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Research Article

DESIGN, SYNTHESIS, AND CHARACTERIZATION AND BIOLOGICAL STUDIES OF SOME BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

Breast cancer is individual of the most vital types of cancer, leading to deaths in the majority of the female population. Globally, various synthetic compounds are being researched for effective treatment and management of breast cancer. In this category, benzimidazole has received the most attention. The present study was carried to synthesis 12-benzimidazole compounds were screened for in vitro anticancer activity against the MCF-7 cancer cell line. A molecular docking study was performed on the data set using gold software (PDB code: 1ZXM) as a possible target for anticancer activity. Molecular docking results showed that compounds N1, N5, N7, N9, and N12 showed good docking scores with better interaction within key amino acids, which correlated with their anti-cancer results. The synthesised molecules were validated using NMR, mass spectrometry, FT-IR, and physicochemical properties. A selected dataset of synthesised benzimidazole compounds was evaluated for their in vitro anticancer activity against cancer cell lines (MCF-7) using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. The anticancer activity of all the synthesised compounds was carried out by the in vitro method to determine the IC50 value. From the results of anticancer activity, compound N10 showed higher sensitivity and a better IC50 value in the range of 7.48 µM MCF-7 cancer cells compared to the standard drug doxorubicin (IC50: 7.90 µM). The ADME results showed that compounds N1, N9, and N12 have significant results in close agreement with Lipinski's rule of five and the Qikprop range rule, and these compounds can be taken as lead molecules for the discovery of new anticancer agents.

Keywords: benzimidazole, anticancer activity, antibacterial, molecular docking, ADME, and doxorubicin.

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INTRODUCTION

Cancer is distinguished by abnormal cell proliferation as well as the ability to spread or invade other cells or tissues. It remains the main cause of death, and although significant progress has been made in understanding the underlying causes of cancer and disease, the mortality and morbidity rates are still high.

Cancer remains a major health problem and a life-threatening disease. It causes the second-highest rate of death in the world after a heart attack.[1]Several risk factors for cancer include environmental factors, tobacco, radiation (UV light and soft and strong X-ray fibres), genetic mutations, and viral infections.[2] We have over 100 types of cancer, with lung cancer, colorectal cancer, and prostate cancer in men, breast cancer in women, and cervical cancer being the most common.[3] Among cancer treatments, chemotherapy plays a role. A significant role is played in the treatment of various types of cancer, but there are numerous challenges, five of which are poor selection, drug resistance, and toxic effects on normal cells.[4] Thus, because of the complications that exist in cancer, the choice is the focus of medical pharmacists.[5] Researchers are looking for heterocyclic systems in order to develop new anti-cancer agents.[6]

Molecular docking is a quick way to identify drug receptor relationships through rational drug design and availability. Benzimidazole, also known as benzimidazole or benzoglyoxaline, is a heterocyclic component of current research options. Because benzimidazole is found in both natural and synthetic chemicals, it has been extensively studied as a bioactive heterocyclic.[7] Benzimidazole is an important structural motif found in a wide range of natural and pharmacologically active compounds12. The benzimidazole ring itself is an emergency pharmacophore nowadays and is used as a special scavenger to combine selected drugs that are of interest in the medical field, including antiulcers, anticancers, antihelmintics, antimicrobials, antihistamines, antioxidants, HIV-RT inhibitors, etc. Selected drugs on the market with the benzimidazole segments (Figure 1) are veliparib (a), glasdegib (b), liarozole (c), pracinostat (d), and abemaciclib (e).[8-9]

Docking using Gold Docking Wizard:

Docking research for designed combinations was done with GOLD Suite 5.0 software. The desired protein mol file was loaded into a gold wizard, and hydrogen atoms were added. The binding site was defined in 1ZXM as X = 6.667, Y = 0.329, and Z = 13.279. The ligand to be

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docked was then added, and docking was carried out using GOLD Suite score as the scoring function. GOLD Suite was used, and the obtained docking score was consider in the present study.

Docking result for compound N1-N12:

On the basis of a literature search, various ligands were prepared and PDB-IDs were obtained from protein data banks based on the mechanism of action. PDB ID 1ZXM for the MCF-7 cell line Chemdraw 2D ultra 8.0 was used to create the ligands, and Chemdraw 3D ultra 8.0 was used to dock the structures. GOLD (Genetic Optimisation for Ligand Docking), a genetic algorithm for docking flexible ligands into protein binding sites, was used to dock the ligands. GOLD Suite has been extensively tested and has shown excellent performance for anticancer activity and good results for virtual screening. Table:1

Table 1: Docking results of synthesized compound structure:

S.No.	Compound Structure	Docking Results 1ZXM
1.	3-(1H-benzo[d]imidazole-2-yl)benzenamine	40.232
2.	2-(3,4-dichlorophenyl)-1H-benzo[d]imidazole	31.724
3.	2-(2-chloro-5-nitrophenyl)-1H-benzo[d]imidazole	38.581
4.	2-(1H-benzo[d]imidazole-2yl)phenol	30.823
5.	4-(1H-benzo[d]imidazole-2-yl)benzenamine	44.491
6.	2-(4-nitrophenyl)-1H-benzo[d]imidazole	33.158
7.	4-(1H-benzo[d]imidazole-2yl)benzene-1,2-diol	51.966
8.	(2,4-dichlorophenyl)-1H-benzo[d]imidazole	36.699
9.	2-(3-dichlorophenyl)-1H-benzo[d]imidazole	42.817
10.	2-(3-methoxyphenyl)-1H-benzo[d]imidazole	39.418
11.	2-(4-fluorophenyl)-1H-benzo[d]imidazole	39.406
12.	4-(1H-benzo[d]imidazole-2yl)benzene-1,2-diamine	40.050
13.	Doxorubicine	40.588

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MATERIALS AND METHODS

O-Phenylenediamine, chloroform, acetone, and methanol were purchased from Spectrochem Pvt. Ltd., Mumbai, India. Benzoic acid and substituted benzoic acid were purchased from Himedia Laboratories Pvt. Ltd. Mumbai, India. TLC aluminium sheets with GF-254 silica gel purchased from Merck Specialities Pvt. Ltd. Mumbai, India.

The melting points of synthesised compounds were measured using the Thiele tube method. The progress of the reaction and purity of the synthesised compounds were checked by TLC using precoated aluminium sheets with GF-254 silica gel and Methanol, chloroform, and acetone (3:2:1) were used as the mobile phase. The spots were seen in a UV light chamber.

The UV analysis of the synthesized compound was done using Shimadzu 1800 UV-visible spectrophotometer and the λ max (nm) of the synthesised compounds were determined.

The FTIR spectra of the synthesised compounds were recorded on an FTIR Alpha Bruker by KBr disc in the range of 400–4000 cm^{-1}.

The proton NMR or ^{1}H NMR (δ, ppm) spectra were recorded using a Bruker Advance II 400 NMR spectrometer.

The mass spectra of synthesised compounds were recorded using the CIF mass facility at IISER Bhopal.

Synthesis of benzimidazole derivatives:

A series of 12 substituted benzimidazole derivatives (N1-N12) were synthesised according to the synthetic route presented in Fig. 1. In RBF, 1.29g of O-phenylenediamine was treated with 4.97g of benzoic acid. Now add 4 N HCl (40 ml) was slowly added to the mixture with constant stirring and refluxed for 4 hours at 250 ^{o}C. The resulting residues were filtered and dried. The obtained crude benzimidazole was separated by filtration and washed with cold water. The pure product was obtained by recrystallization in hot water.

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Synthesis scheme:

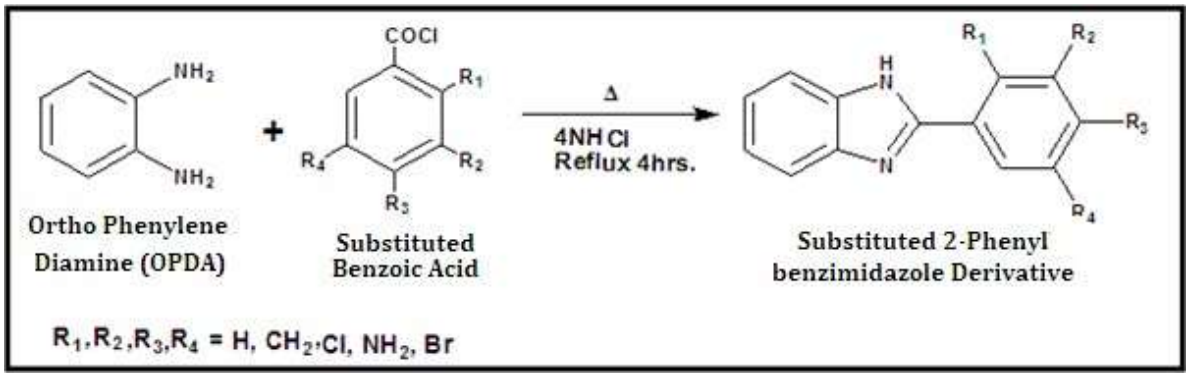

Figure 1: Synthesis scheme of design compound

RESULTS AND DISCUSSION

Spectral data of synthesized compounds:

3-(1H-benzo[d]imidazole-2-yl)benzenamine N1:

FTIR: (-C=C, aromatic)1415, (-C-H, stretch)3185, (-C-H, bend)1441, (-C-N)1032, (-C=N)1631, (N-H)3155., **1H NMR (500MHz, Chloroform);** 6.42- 7.70 (8H, m, ArH); 1.4(1H, s, CH_3); 1.3(2H, s, CH_2); 0.9(3H, s, CH), **m/z:** 210.23 (M+1)

2-(3,4-dichlorophenyl)-1H-benzo[d]imidazole N2:

FTIR: (-C=C, aromatic)1595, (-C-H, stretch)3046, (-C-H, bend)1441, (-C-N)1030, (-C=N)1670, (- N-H)3462, **1H NMR (500MHz, Chloroform):** 6.22 -7.79 (7H, m, ArH); 1.4(1H, s, CH_3); 1.3(2H, s, CH_2); 1 .1-0.9 (3H, s, CH), **m/z:** 263.3 (M^+)

2-(2-chloro-5-nitrophenyl)-1H-benzo[d]imidazole N3:

FTIR: (-C=C, aromatic)1412, (-C-H, stretch)3200, (-C-H, bend)1445, (-C-N)1350, (-C=N)1670, (- N-H)3562, **1H NMR (500MHz, Chloroform);** 8.73-8.92(4H, m, ArH); 7.93-8.32(2H, d, ArH); 7.13-7.92(2H, t, ArH); 6(1H, s, Ar-NH); 1.3(2H, s, CH_2), 0.9(3H, s, CH_3), **m/z:** 274.0 (M+1)

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2-(1H-benzo[d]imidazole-2yl)phenol N4:

FTIR: (-C=C, aromatic)1606, (-C-H, stretch)2933, (-C-H, bend)1443, (-C-N)1355, (-C=N)1668, (- N-H)2990, **1H NMR (500MHz, Chloroform);** 8-7.95(4H, d, ArH); 7.59-7.54(4H, t, ArH); 8-6.5(1H, s, Ar-NH); 1.3(2H, s, CH_2); 0.7-1.2(3H, s, CH_3), **m/z:** 211.1 (M+1)

4-(1H-benzo[d]imidazole-2-yl)benzenamine N5:

FTIR: (-C=C, aromatic)1442, (-C-H, stretch)3082, (-C-H, bend)1442, (-C-N)1008, (-C=N)1635, (- N-H)3107, **1H NMR (500MHz, Chloroform);** 6.53-8.00(9H, m, ArH);); 3.43(2H, s, N-H), **m/z:** 239.2(M^+)

2-(4-nitrophenyl)-1H-benzo[d]imidazole N6:

FTIR: (-C=C, aromatic)1600, (-C-H, stretch)3059, (-C-H, bend)1433, (-C-N)1008, (-C=N)1635, (- N-H)3107, **1H NMR (500MHz, Chloroform);** 7.28-8.40(8H, m, ArH); 1.3-1.7(1H, s, CH); 1.2-1.4(2H, s, CH_2); 0.7-1.1(3H, d, CH_3), **m/z:** 207.5(M-2)

4-(1H-benzo[d]imidazole-2yl)benzene-1,2-diol N7:

FTIR: (-C=C, aromatic)1425, (-C-H, stretch)3039, (-C-H, bend)1454, (-C-N)1033, (-C=N)1656, (N-H)3277.,**1H NMR (500MHz, Chloroform);** 6.627-7.72(7H, m, ArH); 1.5(1H, s, CH); 1.2-1.4(2H, s, CH); 0.9(3H, d, CH_3), **m/z:** 226.3 (M+1)

(2,4-dichlorophenyl)-1H-benzo[d]imidazole N8:

FTIR: (-C=C, aromatic)1413, (-C-H, stretch)3026, (-C-H, bend)1413, (-C-N)1377, (-C=N)1585, (- N-H)3072, **1H NMR (500MHz, Chloroform);** 7.26-7.10(7H, m, ArH); 4-4.5(1H, s, ArNH); 1.4-1.7(1H, s, CH). 1.2-1.3(2H, s, CH_2); 0.7-1.1(3H, s, CH_3), **m/z:** 263.0 (M^+)

2-(3-dichlorophenyl)-1H-benzo[d]imidazole N9:

FTIR: (-C=C, aromatic)1423, (-C-H, stretch)3043, (-C-H, bend)1423, (-C-N)999, (-C=N)1635, (- N-H)3043, **1H NMR (500MHz, Chloroform);**7.32-7.60(8H, m, ArH); 6-8(1H, s, ArNH); 1.3(2H, s, CH_2); 0.9(3H, s, CH_3); **m/z:** 228.1 (M^+)

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2-(3-methoxyphenyl)-1H-benzo[d]imidazole N10:

FTIR: (-C=C, aromatic)1585, (-C-H, stretch)3008, (-C-H, bend)1465, (-C-N)1315, (-C=N)1693, (- N-H)3560, 1H NMR (500MHz, Chloroform); 7.18-7.76(8H, m, ArH); 7.18(1H, s, Ar-NH); 3.9(3H, s, OCH_3); 1.2(2H, s, CH_2), 0.1-0.8(3H, s, CH_3), m/z: 223.2 (M-1)

2-(4-fluorophenyl)-1H-benzo[d]imidazole N11:

FTIR: (-C=C, aromatic)1602, (-C-H, stretch)3078, (-C-H, bend)1427, (-C-N)1012, (-C=N)1676, (- N-H)3684, **1H NMR (500MHz, Chloroform);** 7.14-8.15(8H, m, ArH); 6.87(1H, s, ArNH); 0.8(3H, s, CH_3), **m/z:** 213.3 (M+1)

4-(1H-benzo[d]imidazole-2yl)benzene-1,2-diamine N12:

FTIR: (-C=C, aromatic)1419, (-C-H, stretch)3035, (-C-H, bend)1450, (-C-N)1307, (-C=N)1665, (- N-H)3423, **1H NMR (500MHz, Chloroform**); 6-8.5(7H, m, ArH); 1.4-1.7(1H, s, CH); 1.2-1.4(2H, s,CH_2); 0.9(3H, s, CH_3), m/z: 201.0 (M+10)

Table 2: Physicochemical properties of synthesized compound

S.No.	Comp. code	Mol Wt	Colour	Melting point (^{0}C)	R_f value	λ max	% yield
1	N1	209.25	Sandy brown	160-170	0.31	272	80%
2	N2	263.12	Floral white	175-185	0.38	294	78%
3	N3	273.67	Orange	145-155	0.78	283	50%
4	N4	210.23	White smoke	140-150	0.47	259	50%
5	N5	209.25	Pale yellow	144-153	0.62	265	70%
6	N6	239.23	Pale yellow	175-180	0.22	289	44%
7	N7	226.07	Light golden	170-178	0.73	277	60%
8	N8	263.12	Cream	140-150	0.66	270	51%
9	N9	228.68	Light golden	140-150	0.56	281	60%
10	N10	224.26	White	130-140	0.58	276	45%
11	N11	212.22	Golden yellow	145-151	0.62	268	68%
12	N12	211.24	Sandy brown	*	0.68	285	43%

*R_f: Retardation factor, *: Decomposed*

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Table 3: ADME prediction by QikProp

S.NO.	Donor HB	Acceptor HB	Log S	Qlogp O/W	Mol Wt	Drug likeness	Log P
1	3	1	-3.83	2.538	209.25	-0.91	2.88
2	1	1	-5.59	2.469	263.12	-0.33	4.63
3	1	3	-4.94	2.975	273.67	-0.87	3.65
4	2	2	-3.80	2.482	210.23	-0.57	2.94
5	3	1	-3.89	1.738	209.25	-0.47	2.88
6	1	3	-4.46	2.863	239.23	-0.75	3.05
7	3	3	-3.73	1.749	226.07	-0.36	2.65
8	1	1	-5.46	2.854	263.12	-0.17	4.63
9	1	1	-4.89	2.74	228.68	-0.79	4.04
10	1	2	-4.15	3.209	224.26	-0.62	3.41
11	1	1	-2.65	1.32	212.22	-0.71	2.34
12	5	1	-3.86	1.518	211.24	-0.81	2.32

Log P: Partition Coefficient, Log S: log solubility, ADME: Absorption, Distribution, Metabolism and Excretion

Various modern analytical techniques, such as 1H NMR, IR, and mass spectroscopy, were used to characterise the structures of the synthesised compounds N1-N12. Figure 2,3 & 4

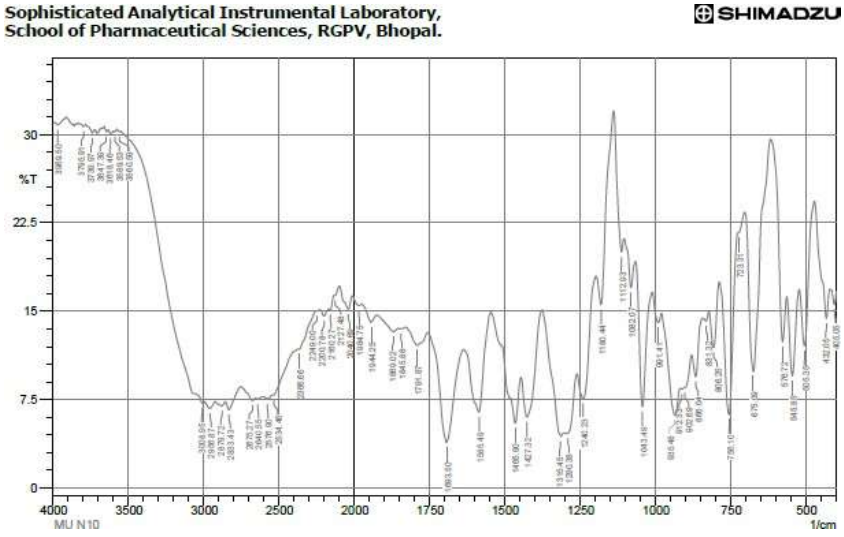

Figure 2: IR spectra of compound N10

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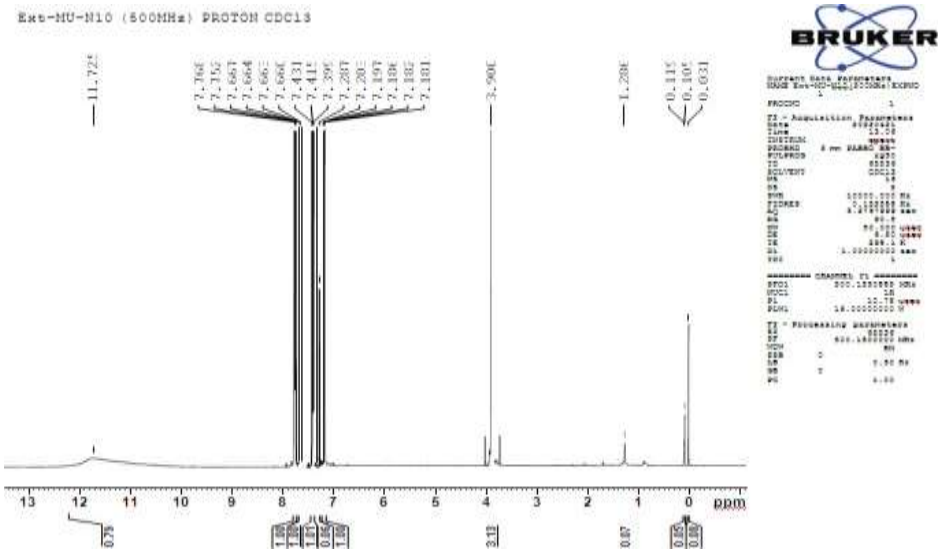

Figure 3: ^{1}HNMR spectra of compound N10

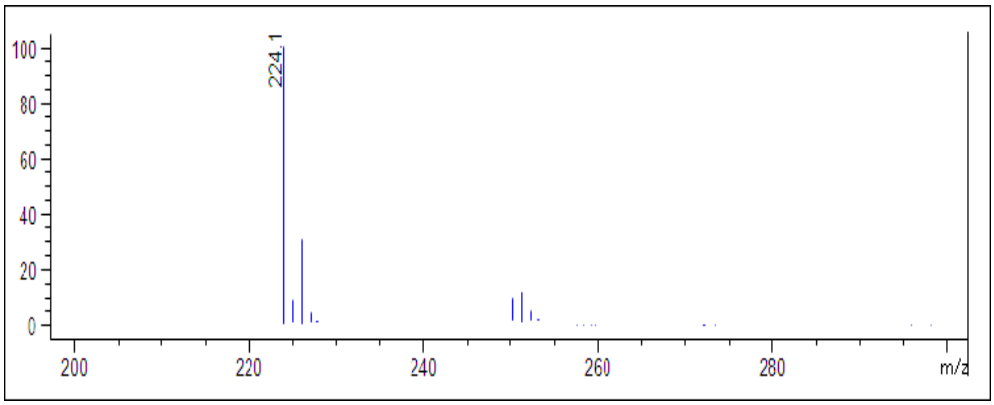

Figure 4: Mass spectroscopy of compound N10

BIOLOGICAL ACTIVITY

In the current study the newly synthesised compound benzimidazole derivatives N1–N12 were synthesised and tested for their anticancer properties using in-vitro method. All synthesised molecules evaluated against breast cancer cell lines revealed sufficient anticancer activities with IC50 values in the micromolar range (7.48–32.24 μM). Among the compounds, N10 was found to be the most potent compound against the MCF-7 cell line. Antitumor activity (MTT Assay) of compounds carried out in the MCF-7 cell line was acquired from Deshpande Laboratories Pvt. Ltd., Bhopal, and was used in the in vitro test. The result is shown in Table 4 and Figure 5.

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Table 4: The growth-inhibitory effects of the tested compounds, Doxorubicin, on MCF-7 human solid tumor cell lines with mean IC_{50}

Compounds	IC_{50} (µM/mL)
N1	18.53
N2	20.13
N3	8.11
N4	**32.24**
N5	10.67
N6	16.91
N7	12.01
N8	20.11
N9	11.25
N10	**7.48**
N11	17.72
N12	11.25
Std. Doxorubicin	**7.90**

**Fifty percent inhibitory Concentration of synthesized compounds µM*

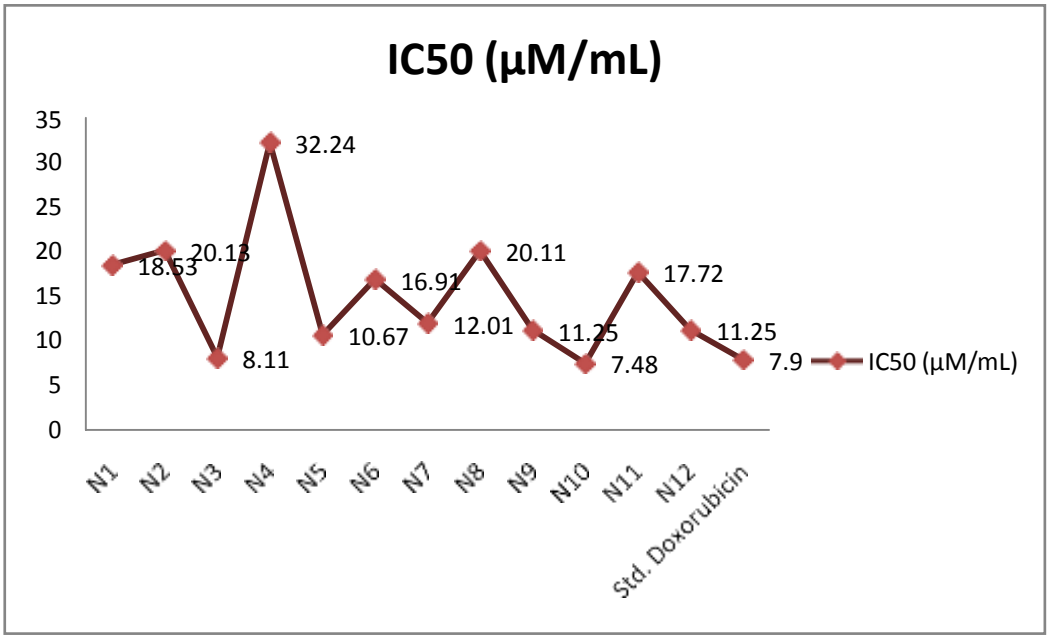

Figure 5: MCF-7 Human Breast Cancer cell line by MTT Assay

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CONCLUSION

In the presence of 4N HCl, the synthesis novel targeted benzimidazole reactions between o-phenylenediamine and substituted benzoic acid was carried out. Spectroscopic analysis confirmed the structures of the newly synthesised benzimidazole derivative. All newly synthesised compounds were evaluated for their anticancer activities. The findings revealed that the anticancer activities of the synthesised compounds were sufficient, with IC50 values in the micromolar range (7.48-32.24µM). In conclusion, compound N10 has remarkable anticancer activity. Pharmacophore elucidation of the compounds was performed based on an in silico ADME evaluation of the tested compounds. The screening results revealed that all compounds follow the accepted rules, meet the drug-likeness criteria, and adhere to Lipinski's rule of five.

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REFERENCE

1. Kumar U, Narang R, Nayak SK, Singh SK, Gupta V. Benzimidazole: structure activity relationship & mechanism of action as antimicrobial agents. Research Journal of Pharmacy and Technology. 2017;10(7).
2. Khalifa ME, Gobouri AA, Kabil FM, Altalhi TA, Almalki ASA and Mohamed MA. Synthesis antibacterial & anti HepG2 cell line human hepatocyte carcinoma activity of some new potentially *benzimidazole-5-(aryl diazenyl)thiazole* derivatives. 2018; 23(12). PP. 3285.
3. Monsuez JJ, Charniot JC, Vignat N, and Artigou JY. Cardiac side effects of cancer chemotherapy. International Journal of Cardiology. 2010; 144 (1). PP.3-15.
4. Kumar D, Patel G, Chavers AK, Chang KH, Shah K. Synthesis of new 1,2,4-oxadiazoles and analogues as anticancer agents. European Journal of Medicinal Chemistry. 2011 Mar 21; 46(7). PP.3085-3092.
5. Patil A, Ganguly S, Surana S. Synthesis and antiulcer activity of 2-[5-substituted-1-*H*-benzo*(d)*imidazol-2-ylsulfinyl]methyl-3-substituted quinazoline-4-(3*H*) ones. J Chem Sci. May 2010; 122 (3). PP. 443-450.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, 2022

6. Ozden S, Atabey D, Yildiz S, Goker H. Synthesis and potent antibacterial activity of several new methyl or ethyl 1H-benzimidazole-5-carboxylate derivatives with amide or amidine groups. Bioorg Med Chem. March 1, 2005; 13. PP.1587-1597.
7. Azam M, Khan AA, Al-Resayes SI, Islam MS, Saxena AK, Dwivedi S, Musarrat J, Trzesowska-Kruszynska A, Kruszynski R. Synthesis and characterization of 2-substituted benzimidazoles and their evaluation as anticancer agent. Spectrochem. Acta Part A. May 5, 2015; 142. PP.286–291.
8. Desai NC, Pandya DD, Joshi VV, Rajpara KM, Vaghani HV, Satodiya HM. Synthesis, characterization and antibacterial screening of hybrid molecules containing benzimidazole-pyrazole and pyridine nuclei. Med Chem Res. 2012; 21. PP.4463-4472.
9. Kumar SV, Subramanian MR, Chinnaiyanc SK. Synthesis, characterisation and evaluation of N-mannich bases of 2-substituted Benzimidazole derivatives. J Young Pharm. 2013 Dec 11; 5(4). PP.154–159. doi: 10.1016/j.jyp.2013.11.004