ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

NOVEL DIARYL-2-MERCAPTOBENZIMIDAZOLE ANALOGUES AS POTENTIAL ANTI-MITOTIC AND ANTI-BACTERIAL AGENTS.

D. Ravi Sankara Reddy*, D. Abhilasha, I. Supriya, T. Mounika

Department of Pharmaceutical Chemistry, University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur 522510, Andhra Pradesh, India.

*Corresponding author E-mail: drsrpharma@gmail.com

ARTICLE INFO

Key words:

Multidrug resistance, Molecular modelling, Diaryl-2-mercaptoBenzimidazole, Anti-mitotic, Autodock vina, Agar disc diffusion.

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

Now a days the drug resistance is a major challenge to the medicinal chemists particularly antibiotics as chemotherapeutic agents. This study aims to design such moiety to combat the drug resistance. One significant pharmacophore in contemporary drug research is the benzimidazole ring. The compounds bearing benzimidazole moiety are reported to possess a number of interesting biological activities like cancer-fighting agent, anti-ulcer and antibacterial effects. The newly synthesized diaryl-2-mercapto benzimidazole compounds are characterized by ^{1}H NMR, ^{13}C NMR, MASS and FTIR spectroscopic techniques and are screened for *in-vitro* anti-mitotic activity by mung bean germination method and antibacterial activity by agar disc diffusion method. Molecular docking studies also performed to newly synthesized compounds to know the binding affinity with protein human epithelial growth factor (3CRD) using Autodock vina software. Derivatives 7c and 7e displayed good antiproliferative response than the aspirin at 100 and 200 µg/ml concentration respectively through % radical growth and % weight gain inhibition .Molecular modelling studies of protein 3RCD revealed that 7c (-9.87 kcal/mol) and 7e (-9.73 kcal/mol) displayed good binding affinity than the standard drug aspirin (-6.2 kcal/mol) due to hydrogen bonding, hydrophobic and electrostatic interactions. The anti-bacterial study results revealed that, among all the 8 novel derivatives 7d (14, 10mm), 7e (13, 10mm) showed moderate activity when compared to streptomycin (15, 12mm) against *E. coli* and *S. aureus* at 50 µg/ml concentration respectively.

INTRODUCTION

Fused imidazole derivatives have gained prominence in medicinal chemistry due to their significant therapeutic properties in clinical applications.[1,2] Because of this, benzimidazole is being studied by the pharmaceutical industry and is crucial in contemporary drug research.[3] Due to their exceptional structural characteristics, the derivatives of substituted benzimidazoles, especially 2-mercaptobenzimidazoles, have also been found to have a variety of medical uses.[4-6] Moreover, structural isosteres of benzimidazole exist spontaneously, which allows nucleotides to interact with the nucleic acid with ease. With a broad range of biological activities, including anti-convulsant,[4] anti-oxidant,[5] anti-cancer,[7] anti-inflammatory,[8] anthelmintic,[8] anti-microbial,[9] anti-hypertensive,[10] anti-viral, HIV-RT inhibitor,[11] anti-ulcer properties.[12]

The benzimidazole-containing compounds have a broad spectrum of pharmacological activities due to the versatile core present in many commercially available drugs like Bendamustin (anti-cancer and anti-fungal),[13,14] Maribavir (anti-viral),[15] Rabeprazole (anti-ulcer),[12] and Telmisartan (anti-hypertensive). New medications to treat bacterial infections and cancer are desperately needed. Cancer continues to be a major cause of death globally, and current treatments frequently face obstacles like resistance, low effectiveness, and numerous adverse effects. In a similar vein, hospital-acquired infections, new infectious illnesses, and growing drug resistance make bacterial infections a serious issue. There is an urgent need for new medications, especially innovative antibiotics, to treat these issues. In order to address this, the current study used artificial intelligence (AI) technologies to screen a novel set of diaryl 2-mercapto benzimidazole derivatives that were evaluated for their ability to inhibit bacterial infections and cell proliferation using *in-silico* and *in-vitro* experiments.

MATERIALS AND METHODS

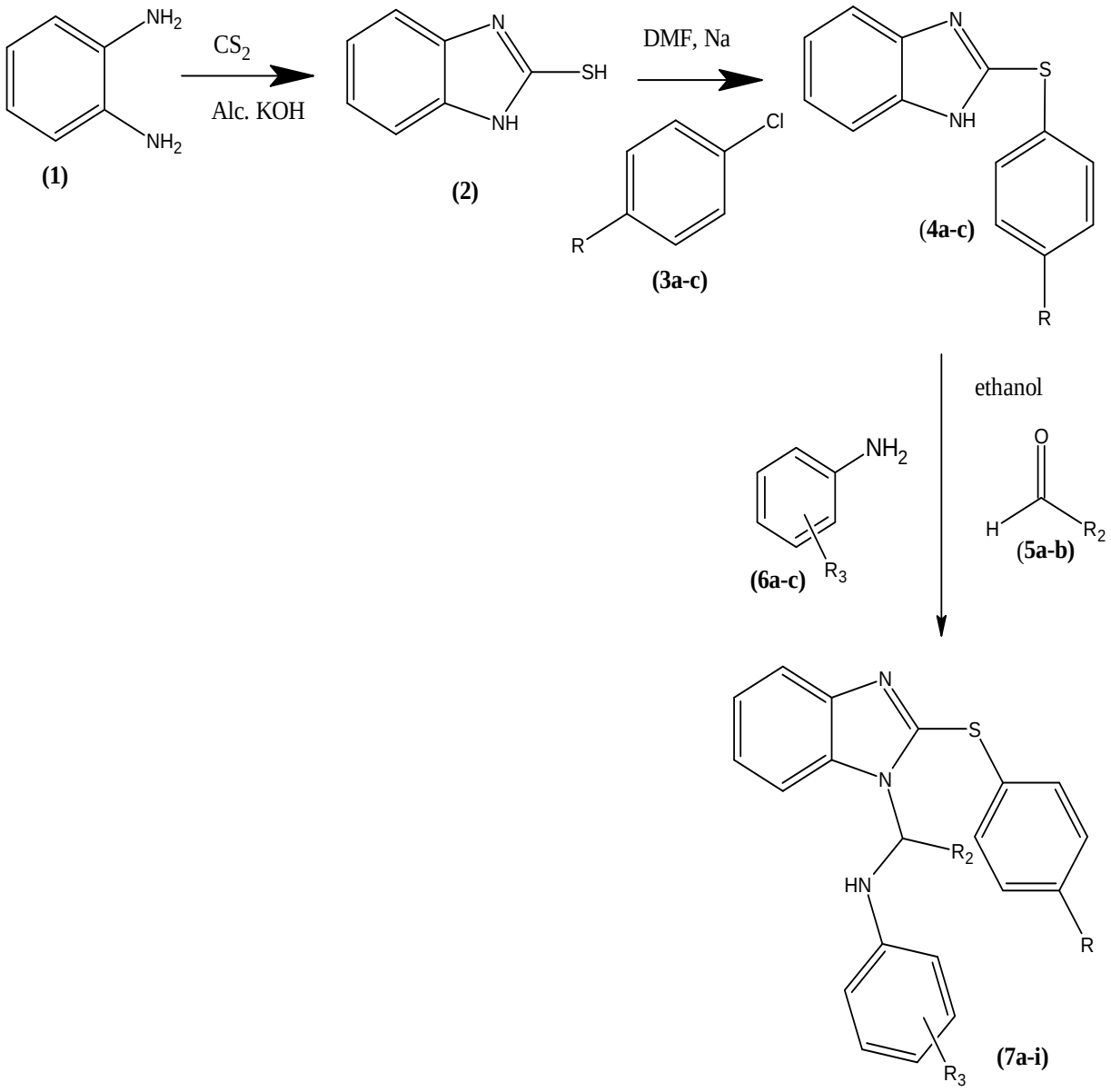
All of the materials used in the current research work were of LR grade and commercial grade from AVRA Synthesis Pvt Ltd, Hyderabad with a purity of more than 95%. Melting ranges of all the synthesised compounds were determined using POLMON (MP96) instrument. ATR technique was used to record the FTIR spectra on a Shimadzu equipment (IRAffinity-1S). Spectra of the ^{13}C and ^{1}H NMR were captured on the BRUCKER (400 MHz) using trimethylsilane as the internal reference in DMSO-d6. The chemical shift values are given in ppm . Employing the ESI-API ionisation method, mass spectra were recorded on an Agilent 6120 spectrometer. Using Merck silica gel 60F254 (105554) plates that were eluted with the appropriate mobile phase and visible under UV light at a wave length of 254 nm, the reactions' progress was tracked. By using a gradient mobile phase and a column chromatography packed with silica (SiO2, 60–120 mesh), all freshly produced compounds were purified. Solvent system used is Chloroform/Ethanol as eluent. The UV chamber displayed the spots. Docking studies were performed on AutoDock vina 1.1.2 software.

Chemistry

General procedure for synthesis of 2-((4-substituted phenyl) -thiolyl) -1H-benzimidazole (4a-c):[16-19] Orthophenylene diamine **1** (3.24g) was added to the mixture of potassium hydroxide (1.9g) in 60ml of 50% ethanol and carbon disulphide (2.7g). The mixture was cooled after refluxed for 4 hrs at 100-110^{0}C and add distilled water. The 2-mercapto benzimidazole **2** gets precipitated by the addition of 50% acetic acid (scheme 1). The product was separated, washed with water and recrystallized by ethanol. Compound **2** (0.75g) in 8ml of Dimethylformamide (DMF) added to the 2.5ml of dry methanolic solution of sodium (0.12g). After 10 min. substituted aryl halides **3a-c** (0.63g) added to the mixture in 2 to 3 portions and stirred for 5 hrs. Then pour the above mixture to an ice bath, solid gets filtered and wash with cold water. The product **4a-c** was recrystallized by methanol (scheme 1).

General procedure for synthesis of 1,2-disubstituted benzimidazole derivatives (7a-i):2-((4-substituted phenyl)-thiolyl)-1H-benzimidazole **4a-c** (0.02 mol) added slowly to the aldehydes **5a-b** (0.02mol), amines **6a-c** (0.02 mol) in 15ml of ethanol with continuous stirring for 45 min. followed by overnight refrigeration (Scheme 1). Filter the solid product and recrystallize by absolute alcohol. Product formation was confirmed by using TLC throughout the synthesis and check for melting points uncorrected (see Table 1).

***4-{[1-(1-anilinoethyl)-1H-benzimidazol-2-yl]sulfanyl}benzaldehyde (7a)*:** 4-((1H-benzo[d]imidazol-2-yl)thio)benzaldehyde **4a** (0.02 mol) added slowly to acetaldehyde **5a** (0.02mol), aniline **6a** (0.02 mol) in 15ml of ethanol with continuous stirring for 45 min. followed by overnight refrigeration. Filter the solid product and recrystallize by absolute alcohol. Yield: 89.94%; mp: 164-166^{o}C; mf: $C_{22}H_{19}N_3OS$; mw: 373.47; Rf: 0.62 ($CHCl_3$: CH_3OH 7:3); FTIR (KBr, cm^{-1}): 3240 (2^0 NH), 3005 (Ar-CH), 2973(CH), 2832(2^{0}CH), 1705 (C=O), 1618 (Ar C=C), 1508 (C=N), 1349 (aldehyde CH), 1257 (C-N), 652(C-S); MS (m/z), M^+ :372.1.



Scheme 1: Synthesis of substituted 1, 2-diaryl benzimidazole derivatives.

4-({1-[1-(4-hydroxyanilino)ethyl]-1H-benzimidazol2-yl}sulfanyl)benzaldehyde (7b): 4-((1H-benzo[d]imidazol-2-)thio)benzaldehyde **4a** (0.02 mol) added slowly to acetaldehyde **5a** (0.02mol), 4-amino phenol **6b** (0.02 mol) in 15ml of ethanol with continuous stirring for 45 min. followed by overnight refrigeration. Filter the solid product and recrystallize by absolute alcohol. Yield: 86.41%; mp: 166-168^{o}C; mf: $C_{22}H_{19}N_3O_2S$; mw: 389.47; Rf: 0.61 ($CHCl_3$: CH_3OH 7:3); FTIR (KBr, cm^{-1}): 3385(OH), 3299(2^0 NH), 3009(Ar-CH), 2972(CH), 2837(2^0CH), 1708(C=O), 1616(Ar C=C), 1504(C=N), 1353(aldehyde CH), 1270(C-N), 1172(C-O), 651 (C-S); MS (m/z), M^+ :387.9

4-({1-[(2-hydroxyanilino)methyl]-1H-benzimidazol-2-yl}sulfanyl)benzaldehyde (7c):4-((1H-benzo[d]imidazol-2 yl)thio)benzaldehyde 4a (0.02 mol) added slowly to formaldehyde 5b (0.02mol), 2-amino phenol 6c (0.02 mol) in 15ml of ethanol with continuous stirring for 45 min. followed by overnight refrigeration. Filter the solid product and recrystallize by absolute alcohol.Yield: 63.97%; mp: 166-168^{o}C; mf: $C_{21}H_{17}N_3O_2S$; mw: 375.44; Rf: 0.63 ($CHCl_3$: CH_3OH 7:3); FTIR (KBr, cm^{-1}): 3377(OH), 3268(2^0 NH),

3001(Ar-CH), 2968(CH), 2859(2^0CH), 1708(C=O), 1593(Ar C=C), 1504(C=N), 1357(aldehyde CH), 1270(C-N), 1174(C-O), 650(C-S); MS (m/z), M^+ :374.2

N-(1-{2-[(4-methylphenyl)sulfanyl]-1H-benzimidazol-1-yl}ethyl)aniline (7d): 2-(p-tolylthio)-1H-benzo[d]imidazole **4b** (0.02 mol) added slowly to acetaldehyde **5a** (0.02mol), aniline **6a** (0.02 mol) in 15ml of ethanol with continuous stirring for 45 min. followed by overnight refrigeration. Filter the solid product and recrystallize by absolute alcohol. Yield: 91.71%; mp: 215-217^{o}C; mf: $C_{22}H_{21}N_3S$; mw: 359.49; Rf: 0.69 ($CHCl_3$:CH_3OH 7:3); FTIR (KBr, cm^{-1}): 3238(2^0 NH), 3072(Ar-CH), 2972(CH_3), 2823(2^0CH), 1600(Ar C=C), 1505(C=N), 1252(C-N), 652(C-S); MS (m/z), M^+ :358.6

4-[(1-{2-[(4-methylphenyl)sulfanyl]-1H-benzimidazol-1-yl}ethyl)amino]phenol (7e):2-(p-tolylthio)-1H-benzo[d]imidazole **4b** (0.02 mol) added slowly to acetaldehyde **5a** (0.02mol), 4-amino phenol **6b** (0.02 mol) in 15ml of ethanol with continuous stirring for 45 min. followed by overnight refrigeration. Filter the solid product and recrystallize by absolute alcohol. Yield: 85.12%; mp: 224-226^{o}C; mf: $C_{22}H_{21}N_3OS$; mw: 375.49; Rf: 0.60 ($CHCl_3$: CH_3OH 7:3); FTIR (KBr, cm^{-1}): 3556(OH), 3151(2^0 NH), 3103(Ar-CH), 2974(CH), 2874(2^0CH), 1598(Ar C=C), 1507(C=N), 1252(C-N), 1172(C-O), 651(C-S); MS (m/z), M^+ :375.01

2-[({2-[(4-methylphenyl)sulfanyl]-1H-benzimidazol-1-yl}methyl)amino]phenol (7f):2-(p-tolylthio)-1H-benzo[d]imidazole **4b** (0.02 mol) added slowly to formaldehyde **5b** (0.02mol), 2-amino phenol **6c** (0.02 mol) in 15ml of ethanol with continuous stirring for 45 min. followed by overnight refrigeration. Filter the solid product and recrystallize by absolute alcohol. Yield: 82.01%; mp: 228-230^{o}C; mf: $C_{21}H_{19}N_3OS$; mw: 361.46; Rf: 0.72 ($CHCl_3$: CH_3OH 7:3); FTIR (KBr, cm^{-1}): 3418OH), 3151(2^0 NH), 3103(Ar-CH), 2978(CH), 2872(2^0CH), 1614(Ar C=C), 1510(C=N), 1299(C-N), 1172(C-O), 652(C-S); MS (m/z), M^+ :360.89

4-{[1-(1-anilinoethyl)-1H-benzimidazol-2-yl]sulfanyl}aniline (7g): 4-((1H-benzo[d]imidazol-2-yl)thio)aniline **4c**(0.02 mol) added slowly to acetaldehyde **5a** (0.02mol), aniline **6a** (0.02 mol) in 15ml of ethanol with continuous stirring for 45 min. followed by overnight refrigeration. Filter the solid product and recrystallize by absolute alcohol. Yield: 73.81%; mp: 205-208 ^{o}C; mf: $C_{21}H_{20}N_4S$; mw: 360.48; Rf: 0.68 ($CHCl_3$: CH_3OH 7:3); FTIR (KBr, cm^{-1}): 3693,3661(Ar-NH 1^0), 3150(2^0 NH), 3114(Ar-CH), 2981(CH), 2838(2^0CH), 1592(Ar C=C), 1505(C=N), 1252(C-N), 649(C-S) ; H^1NMR (DMSO, 300MHz) δ/ppm: 1.9 (CH_3, 3H), 2.50 (1^0 NH, 2H), 2.63 (2^0 NH, 1H), 2.86 (3^0 CH, 1H), 7.09-7.18 (Ar-H, 13H) ; C^{13} NMR ($CDCl_3$, 100MHz) δ/ppm: 20.17 (CH_3, 1C), 64.54 (3^0 CH, 1C), 109-132.25 (Ar-C, 18C), 168 (C=N azole, 1C) ; MS (m/z), M^+ :359.60

4-[(1-{2-[(4-aminophenyl)sulfanyl]-1H-benzimidazol-1-yl}ethyl)amino]phenol (7h):4-((1H-benzo[d]imidazol-2-yl)thio)aniline **4c**(0.02 mol) added slowly to acetaldehyde **5b** (0.02mol), 4-amino phenol **6b** (0.02 mol) in 15ml of ethanol with continuous stirring for 45 min. followed by overnight refrigeration. Filter the solid product and recrystallize by absolute alcohol. Yield: 68.40%; mp: 187-190^{o}C; mf: $C_{21}H_{20}N_4OS$; mw: 376.47; Rf: 0.65 ($CHCl_3$: CH_3OH 7:3); FTIR (KBr, cm^{-1}): 3693,3683(Ar-NH 1^0), 3571(OH), 3151(2^0 NH), 3113(Ar-CH), 2981(CH), 2879(2^0CH), 1611(Ar C=C), 1507(C=N), 1251(C-N), 1173(C-O), 651(C-S); MS (m/z), M^+ :375.61

2-[({2-[(4-aminophenyl)sulfanyl]-1H-benzimidazol-1-yl}methyl)amino]phenol (7i): 4-((1H-benzo[d]imidazol-2-yl)thio)aniline **4c**(0.02 mol) added slowly to acetaldehyde **5b** (0.02mol), 2-amino phenol **6c** (0.02 mol) in 15ml of ethanol with continuous stirring for 45 min. followed by overnight refrigeration. Filter the solid product and recrystallize by absolute alcohol. Yield: 68.16%; mp: 202-206^{o}C; mf: $C_{20}H_{18}N_4OS$; mw: 362.45; Rf: 0.66 ($CHCl_3$: CH_3OH 7:3); FTIR (KBr, cm^{-1}): 3693,3673(Ar-NH 1^0), 3377(OH), 3237(2^0 NH), 3114(Ar-CH), 2972(CH), 2852(2^0CH), 1695(Ar C=C), 1511(C=N), 1260(C-N), 1178(C-O), 650(C-S); MS (m/z), M^+ :361.4.

Pharmacological studies

In-vitro **antimitotic activity by mung bean germination method:**[20]Mung beans weighing 1.54±0.013g used in this investigation were

obtained from local market. They were soaked for 6 hrs in tap water as control group and in benzimidazole derivatives (7a-i),aspirin[21] (standard drug) solutions of varying concentrations (100, 200, 300 µg/ml) as test group. All the drained seedlings were kept moist with those solutions until radicals in the control group had grown to length 1.0-1.5cm and considered as time 0 (t_0). Measure the various parameters like weight variation, radical length, % of seed germination at t_0 and t_{48} (by maintaining the same conditions under room temperature for 48 hrs). The percentage of seed germination was calculated using these formulae.

$$\%\ of\ seed\ germination = \left(\frac{No.of\ seeds\ germinated}{total\ No.of\ seeds\ tested}\right) x\ 100$$

$$\%\ of\ inhibition\ seedling\ weight = \left(\frac{Difference\ in\ test\ seedling\ weight}{Difference\ in\ control\ seedling\ weight}\right) x\ 100$$

$$\%\ inhibition\ radical\ lenght = \left(\frac{difference\ in\ test\ seedling\ radical\ lenght}{difference\ in\ control\ seedling\ lenght}\right) x\ 100$$

***In-silico* antimitotic activity:**[22,23]*In-silico* antimitotic activity was performed by using docking studies with AUTODOCK Vina. All the title compounds 7a-iand standard drug aspirin were assessed for their binding affinities against hEGF (human Epithelial Growth Factor). All the ligands were drawn using Chemsketch software and prepare pdb files by minimising energy using open babel and convert them into pdbqt in Autodock with torsion angles. X-ray crystal structure of hEGF protein (pdb 3RCD) was retrieved from protein data bank with resolution 3.21A^0. Protein cleaning was done with removal of heteroatoms and water molecules, protein preparation was carried out by addition of polar hydrogens and kolmann charges. Make the grid box having dimensions of 40A^0 over the three axis (X,Y,Z) with spacing about 0.375 A^0. The following settings were applied when using the Lamarckian genetic algorithm (LGA) for docking: 25,000,000 energy evaluations as the maximum, a population of 300 randomly placed individuals, a maximum number of 27,000 generations, a mutation rate of 0.2, a crossover rate of 0.80, an elitism value (number of top individuals that automatically survive) of 50 docking runs. Results were clustered according to the root-mean square deviation (RMSD) values. The best docked conformations of ligands were selected as initial active/binding conformations to build the complexes for MD studies. The binding interactions of the molecules were visualized using the Discover studio (BIOVIA).

Antibacterial assay using agar disc diffusion method:[24,25] The antibacterial activity of disubstituted benzimidazole derivatives (**7a-i**) was estimated against 4 bacterial strains using agar disc diffusion method. The 4 strains include Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. DMF used as a control as the derivatives **7a-i**, standard drug was dissolved in DMF by using serial dilutions in sterile test tubes. Sterile filter paper discs with 5mm diameter impregnated with all the test concentrations (50, 100, 200, 400 µg/ml) used for assay. Amoxicillin at 100 µg per disc was used as positive control. The inoculum of all the four strains was prepared from fresh culture using culture media for 24hrs. In the next day, discs placed on petri plates containing 20ml of solidified nutrient broth inoculated with respective bacterial strains using Muller- Hinton Agar medium were incubated overnight at 37±2^{0}C temperature. The inhibition zone diameter of test samples was measured.

RESULTS AND DISCUSSION:

Chemistry: Novel diaryl-2-mercapto benzimidazole derivatives (**7a-7i**) were prepared starting with the cyclization of o-phenylene diamine (**1**) and carbon disulphide in presence of alcoholic potassium hydroxide which acts as a catalyst and solvent, followed by dehydrohalogenation with 4- substituted chloro benzene derivatives (**3a-c**) in presence of DMF, sodium in dry methanol which facilitates hydrogen chloride elimination yields 2-((4-substituted phenyl)thio)-benzimidazole derivatives (**4a-c**). In the next step, (**4a-c**) undergoes condensation with aldehydes (**5a-b**) and 4-substituted anilines (**6a-c**) leaving water molecule as by product yields corresponding diaryl-2-mercapto benzimidazole derivatives.

All derivatives melting points were checked and uncorrected (Table-1). All the diaryl-2-mercapto benzimidazole derivatives displayed characteristic peaks by means of IR, NMR and Mass spectrum, which confirmed the presence of respective functional group. In the IR spectra of all the title compounds peak at 652-649 cm^{-1} for C-S-C bending indicates the condensation between 2-mercapto-1H-benzimidazole and substituted chlorobenzenes. Broad peak at 3299-3150 cm^{-1} for 2^0NH stretching, 1504-1511 cm^{-1} for aromatic C=N stretching, 1251-1299 cm^{-1} for aromatic C-N stretching, 3001-3114 cm^{-1} for aromatic CH stretching and ring stretching (Ar C=C)1592-1618 cm^{-1}indicates the presence of benzimidazole ring. Whereas the spectra at 2823-2972 cm^{-1} for CH stretching indicates presence of alkyl (1^0& 2^0) group confirmed the condensation between aldehyde, aryl primary and secondary amines, nothing but title compounds **7a-7i**.

Pharmacological Screening

In-vitro Antimitotic activity: All the test compounds screened for cell growth inhibition potential 45-55% of mung beans germinated in control group (Fig. 1). Different concentrations (100, 200 and 300µg/ml) of benzimidazole derivatives and standard drugs displayed dose dependant response included in this protocol (Table 2 and Fig. 1). Derivatives except **7g** and **7i**remaining all inhibit the seed germination at high concentration (300 µg/ml) while aspirin inhibit the seed germination at low concentration (100 µg/ml) (see Fig. 2). The extent of water imbibition and seedling growth was indicated by an increase in seedling weight at t_0 when compared to that of dry seeds and t_{48} to t_0 respectively (Table 2). Seedlings at all the concentrations at different time periods (t_0 and t_{48}) showed the gradual increase in its weights and percentage of inhibition ranges from 35-92% (Table 3). Derivative **7a**(69%), **7h** (61.5%), **7i** (69%) and aspirin exhibited maximum inhibition of seed weights at 300 µg/ml concentration, **7d** (82%), **7e** (91.5%)exhibited maximum inhibition of radical length at 200 µg/ml whereas **7b** (69%), **7c** (92%),**7f** (92%), **7g** (80.7%) exhibited maximum inhibition of seed weights at 100µg/ml (Fig.3).The gain in radical length at t_{48} was markedly affected by all the seedlings and the inhibition ranges about 17-86%.Derivatives **7b** (69%),**7c** (56%), **7d** (69%), **7h** (39%)displayed maximum radical growth inhibition at 100 µg/ml, derivative **7a** (47%), **7f** (47%), **7g** (65%), **7i** (47%) and aspirin (56%) displayed maximum radical growth inhibition at 300 µg/ml whereas **7e** (86%) inhibit maximum radical growth at 200 µg/ml.

Table 1-Title compounds substituent's, melting point and yields

Compound code	R	R_2	R_3	Melting point (^{0}C)	% yield
7a	-CHO	$-CH_3$	-H	164-166	89.94
7b	-CHO	$-CH_3$	4-OH	166-168	86.41
7c	-CHO	-H	2-OH	166-168	63.97
7d	$-CH_3$	$-CH_3$	-H	215-217	91.71
7e	$-CH_3$	$-CH_3$	4-OH	224-226	85.12
7f	$-CH_3$	-H	2-OH	228-230	82.01
7g	$-NH_2$	$-CH_3$	-H	205-208	73.81
7h	$-NH_2$	$-CH_3$	4-OH	187-190	68.40
7i	$-NH_2$	-H	2-OH	202-206	68.16

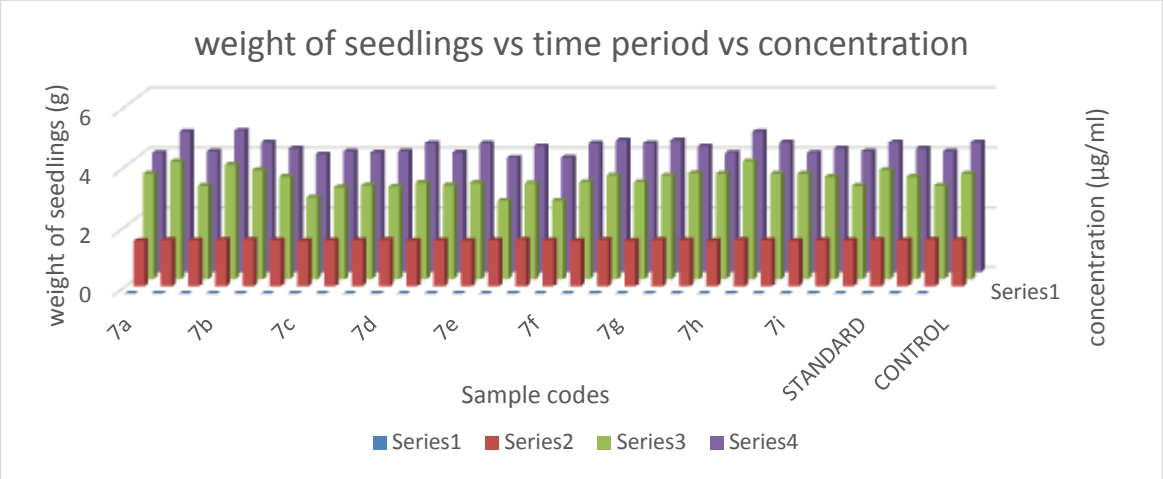


Figure 1- Effect of different concentrations of title compounds (100, 200 and 300 µg/ml) on seedlings weight at different time intervals (initial, t_0 and t_{48}); Series 1- concentrations (100, 200, 300µg/ml), series 2- initial weight of all samples, Series 3- weight of seeds at T_0, series 4- weight of seeds at T_{48}.

Table 2- Antimitotic activity of title compounds 7a-7i by seed germination assay.

Comp. codes	Con. (µg/ml)	Sample code	Weight of the seedling (g)			Radical length (cm)		Seed Germination		% Seed Germination (%)	
			Initial	t_0	t_{48}	t_0	t_{48}	t_0	t_{48}	t_0	t_{48}
7a	100	T_1	1.52	3.52	3.98	0.89	0.98	9	11	45	55
	200	T_2	1.55	3.92	4.68	0.85	0.91	8	11	40	55
	300	T_3	1.54	3.12	4.02	0.84	0.95	9	10	45	50
7b	100	T_4	1.56	3.82	4.72	1.02	1.18	10	11	50	55
	200	T_5	1.56	3.64	4.32	0.52	0.58	7	9	35	45
	300	T_6	1.54	3.42	4.12	0.58	0.62	6	8	30	40
7c	100	T_7	1.52	2.73	3.93	1.32	1.45	10	12	50	60
	200	T_8	1.54	3.07	4.02	1.19	1.28	11	13	55	65
	300	T_9	1.54	3.13	3.99	1.25	1.32	9	11	45	55
7d	100	T_{10}	1.55	3.09	4.02	0.98	1.14	10	12	50	60
	200	T_{11}	1.52	3.22	4.29	1.06	1.15	8	10	40	50
	300	T_{12}	1.54	3.13	3.99	1.12	1.25	9	11	45	55
7e	100	T_{13}	1.52	3.22	4.29	1.06	1.15	8	10	40	50
	200	T_{14}	1.54	2.62	3.81	0.92	1.12	10	11	50	55
	300	T_{15}	1.56	3.21	4.20	0.66	0.76	10	12	50	60
7f	100	T_{16}	1.54	2.62	3.82	0.80	0.87	10	11	50	55
	200	T_{17}	1.52	3.24	4.29	0.71	0.78	9	11	40	50
	300	T_{18}	1.55	3.46	4.39	0.91	1.02	8	10	40	50
7g	100	T_{19}	1.52	3.24	4.29	0.71	0.78	9	11	40	55
	200	T_{20}	1.55	3.46	4.39	0.91	1.02	8	10	40	50
	300	T_{21}	1.54	3.54	4.20	0.82	0.97	9	11	40	55
7h	100	T_{22}	1.52	3.52	3.98	0.89	0.98	9	11	45	55
	200	T_{23}	1.55	3.93	4.68	0.85	0.91	8	11	45	55
	300	T_{24}	1.54	3.52	4.32	0.71	0.98	9	11	40	50
7i	100	T_{25}	1.52	3.52	3.98	0.89	0.95	9	11	45	55
	200	T_{26}	1.54	3.42	4.12	0.56	0.65	6	8	30	40
	300	T_{27}	1.54	3.12	4.02	0.84	0.95	9	10	45	50
Standard	100	T_{28}	1.56	3.64	4.32	0.50	0.54	7	9	35	45
	200	T_{29}	1.54	3.42	4.12	0.56	0.62	6	8	30	40
	300	T_{30}	1.56	3.12	4.02	0.61	0.74	7	9	35	45
Control	-	C	1.56	3.52	4.82	1.21	0.98	9	11	45	55

Table 3- Percentage of growth inhibition of seed weight and radical length of title compounds.

Compound	Sample code	Difference in parameters between time intervals t_0 and t_{48} (t_{48}-t_0)		% of growth inhibition	
		Seedlings weight (g)	Radical length (cm)	Weight (%)	Radical Length (%)
7a	T_1	0.46	0.09	35	39
	T_2	0.76	0.06	58	26
	T_3	0.9	0.11	69	47
7b	T_4	0.9	0.16	69	69
	T_5	0.68	0.06	52	26
	T_6	0.7	0.04	53.8	17
7c	T_7	1.2	0.13	92	56
	T_8	0.95	0.09	73	39
	T_9	0.86	0.07	66	30
7d	T_{10}	0.93	0.16	71	69
	T_{11}	1.07	0.09	82	39
	T_{12}	0.86	0.13	66	56
7e	T_{13}	1.07	0.09	82	39
	T_{14}	1.19	0.2	91.5	86
	T_{15}	0.99	0.1	76	43
7f	T_{16}	1.2	0.07	92	30
	T_{17}	1.05	0.07	80.7	30
	T_{18}	0.93	0.11	71.5	47
7g	T_{19}	1.05	0.07	80.7	30
	T_{20}	0.93	0.11	71.5	47
	T_{21}	0.66	0.15	50.7	65
7h	T_{22}	0.46	0.09	35	39
	T_{23}	0.75	0.06	57.6	26
	T_{24}	0.8	0.27	61.5	17
7i	T_{25}	0.46	0.06	35	39
	T_{26}	0.7	0.09	53.8	26
	T_{27}	0.9	0.11	69	47
Standard (Aspirin)	T_{28}	0.68	0.04	52	17
	T_{29}	0.7	0.06	53.8	26
	T_{30}	0.9	0.13	69	56
Control	C	1.3	0.23	-	-

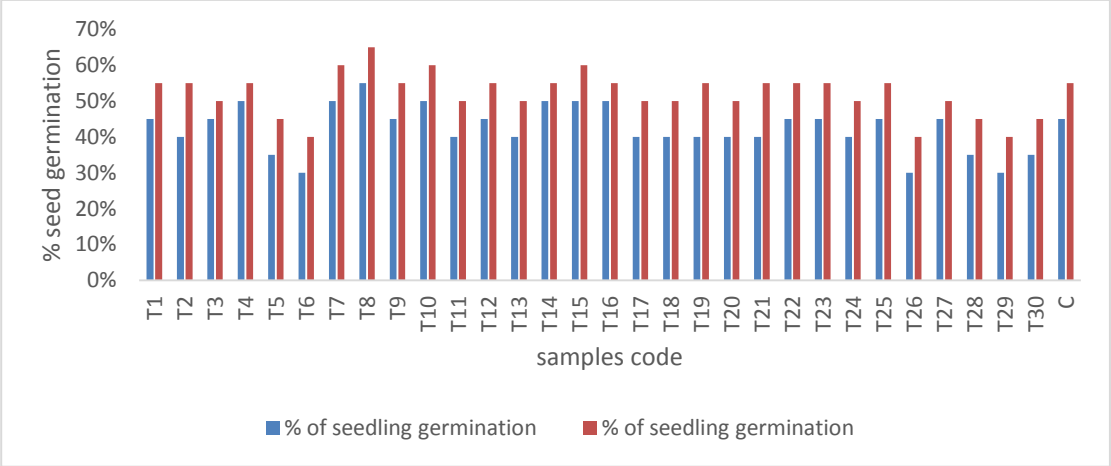


Figure 2- Percentage of seed germination of different concentrations of synthetic derivatives against standard drug aspirin

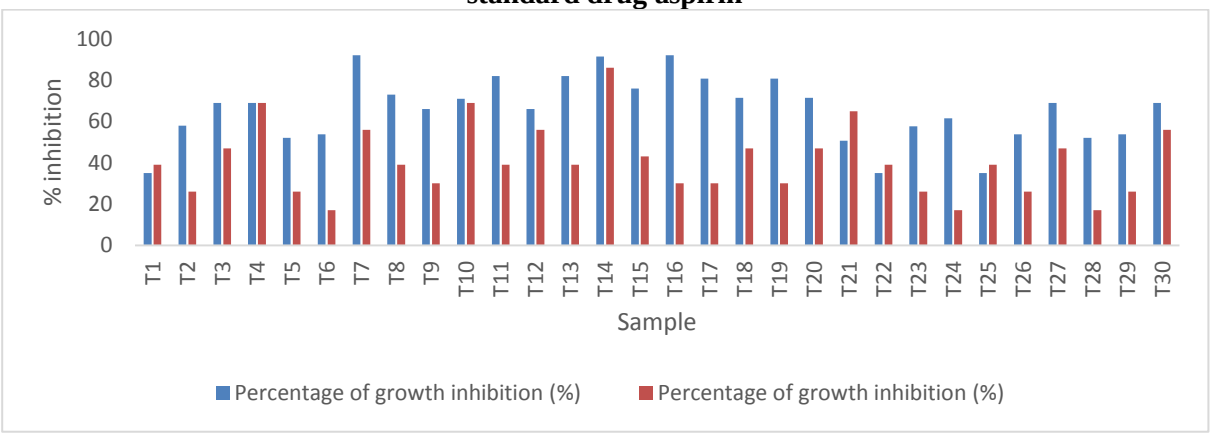


Figure 3- Percentage inhibition of seedling weight and radical length of title compounds (T_1-T_{27}) and aspirin (T_{28}-T_{30}) at different concentrations.

Table 4- Docking results of novel diaryl-2-mercapto benzimidazole compounds.

COMPOUND CODE	Binding Energy (kcal/mol)	Hydrogen bond	Interacting amino acid residue	Interaction atoms	
				ligand	Protein
7a	-8.96	1	Thr A 766	O (aldehyde)	NH (amide)
7b	-9.21	4	Ala A719	O (phenolic)	NH (amide)
			Lys A721	O (aldehyde)	NH (amide)
			Leu A764	O (phenolic)	NH (amide)
			Thr A766	O (phenolic)	NH (amide)
7c	-9.87	1	Met A 769	O (phenolic)	NH (amide)
7d	-8.91	1	LYS A721	N_1(imidazole)	NH (amide)
7e	-9.73	1	LYS A721	N_1(imidazole)	NH (amide)
7f	-7.85	1	MET A769	O (phenolic)	NH (amide)
7g	-8.87	-	-	-	-
7h	-8.05	3	CYS A751	N (aniline)	NH (amide)
			THR A766	O (phenolic)	NH (amide)
			MET A769	O (phenolic)	NH (amide)
7i	-8.41	1	MET A769	O (phenolic)	NH (amide)
Aspirin	-6.2	4	LYS A721	O (carboxyl)	NH (amide)
			THR A766	O (ester)	NH (amide)
			ASP A831	O (carboxyl)	OH (acid)
			ASP A831	O (carboxyl)	NH (amide)

ALA- alanine, ASP- aspartic acid, CYS- cysteine, LEU- leucine, LYS- lysine, MET- methionine, THR- threonine.

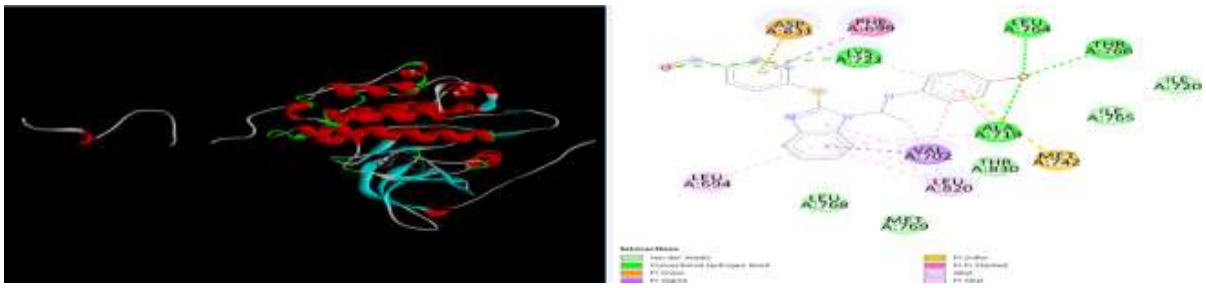


Figure 4- 3d, 2d view and interactions of 7b against 3RCD protein

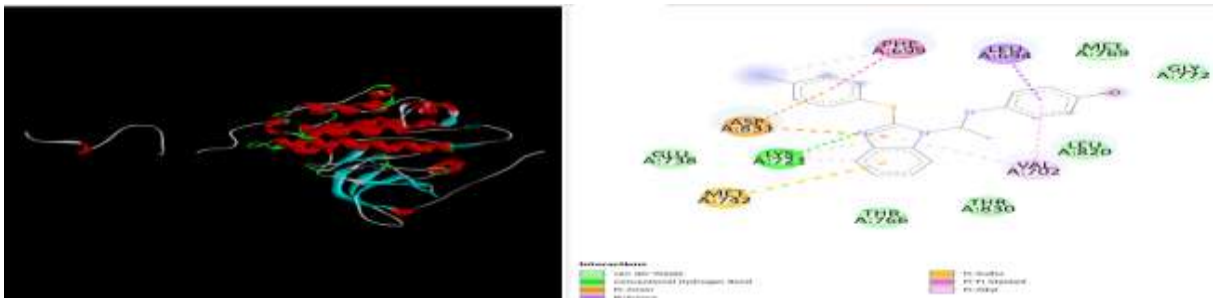


Figure 5- 3d, 2d view and interactions of 7e against 3RCD protein

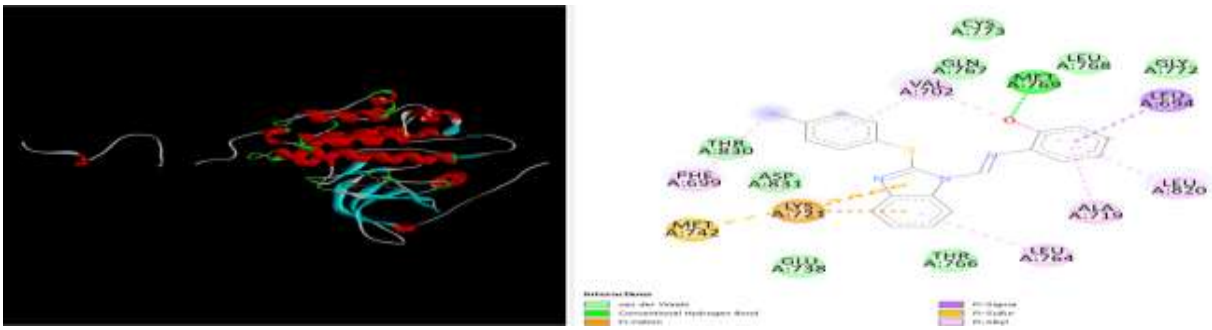


Figure 6- 3d, 2d view and interactions of ligand 7f and 3RCD

Table 5- Antibacterial activity of 1, 2-diaryl substituted benzimidazole derivatives (7a-7c) by agar disc diffusion method.

Compound	ZONE OF INHIBITION (mm) at different concentrations (µg/ml)							
	E. coli (Gram negative)				*S. aureus* (Gram positive)			
	50	100	200	300	50	100	200	300
7a	12	14	15	19	10	12	15	17
7b	11	13	16	17	10	12	14	16
7c	9	12	14	15	9	10	13	15
7d	14	16	19	21	10	12	14	16
7e	13	15	18	20	10	12	13	14
7f	12	13	15	17	10	11	11	12
7g	7	9	12	16	9	10	12	14
7h	-	7	9	14	9	11	12	12
7i	-	-	8	10	9	10	11	11
Streptomycin	15	18	20	23	12	14	16	18
DMF	9				11			

In-silico Antimitotic activity: The special confirmation of ligands **7a-7i** were explored by molecular modelling in order to reveal the affinity against HER2 ranging from -8.05 to -9.87 kcal/mol. with Autodock Vina(Table 4). Post dock analysis established a network of molecular interactions in common among all derivatives such as hydrogen bonds (H-bonds), electrostatic (van der wall (VDW), π-charge), hydrophobic (π-alkyl, π-sigma) and miscellaneous interactions all over the protein molecule. Binding interactions including H-bonds (Lys721, Thr766), VDW (Glu738, Gly 772, Thr 830), π-charge (cation- Lys721, anion- Asp831), π-alkyl (Val702, Ala719), π-sigma (Leu694) and miscellaneous π-sulphur (Met742). The binding energy of standard drug aspirin against HER2 about -6.2 kcal/mol and all the derivatives displayed least binding energy when compared to standard drug (Table 4). Aspirin established H-bonds (Lys721, Thr766, Asp831), VDW (Glu738, Met 742, Val 702, Ala719, Leu820, Thr830) and π-alkyl (Lys721, Leu764). The ligand **7g** was found not to form any H-bonds, but displayed electrostatic VDW (Leu694, Gly695, Ile765, Thr766, Leu764, Leu820, Thr830), π-cation(Lys721), π-anion(Asp831) and hydrophobic interactions π-sigma(Val702), π-alkyl(Val702, Ala719), π- π stacking(Phe699). Among all the derivatives **7c**, **7e**, **7b** showed maximum binding affinity with energies -9.87, -9.73 and -9.21 respectively. Ligand **7b** established H-bond (Ala719, Lys721, Leu764, Thr766), VDW (Ile765, Leu768, Met769, Thr830), π-anion (Asp831), π-sigma (Val702), π-alkyl (Leu694, Val702, Ala719, Leu820), π-π stacking (Phe699), alkyl (Val702, Leu820) and π-sulphur (Met742) (Fig. 4). The ligand **7c** established H-bonds (Met769), VDW (Phe699, Glu738, Thr766, Leu768, Gly772, Thr830, Asp831), π-cation (Lys721), π-alkyl(Val 702, Ala719, Leu764, Leu820), π-sigma (Leu694) and π-sulphur (Met742) (Fig. 5). The ligand **7e** established H-bonds (Lys721), VDW (Glu738, Met769, Gly772, Thr766, Leu820, Thr830), π-anion (Asp831), π-alkyl(Phe699, Val702), π-sigma (Leu694), π- π stacking (Leu699) and π-sulphur (Met742) (Fig. 6).

In-vitro Antibacterial study: All the title compounds screened for anti-bacterial activity by agar disc diffusion method. The results revealed that all the derivatives**7a-7i** gradually increase the zone of inhibition from low concentration to high concentrations (50, 100, 200, 300 µg/ml) against two bacterial strains. Inhibition zone against E. coli (gram negative) ranges from 9 to 21mm and standard drug streptomycin ranges from 15 to 23mmwhereas S. aureus (gram positive) ranges from 9 to 18mm and streptomycin ranges from 12-18mm of varying concentrations. Among all derivatives, **7d** (21mm), **7e**(20mm)and**7a**(19mm)displayed maximum zone of inhibition at high concentration (300 µg/ml) against E. coli (Table 5) and streptomycin (23mm). Whereas **7a** (17mm), **7b** (16mm)and**7c** (15mm)against *S. aureus*(Table 5) and standard drug (18mm).

DISCUSSION

***In-vitro* Antimitotic activity:**

Growth of dormant embryo within a seed starts trough a three-phase process of water uptake by the seed. It ultimately leads to breaking of seed coat and protrusion of radical. Water imbibition in seeds represents that, cells moving from G1 phase to G2 phase, which precedes cell division and the subsequent growth of the radical.[21] The derivatives **7c**(1.21, 1.53, 1.59g), **7e** (1.7, 1.08, 1.65g) and **7f** (1.08, 1.72, 1.91)had minimum change in seed weight before and after soaking at all the three concentrations (100, 200 and 300µg/ml) which decelerate the cell activation. The same derivatives showed maximum percentage of inhibition of seed weight about 92% at 100 µg/ml. the growth retardation brought about by the derivatives was found to be associated with the radical length reduction that means the interruption in cell proliferation at different time intervals. Derivative **7c** at 200µg/ml, **7d** at 100 µg/ml, **7e** at 300 µg/ml were high radical length inhibition. Derivatives **7g** at high concentration (300 µg/ml)had moderate cell proliferation inhibition.

***In-vitro* Antibacterial study by disc diffusion method:** The novel substituted 1,2-diaryl benzimidazole compounds showed significant antibacterial activity against both strains. Series with amine substitution (**7g**, **7h**, **7i**) were almost inactive against *E.coli* whereas slightly active against *S. aureus.*[26] Among these three, hydrogen substituted derivative (**7g**) showed notable antibacterial activity at

300 µg/ml. Series with methyl substitution (**7d**, **7e**, **7f**) were highly active against *E. coli* and moderately active against *S. aureus.*[18] Among these, derivative with 2-hydroxy group (**7f**) showed less activity against two bacterial strains. Compounds with carboxylic acid substitution (**7a**, **7b**, **7c**) displayed moderate activity against both bacterial strains when compared to standard drug streptomycin. *E. coli* was sensitive towards the derivatives **7a**, **7b**, **7d**, **7e** at 300 µg/ml as they have Zone of inhibition values >15mm whereas *S. aureus* was sensitive towards **7a**, **7b** at 300 µg/ml. all the derivatives have intermediately sensitive against gram positive and gram negative at 100 and 200 µg/ml concentration as their zone of inhibition values between 10-15mm. Both gram-positive and gram-negative bacteria were resistant towards the derivatives 7c (COOH & 2-OH),[27] 7g (CH_3& H), 7h (CH_3 & 4-OH), 7i (CH_3 & 2-OH) have zone of inhibition 10mm.

CONCLUSION

All the novel series of substituted 1,2-diaryl benzimidazole derivatives were successfully synthesised with purity and with satisfactory yields, its structures were confirmed by spectral analysis and screened for its therapeutic potency. Anti-mitotic study results disclose that the derivative with carboxylic acid at para position and 2-hydroxy group of phenyl ring (**7c**), methyl & hydroxy group at para position of phenyl ring (**7e**), carboxylic acid & hydroxy group at para position of phenyl ring (**7b**) exhibited high anti-mitotic activity with respect to cell proliferation than the standard drug aspirin in both *in-vitro* and *in-silico* anti-mitotic study and there is correlation of results. The high docking affinity of top 3 ligands (**7c**, **7e**, **7b**) may due to the presence of a greater number of VDW interactions, hydrophobic and hydrogen bonding than the standard drug aspirin. Anti-bacterial study concluded that the derivative with high lipophilicity have high zone of inhibition against both bacterial strains as the lipophilicity of **7d**, **7e**, **7a** were 6.23, 5.75, 5.57 respectively predicted by using Molinspiration. From this study, we can conclude that derivative **7a**, **7c**, **7e** found to be potent compound and further investigation for cytotoxicity and broad anti-bacterial screening may be needed.

Conflict of Interest: The authors declare that they have no known competing conflicts of interest.

Acknowledgements: For providing the facilities needed to carry out this research, the authors would like to thank the University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India, which is financed by DST-FIST.

REFERENCES:

1. Xi N, Huang Q, Liu L. Imidazoles. In: Alan RK, Christopher AR, Eric FVS, Richard JKT, eds. *Comprehensive hetrocyclic chemistry III*, Elsveir; 2008:143-364.
2. Sahar SA, Rasha SS, Khlood A, Khawla K, Dimah A. Imidazoles as a promising medicinal scaffold: Current status and Future direction. Drug Des Devel Ther 2021;15:3289-33112.
3. Ingle R. G, Magar D.D. et al., Heterocyclic chemistry of benzimidazoles and potential activities of benzimidazole derivatives. Int J Drug ResTech 2011;1(1): 26-32.
4. Anandarajagopol K, Tiwari RN, Bothara K, Sunilson JAJ, Dineshkumar C, Promwichit P. 2-mercaptobenzimidazole drivatives: synthesis and anticonvulsant activity. Adv Appl Sci Res 2010;1:132-138.
5. Ali M, Ali S, Khan M, Rashid U, Ahmad M, Khan A, Al-Harrasi A, Ullah F, Latif A. synthesis, biological activities, and molecular docking studies of 2-mercaptobenzimidazole based derivatives. Bioorg Chem 2018;80:472-479.
6. Ali Khan S, Ali M, Latif A, Ahmad M, Khan A, Al-Harrasi A. Mercaptobenzimidazole-based1,3-thiazolidin-4-ones as antidiabetic agents: synthesis, in vitro α-glucosidase inhibition activity, and molecular docking studies. ACS Omega 2022;7(32):28041-28051.
7. Tahlan S, Kumar S, Kakkar S, Balasubramanian N. Benzimidazole scaffolds as promising antiproliferative

agents: A review. BMC Chemistry2019;13:66.
8. Sethi P,Bansal Y, Bansal G. Synthesis and PASS-assisted evaluation of coumarin–benzimidazole derivatives as potential anti-inflammatory and anthelmintic agents. Med Chem Res 2018;27(1):61–71.
9. Philip raja S, Jayamoorthy K, Dhanalekshmi KI, Suresh S. Mn_3O_4 nanoparticles bearing 5-amino-2-mercapto benzimidazole moiety as antibacterial and antifungal agents. J Biomol Struct Dyn 2022;40(15):7084-7090.
10. Iqbal H,Verma AK, Yadav P, Alam S, Shafiq M, Mishra D, Khan F, Hanif K, Negi AS, Chandra D. antihypertensive effect of a novel angiotensin II receptor blocker Fluorophenyl Benzimidazole: contribution of cGMP, voltage-dependent calcium channels, and BKCa channels to vasorelaxant mechanism. Front Pharmacol2021;30:611109.
11. Musa A, Abulkair HS, Aljuhani A, Rezki N, Abdelgawad MA, Shalaby K, El-Ghorab AH, Aouad MR. phenylpyrazolone-1,2,3-triazole hybrids as potent antiviral agenys with promising SARS-CoV 2 main protease inhibition potential. Pharmaceuticals 2023;16:463.
12. Noor A, Qazi NG, Nadeem H, Khan AU, Paracha RZ, Ali F, Saeed A. Synthesis, characterization, anti-ulcer action and molecular docking evaluation of novel benzimidazole-pyrazole hybrids. Chem Cent J 2017;11(1):85.
13. Lalic H, Aurer I, Batinic D, Visnjic D, Smoljo T, Babic A. Bendamustine: A review of pharmacology, clinical use and immunological effects (Review). Oncol Rep 2022;47(6):114. https://doi.org/10.3892/or.2022.8325.
14. Wu TKY, Tang KHK, Hwang YY, Chan TSY, Tse E, Kwong Y. Bendamustine treatment of hematological malignancies: significant risks of opportunistic viral, fungal and bacterial infections. Hematology 2022;27(1):535-542.
15. Koff A, Malinis M. Drug evaluation: maribavir as a cytomegalovirus antiviral agent. Future Virol 2024:1-13.
16. Gurrala S, Babu YR, Rao GV, Latha BM. Symmetrical coupling of 2-mercaptobenzimidazole derivatives and their antimicrobial activity. Int J Pharm Pharm Sci 2011;3:230-236.
17. Anandarajagopal K, Tiwari RN, Venkateshan N, Promwichit. Synthesis and characterization of 2-mercaptobenzimidazole derivatives as potential analgesic agents. Int J Chem Pharm Res 2010;2(3):230-236.
18. Deepkumar J, Kalpesh P. Synthesis and evaluation of novel benzimidazole derivatives as antimicrobial agents. Med Chem Res 2014;23:1290-1299.
19. Sumit T, Balasubramanian N, Siong ML, Kalavathy R, Vasudevan M, Syed AAS. 2-mercaptobenzimidazole Schiff Bases: Design, Synthesis, Antimicrobial studies and Anticancer activity on HCT-116 Cell line. Mini RevMed Chem 2019;19(13):1080-1092. https://doi.org/10.2174/1389557518666181009151008
20. Vigna radiata (Fabaceae), as a model for the preliminary evaluation of cytotoxic effects of drugs. Bio Cell 2009;33(1):19-24.
21. A novel mechanism for the Anti-cancer activity of aspirin and salicylates.
22. Ishikawa T, Seto M, Banno H, Kawakita Y, Oorui M, Taniguchi T, Nakayama A, Miki H, Kamiguchi H, Tanaka T, Habuka N, Sogabe S, Yano J, Aertgeerts K, Kamiyama K. Design and synthesis of novel human epidermal growth factor receptor 2 (HER2)/Epidermal growth factor receptor (EGFR) Dual Inhibitors bearing a pyrrolo[3,2-d]pyrimidine scaffold. J Med Chem 2011;54:8030-8050.
23. Hussein HE, Fahad MA, Mohamed NE, Hanem MA, Eman SN, Wael AE. Design, Synthesis, Anticancer activity and Molecular docking of new 1,2,3-

Triazole-Based Glycosides bearing 1,3,4-Thiadiazoyl, Indolyl and Arylacetamide Scaffolds. Molecules 2022;27:6960.

24. MavrovaATs, Yancheva D, AnastassovaN, AnichinaK,Zvezdanovic J, Djordjevic A, Markovic D, SmelcerovicA. Synthesis, electronic properties, antioxidant and antibacterial activity of some new benzimidazoles. Bioorg Med Chem 2015;23(19):6317-26.
25. Pardeshi VA, Pathan S, Bhargava A, Chundawat NS, Singh GP. Synthesis and evaluation of novel Benzimidazole derivatives as potent antibacterial and antifungal agents. EJBAS 2021;8(1):330-344.
26. Kiran MK, Sagar AJ, Pramod PP, Vikas RD, Shitalkumar SP. Synthesis and microbial screening of 2-mercaptobenzimidazole derivatives. Der Pharma Chemica 2016;8(4):1-5.
27. Chandrasekar S, Rajam S, Ruby SC, Maruthamuthu. Synthesis, characterization and antimicrobial activity of N-substituted 2-substituted-benzimidazole derivatives. JOCPR 2012;4(11):4937-4940.