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ORIGINAL ARTICLE **OPEN ACCESS**

Synthesis, Characterization and Evaluation of Cytotoxic Activity of Some Benzimidazole-Oxazole Derivatives

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ABSTRACT

Cancer, the uncontrolled, rapid, and pathological proliferation of abnormal cells, is one of the most formidable afflictions in the world jeopardizing human life and health. Cancer is the second most common cause death in the worldwide, accounting for an estimated 9.6 million deaths, or one in six deaths, in 2018.Heterocyclic compounds occupy a central position in medicinal chemistry and are of particular interest and significant importance in the search for new bioactive molecules in the pharmaceutical industry. Benzimidazole is heterocyclic, aromatic compound, it is a biologically active scaffold which possess anticancer, antitumour and antiproliferative properties along with other useful biological action. oxazole is one such moiety which has gained attention in recent times due to its increasing importance in the field of medicinal chemistry. The main objective was to synthesize novel 2 substituted benzimidazole derivatives which shows the good cytotoxic activity and then characterized by the IR, ^1H NMR and MS. Finally, to check the pharmacological activity of these complexes, brine shrimp lethality bioassay is carried out. Bioassay shows comparative studied of cytotoxicity of synthesized compounds and standard drug i.e. bendamustine.In this study it was found that from synthesized 4 derivatives, the derivative D2 shown excellent anticancer activity, LC_{50} is at 689.26 in comparison with standard drug bendamustine. Where derivative D1 shows the least cytotoxic activity, LC_{50} is at 1022.82.

Keywords:*Cancer, Benzimidazole, Oxazole, Cytotoxic, Bendamustine.*

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INTRODUCTION

History of cancer

The history of cancer is a story of theories about the causes and effects of the disease as well as of the continuing specific discoveries regarding the disease's structure, treatments, and methods for diagnosis. Thedoctor Hippocrates (460–370 B.C.E.) left several detailed descriptions of various diseases, including descriptions of lesions that had affected skin, breasts, stomach, cervix, and rectum. He also classified these into cancers that were in an early stage and others that were "occult."As for treating early-stage cancers, the options were to cauterize and to apply several ointments. Hippocrates is also credited with the word cancer because he used the term karkinoma to describe ulcers or growths that appeared to be malignant tumors.Hippocrates's theory about cancer was to persist for more than 1,300 years and was based on his overall theory of the four types of body fluid, or humors, that a human body had. These were blood, phlegm, yellow bile, and black bile. A person was healthy when the humors were balanced. An excess of the black bile humor, however, was the cause of cancer.

The Roman doctor Aulus Cornelius Celsus (25 B.C.E.–50 C.E.) elaborated on the Hippocrates theory and divided cancers into different stages. He called the first stage cacoethes (malignant), and only this stage was receptive to treatment. The doctor and philosopher Claudius Galenus (130–200) introduced the Greek word onkos, meaning a bulk or a mass, for referring to a growth or a tumor that appeared to be malignant, and is thus credited as the originator of the term oncology. Approaching the 17th century,

there were several theories contesting the humor theory, including hypotheses proposed by Zacutus Lusitani (1575–1642) and Nicholas Tulp (1593–1674), who published their hypotheses in 1649 and 1652, respectively, that cancer was contagious based on observations of breast cancer cases within the same household.[1-3]

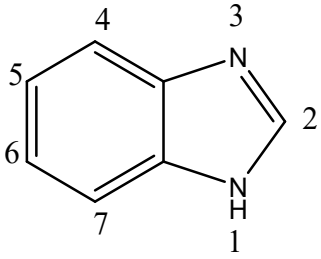
Basics about cancer:

Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and multiply (through a process called cell division) to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place.[4]

Sometimes this orderly process breaks down, and abnormal or damaged cells grow and multiply when they should not. These cells may form tumors, which are lumps of tissue. Tumors can be cancerous or not cancerous (benign). Cancerous tumors spread into, or invade, nearby tissues and can travel to distant places in the body to form new tumors (a process called metastasis). Cancerous tumors may also be called malignant tumors.

Benzimidazole:

Benzimidazole is a heterocyclic compound which formed by fusion of benzene and imidazole. Benzimidazole contains two nitrogens as heteroatom.First benzimidazole derivative synthesized by Hobrecker in 1872. The first research paper on pharmacological properties of benzimidazole published by Goodman and Nancy Hart in 1943. Then Woolley reported the antibacterial activity of some benzimidazole derivatives in 1944. After long research, it concluded that benzimidazole is important heterocyclic system because it exhibits biological activity against several pathogens and physical disorders. Benzimidazole derivatives plays an active role in therapeutic agents like antiviral anticancer, anthelmintics, anti-inflammatory agents, analgesics, antihistaminic, antiparasitic, anticonvulsants, antiulcer, antihypertensives, antifungals, proton pump inhibitors and anticoagulants.[5]



Its IUPAC name is 1H-benzimidazole and it is also referred to as 1H-1,3-Benzimidazole or 1H-Benzo[d]imidazole. The most important positions or sites effecting drug acting of benzimidazole substituents is 1st and 2nd position.

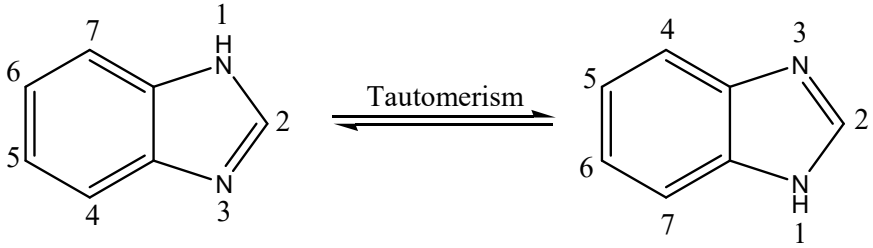
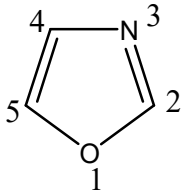


Figure 2: Tautomers of benzimidazole

Benzimidazole is bicyclic heterocyclic aromatic compound in which benzene ring fused to 4 and 5 position of imidazole ring, it contains two nitrogen atoms at 1 and 3 position exhibits both acidic and basic nature called amphotericin nature and exists in two equivalent tautomeric forms, when the hydrogen present at first position nitrogen atom possess acidic nature, when the hydrogen present at third position nitrogen atom possess basic nature.

Chemistry of Oxazole:



Structure of Oxazole

Oxazoles confirm their importance in medicinal chemistry and in all those processes connected to the synthesis of bioavailable products and their derivatives in the attempt to find new effective drugs.Heterocyclic systems are a part of large number of drugs and biologically relevant molecules. The chemistry and biological study of heterocyclic compounds has been interesting field for a long time and oxazole is one such moiety which has gained attention in recent times due to its increasing importance in the field of medicinal chemistry.Oxazoles is a doubly unsaturated 5-membered ring having one oxygen atom at position 1 and a nitrogen at position 3 separated by a carbon in-between. It was first prepared in 1947, has a boiling point of 69 °C and is a stable liquid at room temperature.Substitution pattern in oxazole derivatives play a pivotal role in delineating the biological activities like antimicrobial, anticancer, antitubercular, anti-inflammatory, antidiabetic, antiobesity and antioxidant etc.[6]

MATERIAL AND METHODS

All chemicals used were of Sigma Aldrich, SD Fine Chemicals and Thomas baker.All solvents used were of reagent grade and ordered.Thin-layer Chromatography (TLC) was performed on 60 F_{254} precoated silica gel plates (Merck) to establish identity of reactants and products monitored in between reactions as well at the end for completion of reaction. The spots were visualized in UV chamber or by iodine vapors in an enclosed chamber.All the melting points were determined with melting point apparatus and are corrected.IR spectra were recorded on KBr pellets on a shimadzu 1000 FTIR spectrometer in the range of 4000-400cm^{-1}, Resolution 8.0 No. of scan-45. Apodization; Happ-Genzel.Proton resonance magnetic spectra (^{1}H NMR) were recorded on Brucker 4000MHz spectrometer using d_6-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.

EXPERIMENTAL

Synthesis of reaction intermediates are as follows

Step1:

General procedure for the synthesis of 2-(4-chlorophenyl)-1H-benzimidazole

O-Phenylene diamine (0.01 mole) and p-chloro-benzoic acid (0.01) both in stoichiometric proportion were taken in ethanol as solvent in presence of NH_4Cl as a catalyst. The reaction mixture was stirred for 4hr at 80°C on hot plate. After the completion reaction, the reaction mixture was cooled and poured in ice-cold water. The granular solid was obtained. It was crystalized from ethanol.[7]

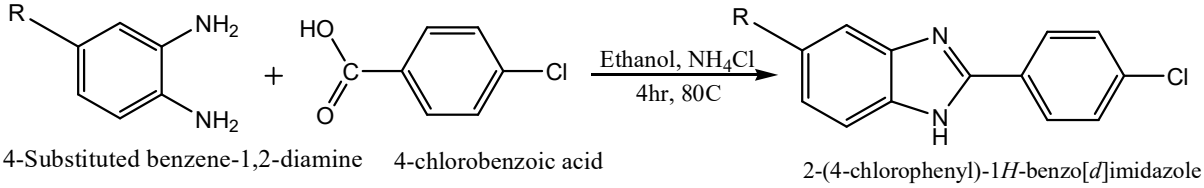


Figure 3: Synthesis of 2-(4-chlophenyl)-1H-benzimidazole

R:CH_3, NO_2, Cl

Step 2:

General method for the Synthesis 2- Chloro-N-phenyl acetamide

5 ml substituted aniline is mixed with 7 ml of ethanol were shaken in a magnetic stirrer for half an hour. 4ml chloro-acetyl-chloride was added drop by drop to the above mixture. The mixture was then stirred for half an hour with heating. The stirred mixture was then poured into ice cold water. The mass obtained was filtered and recrystallized from ethanol.[8]

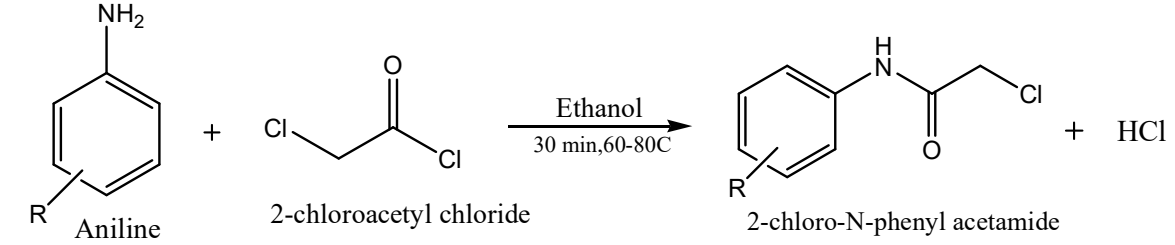


Figure 4: Synthesis of 2- Chloro-N-phenyl acetamide

Synthesis of substituted N-phenyl-oxazole-2,4-diamine

A mixture of substituted 2-chloro-N-phenylacetamide (1.6 gm) and urea (0.6 gm) were dissolved in 10 ml ethanol and the reaction mixture stirred and reflux for 2 hours and poured into ice cold water and filtered.

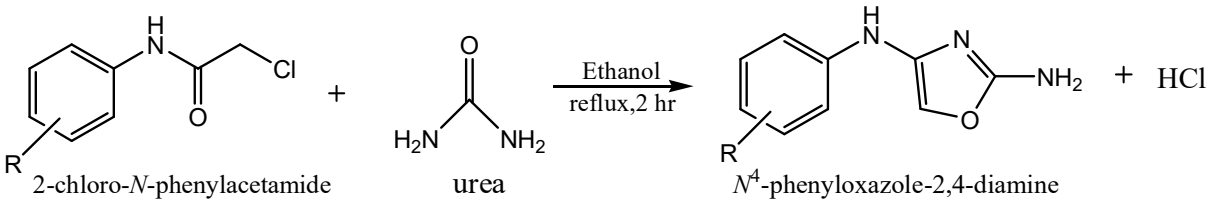


Figure 5: Synthesis of N-phenyl-oxazole-2,4-diamine

Synthesis of Derivatives

Step 3

General procedure for the synthesis N-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-N-phenyloxazole-2,4-diamine

In an RBF 2-(4-chlorophenyl)-1H-benzimidazole(7.5mmol, 0.198ml) and N-phenyl-oxazole-2,4-diamine (5mmol,500mg) was dissolved in 1,4-dioxane after TEA was added and the reaction mixture were refluxed at 102℃ for 5hr. The reaction was filtrated and extracted with dichloromethane and water. The organic layer was saturated by NaCl solution, dried using $NaSO_4$ and evaporated to get desired compound. Recrystallize with ethanol.[9]

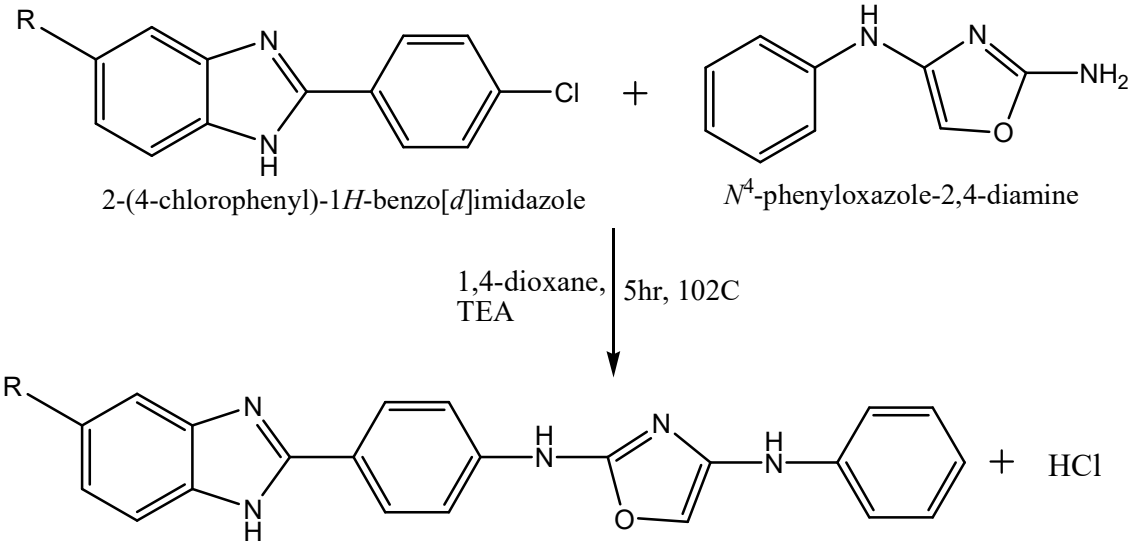


Figure 6: Synthesis of N-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-N-phenyloxazole-2,4-diamine

R**:** CH_3, NO_2, Cl

Molecular docking:

Molecular docking study was applied to investigate thebinding mode of compound with selected PDB ID for cancer cell lines. Docking score obtained from Pyrx software and binding site was targeted and the grid was created. The active site grid covered the important amino acids interacting with receptor. The 3D structure of the protein was obtained from protein data bank using their specific (PDB code: 5FGK). A data set of benzimidazoles was used as ligands and their structures were drawn using the Chem draw and were converted to 3D form for the docking studies using Avogadro software. The collected ligands were prepared for docking. Then the prepared ligands were docked into the generated grid in the prepared protein.[10]

Preparation of ligand:

Ligand structure was drawn using Chem draw software and the structure was cleaned using the clean structure tool.The structure was saved in the working folder as .mol file in a working folder. The .mol file present in working folder was then accessed in Avogadro software and structure was optimized using optimization tool. The optimized structure was exported in the working folder as F1.pdb – F15.pdb file format.[11]

Preparation of receptor:
Target protein i.e. human cyclin-dependent kinase CDK-8 (PDB code: 5-FGK) were obtained using protein data bank (PDB) http://www.rcsb.org/pdb/home/home.do, the protein structure were prepared using DS visualizer. All water molecules were removed and the polar hydrogen are added.

CYTOTOXIC ACTIVITY

Brine shrimp lethality bioassay
Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of synthesized compound. Brine shrimp 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.[12]
This in vitro lethality test has been successfully used as a preliminary study of antitumor agents.

Preparation of brine solution
38 g of iodize sodium chloride was weighed, dissolved in 1000 ml of distilled water, and filled to obtain a clear solution.

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

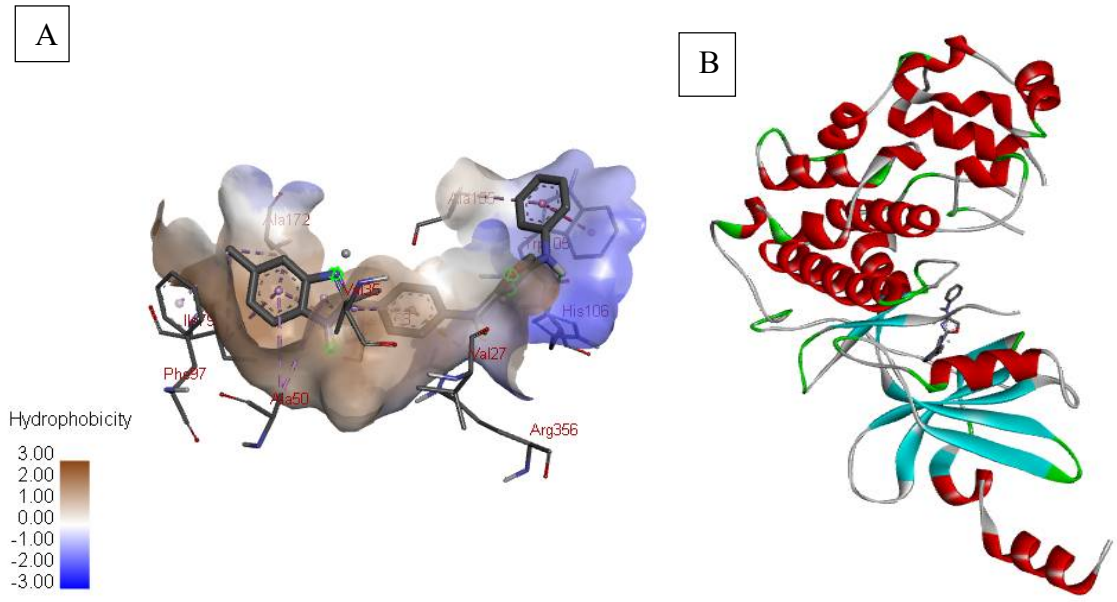
Preparation of sample solution
10 mg of compounds was dissolved in 10 ml of DMSO to obtain the stock concentration of 1000µg/ml. In order to prevent the toxicity results from possible false effect originated from DMSO's toxicity, stock solutions of the compounds were prepared according to suggested volume range by dissolving in DMSO. Pure DMSO was used as a positive control for the toxicity assay.

Application of test solution and larvae to the test tubes
About 5 ml of brine solution was taken into each test tubes. Suitable dilutions of the test substances were made as per the concentration. The 0.05 ml diluted test solution was added to the test tubes.

- 10 active shrimps (larvae) were added into each test tubes
- The solution should be mixed thoroughly
- The surviving (larvae) shrimps were counted after 24 hours and lethality concentration LC50 was assessed.

RESULT AND DISCUSSION

Molecular docking study
The all synthesize derivatives (D1-D4) were evaluated for cytotoxic activity.The docking score of compounds (D1-D6) are shown in table and the compound code D2 shows dock score is found to be -9.9. Which shows minimum dock score than other 3 derivatives. We compared results of derivatives D2 have good binding affinity to receptor (PDB code- 5FGK).



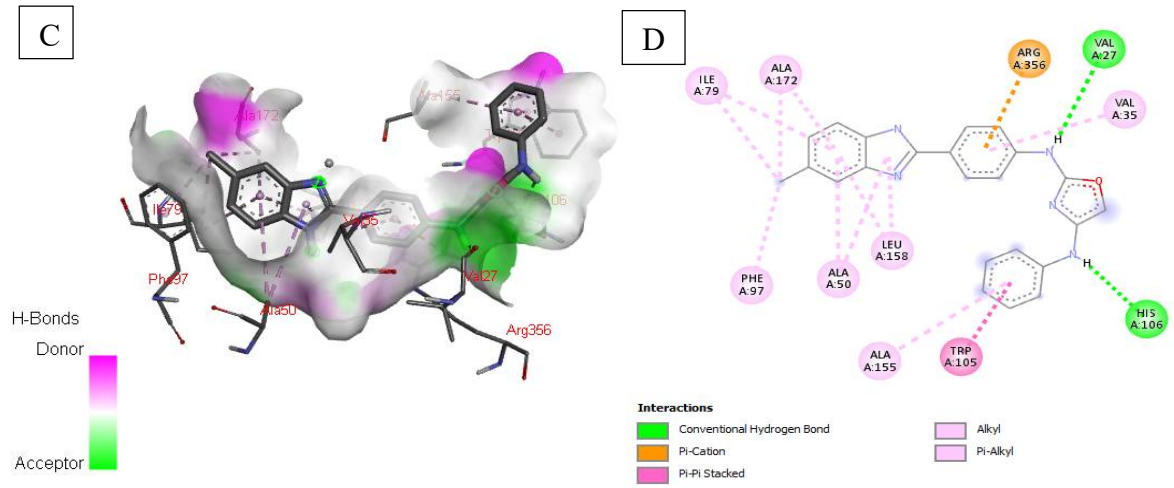


Figure 7: Visualization of docking analysis of compound D3 binding with 5FGK receptor (A) visualization of hydrophobic interaction (B) interaction of compound D3 with 5FGK receptor (C) visualization of hydrogen bond (D) 2D representation describing binding of compound D3 bindings with active site of 5FGK receptor.

The above figures show hydrophobic interaction within the range of 4.01-5.48Å, hydrogen bond interaction within the range of 2.41-2.49Å and the residue interacted are ILE 79A, ALA 172A, ALA 50A, LEU 158A, ALA 155A, VAL 35A, PHE 97A, VAL 27A and HIS 106A.

Table 1: Results of binding affinity of molecule

Sr. No.	Compound code	Docking score
1.	D1	-9.4
2.	D2	-9.9
3.	D3	-9.8
4.	D4	-9.7
5.	Standard	-6.9

Spectral analysis of Derivatives

Total 4 Derivatives were synthesized. % yield, melting point and Rf value (TLC) were calculated for all 4 Derivatives. The Derivatives were characterized by IR,^{1}H NMR and MS.

Derivatives

D1- N-(4-(1H-benzo[d]imidazol-2-yl) phenyl)-N-phenyloxazole-2,4-diamine

M.P: 204-206°C, % yield:61.20%, IR (KBr) (cm-1): 3267 (N-H), 3072 (C-H), 1598 (C=C), 1120 (C-N), 1666 (C=N), 1273 (C-O).^{1}H NMR δ ppm:7.108-Ar-H (Ha), 7.352-Ar-H (Hb), 7.558-Ar-H (Hc), 7.663-Ar-H (Hd), 4.254-Ar-NH.

D2-N-(4-(5-methyl-1H-benzo[d]imidazol-2-yl) phenyl)-N-phenyloxazole-2,4-diamine

M.P: 208-210°C, % yield: 70.35%, IR (KBr) (cm-1): 3257 (N-H), 3061 (C-H), 1597 (C=C), 1122 (C-N), 1681(C=N), 1274 (C-O).^{1}H NMR δ ppm: 2.450-Ar-CH_3 (Ha), 6.605-Ar-H (Hb), 7.644-Ar-H (Hc), 7.669-Ar-H (Hd), 7.142-Ar-H (He), 5.004-Ar-NH. ESI-MS m/z: 381.158, 366.188, 222.103, 207.092, 174.067, 192.127, 116.04, 76.03.

D3-N-(4-(5-nitro-1H-benzo[d]imidazol-2-yl) phenyl)-N-phenyloxazole-2,4-diamine

M.P- 218-220°C, % yield- 75.50%, IR (KBr) (cm-1): 3292 (N-H), 3070 (C-H), 1598 (C=C), 1523 (R-NO_2), 1336 (C-N), 1687 (C=N), 1288 (C-O). ^{1}H NMR δ ppm: 8.601-Ar-H(Ha), 6.592-Ar-H(Hc), 7.599-Ar-H(Hd), 4.501-Ar-NH.

D4-N-(4-(5-chloro-1H-benzo[d]imidazol-2-yl) phenyl)-N-phenyloxazole-2,4-diamine

M.P- 202-204°C, % yield- 65.40%, IR (KBr) (cm-1): 3263 (N-H), 3097 (C-H), 1481 (C=C), 731 (C-Cl), 1301 (C-N), 1674 (C=N), 1247 (C-O).

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 LC_{50} was calculated. These results were compared with standards drugs i.e., bendamustine. the positive control was done with DMSO.

- 10 active shrimps (larvae) were added into each test tube

- The surviving (larvae) shrimps were counted after 24 hours and lethality concentration LC_{50} was assessed.

Table 2: Brine shrimp lethality bioassay

Sr. No.	Compound code	LC_{50} (µg/ml)
1.	D1	1022.82
2.	D2	689.26
3.	D3	738.70
4.	D4	853.30
5.	Bendamustine	670.07

Positive control with DMSO has shown mortality of 1 shrimps.
No. of shrimps taken: 30

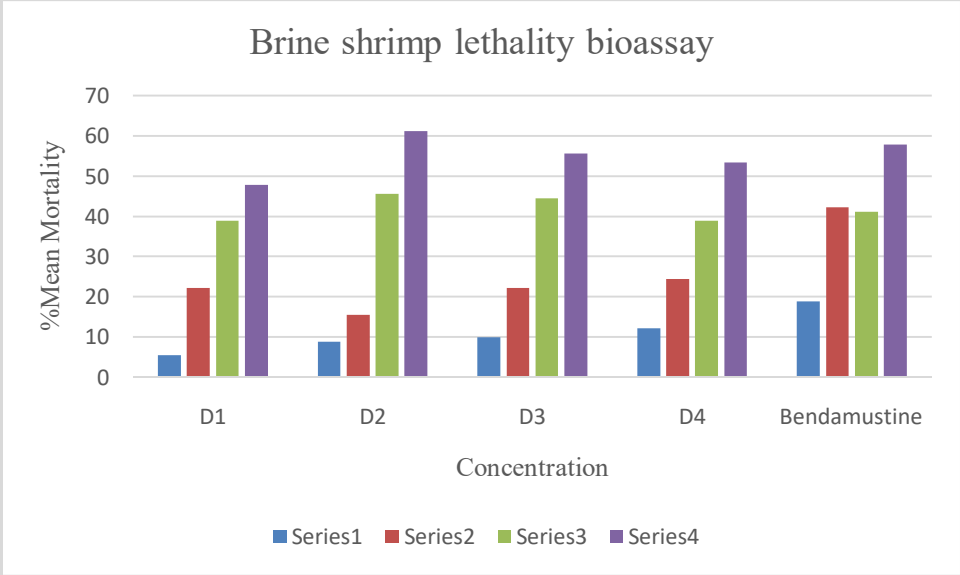


Figure 8: Graphical representation showing activity of derivatives concerning Bendamustine

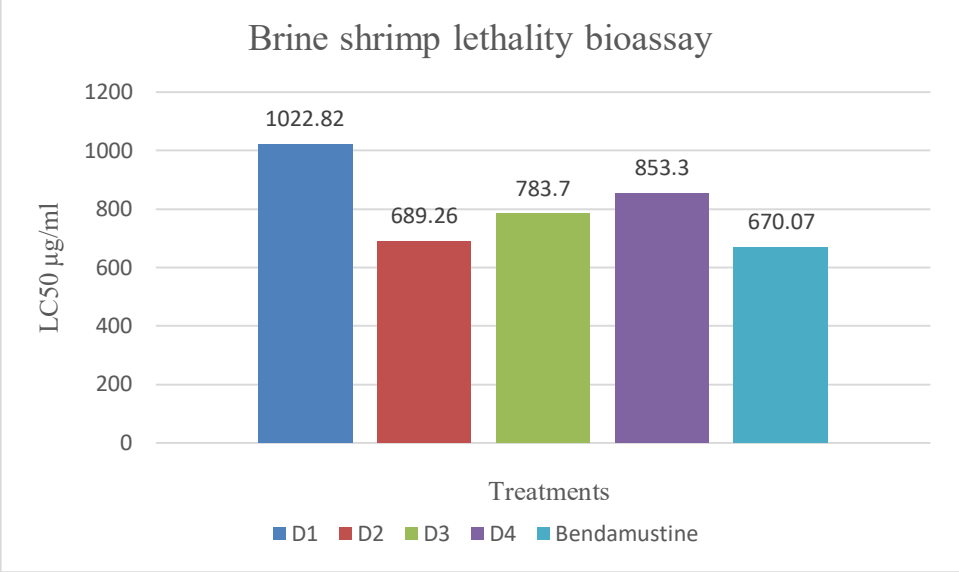


Figure 9: Graphical representation showing LC_{50} values of derivatives and Bendamustine.

CONCLUSION

- The molecular docking studies were performed and the compounds selection were done based on the docking score of the molecule.
- The synthesized derivatives were characterized by IR, ^1H NMR, and MS All 4 synthesized derivatives were evaluated for cytotoxic activity by brine shrimp lethality bioassay. Cytotoxicity

was calculated in terms of LC_{50}. Bendamustine was taken as standard for the biological evaluation.

- In conclusion, it was found that substituted O-phenylenediamine gives more % yield than unsubstituted one. The synthesized compounds were adequately confirmed by ^1H NMR.
- All derivatives show good cytotoxic activity. Among all derivatives, derivative **D2** showed more cytotoxic activity than any compound with standard drug bendamustine. Derivative **D1** shows the least cytotoxic activity,where derivatives **D3**and **D4** show moderate cytotoxic activity as compared to bendamustine.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Gronning, T. (2015). History of Cancer, in Graham A. Colditz (ed.) The SAGE Encyclopediaof Cancer and Society, 2nd Edition. Thousand Oaks, CA: Sage Publications.
2. Leslie Foulds, (1958). The natural history of cancer, Journal of Chronic Diseases, Volume 8, Issue 1 Pages 2-37, ISSN 0021-9681.
3. "The History of Cancer". American Cancer Society. 2009.
4. https://www.cancer.gov/about-cancer/understanding/what-is-cancer
5. Garvit Satija, Barkha Sharma, Anish Madan; Benzimidazole based derivatives as anticancer agents: Structure activity relationship analysis for various targets, Journal of Heterocyclic Chemistry Volume 59, Issue 1,2022, Pages 22-66, ISSN 0022-152X.
6. Nancy Y. Guerrero-Pepinosa, María C. Cardona-Trujillo, Sandra C. Garzón-Castaño; Antiproliferative activity of thiazole and oxazole derivatives: A systematic review of in vitro and in vivo studies, Biomedicine & Pharmacotherapy, Volume 138, 2021,111495, ISSN 0753-3322.
7. Rithe, S.R. & Jagtap, R.S. & Ubarhande, S.S. (2015). One pot synthesis of substituted benzimidazole derivatives and their characterization. Rasayan Journal of Chemistry.8. 213-217.
8. Chantika Octaviani Putri, Antonius Herry Cahyana; (2022). Synthesis and antioxidant activity screening of thiazole and oxazole derivative compounds. AIP Conference Proceedings. 2391 (1): 050004.
9. Karmaker, Nilima& Lira, Dilshad& Das, Biplab& Kumar. (2018). Synthesis and Antioxidant Activity of Some Novel Benzimidazole Derivatives. Dhaka University Journal of Pharmaceutical Sciences. 16. 245.
10. Surti, Malvi and Patel, Mitesh and Adnan, Mohd and Moin et.al. Ilimaquinone (marine sponge metabolite) as a novel inhibitor of SARS-CoV-2 key target proteins in comparison with suggested COVID-19 drugs: designing docking and molecular dynamics simulation study","RSC Adv.","2020","10""37707-37720".
11. Pratyush , K., Asnani , A., Chaple , D., & Bais , A. (2020). In silico screening and molecular docking of bioactive agents towards human coronavirus receptor. GSC Biological and Pharmaceutical Sciences, 11(1), 132–140.
12. Sarah, Quazi& Anny, Fatema& Misbahuddin, Mir. (2017). Brine shrimp lethality assay. Bangladesh Journal of Pharmacology. 12. 5.
13. Jian, Fuji& Yu, Huan-Qing& Yuanbiao, Qiao& Zhao. (2006). 2-(4-Chloro-phenyl)-1H-benzimidazole. Acta Crystallographica Section E. 62.
14. Yadav, S., Narasimhan, B., & Kaur, H. (2016). Perspectives of Benzimidazole Derivatives as Anticancer Agents in the New Era. Anti-cancer agents in medicinal chemistry, 16(11), 1403–1425.
15. Poddar, Saikat& Saqueeb, Nazmus& Rahman, S. M. (2016). Synthesis and Biological Evaluation of 2-methyl-1H-benzimidazole and 1H-benzimidazol-2-yl-methanol. Dhaka University Journal of Pharmaceutical Sciences. 15. 83-87.
16. Leslie Foulds, The natural history of cancer, Journal of Chronic Diseases, Volume 8, Issue 1 1958, Pages 2-37, ISSN 0021-9681.
17. Ibrahim, H.A., Refaat, H.M. Versatile mechanisms of 2-substituted benzimidazoles in targeted cancer therapy. Futur J Pharm Sci 6, 41 (2020).
18. Pham, E.C., Thi Le, T.V., & Truong, T.N. (2022). Design, synthesis, bio-evaluation, and in silico studies of some N-substituted 6-(chloro/nitro)-1H-benzimidazole derivatives as antimicrobial and anticancer agents. RSC Advances, 12, 21621 - 21646.
19. Siddig, L. A., Khasawneh, M. A., Samadi, A., Saadeh, H., A. (2021). Synthesis of novel thiourea-/urea-benzimidazole derivatives as anticancer agents. Open Chemistry, 19(1), 1062-1073.
20. Sachin V. Patil, Sachin S. Patil, Vivek D. Bobade, A simple and efficient approach to the synthesis of 2-substituted benzimidazole via sp3 C–H functionalization, Arabian Journal of Chemistry, Volume 9, Supplement 1,2016, Pages S515-S521, ISSN 1878-5352.
21. Nerella, Sridhar Goud& Kumar, Pardeep& Bharath, Rose. (2020).Recent Developments of Target-Based Benzimidazole Derivatives as Potential Anticancer Agents. 10.5772/intechopen.90758.

22. Jian, Fuji& Yu, Huan-Qing& Yuanbiao, Qiao& Zhao. (2006). 2-(4-Chloro-phenyl)-1H-benzimidazole. Acta Crystallographica Section E. 62.

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