INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Paper

Design, Synthesis and Evaluation of New 2, 5-Di Substituted Benzimidazole Derivatives

M. Chennarao[1], S. Shobharani[2], G. Sai Krishna*[3]

[1] *Vaagdevi College of Pharmacy, Ramnagar, Hanamakonda, Warangal*
[2, 3] *Centre for Pharmaceutical Sciences, UCESTH, JNTUH, Kukatpally, Hyderabad.*

ARTICLE INFO

Published: 07 May 2026
Keywords:
ADME; Anticancer activity; Benzimidazoles; CDK-8; Docking; ER-alpha
DOI:
10.5281/zenodo.20068213

ABSTRACT

Benzimidazole is a significant pharmacophore in medicinal chemistry, with a wide range of biological activities. The molecular docking technique is widely employed in current drug research to investigate the drug-receptor interaction. The selected data set of synthesized benzimidazole compounds was tested for in vitro antibacterial activity against S. aureus, S. typhi, E. coli, and M. luteus. Furthermore, Autodock 4.2 was used to conduct a molecular docking investigation on the data set, with FtsZ protein (PDB: 3V08) and FtsZ protein (PDB: 3V08) as potential antimicrobial targets. Molecular docking data showed that compounds 3a, 3b, 3c, 3e, and 3g had a high docking score, indicating improved interaction within key amino acids, which correlates to their antibacterial properties. Insilico studies showed that significant results were within the range of Lipinski's rule of five, with PASS and osiris serving as lead molecules for the identification of novel antimicrobial medicines. In vitro findings showed that compounds 3b and 3e have a larger zone of inhibition than the standard (ciprofloxacin).

INTRODUCTION

Antimicrobial resistance is a persistent and growing threat to global public health, significantly lowering the efficacy of many standard antibacterial and antifungal treatments and emphasizing the need for novel bioactive chemical scaffolds. Along with the rapid rise of multidrug-resistant bacterial strains, invasive and opportunistic fungal infections have become more clinically important. Microbial pathogens, which include Gram-positive and Gram-negative bacteria, as well as pathogenic fungi, cause a wide range of infections, from moderate and localized to severe, systemic, and possibly lethal. These infections disproportionately affect immune-compromised people, critically ill patients, and those undergoing lengthy hospital stays or

***Corresponding Author:** G. Sai Krishna
Address: *Centre for Pharmaceutical Sciences, UCESTH, JNTUH, Kukatpally, Hyderabad*
Email ✉: saikrishnaguduru095@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

invasive medical procedures. The increasing prevalence of drug-resistant bacterial and fungal species, combined with the limited effectiveness and safety concerns of current antimicrobial agents, highlights the critical need for the development of new broad-spectrum antimicrobial compounds with improved efficacy and resilience to resistance-modulating capabilities.

Within this context, benzimidazole has emerged as a highly important and adaptable scaffold in medicinal chemistry, providing a foundation for the design and development of a wide range of therapeutic medicines. Because of their structural closeness to purine nucleobases, benzimidazole motifs can interact successfully with a wide range of biological targets, which contributes to the diverse spectrum of biological activity documented for this family of molecules. Benzimidazole-containing compounds have been extensively studied and developed as antibacterial, antifungal, antiviral, and anticancer drugs. Furthermore, the benzimidazole core's ease of chemical modification makes it ideal for the rational creation of novel bioactive compounds. A recent study found that benzimidazole compounds have notable antibacterial activity against both fungal and bacterial infections.

MATERIALS AND METHODS:

Insilico screening:

DESIGNING OF MOLECULES

Chem Draw version 12.0 was used to design the molecules, various substitutions were done using structure mode in the softwares.

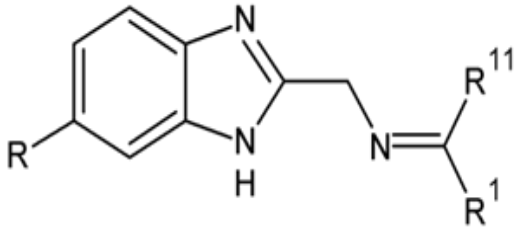


Table:1- Designed Molecules

s.no	Molecule	R	R^1	R^{11}
1	1	-H	$-C_6H_5$	H
2	2	-H	$-C_6H_4Cl(p)$	-H
3	3	-H	$-C_6H_4OH(m)$	-H
4	4	-H	$-C_6H_4NO_2(p)$	-H
5	5	-H	$-C_6H_4OH(p)$	-H
6	6	-H	$-C_6H_4N(CH_3)_2(p)$	-H
7	7	-H	$-C_6H_4CH_3(p)$	-H
8	8	-H	$-C_6H_4OCH_3(p)$	-H
9	9	-H	$-C_6H_4Cl(o)$	-H
10	10	-H	$-C_4H_3O$	-H
11	11	-H	$-C_5H_5N$	-H
12	12	-H	$-CH_2CH_3$	-H
13	13	-H	$-C_6H_5$	-CH3
14	14	-H	$-C_6H_4NH_2(p)$	-CH3
15	15	-H	$-C_6H_3Cl(o,p)$	-CH3
16	16	-H	$-C_6H_4NO_2(m)$	-CH3
17	17	-H	$-C_6H_4NO_2(p)$	-CH3
18	18	-H	$-C_6H_4CH_3(p)$	-CH3
19	19	$-CH_3$	$-C_6H_5$	-H
20	20	$-CH_3$	$-C_6H_4Cl(p)$	-H
21	21	$-CH_3$	$-C_6H_4OH(m)$	-H
22	22	$-CH_3$	$-C_6H_4NO_2(p)$	-H
23	23	$-CH_3$	$-C_6H_4OH(p)$	-H

24	24	$-CH_3$	$-C_6H_4N(CH_3)_2$(p)	-H
25	25	$-CH_3$	$-C_6H_4CH_3$(p)	-H
26	26	$-CH_3$	$-C_6H_4OCH_3$(p)	-H
27	27	$-CH_3$	$-C_6H_4Cl$(o)	-H
28	28	$-CH_3$	$-C_4H_3O$	-H
29	29	$-CH_3$	$-C_5H_5N$	-H
30	30	$-CH_3$	$-CH_2CH_3$	-H
31	31	$-CH_3$	$-C_6H_5$	$-CH_3$
32	32	$-CH_3$	$-C_6H_4NH_2$(p)	$-CH_3$
33	33	$-CH_3$	$-C_6H_3Cl$(o,p)	$-CH_3$
34	34	$-CH_3$	$-C_6H_4NO_2$(m)	$-CH_3$
35	35	$-CH_3$	$-C_6H_4NO_2$(p)	$-CH_3$
36	36	$-CH_3$	$-C_6H_4CH_3$(p)	$-CH_3$
37	37	-Cl	$-C_6H_5$	-H
38	38	-Cl	$-C_6H_4Cl$(p)	-H
39	39	-Cl	$-C_6H_4OH$(m)	-H
40	40	-Cl	$-C_6H_4NO_2$(p)	-H
41	41	-Cl	$-C_6H_4OH$(p)	-H
42	42	-Cl	$-C_6H_4N(CH_3)_2$(p)	-H
43	43	-Cl	$-C_6H_4CH_3$(p)	-H
44	44	-Cl	$-C_6H_4OCH_3$(p)	-H
45	45	-Cl	$-C_6H_4Cl$(o)	-H
46	46	-Cl	$-C_4H_3O$	-H
47	47	-Cl	$-C_5H_5N$	-H
48	48	-Cl	$-CH_2CH_3$	-H
49	49	-Cl	$-C_6H_5$	$-CH_3$
50	50	-Cl	$-C_6H_4NH_2$(p)	$-CH_3$
51	51	-Cl	$-C_6H_3Cl$(o,p)	$-CH_3$
52	52	-Cl	$-C_6H_4NO_2$(m)	$-CH_3$
53	53	-Cl	$-C_6H_4NO_2$(p)	$-CH_3$
54	54	-Cl	$-C_6H_4CH_3$(p)	$-CH_3$
55	55	$-NO_2$	$-C_6H_5$	-H
56	56	$-NO_2$	$-C_6H4Cl$(p)	-H
57	57	$-NO_2$	$-C_6H_4OH$(m)	-H
58	58	$-NO_2$	$-C_6H_4NO_2$(p)	-H
59	59	$-NO_2$	$-C_6H_4OH$(p)	-H
60	60	$-NO_2$	$-C_6H_4N(CH_3)_2$(p)	-H
61	61	$-NO_2$	$-C_6H_4CH_3$(p)	-H
62	62	$-NO_2$	$-C_6H_4OCH_3$(p)	-H
63	63	$-NO_2$	$-C_6H_4Cl$(o)	-H
64	64	$-NO_2$	$-C_4H_3O$	-H
65	65	$-NO_2$	$-C_5H_5N$	-H
66	66	$-NO_2$	$-CH_2CH_3$	-H
67	67	$-NO_2$	$-C_6H_5$	$-CH_3$
68	68	$-NO_2$	$-C_6H_4NH_2$(p)	$-CH_3$
69	69	$-NO_2$	$-C_6H_3Cl$(o,p)	$-CH_3$
70	70	$-NO_2$	$-C_6H_4NO_2$(m)	$-CH_3$

Lipinski's rule of 5 filtration:

The files were inserted in *.pdb, *.mol, *.mol2, *.xyz, *.sdf, or .smile formats. Care was taken to

avoid whitespace(s) in the input file name. The window opened and the files were uploaded in the above mentioned formats. pH was adjusted from 0-14 as required. Upon submission, results were obtained.

OSIRIS property explorer (version 2):

OSIRIS property explore version 2 (which requires JAVA platform to run) was used in the present study. The structure of designed molecule when drawn in or when pasted in smiles format will show the results at right side with colour coding. A green colour indicates non-toxic and red indicates toxicity.

Prediction of activity spectra for substances (PASS):

Molecules which have been filtered through Lipinski rule were subjected to online PASS software to predict their biological activities. The Pa and Pi values from 0.000 to 1.000. To define the threshold for selecting type of activity to be predicted.

Molsoft property explorer (version v.3.7-2):

Molsoft property explorer version v.3.7-2 was used in the present study. The structure when drawn directly on the window or when inserted in mol, Inch, smiles formats will calculate properties like MlogP, MlodS.

Docking (version 4.2):

AutoDock is a molecule modeling simulation software. It is especially effective for protein ligand docking. It has two versions Auto Dock 4.2, vina. Vina is a advanced version

Docking is used to find the exact binding conformation and orientation of the ligand molecule into the active site of the protein. The synthesized five benzimidazole compounds and standard (ciprofloxacin) were docked against beta tubulin using Auto-Dock Tool 4.0, an automated docking tool.

The docking process involves four main steps,

(i) Protein preparation

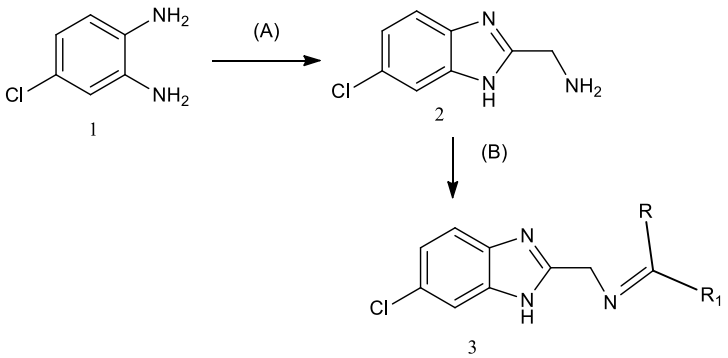
(ii) Ligand preparation

(iii) Grid preparation and

(iv) Docking.

The Lamarckian genetic algorithm has been used as the search algorithm to search for the best conformers. The initial population size was set randomly as 150 individuals and ten generations was set for each genetic algorithm run and the maximum number of energy evaluations was set to 2,500,000. The grid box size was set as to include all the active site residues present in rigid macromolecules. The grid box was centered at 8.671 Å x -8.036 Å x 0.67 Å and the dimensions of the grid box have been set as 40, 40, 40 (X,Y,Z co-ordinates) so as to include all the active site residues.

SCHEME :



A= Glycine , HCl reflux for 12hrs

B= Different aldehydes with ethanol reflux for 14hrs

General procedure for the synthesis of 2,5-Di substituted benzimidazole:

Place 5gms of 4-chloro Phenylenediamine (OPDA) in a 250ml round bottomed flask add 19.7gm of glycine and add hydrochloric acid(4N). Heat the mixture on a water bath at 100^0C for 12 hrs. The progess of the reaction was monitored by TLC(chloroform : ethanol in the ratio of 1:1). Cool and add sodium hydroxide (10%)solution slowly with constant stirring of the solution until the mixture is just alkaline to litmus. Filter off the crude product, wash with ice-cold water, drain well and again wash with 20ml of cold water. The obtained solid was recrystallized from water to give a pure colour (brown,2,5-disubstitued bezimidazole)compound.

Synthesis of *N*-[(6-chloro-1*H*-benzimidazol-2-yl)methyl]-1- substituted phenylmethanimine (3a,3b,3e):

A mixture of compound 2 (0.01mol)and 3a, 3b, 3e with aromatic benzaldehyde(substituted 4-chloro benzaldehyde and 2-chloro benzaldehyde) (0.01 mol) in ethanol (30 ml) was refluxed under stirring for 5-14 hours and monitered by TLC (chloroform : ethanol in the ratio of 0.2:1.8) after that the mixture cooled at room temperature, then poured on crushed ice. The precipitate was collected by filtration and gives orange to brown crystals.

EVALUTION OF ANTIMICROBIAL ACTIVITY

Microbes are living organisams that multiply frequently and spread rapidly. An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms against which they are primarily effective. Antibacterials (commonly known as antibiotics) are used against bacteria and fungals are used against fungi. Drugs have been developed to achieve better pharmacokinetic and pharmacodynamic properties. In addition antimicrobial agents that are associated with serious side effects have been replaced by other safe drugs.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of the test compounds was assayed systematically against four non-pathogenic strains of bacteria i.e. *S.aureus ,S.typhi, E.coli* and *M.luteus* The antibacterial activity of a compound is its ability to inhibit the growth of bacteria in nutrient broth or agar. The method used in this present investigation was diffusion method.

NUTRIENT AGAR COMPOSITION

Beef extract : 10g
Peptone : 10g
Sodium chloride : 5g
Distilled water : 100ml

Media was prepared by dissolving required quantity of nutrient agar (28g for 1Lit) in distilled water by heating water bath if required and pH was adjusted to 7.0-7.2. The prepared media was sterilized by autoclaving at 121^0C (151b\in^2) for about 15 min and transferred to each petri plate.

CUP PLATE METHOD

The test compounds in the concentration of 75, 100µg/mL were prepared by dissolving in DMSO. Sterilized media was cooled to 40^0C, inoculated with respective bacteria and poured into petri plates. After solidification of the medium at room temperature, cups of 4mm diameter were made in each plate with sterile with borer. Accurately 0.01mL of test solution was transferred to cups and labelled accordingly. The plates were kept undisturbed for at least two hours at room temperature to allow diffusion properly. Incubation of the petri plates was done at 37 ± 1^0C for 24h. The growth/inhibition of bacteria was observed the solvent effects. The diameter of zone of inhibition was read and results were calculated. Ciprofloxacin and Streptomycin were chosen as standards.

MINIMUM INHIBITORY CONCENTRATION-BROTH DILUTION METHOD

PRINCIPLE

The minimum inhibitory concentration Assay is a technique used to determine the lowest concentration of a particular antibiotic needed to kill bacteria. This assay is typically performed on planktonic (free floating) bacterial cells.

PROCEDURE

- Take clean and dry test tubes, sterilize and label them.
- Prepare dilutions for different concentrations (25, 50, 100µg/mL) of test compounds
- Then nutrient broth was prepared, sterilized and allowed to cool to room temperature, it was transferred to test tubes, 10-15ml in each tube
- The bacterial culture was inoculated into the test tubes in laminar chamber at aseptic conditions to avoid contamination. The test tubes were shaken to allow proper mixing.
- Various concentrations of test compounds were added to the test tubes.
- The test tubes were shaken well and tightly closed with cotton plug.
- The tubes were allowed to incubate overnight (18-24hrs).
- Broth tubes were appeared turbid are indicative of bacterial growth while tubes that remained clear indicate no growth.
- MIC was calculated by using following formula.

MIC= Highest conc. that inhibit growth + Lowest conc. that allow growth of microorganism

RESULTS AND DISCUSSION:

Table-2: Physical data of synthesized compounds:

Sym	Chemical Name	Chemical Formula	Percentage Yield	m.p.	Rf value
3b	*N*-[(6-chloro-1*H*-benzimidazol-2-yl)methyl]-1-(4-chlorophenyl) methanimine	$C_{15}H_{11}N_3Cl_2$	40.2%	241-250°C	0.8
3c	*N*-[(6-chloro-1*H*-benzimidazol-2-yl)methyl]-1-(3-chlorophenyl) methanimine	$C_{16}H_{12}Cl_2N_2$	60.8%	272-275°C	0.6

Table-3: Spectral Data:

Syn	IR Spectra Characteristics	1H NMR Characteristics
3b	3410 (N-H, str), 2853(C-H, str), 1690(C=N,str),1623(C=C,str), 1458(C-H, bend),1358(C-H, bend)713(C-Cl, str)	2.25(s,Aliphatic-H) 7.77(s,Ar-H)), 7.549(d,Ar-H),7.439(d,Ar-H).
3e	3427.17 (N-H str), 2850(C-H str),1615(C=C str), 1465(C-H bend), 1385(C-H bend), 1089(C=N str), 593(C-Cl str)	2.25(s,Aliphatic-H) 7.77(s,Ar-H)), 7.549(d,Ar-H),7.439(d,Ar-H).

Insilico screening:

Table-4: Results of lipinski's rule of 5 filtration:

S.NO	MOLECULE	MW	HBD	HBA	LogP	M.R
1	**1**	**234**	**0**	**2**	**0.00**	**0.00**
2	2	257.5	0	3	1.28	62.37
3	3	238	0	4	0.15	62.23
4	4	268	0	5	0.93	65.00
5	5	238	0	4	0.21	62.50
6	6	260	0	4	1.04	67.31
7	7	234	0	3	1.49	63.34
8	8	250	0	4	1.44	63.31
9	9	252.7	0	3	1.28	62.23
10	10	214	0	4	0.60	53.78
11	11	224	0	4	0.67	58.57
12	12	174	0	3	0.66	44.91
13	13	234	0	3	1.07	61.13
14	14	248	0	4	0.89	65.32
15	15	305	0	3	1.23	67.61
16	16	280	0	5	0.79	68.12
17	17	280	0	5	0.79	68.12
18	18	246	0	3	1.33	65.48
19	19	234	0	3	1.49	63.34
20	20	269	0	3	1.58	66.58
21	21	250	0	4	0.45	66.58
22	22	280	0	5	1.22	70.33
23	23	250	0	4	0.50	66.84
24	**24**	**272**	**0**	**4**	**0.00**	**0.00**
25	25	246	0	3	1.79	67.78
26	26	262	0	4	1.73	67.73
27	27	269.5	0	3	1.50	66.58
28	28	220	0	4	0.89	58.12
29	29	236	0	4	0.97	62.91
30	30	186	0	3	0.96	49.26
31	31	246	0	3	1.36	65.48
32	32	260	0	4	1.19	69.67
33	33	317	0	3	1.52	71.96
34	34	292	0	5	1.09	72.47
35	35	292	0	5	1.09	72.47
36	36	258	0	3	1.66	69.82
37	37	257	0	3	1.28	62.23
38	38	293	0	3	1.36	65.47
39	39	273.5	0	4	0.24	65.47
40	40	273.5	0	4	0.29	65.47
41	41	303	0	5	1.01	69.23
42	**42**	**295**	**0**	**4**	**0.00**	**0.00**
43	43	269.5	0	3	1.58	66.58

44	44	293	0	3	1.36	65.47
45	45	249.5	0	4	0.68	57.02
46	46	285	0	4	1.25	66.55
47	47	259.5	0	4	0.75	61.81
48	48	209.5	0	3	0.74	48.16
49	49	269.5	0	3	1.15	64.37
50	50	283.5	0	4	0.97	68.53
51	51	340.5	0	3	1.13	70.86
52	52	315.5	0	5	0.88	71.37
53	53	315.5	0	5	0.88	71.37
54	54	281.5	0	3	1.44	68.72
55	55	268.5	0	5	0.93	65.98
56	56	284	0	6	0.058	69.49
57	57	284	0	6	0.42	69.22
58	58	303.5	0	5	1.01	69.23
59	59	314	0	7	0.65	72.98
60	60	306	0	5	0.77	74.67
61	61	280	0	5	1.22	70.33
62	62	296	0	6	1.16	70.34
63	63	260	0	6	0.32	70.34
64	64	270	0	6	0.40	60.77
65	65	303.5	0	5	1.01	65.66
66	66	220	0	5	0.39	69.23
67	67	280	0	5	0.79	51.91
68	68	294	0	6	0.62	68.12
69	69	351	0	5	0.96	72.32
70	70	326	0	7	0.52	74.61

Based on the results of Lipinski rule of 5 out of 70 compounds 67 compounds were obeyed Lipinski rule of 5. Those 67 compounds are subjected to OSIRIS to know the toxicity

Table-5: Results of OSIRIS property explorer (version 2):

S.NO	Molecule	Toxicity	c log P	Solubility	MW	TPSA	DL	DS
1	**2**	**NO**	**3.23**	**-3.55**	**257.5**	**41.04**	**2.93**	**0.79**
2	**3**	**NO**	**2.28**	**-2.52**	**238**	**61.27**	**2.07**	**0.86**
3	**4**	**YES**	**1.31**	**-3.27**	**268**	**86.86**	**-8.47**	**0.44**
4	**5**	**NO**	**0.28**	**-2.52**	**238**	**61.27**	**2.07**	**0.86**
5	**6**	**YES**	**2.52**	**-2.85**	**260**	**44.28**	**0.99**	**0.46**
6	**7**	**YES**	**2.97**	**-3.16**	**234**	**41.04**	**0.42**	**0.69**
7	**8**	**NO**	**2.56**	**-2.83**	**250**	**50.22**	**2.01**	**0.83**
8	**9**	**NO**	**3.23**	**-3.55**	**257.5**	**41.04**	**2.42**	**0.78**
9	**10**	**NO**	**1.82**	**-2.25**	**214**	**54.16**	**1.76**	**0.86**
10	**11**	**NO**	**1.68**	**-2.04**	**224**	**53.43**	**1.99**	**0.89**
11	**12**	**NO**	**1.33**	**-1.84**	**174**	**41.04**	**1.47**	**0.87**

12	13	NO	2.56	-3.17	248	41.04	2.21	0.83
13	14	YES	1.89	-3.25	305	67.06	0.14	0.68
14	15	YES	3.78	-4.65	305	41.04	2.57	0.64
15	16	YES	1.25	-3.63	280	86.86	-4.16	0.43
16	17	YES	1.25	-3.63	280	86.86	-8.91	0.43
17	18	YES	2.91	-3.52	246	41.04	0.03	0.5
18	19	NO	2.97	-3.16	234	41.04	1.13	0.75
19	20	NO	3.58	-3.89	269	41.04	2.2	0.73
20	21	NO	2.62	-2.86	250	61.27	1.28	0.79
21	22	YES	1.65	-3.62	280	86.86	-9.25	0.43
22	23	NO	2.62	-2.86	250	61.27	1.38	0.79
23	25	YES	3.31	-3.5	246	41.04	-0.19	0.59
24	26	NO	2.9	-3.18	262	50.27	1.32	0.76
25	27	NO	3.58	-3.89	269.5	41.04	1.67	0.70
26	28	NO	2.16	-2.84	220	54.18	1.08	0.79
27	29	NO	2.02	-2.39	236	53.9	1.30	0.82
28	30	NO	1.67	-2.08	186	41.04	0.89	0.81
29	31	NO	2.91	-3.52	246	41.04	1.54	0.76
30	32	YES	2.23	-3.59	260	67.0	-0.47	0.59
31	33	YES	4.12	-4.99	317	41.04	1.89	0.56
32	34	YES	1.59	-3.98	292	86.88	-9.51	0.41
33	35	YES	1.09	-3.96	292	86.86	-4.74	0.41
34	36	YES	3.25	-3.86	258	41.04	-0.56	0.43
35	37	NO	3.23	-3.25	257	41.04	2.78	0.79
36	38	NO	3.84	-4.29	293	41.04	3.79	0.7
37	39	NO	2.89	-3.25	273.5	61.27	2.91	0.82
38	40	NO	2.89	-3.25	273.5	61.27	3.0	0.82
39	41	YES	1.92	-4.01	303	86.89	-7.51	0.4
40	43	NO	3.58	-3.89	269.5	41.04	1.39	0.69
41	44	NO	3.84	-4.29	293	41.04	3.26	0.69
42	45	NO	2.42	-3.23	249.5	54.16	2.66	0.84
43	46	NO	3.16	-3.57	285	50.27	2.92	0.79
44	47	NO	2.29	-2.78	259.5	53.93	2.95	0.85
45	48	NO	1.93	-2.57	209.5	41.04	2.48	0.89
46	49	NO	3.17	-3.91	269.5	41.04	3.13	0.72
47	50	NO	2.49	-3.99	283.5	67.06	1.9	0.7
48	51	YES	4.38	-5.38	340.5	41.04	2.95	0.52
49	52	YES	1.85	-4.37	315.5	86.86	-3.13	0.4
50	53	YES	1.85	-4.37	315.5	86.86	-7.92	0.38
51	54	YES	3.51	-4.51	281.5	41.04	-1.03	0.51
52	55	YES	1.31	-3.27	268.5	86.86	-4.44	0.45
53	56	YES	0.96	-2.98	284	107.0	-4.1	0.46
54	57	YES	0.96	-2.98	284	107.0	-4.2	0.45
55	58	YES	1.92	-4.01	303.5	86.86	-3.36	0.42
56	59	YES	0.39	-3.73	314	132.5	-9.5	0.42

57	**60**	**YES**	**1.21**	**-3.31**	**306**	**90.1**	**-5.2**	**0.26**
58	**61**	**YES**	**1.65**	**-3.62**	**280**	**80.86**	**-5.78**	**0.43**
59	**62**	**YES**	**1.24**	**-3.29**	**296**	**96.09**	**-4.13**	**0.44**
60	**63**	**YES**	**0.5**	**-2.96**	**260**	**100**	**-4.33**	**0.46**
61	**64**	**YES**	**0.36**	**-2.5**	**270**	**99.75**	**-4.27**	**0.47**
62	**65**	**YES**	**1.92**	**-4.01**	**303.5**	**86.86**	**-3.86**	**0.41**
63	**66**	**YES**	**0.01**	**-2.3**	**220**	**86.86**	**-4.73**	**0.48**
64	**67**	**YES**	**1.25**	**-3.68**	**280**	**86.06**	**-4.0**	**0.43**
65	**68**	**YES**	**0.57**	**-3.71**	**294**	**112.0**	**-6.02**	**0.42**
66	**69**	**YES**	**2.46**	**-5.4**	**351**	**86.86**	**-3.7**	**0.33**
67	**70**	**YES**	**0.33**	**-4.09**	**326**	**132.6**	**-5.16**	**0.4**
68	**71**	**YES**	**0.33**	**-4.09**	**326**	**132.6**	**-9.92**	**0.4**
69	**72**	**YES**	**1.09**	**-3.98**	**292**	**86.86**	**-6.132**	**0.33**

By the results obtained for OSIRIS Molecular Property explorer out of 69 compounds only 31 compounds shows non toxicity These 31 compounds we are go for PASS to check biological activity of the compounds

Table-6: Results of Prediction of activity spectra for substances (PASS):

S.No	Molecule	Romans	FtsZ polymerase inhibitor	
			Pa	Pi
1	37	3a	0.036	0.027
2	38	3b	0.036	0.028
3	39	3c	0.043	0.017
4	40	3d	0.046	0.015
5	44	3e	0.034	0.031
6	48	3f	0.039	0.023
7	49	3g	0.038	0.024
8	--	Ciprofloxacin (Standard)	0.058	0.022

Among the 31 molecules 7 molecules exhibit FtsZ Polymerase Inhibitor activity

Docking Analysis:

PDB ID: **3VO8 , 1W59**

Docking studies showed that all ligands chosen for analysis possessed a least binding affinity with the target protein Ftsz. The protein ligand interactions were studied in terms of minimum binding energy (Kcal/mol) and the number of hydrogen bonds formed with active site residues. The docking interactions of the 5 ligands and the protein Ftsz were visualised using Chimera 1.13.1 viewer and shown in Fig.10-17. The final docked confirmation obtained for the different ligands based on the binding energy, number of hydrogen bonds formed, bond distance and the interacting residues were shown in (Table 13&14), 3e and ciprofloxacin show a least binding energy with the docking score of -7.52 Kcal/mol (forms H-bond with Lys), when docked against FtsZ(PDB id:3VO8). 3g & Ciprofloxacin exhibit least binding energy with the score of -7.92 & -7.14 k.cl/mol ,docked against FtsZ(PDB ID: 1W59)

The length of the hydrogen bonds formed with interacting residues for all the ligands, which shows that the bonding was good. Most of the key residues shown in the Table 13&14 are the active

site residues of the target protein predicted by PDB. Based on the docking score all the ligands have docking interactions with the protein FtsZ.

Table-7: Docking results of all synthesized compounds & standard(Ciprofloxcin) against FtsZ protein (PDB: 3V08)

Compound	Key Residues	Distance (A°)	No.of hydrogens	Binding energy(Kcal/Mol)
3a	Lys	3.24	1	-7.34
3b	Lys Lys	2.919 3.105	2	-7.60
3e	Lys	2.979	1	-7.52
Standared(ciprofloxacin)	Lys Lys	2.734 2.665	2	-7.52

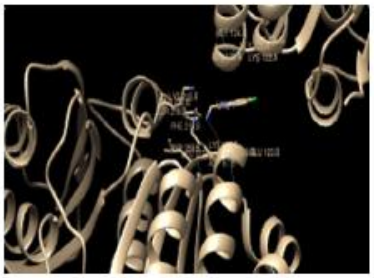


Fig-1:Docking result of 3a

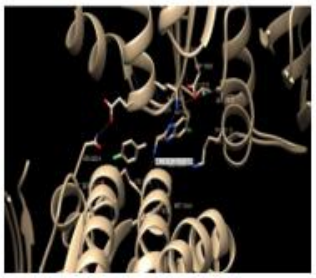


Fig-2: Docking result of 3b

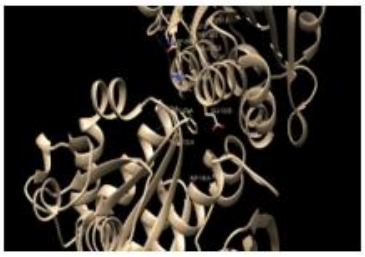


Fig-3 :Docking result of 3e

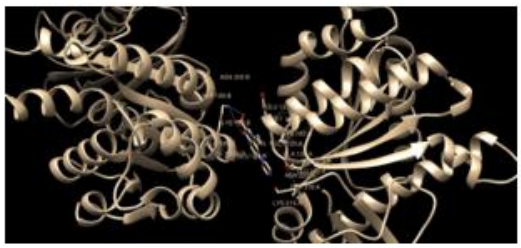


Fig-4:Docking result of standard drug(Ciprofloxacin)

Table-8: Docking results of all synthesized compounds & standard(Ciprofloxacin) against FtsZ protein (1W59)

Compound	Key Residues	Distance (A°)	No.of hydrogens	Docking Score(Kcal/Mol)
3c	TRP O-ASN	2.99 3.029	8	-8.23
3e	TRP	2.777	8	-8.11
3g	TRP	3.114	21	-7.92
Standared(ciprofloxacin)	TRP GLY THR	3.217 3.46 3.207	4	-7.14

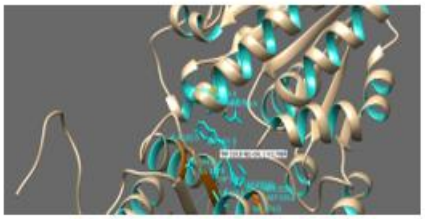


Fig-5: Docking result of 3c

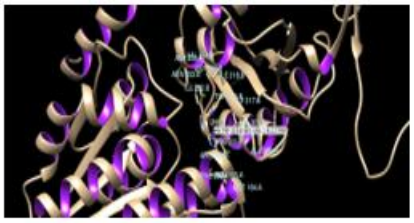


Fig-6: Docking result of 3e

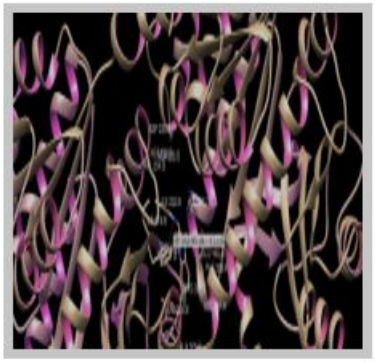


Fig-7: Docking result of 3g

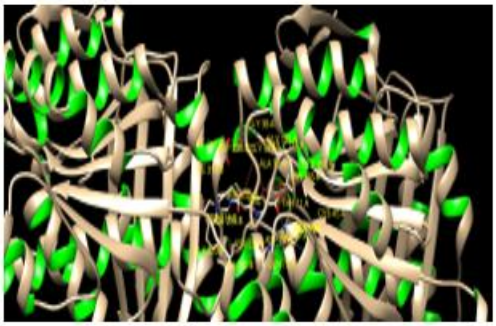


Fig-8: Docking result of standard drug(Ciprofloxacin)

ANTIBACTERIAL ACTIVITY DETERMINATION BY CUP-PLATE METHOD:

The 2 derivatives 3b and 3e were screened for their antibacterial activity against *S.aureus* ,*S.typhi, E.coli* and *M.luteus* by cup-plate method and broth dilution, the results were compared with the standard(Ciprofloxacin). Results of the study indicated that 2 compounds not exhibiting antibacterial activity at 25µg/ml, 75µg/ml and 100µg/ml

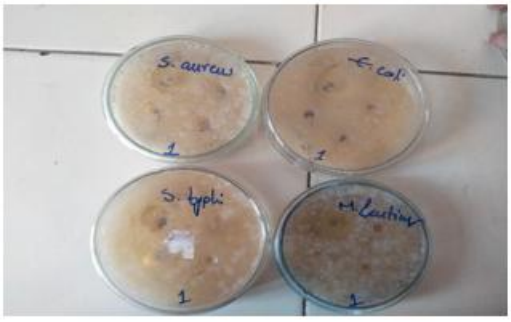


Fig-9: zone of inhibition of 3b

Fig-10: zone of inhibition of 3e

M.leuteum is showing little acivity with 3e at 100µg/ml but it is not considarable.

CONCLUSION

Benzimidazole derivatives are clinically approved for number of pharmacological activities like antiparasitics, anticonvulsants, anti ulcers, antivirals, antihypertensives, anticancers, antifungals, antiinflammatory agents and anticoagulants etc. Upto now there is no clinically approved drug for antibacterial activity which are active against different bacterial species.From the Insilico studies, amongst the 72 deigned moleules, 69 molecules satisfy the LIPINSKI rule of five and OSIRIS:- out of 69 molecules only 31 molecules shows the green colour and these molecules are non-toxic. PASS:- out of 31 compounds 7 compound shows the Prediction activity on FtsZ

enzyme. Docking:-1.Compound 3a have good binding affinity compared to the standard Ciprofloxacin with FtsZ protein(3vo8). Compound 3e and ciprofloxacin exhibit equal binding affinity to that of FstZ(3vo8). Among the three compounds Compound 3g exhibit low binding affinity to that of the FtsZ protein (PDB ID:1w59). Compound 3b & 3e were screend for antibacterial activity against (S.aureus ,S.typhi, E.coli and M.luteus) by cup-plate method by taking Ciprofloxacin as standard drug 25,50,100µg/ml concentrations didn't exhibit the zone of inhibition. Among the three compounds, 3b compound obtained with more percentage yield and less time.

ACKNOWLEDGEMENT

The authors are thankful to Head, Teaching and non teaching staff of Centre for Pharmaceutical Sciences, University College of Engineering, Science and Technology, JNTUH, Kukatpally, Hyderabad, Telangana, India.

REFERENCES

1. Anason BD, Junyi MH, Qiang H, Is the medical research right track, Global journal of medical research, 2009, 16(6), 34-35.
2. Paul MS, Daniel SM, Christopher TD, Charles CP, Bernard HM, Stacy R. Lindborg, Aaron L. Schacht, How to improve RD productivity, The pharmaceutical industry's grand challenge, Washington university school of medicine in st. louis, 2010 (9), pp:203-214.
3. Neil OM, Smith JM, Heckman PE (Eds), International journal of pharma bio sciences, 2014, 5(4), 1-239.
4. Brown KL, Chemical review on vit B 12 , Chemical Reviws, 2005, pp:105-2075.
5. Skalova BV, Mass LR, De-Vrieze FM, Bull GS, Fink G, Journal of research veterinary sciences, 2003, pp:75-61.
6. Habib NS, Soliman RA, El-Taiebi FA, Synthesis and antimicrobial testing of novel oxadiazolybenzimidazole derivatives, Pharmazie, 1997, pp:52-746.
7. Wang HG, Lown,RJ, Anticancer Drug Deseases, 1994, pp:9-153.
8. Spasov AA, Yozhitsa IN, Bugaeva LI, Anisimova VA, Benzimidazole derivatives: spectrum of pharmacological activity and toxicological properties, Pharamceutical chemistry journal, 1999, 33, 232-243.
9. Hossein N, Nasrin A, A simple, mild and efficient one-pot synthesis of 2- substituted benzimidazoles in the presence of H 2 O 2 /HCl under microwave irradiation, Journal of the Chinese chemical society, 2012, 59(8), pp: 1001-1005.
10. Lucio R, Silvano G, Nicola D, Jochen W, vitamin B 12 : Unique metalorganic compounds and the most complex vitamins, 2010, 15(5), pp: 3228-3259.
11. Sharma P, Kumar A, sharma M, Jitendra S, Prabal B, Manisha S, Kaushik MP, Syntheis and exploration of QSAR model of 2-methyl-3-[2-(2-methylprop-1-en-1-yl)-1H-benzimidazol-1-yl] pyrimidol[1,2-a] benzimidazol-4(3H)-one as potential antibacterial agents, Journal of enzyme inhibition and medicinal chemistry, 27(2), pp: 294-301.
12. Hassan YA, Ahmed AR, Benzimidazole derivatives as antidiabetic agents, Medicinal chemistry,2015, 5(7), pp:318-325.
13. Kumaraswami G, Brahmeshwari G, Ravichander M, Uma K, Synthesis, antibacterial and molecular docking studies of new benzimidazole derivatives, Egyptian Journal of Basic and Applied Sciences, 2017, 4(4), pp: 303-309.

14. Walia R, Dhamija K, Vandana MD, Akhtar J, Lamba HS, Synthesis of novel substituted benzimidazole derivatives as potential antimicrobial agents, International Journal of Pharmaceutical, Chemical andBiological Sciences, 2012, 2(3), 293-298.
15. Song D, Shutao MA, Recent development of benzimidazole containing antibacterial agents, chem medchem,2016,11, 646-659
16. Rathee PS, dhanker R, Bharadhwaj S, Mounika Kumar R, Synthesis and antimicrobial studies of novel benzimidazole derivatives, Journal of pharmaceutical science,2011,1(4), 127-130
17. Oren I, Yalcin I, Sener E, Ucarturk N, Synthesis and structure activity relationship of new antimicrobial active multisubstituted benzimidazole derivatives, European journal of chemistry,2004,39, 291-298.
18. Prashanth K, Kumar R, Green synthesis of benzimidazole derivatives: an overview of bulk drug synthesis, International journal of pharma technology research, 2015,8(9), 60-68.
19. Rithe S, Jagtap RS, Ubarhande SS, One pot synthesis of substituted benzimidazole derivatives and their charecterisation, Rasayan journal of chemistry, 2015,8(2),pp:213- 217.
20. Vinod K, Misra A, khalid M and khalid M and Singh S, Synthesis, characterisation and antimicrobial evaluation of some novel cyclic and acyclic terpenoid substituted benzimidazole-semicarbazone derivatives, Journal of pharmaceutical,2016,4(3), 350-358.
21. Joshi D, Kalpesh P, Synthesis and evaluation of novel benzimidazole derivatives as antimicrobial agents, Medicinal chemistry of research, 2014,23,1290-1293.
22. Ajani O, Aderohunmu D, Olorunshola S, Ikpo O, Olanrewaju O, Facile synthesis, charecterisation and antimicrobial activity of 2-alkanamino benzimidazole derivatives, Oriental journal of chemistry, 2016,32(1),109-120.
23. Janeczko M, Kazimierczuk Z, Niewiadomy A, Krol E, Szyszka and Maslyk M, In search of antimicrobial potential of benzimidazole derivatives, Polish journal of microbiology, 2016,65(3),359-364.

HOW TO CITE: M. Chennarao, S. Shobharani, G. Sai Krishna, Design, Synthesis and Evaluation of New 2, 5-Di Substituted Benzimidazole Derivatives, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 5, 1419-1432, https://doi.org/10.5281/zenodo.20068213