4-nitrophenyl)-1H-benzo[d]imidazole-5-carboxylic acid were synthesized and further investigated for *in vitro* antimicrobial. *In-silico* studies and *in vitro* results comply with each other.

PICTORIAL ABSTRACT