Synthesis, Molecular Docking Studies and Cytotoxic Screening of Novel 2-Substituted Benzimidazole Derivatives

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Abstracts: Cancer is one of the most serious lifethreatening diseases for which there is presently no cure. According to current World Health Organization (WHO) reports cancer is responsible for one out of every six deaths worldwide. Benzimidazole is a heterocyclic, aromatic molecule that acts as a biological scaffold with anticancer, antitumor, and antiproliferative activities, among other biological effects. benzimidazole and oxazole are the important pharmacophore in the medicinal chemistry, due to their widespread pharmacological activities. So, to exploit their anticancer potential we have selected these two for our research work. So, in order to develop potential anticancer agents, it was considered of interest to synthesize some benzimidazole derivatives. Before synthesis of molecule, Molecular docking was done and afterwards the compound with lowest dock score selected for synthesis. Synthesized derivatives Characterized By Proton Nuclear Magnetic Resonance (1H NMR), Infra-Red (IR), And Mass Spectroscopy (MS) and screen for cytotoxic activity.

Keywords: Anticancer, Benzimidazole, Bendamustine, Cytotoxic Activity, Oxazole, 5FGK.

1. INTRODUCTION

A group of illnesses known as cancers are defined by the unchecked development and division of aberrant cells. Metastasis, the term for the spread of cancer cells at this stage, if unchecked, can be fatal. Tobacco, chemicals, radiation, infectious agents, and other environmental elements, in addition to some internal ones, all contribute to the development of cancer (inherited mutations, hormones, immune conditions and random mutations)[1-2]. Cancer has a wide variety of complex, poorly understood causes. Many factors, such as dietary elements, specific illnesses, a lack of physical exercise, obesity, and environmental contaminants are known to raise the risk of cancer[1]. Several elements may interact to start or encourage carcinogenesis in humans, making cancer the major cause of mortality. In India, cancer is become one of the leading causes of mortality. There are thought to be between 2 and 2.5 million cancer cases worldwide at any given moment. Annually, there are more than 7 lakh new instances of cancer and 3 lakh deaths from it. At any given time, about 15 lakh patients need access to facilities for diagnosis, treatment, and follow-up[3-4].

1.1. Difference Between Normal Cell and Cancer Cell

Cancer cells ignore signals from the body to stop dividing. Apoptosis, or programmed cell death, is a built- in process in your body that tells it to get rid of cells it no longer needs. Normal cells are better at listening: they detect cues from the body and stop reproducing when there are enough cells[1-3].Normal cells differentiate into distinct cell types. These various cell types serve specific purposes. For example, liver cells assist your body in metabolizing proteins, fats, and carbohydrates, as well as removing alcohol from your blood. Cancerous cells divide

so quickly that they never have a chance to mature and become the specialized cells that they were designed to be[2-4].

In the vicinity of a tumour, cancer cells may affect the behaviour of healthy blood vessels, molecules, and cells. For instance, cancer cells may enlist the help of normal cells to create a new blood vessel. These veins give the tumour the oxygen and nourishment it needs to stay alive and grow. The immune system often gets rid of aberrant or damaged cells because cancer cells resist signals from the body to cease dividing. They invade the surrounding tissues. Normal cells locate their proper locations in your body and remain there. Metastatic cancer cells are those that have spread to other areas of the body. For instance, cancer might start in the lungs and progress to the liver. Instead of liver cancer, it is referred to as metastatic lung cancer if this spread takes place [5].

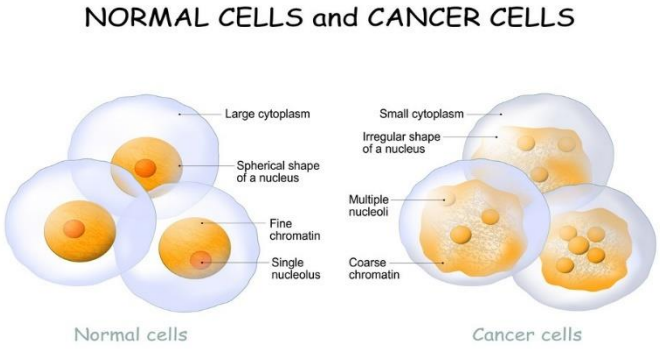


Fig.1. Difference between normal cell and cancer cell

1.2. Genetics of Cancer

Cancer is a genetic disease, which means that it is caused by alterations in genes that control how our cells behave, particularly how they grow and divide. Proteins do a lot of the work in our cells, and genes hold the instructions for making them. Certain gene mutations can cause cells to escape cell.

Normal growth regulators and turn cancerous. If the mutations are present in germ cells, the body's reproductive cells, we can inherit cancer-causing genetic abnormalities from our parents (eggs and sperm). These mutations, known as germline alterations, can be identified in every cell of the progeny[5].

Only a small percentage of the human genome's approximately 35,000 genes have been linked to cancer. Changes in the same gene are frequently linked to distinct types of cancer. These faulty genes can be divided into three categories. Proto-oncogenes, for example, produce protein products that ordinarily promote cell division or prevent normal cell death. Oncogenes are the mutant versions of these genes. Tumor suppressors, on the other hand, generate proteins that generally block cell division or cause cell death. DNA repair genes are included in the third group, and they help to avoid cancer-causing mutations.

Similarly, proto-oncogenes, which speed up cell growth, and tumour suppressor genes are regulated to keep cell growth under controlwhich inhibits cell development Oncogene mutations speed up growth, while tumour suppressor mutations prevent growth from being inhibited normally[5-6].

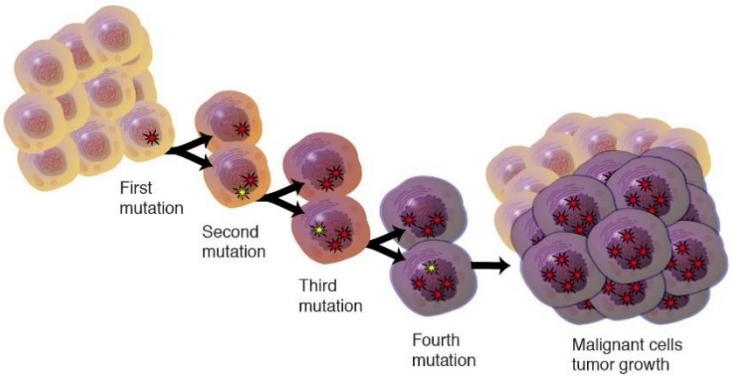


Fig. 2. Genetics of cancer cell

1.3. Benzimidazole

Benzimidazole is a heterocyclic aromatic organic compound. Benzimidazole is bicyclic ring which consists of the fusion of two ring- benzene and imidazole. This important group of compounds has found number of Practical applications in a Number of Fields[6].

Benzimidazole Scaffold is Isosteric with indole and purine Nuclei. In early 1950's, was an important period regarding the biological significance of benzimidazole and closely related purines; the vital role of purines in the biological significance of benzimidazole and the closely related purines; the vital role of purines in the biological system was established and it was discovered that 5, 6- dimethyl- 1- D- ribofuranosyl) benzimidazole is an integral part of structure of Vit. B12[6-7].

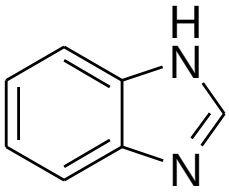


Fig. No. 3: Structure of Benzimidazole

Benzimidazole Contain a hydrogen atom attached to nitrogen atom attached to nitrogen in the position-1 which is readily tautomerize. This tautomerism is analogous to that found in the imidazole's and amidines. This may be depicted as below:

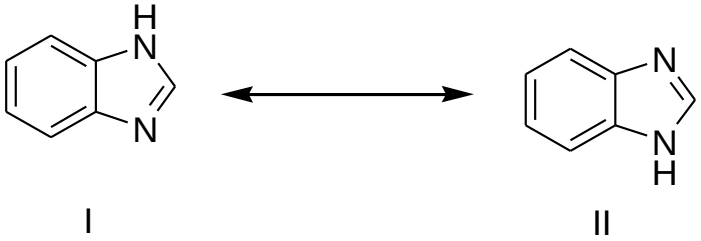


Fig. No. 4: Tautomerism of Benzimidazole

Benzimidazole is depicted as (I) Possessing the proton at N1, there exist a rapid exchange between -NH and =N-nitrogen atoms. Tautomerization(I) and (II) occur through intermolecular process involving two or more

benzimidazole molecules or through interaction with a protic solvent such as water. The groups at position C5 and C6 in the ring system are chemically Equivalent [8].

1.4. Chemistry of Oxazole

Oxazole is the parent compound for a vast class of heterocyclic aromatic organic compounds. These are azoles with an oxygen and a nitrogen separated by one carbon[9]. Oxazoles are aromatic compounds but less so than the thiazoles. Oxazole is a weak base; its conjugate acid has a pK_a of 0.8, compared to 7 for imidazole[10-13].

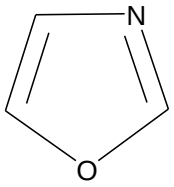


Fig. No. 5. Structure of Oxazole

2. MATERIAL AND METHODS

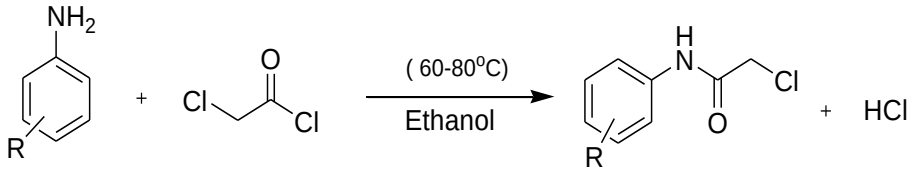
All of the compounds were from Thomas Baker, Sigma Aldrich, and SD Fine compounds. All of the solvents were reagent grade and had been ordered. On 60 F254 precoated silica gel plates from Merck, thin-layer chromatography (TLC) was used to identify the reactants and products that were being monitored both during and after reactions to ensure that the reaction was complete. The dots were seen in an enclosed chamber with iodine vapors or a UV chamber. The melting points of each material were all calculated using the uncorrected Thief's tube melting/boiling point instrument. On KBr pellets, IR spectra were captured using a Shimadzu 1000 FTIR spectrometer in the 4000-400 cm-1 range, Resolution 8.0, and Scanning No. 45. Proton resonance magnetic spectra (1H NMR) were recorded on Bruker 400MHz spectrophotometer using d6-DMSO as solvent and chemical shifts were expressed in parts per million (δ ppm), downfield from TMS as an internal standard. Mass spectra (MS) were recorded on LCMS instrument.

3.1. Experimental

Part A:

3.1.1. Synthesis of 2-Chloro-N-Phenylacetamide Derivatives

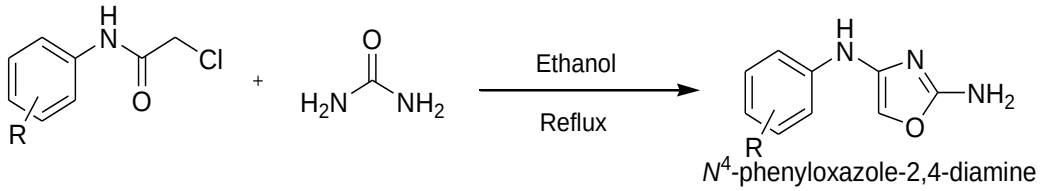
Aniline derivatives (0.1 mole) in 120 ml of ethanol were shaken in a magnetic stirrer for 2-3 hours. Chloroacetyl chloride (0.1 mole) was added drop by drop to the above mixture. The mixture was then stirred for 1-2 hours. DMF was used as a solvent with K_2CrO_3 as a base. The stirred mixture was then refluxed for 2-2.5 hours and poured into ice cold water. The mass obtained was filtered and recrystallized from ethanol[9].



2-chloro N-phenyl acetamide

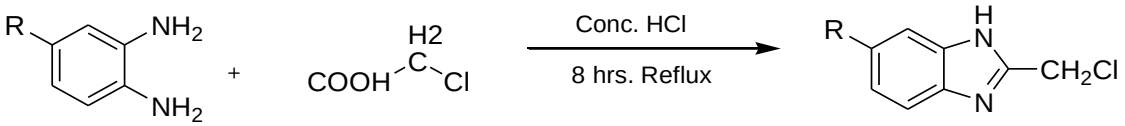
3.1.2. Synthesis of N4 -Phenyloxazole-2,4-Diamine

2-Chloro- N- phenylacetamide (0.01 mole) and Urea (0.01 mole) were dissolved in ethanol, the mixture was irradiated in microwave oven at a low power for 15 minutes. The solid mass was recrystallized using ethanol to produce 60 % yield.



N^4-phenyloxazole-2,4-diamine

Part B:

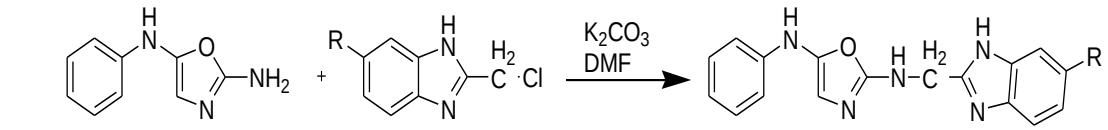


Monochloroacetic acid

R= CH3, Cl, F, NO2
Acids: Monochloroacetic acid
Chloropropionic acid

1. 2 gm of Methyl-OPD and 4 gm of monochloroacetic acid were weighed separately and transferred to RBF.
2. To the above mixture 6-10 ml of conc. HCL was added and Refluxed for 8 hrs. Reaction Completion was monitored by TLC.
3. After the completion of the Reaction, the solution was washed with ether in a separating funnel.
4. The solution was poured in ice cold water with stirring and basified with concentrated Ammonia solution until the ppt was obtained.
5. The above solid ppt was filtered and dried.
6. Recrystallisation Was done with ethanol by addition of Charcoal.

Part C:



N^5-phenyloxazole-2,5-diamine

2-(chloromethyl)-1*H*-benzo[*d*]imidazole

N^2-((1*H*-benzo[*d*]imidazol-2-yl)methyl)-N^5-phenyloxazole-2,5-diamine

R= CH_3, Cl, F, NO_2

1. A Solution of 2-(Chloromethyl)-1*H*-Benzimidazole and N^5- Phenyl Oxazole- 2, 5- Diamine in N, N Dimethylformamide was taken in a RBF.
2. K_2CrO_3 Was added to the Reaction mixture. The Reaction Mixture was stirred for 8 hrs at 80^oC on a magnetic stirrer (heat+ Stirring). The Progress of the was monitored by thin layer chromatography (TLC)[14-17].
3. Upon completion of the Reaction, water was added to the Reaction mixture and the Product extracted by shaking the Reaction mixture with Ethyl Acetate in a separating funnel.
4. The Ethyl Acetate layer was washed with water and brine before being dried over anhydrous sodium sulphate.
5. Evaporation of the solvent gave the Product. Recrystallized with Chloroform.

3.2. Molecular Docking

All Molecular docking was performed using the molecular modeling software (PyRx) version 0.8. It provided a facility to dock different ligands in protein binding sites chosen by the user.PyRx has provided rigid (no torsional flexibility for a protein as well as a ligand) and flexible (torsional flexibility to a ligand with a rigid protein) docking of the molecules. In Ligand preparation 2D structure of substituted benzimidazole derivatives was drawn using ChemDraw software. All 2D structures were converted into 3D structures and optimized. All the 3D structures were optimized using Merck molecular force field (MMFF) with distance-dependent dielectric function and energy gradient of 0.01 kcal/mol A with 10000 numbers of cycles. The conformers for all structures were generated and the low-energy conformer for each compound was selected and used for further study [18-19].

Preparation of ligands: The ligands (substituted benzimidazole derivatives) were studied for their binding activities. The 2D structures were drawn using ChemDraw software and converted to 3D conformations. The conformers thus obtained were optimized (MMFF) till they reached an rms gradient energy of 0.001 kcal/mol[20].

Preparation of protein: Target protein i.e. human cyclin-dependent kinase CDK-8 **(PDB code: 5-FGK)** were obtained using a protein data bank (PDB). The protein structure was prepared using preparation wizards. All water molecules were removed and the polar hydrogen are added[21].

3.3. Cytotoxic Evaluation

3.3.1. Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of synthesis compound. Brine shrimp lethality bioassay is easily mastered, costs little and it utilizes small amount of test compound. This provides a front line screen that can be backed up by more specific and expensive bioassay. This in vitro lethality test has been successfully used as a preliminary study of antitumor agents[23-26].

3.3.2. Preparation of Brine Solution

38 g of iodize sodium chloride was weighed, dissolved in 1000 ml of distilled water.

and filtered to obtain a clear solution.

3.3.3. Hatching of Artemia Salina Shrimps

Brine shrimp (Artemia salina) were hatched using brine shrimp eggs in a vessel filled with artificial sea water under constant aeration for 48 hours. The active shrimps (nauplii, larvae) were collected and used for the assay[29].

3.3.4. Preparation of Sample Solution

38 mg each of compounds was dissolved in 10 ml of DMSO to obtain the stock concentration of 1000 µg/ml and then stock solution was diluted to various concentrations 100, 10, 1 µg/mL. In order to prevent the toxicity results from possible false effect originated from DMSO's toxicity, stock solutions of the compounds were prepared according to suggested volume range by dissolving in DMSO. Pure DMSO used as a positive control for the toxicity assay[27-28].

4. RESULT AND DISCUSSION

In first step, N4 -phenyloxazole-2,4-diamine is prepare by N-phenyl acetamide and urea, progress of reaction is monitored by TLC. In second step 2-substitued benzimidazole is prepare by substituted OPD reacted with chloroacetic acid and chloropropionic acid. In third step final derivatives prepared by condensation of N4-phenyloxazole-2,4-diamine with 2-substituted benzimidazole by using catalyst K_2CO_3 and DMF reaction were monitored by TLC. Product were recrystallized by ethanol. The final derivatives were characterized by IR, NMR and Mass spectrometry.

4.1. Molecular Docking Study

The all synthesize derivatives (PD1-PD8) were evaluated for cytotoxic activity. The docking score of compounds (PD1-PD8) are shown in table and the compound code PD8 shows dock score is found to be -8. Which shows minimum dock score than other7 derivatives. We compared results of derivatives PD8 with std. bendamustine have good binding affinity to receptor (PDB code -5FGK).

Table 1: Docking Score Of Molecules

Sr. No.	Derivatives	Dock Score Kcal/mol
1.	Bendamustine	-6.6
2.	PD1	-8.4
3.	PD2	-8.3
4.	PD3	-8.4
5.	PD4	-8.1
6.	PD5	-8.2
7.	PD6	-8.5
8.	PD7	-8.4
9.	PD8	-8

Table 2: Type of interaction and its interaction distance (range)

Sr. No.	Type of Interaction	Compound code (PD1-PD8) Interaction distance
1.	Hydrophobic	3.79-5.39 A^0
2.	Hydrogen Bond	2.16-5.36 A^0
3.	Charge	3.81-5.09 A^0
4.	Pi- Stacked	3.85-5.33 A^0

4.2. Spectral Analysis of Derivatives

The Derivatives were synthesized according to the literature procedure from intermediates (OPD + Substituted acids). Total 8 Derivatives were synthesized. % Yield, melting point and Rf values (TLC) were calculated for all 8 Derivatives. The Derivatives were characterized by IR, 1H NMR and MS. The IR spectra of the complexes were measured in the region 4000-200 cm^{-1}. The N-H and CN (Ar-H) Vibrations are considered characteristics for the formation of benzimidazole moiety.

The IR spectra of N-H at particular values of derivatives are useful in confirmation of piperazine nucleus in benzimidazole structure.

The insolubility/ partial solubility of the compounds in the other organic solvents made it necessary to record 1H NMR spectra in d_6-DMSO. All 1H NMR measurements were recorded in d_6-DMSO.

4.3. Derivatives

PD1-N^2-((6-methyl-1*H*-benzo[d]imidazole-2-yl)methyl-N^4-phenyloxazole-2,4-diamine

M.P-115-122^{o}c,% yield-48.7%,IR(KBr): 2945(C-H),3275/3199(N-H), 1444(C-N), 1311.59(C-O), 1674(C=N), 1600(C=C);1H NMR δ ppm: 7.4(m, 8H), 3.9(s, 3H), 4.26(d, 2H), 2.35(s, 3H)

ESI- MS m/z: 319.36,304,227,174,145,77

PD2- N^2-((6-chloro-1*H*-benzo[d]imidazole-2-yl)methyl-N^4-phenyloxazole-2,4-diamine

M.P-110-113^{o}c,% yield- 40%,IR(KBr): 2879 (C-H),3277/3199(N-H), 1444(C-N), 1311.59(C-O), 1674(C=N), 1600(C=C), 754/542(c-cl) ;1H NMR δ ppm: 7.5(m, 9H), 4.22(d, 2H), 2.35(s, 3H)

ESI- MS m/z: 340.36,320,227,174,145,77

PD3- N^2-((6-nitro-1*H*-benzo[d]imidazole-2-yl)methyl-N^4-phenyloxazole-2,4-diamine

M.P-100-104^{o}c,% yield- 62%,IR(KBr): 2829 (C-H), 3313(N-H), 1444(C-N), 1338 (C-O), 1674(C=N), 1600(C=C),1530(R-NO2);1H NMR δ ppm: 7.09(m, 9H), 4.0(d, 2H), 5.0(s, 3H), 4.32(d, 2H)

ESI- MS m/z: 350.11, 342,298,174,145,77

PD4- N^2-((1*H*- benzo [d]imidazole-2-yl)methyl-N^4-phenyloxazole-2,4-diamine

M.P-85-88^{o}c,% yield- 65%,IR(KBr): 2831 (C-H), 3278 (N-H), 1442(C-N), 1313 (C-O), 1670(C=N), 1600(C=C);1H NMR δ ppm: 7.0/7.31(m, 9H), 4.0(d, 2H), 5.22(s, 1H)

ESI- MS m/z: 305.13,172,145,77

PD5-N^2-((6-Fluoro-1*H*-benzo[d]imidazole-2-yl)methyl-N^4-phenyloxazole-2,4-diamine

M.P-80-83^{o}c,% yield- 54.2%,IR(KBr): 2879 (C-H), 3277 (N-H), 1444(C-N), 1311(C-O), 754/542(C-F), 1600(C=C);1H NMR δ ppm: 7.0/7.61(m, 9H), 4.32(d, 2H), 5.0(s, 1H), 4.0(s, 1H)

ESI- MS m/z: 323,302,174,145,77

PD6-N^2-(2-(6-methyl-1*H*-benzo[d]imidazole-2-yl)ethyl)-N^4-phenyloxazole-2,4-diamine

M.P-85-90^{o}c,% yield- 48.2%,IR(KBr): 2945 (C-H), 3275 (N-H), 1444(C-N), 1311(C-O), 1600(C=C), 1674(C=N) ;1H NMR δ ppm: 7.0/7.51(m, 9H), 4.0(d, 1H), 5.0(s, 1H), 3.39(m,3H), 2.78(m,3H).

ESI- MS m/z: 333.39, 323.12,305,172,145,77

PD7-N^2-(2-(6-nitro-1*H*-benzo[d]imidazol-2-yl)ethyl)-N^4-phenyloxazole-2,4-diamine

M.P-125-129^{o}c,% yield- 50%,IR(KBr): 2945 (C-H), 3275 (N-H), 1444(C-N), 1311(C-O), 1600(C=C), 1674(C=N) ;1H NMR δ ppm: 7.0/7.51(m, 9H), 5.0(s, 1H), 3.84(m,3H), 2.58(m,1H).

ESI- MS m/z: 364,318.13,293.12,272,190,174,77

PD8-N^2-(2-(1*H*-benzo[d]imidazole-2-yl)ethyl)-N^4-phenyloxazole-2,4-diamine

M.P-95-98^{o}c,% yield- 38.2%,IR(KBr): 2831 (C-H), 3278(N-H), 1444(C-N), 1311(C-O), 1600(C=C), 1674(C=N) ;1H NMR δ ppm: 7.0/7.71(m, 9H), 4.26(d, 1H), 3.9(s, 3H), 2.51(m,3H).

ESI- MS m/z: 319.36,302,218,174,145,77

4.4. Cytotoxic Evaluation

Brine shrimp lethality bioassay was performed in laboratory. Complexes has solubility problem so it should be dissolved in DMSO for the preparation of drug solution. Following results were obtained by which LC50 was calculated. These results were compared with standard drugs i.e. bendamustine. The positive control was done with DMSO.

- 30 active shrimps (larvae) were added into each test tube
- The surviving (larvae) shrimps were counted after 24 hours and lethality concentration LC50 was assessed.

Table No. 3 Brine Shrimp Lethality Bioassay

Comp.	Conc. Ppm	Mortality of shrimps			% Mean mortality	Lc_{50} µg/mL
		I	II	III		
1	1000	13	15	13	45.55	1127.69
	100	10	11	9	33.33	
	10	7	7	4	20	
	1	2	4	4	11	
2	1000	16	15	15	51.11	914.10
	100	15	12	10	41.11	
	10	7	4	5	17.77	
	1	5	3	3	14.44	
3	1000	14	14	15	47.77	1027
	100	11	11	12	37.77	
	10	8	5	3	17.77	
	1	2	3	4	10	
4	1000	15	16	15	51.11	910.46
	100	11	12	15	42.22	
	10	5	5	4	15.55	

	1	2	3	3	8.88	
5.	1000	14	15	17	51.11	908.39
	100	10	16	12	42.22	
	10	7	5	5	18.88	
	1	3	5	2	7.77	
6.	1000	17	18	18	58.88	713.34
	100	11	11	12	37.77	
	10	7	6	4	18.88	
	1	5	3	4	13.33	
7.	1000	16	17	17	55.55	765.95
	100	11	11	13	38.88	
	10	7	8	7	24.44	
	1	5	2	3	11.11	
8.	1000	12	14	14	44.44	1221.29
	100	10	11	11	35.55	
	10	9	7	8	26.66	
	1	5	4	4	14.44	
Bendamustine	1000	17	18	19	60	647.82
	100	11	13	13	41.11	
	10	12	12	11	38.88	
	1	5	4	4	14.44	

CONCLUSION

The benzimidazole derivatives were subjected to cytotoxic activity and it was found that all derivatives show promising cytotoxic activity on brine shrimp. Molecular docking study shows that compound have binding affinity towards receptor 5-FGK, among all derivative-8 shows best score. Docking score of all derivatives are more than the standard drug hence all derivatives have anticancer properties. And having future scope to synthesize various derivatives of this series. All novel derivatives are synthesized by conventional method. Brine shrimp lethality bioassay is considered as useful tool for the preliminary assessment of cytotoxicity of derivatives**.**

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