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***IN SILICO* DESIGN AND SYNTHESIS OF N-HETEROCYCLIC COMPOUNDS AS ANTI-CANCER AND ANTI-MICROBIAL AGENTS**

Mangala K J¹, Nikam Anil Ishwar², Surya Pratap Singh³, M.Keerthi⁴, Pavankumar D Chopade⁵, Kamalesh Tripathi⁶, Krishna Chandra Panda⁷, Priyanka Kamaria^{8*}

1. Assistant Professor, Department of Biotechnology, Government First Grade College Ramanagara, BM Road, Ramanagara-562159. Karnataka, India.
2. Assistant Professor in Chemistry & HOD, SKN Sinhgad College of Engineering, Pandharpur Dist: Solapur, Maharashtra. PIN Code: 413304
3. Research Scientist-1, Multidisciplinary Research Unit, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Pin-206130, UP
4. Assistant Professor, MB School of Pharmaceutical Sciences, Sree sainath nagar, A. Rangampeta, Tirupati, Pincode:517102 Andhrapradesh.
5. Assistant Professor, Oriental College Of Pharmacy Sanpada Navi Mumbai Maharashtra India 400703
6. Associate professor, Dr. APJ Abdul Kalam Technical University Lucknow
7. Associate Professor, Roland Institute of Pharmaceutical Sciences, Khodasingi, Berhampur, Odisha, Pin-760010
8. Assistant Professor, KLE College Of Pharmacy, Bengaluru Affiliated to KLE Academy of Higher Education And Research, Belgaum, Rajaji Nagar, 2nd block, Bengaluru, Pin-560010

Corresponding Author: Dr. Priyanka Kamaria

Designation and Affiliation: Assistant Professor, KLE College Of Pharmacy, Bengaluru Affiliated to KLE Academy of Higher Education And Research, Belgaum, Rajaji Nagar, 2nd block, Bengaluru, Pin-560010

Email id: kamaria.priyanka@gmail.com

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ABSTRACT: The present work aims to design, synthesize some novel N containing heterocyclic derivatives and to perform in vitro evaluation of their anticancer and antimicrobial activity. In silico modelling of N-heterocyclic derivatives was carried out using various Softwares such as, ACD Labs ChemsSketch, Molinspiration, PASS(Prediction of activity spectra for substances) and Schrodinger Glide XP (Grid based ligand docking with energetics).

Nine derivatives compound (BT-A1, A2, A3; BT-B1, B2, B3; BT-C1, C2, C3) were designed. The designed molecules with required physicochemical properties, drug likeness and obeying Lipinski's rule of three were selected for the synthesis. The synthesized compounds were subjected to TLC, melting point determination, FTIR and mass spectral studies. All the synthesized compounds showed characteristic peak in FTIR and Mass spectroscopic studies. As per the PASS score and GLIDE score the compounds were selected for biological studies like anticancer and antimicrobial activity. These results were useful for further investigation in the future. All the selected derivatives showed better activity, when compared with standard drugs.

INTRODUCTION:

The progress of drug resistance among human pathogens continues to be a serious health problem and leads to the importance of discovering and developing new drugs and synthesizing new compounds with active pharmacophore. The incidence of antimicrobial resistance (AMR) is increasing and a growing serious threat to public health burden worldwide [1]. In the recent few years, the expression of resistance related complications and therapeutic difficulties by microorganisms such as *Staphylococcus aureus*, *Enterococcus faecium*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis* (*M. tuberculosis*), nontuberculous (atypical) mycobacteria, and gram-positive and gram-negative bacteria against the marketed antibacterial agents, therefore, there is a great need for providing an effective approach in drug design of novel antimicrobial molecules and modification of their mechanisms against microbes [2, 3]. A number of nitrogen containing heterocyclic groups are present in many of the biologically active compounds. Hence, they were subject of interest to synthesize and study some new heterocyclic derivatives. This along with tremendous success of synthetic heterocyclic drugs has attracted medicinal chemist's attention to this field. Designing of new hetero compounds are done by incorporating heterocyclic moiety into lead molecule. [4, 5, 6]

The nucleus selected for the present work is a combination of two hetero aromatic molecules benzimidazole and imidazole. [7] There are numerous reports in the literature about the wide spectrum of biological activities possessed by these lead molecules. The benzimidazole moiety is a versatile lead molecule in pharmaceutical development. It has a wide range of biological activities such as anti-inflammatory, antioxidant, antimicrobial [8, 9], anthelmintic, antitubercular [10, 11], antiviral [12, 13] etc. The synthetic versatility of benzimidazole has lead to the extensive use of this compound in organic synthesis.

In silico molecular modeling studies are the most important step in drug design. The in silicomodeling of all the proposed derivatives were carried out by using different computational software in order to predict the physiological and biological parameters. The

Softwares used for in silico studies includes Molinspiration, ACD lab Chems sketch and Marvin sketch, PASS and Schrodinger.

MATERIALS AND METHODS:

Molecular Docking: Molecular docking is usually performed between a small molecule and a target macromolecule. This is often referred to as ligand–protein docking. Docking is the computational simulation of a candidate ligand binding to a receptor. During docking a pose is generated, scored and compared with a previous pose.

Methodology of Docking:

Target Identification and Retrieval: Crystallographic structures of the targets of interests were obtained from PDB (Protein Data Bank) and saved in standard 3D coordinate format. The targets used for the present study are membrane protein of Mycobacterium tuberculosis (2OAR) and Dihydrofolate reductase (3I8A)

Protein Preparation: The procedure started with a protein and a co-crystallized ligand. It was finished with a partially optimized protein-ligand complex, to which hydrogens were added subjected to protonation states for ionizable residues, modification of tautomeric forms and the repositioning of the reorientable hydrogens.

- The first step was to prepare the co-crystallized ligands by correctly defining multiple bonds and adding hydrogens. Normally, proteins are provided without attached hydrogens. When hydrogens are present, all are deleted except those in peptide bond.
- The second step was to neutralize residues that did not participate in the salt bridges and that were more than a specified distance from nearest ligand atom. The script also set the tautomeric state which was assumed to be neutral, by considering potential metal ligation and the hydrogen interactions.
- The third step was to preprocess the receptor before grid preparation. This is necessary as the judgment made by the preparation procedure need not to be correct always.
- In the fourth step the optimization of the protein was carried out by adding hydrogens to the protein, to any cofactors and to any added structural waters and the final step carried out series of restrained minimizations on the protein-ligand complex. [14]

Ligprep: Ligprep generates energy minimized 3D molecular structures. It is used for the versatile generation of accurate 3D molecular models. Ligprep went far beyond simple 2D to 3D structure conversions by including tautomeric, stereo chemical and ionization variations as well as energy minimization and flexible filters to generate fully customized ligand libraries that were optimized for further computational analyses.

Docking: The generated structures of various conformations of drug like molecules using Ligprep was then docked in to the binding site of receptor after generating a receptor grid around the site using glide. The docking was conducted using XP GLIDE (Extra Precision). [15]

Procedure for Synthesis:

Synthesis of Benzimidazoles: Monochloroacetic acid / Lactic acid / p- Chlorobenzoic acid and o-phenylene diamine were dissolved in 50 ml of 5N HCl and refluxed for seven hours with stirring. It was then cooled to 0 - 5°C, and was neutralised with aqueous ammonium hydroxide. The precipitated product was collected by vacuum filtration, washed with water and dried in air. [16, 17]

Synthesis of Thioimidazole: A mixture of benzoin and thiourea was dissolved in a mixture of chloroform and ethanol (3:1). The reaction mixture was stirred thoroughly and refluxed for 12 h. The product obtained was recrystallised from methanol. [18]

Condensation of Thioimidazole and various Benzimidazoles to yield Thioethers: The

thioimidazole (0.01mol) was dissolved in 25 ml of aqueous sodium hydroxide solution by stirring at room temperature. To this clear solution it was added a pinch of cetrimide and stirring was further continued for 10 min. To this a solution of various benzimidazoles (2-chloromethylbenzimidazole / 2-(1-chloroethyl)-1H-benzimidazole / 2-(4-chlorophenyl)-1H-benzimidazole) (0.01mol) in 25 ml ethanol was added over a period of 15- 20 min. The reaction mixture was further stirred for 2 hrs. It was then cooled to 5- 10°C for 1/2 h. and the separated solid was filtered, washed with cold water and dried. The crude product on recrystallization from chloroform and ethanol yielded a yellowish crystalline solid. Products obtained were named BT (A1, A2, A3). [16]

Mild Oxidation of Various Thioethers to Yield Various SulfinylBenzimidazoles: Various thioethers (2-[[[(4,5-diphenyl-2H-imidazol-2-yl)sulfanyl]methyl]-1H-benzimidazole /2-{1-[[[(4,5-diphenyl-2H-imidazol-2-yl)sulfanyl]ethyl]-1H-benzimidazole/2-{1-[(4,5diphenyl-2H-imidazol-2-yl)sulfanyl]benzyl]-1H-benzimidazole) were dissolved in 20ml iso-propanol by stirring at room temperature. The reaction mixture was chilled thereafter in an ice salt bath maintaining its temperature between 0 – 2°C, to this a solution of meta-chloroperbenzoic acid (m-CPBA) (0.02 mol) was added with stirring. The stirring was continued for 2 – 3 h. After completion of the reaction the mixture was washed with 10% sodium bicarbonate and extracted with chloroform (30 ml). The organic layer was dried with anhydrous sodium sulphate and solvent was distilled off under reduced pressure at room temperature. The crude product was purified by column chromatography using toluene: methanol (4.5:0.5) as the mobile phase. Products obtained were named (BT-B1, B2, B3). [16]

Oxidation of Sulfinyl Benzimidazoles to Yield Various Sulfonyl Benzimidazoles: Various sulfinyl benzimidazoles were treated with 15 ml of 30% aqueous Hydrogen peroxide in 20 ml acetic acid and stirred at ambient temperature for 20 h. The reaction mixture was poured over 200 ml of ether containing 10 ml of hydrogen chloride – saturated alcohol and the mixture was stirred vigorously. The supernatant solvent was decanted and a moderate amount of ethanol was added. The mixture was stirred until a crystalline solid was formed. The product was collected, washed with ether and recrystallised. The product was again purified by column chromatography using toluene: methanol (4.5:0.5) as the mobile phase. Products obtained were named (BT-C1, C2, C3). [16]

Characterization: The synthesized 2-substituted benzimidazole analogues were characterized by various analytical techniques like,

- a) Melting point determination,
- b) Thin Layer Chromatography (TLC),
- c) Infrared Spectroscopy (IR), and
- d) MASS spectroscopy (MS).

As per the PASS score and GLIDE score the analogues designed were selected for biological studies like anticancer, antibacterial etc. The designed benzimidazole analogues which were selected for various studies were compared with standard drugs for any violation of Lipinski rule of five and drug likeness score. [17]

Anticancer Activity: Anticancer screening was done on novel benzimidazole analogues (BT-C1, C2, C3). A protocol of 48 hr continuous drug exposure was used and an SRB cell proliferation assay was used to estimate cell viability or growth. All the cell lines were grown in Dulbecco's modified Eagle's medium (containing 10% FBS in a humidified atmosphere of 5% CO₂ at 37 °C). Cells were trypsinized when sub-confluent from T25 flasks/60 mm dishes and seeded in 96-well plates in 100 µL aliquots at plating densities depending on the doubling

time of individual cell lines. The micro titre plates were incubated at 37 °C, 5% CO₂, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs and were incubated for 48 hrs with different doses (0.01, 0.1, 1, 10, 100µM) of prepared derivatives. After 48 hours incubation at 37 °C, cell mono layers were fixed by the addition of 10% (wt/ vol) cold trichloroacetic acid and incubated at 4 °C for 1h and were then stained with 0.057% SRB dissolved in 1% acetic acid for 30 min at room temperature. Unbound SRB was washed with 1% acetic acid. The protein bound dye was dissolved in 10mM Tris base solution for OD determination at 510 nm using a micro plate reader (Enspire, Perkin Elmer, and USA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$$[(Ti-Tz)/(C-Tz)] \times 100 \text{ for concentrations for which } Ti \geq Tz$$

$$[(Ti-Tz)/Tz] \times 100 \text{ for concentrations for which } Ti < Tz.$$

The dose response parameter, growth inhibition of 50 % (GI50) was calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Values were calculated for this parameter if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested. [18, 19]

Antibacterial Screening: Antibacterial screening was done on novel benzimidazole analogues (BT-A1, A2 and A3). The organisms used were Staphylococcus aureus ATCC 25923 (Gram +ve) and Escherichia coli ATCC 25922 (Gram -ve). The test solutions were prepared in chloroform. The concentrations used for antibacterial screening were 100, 200µg/mL. Standard drug solution of Amikacin (100µg/mL) was prepared in distilled water. Using a sterile cork borer of about 5 mm diameters, 4 wells were made in each petri dish. Numbers were marked on the bottom of petri dish to identify each cup. The test solutions (single and double strength), standard solution and the vehicle control (chloroform) were placed in each cup of each petri dish and incubated at $37 \pm 0.5^\circ\text{C}$ for 24 h. The presence of a definite zone of inhibition of any size was observed and compared with that of standard drug solution. [20, 21]

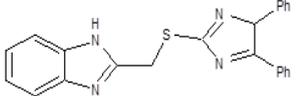
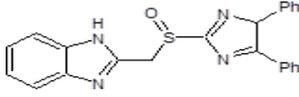
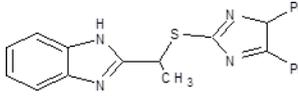
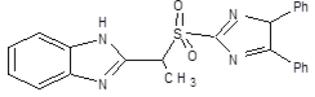
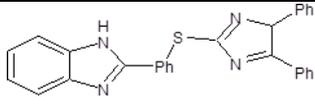
RESULTS:

In the present study, in silico design of the proposed derivatives was carried out by using different software. 3D drawing, optimizing and calculating various molecular descriptors of the proposed and derivatives were done using ACD labs chemsketch. The results are shown in **Table 1**. With the help of Molinspiration software the log P values, any violation of Lipinski rule of five and drug likeness were studied by comparing with the existing standard drugs. The results are shown in Tables 2 and 3. Nine benzimidazole derivatives were prepared through a series of five steps. The synthesized compounds were named (BT-A1, A2, A3; BT-B1, B2, B3; BT-C1, C2, C3). Purity of the compounds was ascertained by TLC and melting point determination. The results are shown in table 4. The results of characterization by IR and MASS are shown in table 5.

Docking studies were performed using Schrodinger Glide XP software. Compounds having high (-) value is considered as the best one. Dock scores of proposed benzimidazole derivatives with Dihydrofolate reductase (3I8A) are given in Table 6 and 7 and the images of docked complex are shown in Figure 1 and 2 respectively. Based on the Schrodinger Glide XP score, (BT-C1, C2, C3) were selected for in vitro anticancer evaluation and (BT-A1, A2, A3) were selected for antibacterial screening. The results of anti-cancer, antibacterial activities are shown

in Tables 8 & 9 respectively.

Table 1: Various derivatives designed and drawn using ACD Labs Chem Sketch

Compound	Analogues	Smiles Notation
BT-A1		<chem>c5ccc(C3=NC(SCC2Nc1ccccc1N2)N=C3c4ccccc4)cc5</chem>
BT-A2		<chem>O=S(Cc2nc1ccccc1[nH]2)C5N=C(c3ccccc3)C(c4ccccc4)=N5</chem>
BT-A3		<chem>O=S(=O)(Cc2nc1ccccc1[nH]2)C5N=C(c3ccccc3)C(c4ccccc4)=N5</chem>
BT-B1		<chem>CC(SC3N=C(c1ccccc1)C(c2ccccc2)=N3)c5nc4ccccc4[nH]5</chem>
BT-B2		<chem>CC(c2nc1ccccc1[nH]2)S(=O)C5N=C(c3ccccc3)C(c4ccccc4)=N5</chem>
BT-B3		<chem>CC(c2nc1ccccc1[nH]2)S(=O)(=O)C5N=C(c3ccccc3)C(c4ccccc4)=N5</chem>
BT-C1		<chem>c6ccc(C4=NC(Sc3ccc(c2nc1ccccc1[nH]2)cc3)N=C4c5ccccc5)cc6</chem>

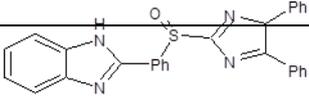
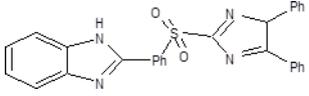
BT-C2		<chem>O=S(c3ccc(c2nc1cccc1[nH]2)cc3)C6N=C(c4ccccc4)C(c5ccccc5)=N6</chem>
BT-C3		<chem>O=S(=O)(c3ccc(c2nc1cccc1[nH]2)cc3)C6N=C(c4ccccc4)C(c5ccccc5)=N6</chem>

Table 2: Analysis of Lipinski rule of five of standard drugs and the proposed derivatives

Derivative	LOG P	Mol. Wt.	nHDon	nHAcc	nrotb	Lipinski rule alert index
BT-A1	4.861	382.48	2	4	5	0
BT-A2	3.397	398.48	1	6	6	0
BT-A3	3.792	414.48	1	5	4	0
BT-B1	5.286	396.51	2	6	5	1
BT-B2	3.725	412.52	1	5	5	0
BT-B3	4.134	428.51	1	4	6	0
BT-C1	6.845	444.55	1	6	5	1
BT-C2	5.246	460.55	2	5	6	1
BT-C3	5.312	476.55	1	5	5	1
Isoniazid	-0.947	137.14	4	4	2	0
Amoxicillin	-1.272	365.4	6	8	4	0

Table 3: Analysis of Drug Likeness Score of standard drugs and the proposed derivatives

Compound	GPCR ligand	Ion Channel modulator	Kinase inhibitor	Nuclear receptor ligand
BT-A1	-0.14	-0.16	-0.32	-0.31
BT-A2	0.01	-0.15	-0.52	-0.81

BT-A3	-0.01	-0.18	-0.34	-0.63
BT-B1	-0.05	-0.12	-0.44	-0.56
BT-B2	-0.04	-0.07	-0.57	-0.73
BT-B3	-0.06	-0.14	-0.39	-0.56
BT-C1	0.01	-0.30	-0.12	-0.78
BT-C2	0.01	-0.32	-0.18	-0.78
BT-C3	0.08	-0.42	-0.1	-0.75
Doxorubicin	-1.37	-1.36	-0.65	-2.86
Amoxicillin	0.71	-0.42	-0.65	-0.48

Table 4: Melting point and R_f value of the synthesized derivatives

Compound	Molecular formula	Yield (%)	Melting point (°C)	R _f
BT-A1	C ₂₃ H ₁₈ N ₄ S	94	182	0.42
BT-A2	C ₂₃ H ₁₈ N ₄ OS	89	166	0.59
BT-A3	C ₂₃ H ₁₈ N ₄ O ₂ S	73	294	0.61
BT-B1	C ₂₄ H ₂₀ N ₄ S	91	184	0.38
BT-B2	C ₂₄ H ₂₀ N ₄ OS	88	168	0.48
BT-B3	C ₂₄ H ₂₀ N ₄ O ₂ S	76	269	0.72
BT-C1	C ₂₈ H ₂₀ N ₄ S	96	274	0.39
BT-C2	C ₂₈ H ₂₀ N ₄ OS	86	245	0.46
BT-C3	C ₂₈ H ₂₀ N ₄ O ₂ S	73	245	0.73

Table 5: Spectral results of synthesized benzimidazole derivatives

Compound	IR spectral analysis	Mass M+ peak
BT-A1	3383 (NH str), 1547(C=N str), 769 (C-S str), 3761 (Ar-CH str)	367
BT-A2	2987 (NH str), 762 (C-S str), 3242 (Ar-H str)	343
BT-A3	3395 (NH str), 1592 (C=N str), 785 (C-S str), 852 (S-Ostr)	398
BT-C1	3453 (NH str), 1603 (C=N str), 1687 (Ar C=C str), 774 (C-S str)	421
BT-C2	3453 (NH str), 1692 (Ar C=C str), 1543 (C=n str) 856 (S-O), 1089 (SO ₂ , symstr), 746 (Ar C-C v)	454
BT-C3	3403 (NH str), 1604 (C=N str), 775 (C-S str) 809 (S-O), 1129 (SO ₂ , symstr), 732 (Ar C-C v)	428

Table 6: Glide Score with 3I8A (Dihydrofolate reductase)

Compound	glide score	glide energy (Kcal/mol)
BT-A1	-9.16	-52.62
BT-A2	-9.08	-55.63
BT-A3	-9.02	-53.65
BT-B1	-8.94	-58.23
BT-B2	-8.84	-55.45
BT-B3	-8.86	-53.48
BT-C1	-8.76	-52.82
BT-C2	-8.23	-57.45
BT-C3	-7.17	-58.85
Doxorubicin	-8.52	-52.45

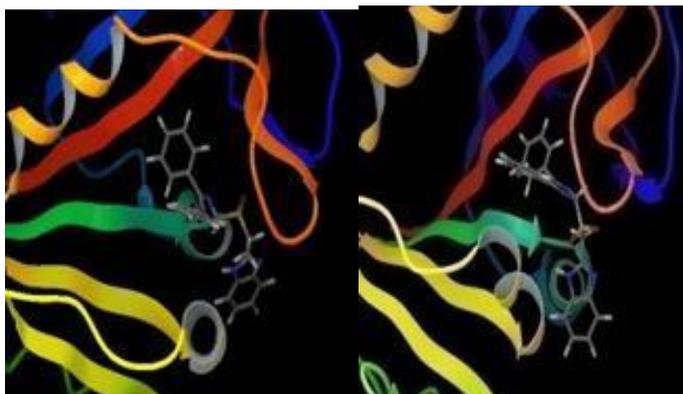


Fig. 1: Docking image of BT-C1 and BT-C3 to 3I8A

Table 7: Glide Score with 3I8A (Dihydrofolate reductase)

Compound	Glide score	Glide energy (Kcal/mol)
BT-A1	-6.86	-41.75
BT-A2	-5.23	-43.76
BT-A3	-5.75	-45.43
BT-B1	-5.77	-43.88
BT-B2	-5.54	-31.45
BT-B3	-5.12	-42.16
BT-C1	-5.02	-38.93
BT-C2	-4.79	-41.65
BT-C3	-2.89	-35.45
Amoxicillin	-5.91	-42.45

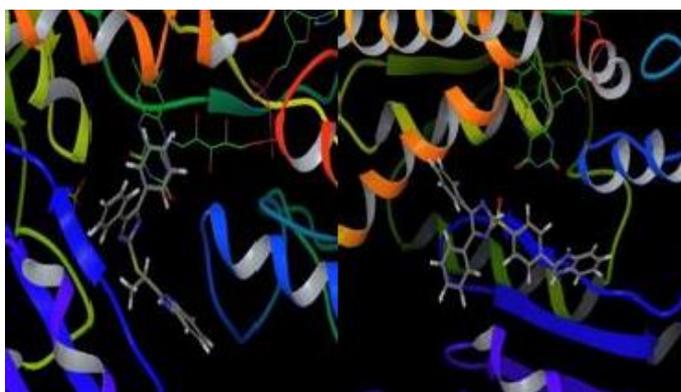


Fig. 2: Docking image of BT-A1 & BT-A3 to 3I8A

Table 8: (GI₅₀/μM) values of the tested compounds BT-C1, C2, C3 against four human cancer cell lines.

Sample	HeLa	MIAPACA	MDA MB 231	IMR 32
BT-C1	0.97±0.06	59.2±0.5	2.0±0.06	>100
BT-C2	5.7±0.51	>100	20.4±0.1	1.4±0.07
BT-C3	9.2±0.8	0.7±0.01	0.29±0.02	26.5±0.08
Doxorubicin	0.09±0.002	0.086±0.003	0.087±0.001	0.03±0.008

Table 9: Antibacterial activity of selected derivatives (BT-A1, A2, A3)

Serial No	Sample	Zone of Inhibition(mm)			
		<i>E. coli</i>		<i>S. aureus</i>	
		100 μg	200 μg	100 μg	200 μg
1	BT-A1	15±0.33	18±1.64	18±1.76	18±0.82
2	BT-A2	15±0.65	17±1.84	17±.28	17±0.76
3	BT-A3	16±0.86	16±0.62	15±1.65	15±0.62
4	Control	12	-	10	-
5	Standard	19	-	18	-

DISCUSSION: In silico design of all the proposed derivatives were carried out using ACD Labs ChemsSketch, Molinspiration, PASS and Schrodinger Glide XP. The compounds were synthesized by conventional method and were characterized by TLC, melting point determination, FTIR, and MASS. The synthesized derivatives were screened for the biological activities on the basis of Pass and Glide score. The in vitro anticancer and antibacterial screening of all the selected derivatives exhibited better activity. Compound BT-C2 showed significant anticancer activity and the compound BT-A2 showed significant activity when compared with the standard. These compounds BT-C2 and BT-A2 needs further studies, so that these compounds give better results. So it is evident that further work on these derivatives has to be done in future for the development of clinically useful antimicrobial agents.

CONCLUSION: In summary, the main objective of the present work was to design, synthesize and biologically screen some novel benzimidazole derivatives. Various benzimidazole derivatives were synthesized and characterized by spectral studies. In the in vitro anticancer, and antibacterial screening, compound BT-C2 showed significant anticancer activity and the compound BT-A2 showed significant antibacterial activity. Substitution of the benzimidazole moiety with hetero aromatic, sulfonyl group and sulphur atom might be responsible for the enhancement of activity. So, these derivatives (BT-C2 and BT-A2) can be subjected to further studies for consideration as a drug candidate.

REFERENCES:

1. Chopra I., Hodgson J., Metcalf B., Poste G. The search for antimicrobial agents

- effective against bacteria resistant to multiple antibiotics, *Antimicrob. Agents Ch.* 41 (1997) 497–503.
2. Kr'atky M., Vin'sov'a J., Volkov'a M., Buchta V., Trejtnar F., Stola'rikov'a J. Antimicrobial activity of sulfonamides containing 5-chloro-2-hydroxybenzaldehyde and 5-chloro-2-hydroxybenzoic acid scaffold, *Eur. J. Med. Chem.* 50 (2012) 433–440.
 3. Huang T.S., Kunin C.M., Yan B.S., Chen Y.S., Lee S.S.J., Syu W. Susceptibility of *Mycobacterium tuberculosis* to sulfamethoxazole, trimethoprim and their combination over a 12 year period in Taiwan, *J. Antimicrob. Chemother.* 67 (2011) 633–637.
 4. Song D.Q., Wang Y., Wu L.Z., Yang P., Wang Y.M., Gao L.M., Li Y., Qu J.R., Wang Y.H., Li Y.H., Du N.N., Han Y.X., Zhang Z.P., Jiang J.D. Benzoylurea Derivatives as a Novel Class of Antimitotic Agents: Synthesis, Anticancer Activity, and Structure Activity Relationships, *J. Med. Chem.* 51 (2008) 3094–3103.
 5. Abovyan L.S., Shkulev V.A., Stepanyan N.O., Agaronyan A.S., Mndzhoyan O.L. Synthesis and hypoglycemic activity of N-(5-carboalkoxyfuran-2-sulfonyl)-N'-butyl urea, *Pharm. Chem. J.* 17 (1983) 410–413.
 6. Huang P.P., Randolph J.T., Klein L.L., Vasavanonda S., Dekhtyar T., Stoll V.S., Kempf D. J. Synthesis and antiviral activity of P1' arylsulfonamide azacyclic urea HIV protease inhibitors, *Bioorg. Med. Chem. Lett.* 14 (2004) 4075–4078.
 7. Shimshoni J.A., Bialer M., Wlodarczyk B., Finnell R.H., Yagen B. Potent Anticonvulsant Urea Derivatives of Constitutional Isomers of Valproic Acid, *J. Med. Chem.* 50 (2007) 6419–6427.
 8. Ramanpreet W, Hedaitullah M and Naaz S F: Benzimidazole derivatives – an overview. *International Journal of Research in Pharmacy and Chemistry* 2011; 1(3): 565- 574.
 9. Alasmary A S, Snelling A M and Zain M E: Synthesis and Evaluation of Selected Benzimidazole Derivatives as Potential Antimicrobial Agents. *Molecules* 2015; 20: 15206-15223.
 10. Krishna Prasad P M and Sundararajan R: Design, synthesis, antitubercular and antimicrobial activities of novel thiazole substituted benzimidazole derivatives. *Der Pharmacia Lettre* 2017; 9 (6): 270-284.
 11. Gong J, Ya He and Cui Z L: Synthesis, spectral characterization, and antituberculosis activity of thiazino[3,2-A]benzimidazole derivatives. *Phosphorus, Sulfur, and Silicon and the Related Elements* 2016; 191(7): 1036- 1041.
 12. Nomi S, Janmajoy B and Sujiti S: A review on chemistry and biological significance of benzimidaole nucleus. *IOSR Journal of Pharmacy* 2014; 4(12): 28- 41.
 13. Singh G, Kaur M and Chander M: Benzimidazoles: The latest information on biological activities. *International Research Journal of Pharmacy* 2013; 4(1): 82- 87.
 14. Venugopal S, Kaur B, Verma A, Wadhwa P, Sahu SK. A Review on Modern Approaches to Benzimidazole Synthesis. *Curr Org Synth.* 2023;20(6):595-605. doi: 10.2174/1570179420666221010091157. PMID: 36221870.
 15. Manju P T, Anton Smith A and Padmaja V: Insilico Design of an SGLT2 Inhibitor in Diabetes mellitus. *Middle-East Journal of Scientific Research* 2016; 24 (10): 3108-3112.
 16. Bhaskar V, Kumar S, Nair AS, Rajappan KP, Sudevan ST, Parambi DGT, Al-Sehemi AG, Zachariah SM, Pappachen LK. A Review on Benzimidazole Scaffolds as Inhibitors of *Mycobacterium tuberculosis* Mycolyl-arabinogalactan-peptidoglycan Complex Biosynthesis. *Comb Chem High Throughput Screen.* 2023;26(4):668-681. doi: 10.2174/1386207325666220415144511. PMID: 35430964.

17. Bariwal JB, Shan AK, Kathiravan MK, Somani RS and Jain KS: Synthesis and antiulcer activity of novel pyrimidylthiomethyl- and pyrimidylsulfinylmethyl- benzimidazoles as potential reversible proton pump inhibitors. *Indian Journal of Pharmaceutical Education and Research* 2008; 42: 225 -229.
18. Abdelhameid, M.K.; Zaki, I.; Mohammed, M.R.; Mohamed, K.O. Design, synthesis, and cytotoxic screening of novel azole derivatives on hepatocellular carcinoma (HepG2 Cells). *Bioorganic Chem.* 2020, 101, 103995. [CrossRef]
19. Yadagiri B, Uma Devi H, Rajashaker B, Lingaiah N, Vijayacharan G, Sowjanya P, and Nishanth Jain: Rational Design, synthesis and anti-proliferative evaluation of novel benzosuberone tethered with hydrazide-hydrazones. *Bioorg. Med. Chem.* 2014, 24: 5041.
20. Desai, N.; Joshi, V.; Rajpara, K.; Makwana, A.H. A new synthetic approach and in vitro antimicrobial evaluation of novel imidazole incorporated 4-thiazolidinone motifs. *Arab. J. Chem.* 2017, 10, S589–S599. [CrossRef]
21. Hawser, S.; Lociuero, S.; Islam, K. Dihydrofolate reductase inhibitors as antibacterial agents. *Biochem. Pharmacol.* 2006, 71, 941–948.
22. Anshul C, Ashu S and Anil kumar S: Review: A convenient approach for the synthesis of imidazole derivatives using microwaves. *Der Pharma Chemica* 2012; 4 (1): 116-140.