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Synthesis, molecular docking studies and cytotoxic screening of novel 2-substituted benzimidazole derivatives

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Abstract

2-substituted benzimidazole derivatives were prepared by different substituted orthopheneledіamine and substituted chloro-benzoic acid which are evaluated for cytotoxic activity. The compounds where synthesize from orthopheneledіamine and were characterize by spectral analysis i.e., IR, 1HNMR and mass spectrometry. all novel synthesizes derivativities code D1- D6 were further evaluated for cytotoxic activity using brine shrimp lethality bioassay. The molecular docking studies were performed by using VLife-MDS 4.3 software. Derivative code D3 exhibited good docking results of -39.5844 than the standard drug bendamustin. The cytotoxic activity and docking results of all synthesize derivatives shows that derivative D3 has more promising results at

5-FGK receptor and while comparing docking results of all derivatives to standard drug highlight the fact that synthesize derivatives considered as possible hit as therapeutic agents.

Keywords: cancer, benzimidazole, molecular docking, cytotoxic, 5-FGK, ligand etc.

Introduction

Cancer:

Egypt and dates back to about 3000 BC was discovered word cancer. The origin of word cancer is credited to the Greek physician Hippocrates (460- 370BC), who term as father of medicine, He left a number of detailed information of various diseases. Hippocrates is also credited with word cancer because he used the term karkinomas to describe were or growth that appeared to be malignant[1]. Cancer is abnormal, uncontrolled cells growth in body. When the damaged cell/ unrepaired cells in body do not die and later become cancerous cells and they show uncontrolled cell division and cell growth - a mass of cancer cells start to develop.Cancer cells can break away from its original clusters of cells, which travel through the blood and lymph systems.For example, if breast cancer cells spread to a bone, it means that the individual has metastatic breast cancer to bone[2]. Cancer is disease which often cause by gene or called genetic disease. That is cancer caused to gene that control the way our cell's function.Over the past few years, there is a considerable interest in the development and pharmacology of heterocyclic organic compounds, such as benzimidazoles, benzothiazoles, indole, acridine, oxadiazole, imidazole, isoxazole, pyrazole, triazoles, quinolines and quinazolines for their diverse

activities[3].

Benzimidazole

Benzimidazole is very versatile class of heterocyclic compounds in medicinal chemistry. Synthesis of benzimidazole has received considerable interest in past few decades[4]. Benzimidazole is benzo derivative of imidazole, this belongs to bicyclic (Consist of benzene and imidazole rings) aromatic organic compound. Benzimidazole is synthesis from orthophenelenediamine with mono & di basic acids[5].This vital pharmacophore having many biologically active heterocyclic compounds with variety of activities. Benzimidazole consist of NH group is highly acidic and weakly basic nature[6].This pharmacophore having different pharmacological activities like anticancer, antifungal[7], antihypertensive, antimalarial[8], antimicrobial[9], antiviral[10], antiprotozoal, antitubercular etc.

Chemistry:

Its IUPAC name is 1H-benzimidazole and it is also referred to as 1H-1,3-Benzimidazole or 1H-Benzo[d]imidazole. It was first prepared by Hobrecker[11] in 1872. Benzimidazole scaffold is isosteric with indole and purine nuclei. Important period in the pharmacological significance of benzimidazoles moeity and the closely related structure purine was in early 1950's.It was discovered that 5,6-dimethyl-1- (D-ribofuranosyl) benzimidazole is an integral part of structure of Vit.B12[12-13].

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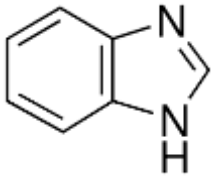
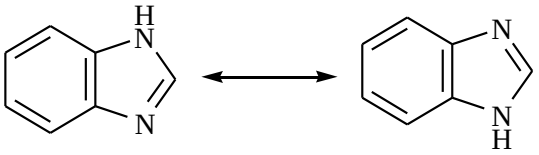


Benzimidazoles compounds contain a hydrogen atom which attached to nitrogen in the position-1 which is readily tautomerize. In tautomerism is the resemble to that found in the two groups- imidazoles and amidines. This may be shown as below:



I II

Benzimidazole is depicted as (I) possessing the proton at N1, there exist a rapid exchange between –NH and =N- nitrogen atoms. The groups at position C5 and C6 in the ring system are chemically equivalent[14].

2. 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 thief's tube melting/boiling point apparatus and are uncorrected.IR spectra were recorded on KBr pellets on a shimadzu 1000 FTIR spectrometer in the range of 4000-400 cm^{-1}, Resolution 8.0 with No. of scan- 45. Apodization; Happ-Genzel. Proton resonance

magnetic spectra (^{1}H NMR) were recorded on Bruker 400MHz spectrophotometer 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.

2.1 Synthesis of Intermediates- Step1:

2.1.1 General procedure for the synthesis of 2-(4-chlorophenyl)-1H-benzo imidazole[15]

O-Phenylene diamine (0.01 mole) and p- chloro-benzoic acid (0.01 mole) both in stoichiometric proportion were taken in ethanol as solvent in presence of NH4Cl as a catalyst. The reaction mixture was stirred for 4 h at 80 ^{0}C on hot plate. After the completion of reaction, the reaction mixture was cooled and poured in the ice-cold water. The granular solid was obtained. It was crystallized from the ethanol, yield 70.00%, M.P. 260 ^{0}C.

2.1.2 Synthesis of 5-methyl-2-chlorophenyl benzimidazole

4methyl orthopheneledіamine 0.01mole (6.18gm), Chloro- benzoic acid 0.01mole (7.6gm) both in stoichiometric proportion were taken in ethanol as solvent in presence of NH4Cl as a catalyst. The reaction mixture was stirred for 4 h at 80 ^{0}C on hot plate. After the completion of reaction, the reaction mixture was cooled and poured in the ice-cold water. The granular solid was obtained. It was crystallized from the ethanol, yield 78.90%, M.P

2.1.3 Synthesis of 5 nitro-2- chloro- ethyl benzimidazole[16]:

A Mixture of 4-nitroOrthophenelendiamine (0.01mole) and chloro-Butyric acid

(0.01mole) was heated at 100 C for 4-5 hours in 15ml of 4N HCl and reaction progress examined under thin layer chromatography (TLC). After the reaction was completed, the mixture was completed the mixture was cooled to room temperature and then poured into ice cold water. Stirring was cooled to room temperature and poured into ice cold water. Stirring was continuous for few minutes and mixture was neutralized with aq. Ammonia. Filter the product and recrystallized using ethanol.mp 115 C, % Yield- 44.44%.

2.1.4 Synthesis of 5 nitro 2- chloro- phenyl benzimidazole[17]

A solution of 2-nitrobenzaldehyde (1 equiv.) in ethanol (60ml) and the 4- nitro Orthopheneledіamine (1eqiv.) is stirred at 80 C for 24 hours. The reaction mixture is then poured into water. The precipitate is then purified by recrystallization from ethanol, isolating the corresponding benzimidazole. MP- 270C, %Yield- 64.48 %.

2.2 Synthesis of Step-2

2.2.1 D3- 2-(4-(4-ethylpiperazin-1-yl) phenyl)-1H benzimidazole[18]

2-(4-chlorophenyl)-1H-benzo imidazole (step1 product) 0.01 mole was dissolved in ethanol (25ml) followed by addition of ethyl piperazine derivatives (0.01mole) and formaldehyde solution (40% w/v) (0.0105mole) to undergo mannich reaction. The reactant was refluxed for 10 hours, after completion of reaction mixture kept in refrigerator overnight after that add dil. HCl into that liquid to get precipitate, filter and dry, M P- 224-226.

2.2.2 D1- 5-Methyl-2-(4-(piperazin-1-yl) phenyl)-1H-benzimidazole[19]

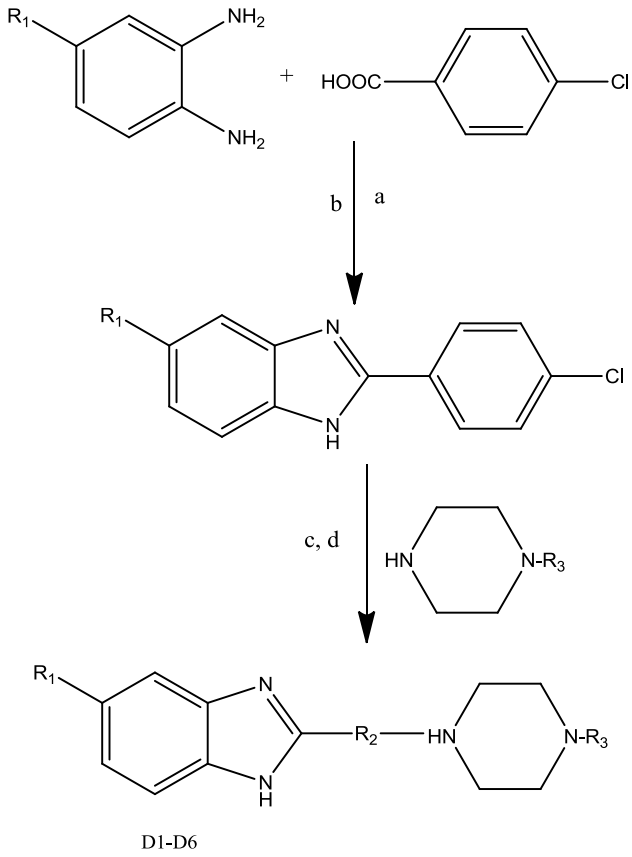
A solution of 5- methyl-2-(4-chlorophenyl)-1H-benzo imidazole (step1 product), (0.37g,0.01051mol) and piperazine (0.44,0.0105mol) in dimethylformamide was taken in a RBF.K_2CO_3 (2gm,) was added to the reaction mixture. The reaction mixture was stirred for 8h at 80^oC on a magnetic stirrer (heat + stirring). The progress of the reaction was monitored by thin layer chromatography (TLC). Upon completion of the reaction, water was added to the reaction mixture and the product extracted by shaking the reaction mixture with dichloromethane in a separating funnel. The dichloromethane layer was washed successively with water and brine, dried. over anhydrous sodium sulfate. Evaporation of the solvent gave the product.

Recrystallized with chloroform, Mp- 270-272 C.

2.2.3 D4: 5-Nitro-2-(4-piperazin-1yl) phenyl)-1H benzimidazole

A solution of 5- Nitro-2-(4-chlorophenyl)-1H-benzo imidazole (step1 product), (0.51, 0.01051mol) and piperazine (0.11, 0.0105mol) in dimethylformamide was taken in a RBF. K_2CO_3 (1gm) was added to the reaction mixture. The reaction mixture was stirred for 8h at 80^oC on a magnetic stirrer (heat + stirring). The progress of the reaction was monitored by thin layer chromatography (TLC). Upon completion of the reaction, water was added to the reaction mixture and the product extracted by shaking the reaction mixture with dichloromethane in a separating funnel. The dichloromethane layer was washed successively with water and brine, dried over anhydrous sodium sulfate. Evaporation of the solvent gave the product.

Recrystallized with chloroform, Mp- 212-214C



Code	R1	R2	R3
D1	CH3	Phenyl	-H
D2	CH3	Phenyl	-CH3
D3	-H	Phenyl	C2H5
D4	-NO2	Phenyl	-H
D5	- NO2	Phenyl	-CH3
D6	-NO2	C3H7	-H

Scheme1- synthetic route for preparation of benzimidazole derivatives.

2.3 Analytical data of synthesize benzimidazole derivatives.

2.3.1 Derivatives D1-5-Methyl-2-(4-(piperazin-1-yl) phenyl)-1H-benzimidazole.

M.P- 270-272℃, % yield- 63.15%, IR (KBr) (cm-1) – 3263 (N-H), 2854 and 2900 (C-H) stretch, 1550 Ar (C=C) stretch, 771- (o-substituted-benzene), 817 – Aromatic substitution (2- adjacent H atom), 1273 and 1365 Aromatic C-N Vibration. NMR- 2.29-Ar-H (Ha), 7.27- Ar- H (Hb), 7.057- Ar-H (Hc), 7.261-Ar-H (Hd), 7.26- Ar-H (Hf, He), 3.11-H (Hg), 2.817-H (Hh).

2.3.2 Derivatives D2-5-Methyl-2-(4-(4-methylpiperazin-1-yl) phenyl)-1H benzimidazole.

M.P-260-262℃, % yield-80.90%, IR (KBr) (cm-1) –3221-Ar-(N-H), 3178- (C-H) stretch, 1589-(C=C),1346-(C-N). NMR- 2.20-Ar-H(Ha),7.16-Ar-H(Hb), 7.51-Ar-H(Hc),7.6-Ar-H(Hd),7.63-H(Hf,He),3.14-H(Hg),2.502-H(Hb),2.49-H(Hi).

2.3.3 Derivatives D3-2-(4-(4 ethyl piperazin-1-yl) phenyl)-1H-benzimidazole

M.P-224-226℃, % yield-48.85%, IR (KBr) (cm-1) –3352 (N-H), 3178 (C-H), 1589 (C=C), 1346 (C-N), 775 (Cl-Halogen)). NMR-7.28-Ar-H(Ha), 7.2-Ar-H(Hb),7.169-Ar-H(Hc),7.873-Ar-H(Hd), 7.82-Ar-H(Hf, He), 3.11-H(Hg), 2.52-H(Hh).2.49- H(Hi).

2.3.4 Derivatives D4-5-Nitro-2-(4-piperazin-1yl) phenyl)-1H benzimidazole

M.P-212-214℃, % yield-87.27%, IR (KBr) (cm-1) – 3197 (N-H), 3097 (C-H), 1558 (C=C), 1570and 1338 – (Ar, NO2)

2.3.5 Derivatives D5-5-Nitro-2-(4-methylpiperazin-1yl) phenyl)-1H benzimidazole

M.P-254-256℃, % yield-83.76%, IR (KBr) (cm-1) – 3290 (N-H), 3078 (C-H) stretch, 1500 (C=Cl), 1570 and 1338 ($-NO_2$), 2800 and 2897- SP^3 (C-H) stretch.

2.3.6 Derivatives D6-5-Nitro-2-(piperazin-1yl) Propyl-1H benzimidazole

M.P-232-234℃, % yield-42.42%, IR (KBr) (cm-1) - 3178 –Ar- N-H, 1566-(C=Cl), 1566 and 1350-Ar, $-NO_2$, R_2-CH-NO_2

2.4 Molecular docking

All Molecular docking were performed using the molecular modeling software (VLifeMDS) version 4.3. It provided a facility to dock different ligands in protein

binding sites chosen by the user. VLifeMDS 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. The target or receptor was either experimentally known or theoretically generated through homology modelling or knowledge-based protein modeling[20].The molecular docking tool has been developed to obtain a preferred geometry of interaction of ligand–receptor complexes having minimum interaction energy based on different scoring functions viz. only the dock score, electrostatics and the sum of steric and electrostatic (parameters from the force field). This utility allowed us to screen a set of compounds for the purpose of lead optimization. VLifeMDS uses the Piecewise Linear Pair Wise Potential (PLP), genetic algorithm and Grid algorithms to minimize the interaction energy between the ligand and receptor protein.

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[21-22].

2.4.1 Preparation of ligands:

The ligands (substituted benzimidazole derivatives) were studied for their binding activities. The 2D structures of were drawn using chemdraw software and converted to 3D conformations. The conformers thus obtained, were optimized (MMFF) till they

reached a rms gradient energy of 0.001 kcal/mol.

2.4.2 Preparation of protein:

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 preparation wizards. All water molecules were removed and the polar hydrogen are added.

2.4.3 Cytotoxic activity:

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].

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.

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.

Preparation of sample solution

10 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 was used as a positive control for the toxicity assay[24].

Application of test solution and larvae to the test tubes

About 5 mL of brine solution was taken into each test tube. Suitable dilutions of the test substance 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 tube
- The solution should be mixed thoroughly

The surviving (larvae) shrimps were counted after 24 hours and lethality concentration LC50 was assessed.

3. result and disscusion

3.1 Chemistry:

In first step, 2-substituted benzimidazole is prepare by substituted orthophenelediamine reacted with chloro-benzoic acid or chloro- substituted acidsby addition of ethanol and ammonium chloride at 80C with reflux, progress of reaction is monitored by TLC. In second step final derivatives were prepared by addition of substituted piperazine in 2-substituted benzimidazole by using catalyst K2CO3 and DMF reaction were

monitored by TLC. Product were recrystallized by ethanol. The final derivatives were characterized by IR, NMR and Mass spectrometry.

3.2 Molecular docking study:

The all synthesize derivatives (D1-D6) were evaluated for cytotoxic activity. The docking score of compounds (D1-D6) are shown in table and the compound code D3 shows dock score is found to

be -39.5844

Which shows minimum dock score than other 5 derivatives. We compared results of derivatives D3 have good binding affinity to receptor (PDB code -5FGK). The best pose for docking results is shown in fig-3, where main interaction between receptor can be observed in fig-2.

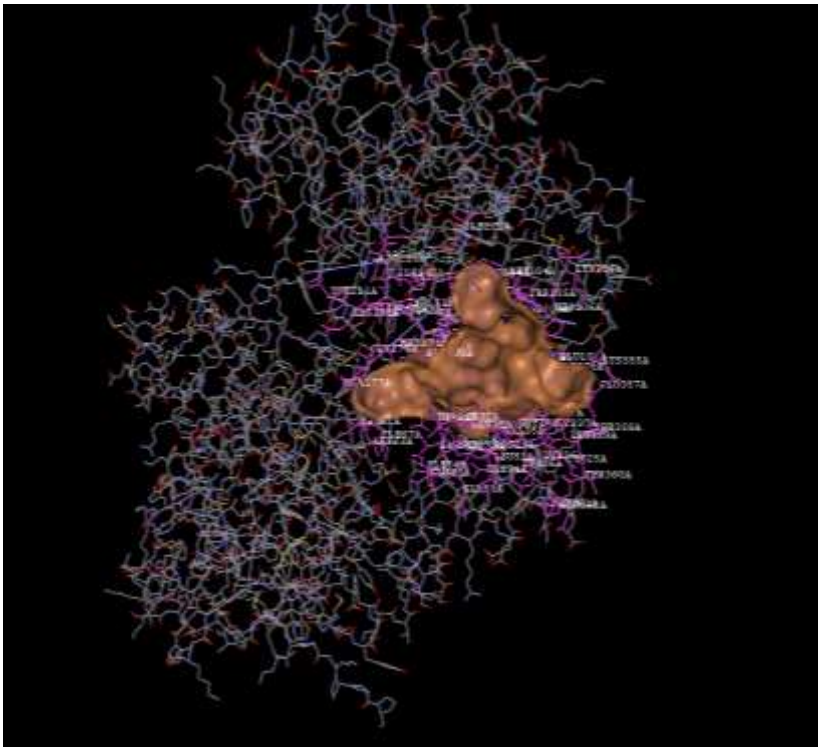


Fig1- Docking representation of ligand with receptor

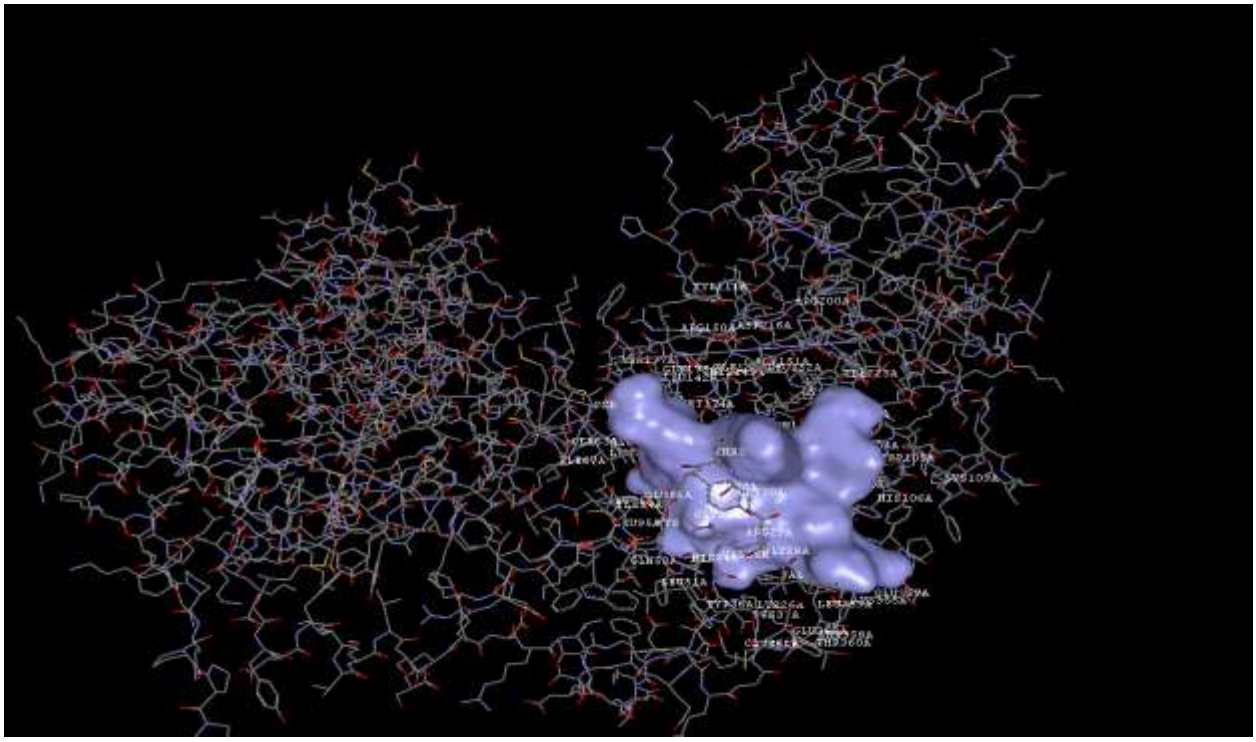


Fig2- 3D representation of Receptor-ligand interaction in cavity 1

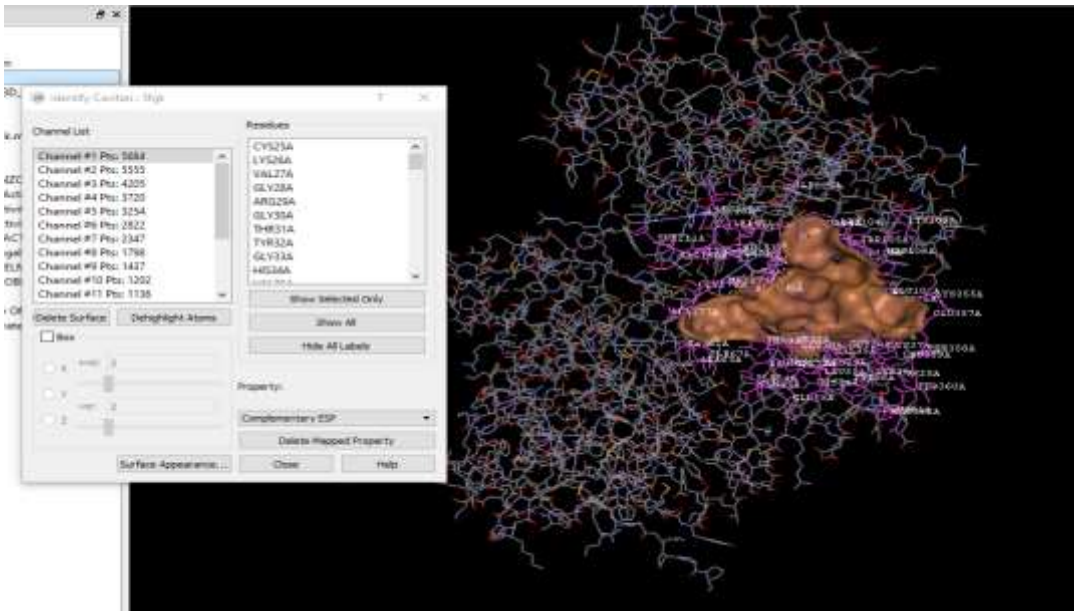


Fig3- 3D representation of docking score with cavity poses.

Table1: molecular docking results of benzimidazole derivatives for their anticancer activity.

Sr. No	Compound score	Docking score (Kcal/mol)
1.	D1	-42.0330
2.	D2	-41.504601
3.	D3	-39.5844
4.	D4	-46.57
5.	D5	-41.94
6.	D6	-51.30
7.	Bendamustin	-52.576277

3.3 Pharmacological 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 standard drugs i.e. cisplatin. 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	625.68
2.	D2	800.26
3.	D3	609.60
4.	D4	639.40
5.	D5	783.40
6.	D6	645.82
7.	Cisplatin	436.55

Positive control with DMSO has shown mortality of 1 shrimps.

No. of shrimp taken

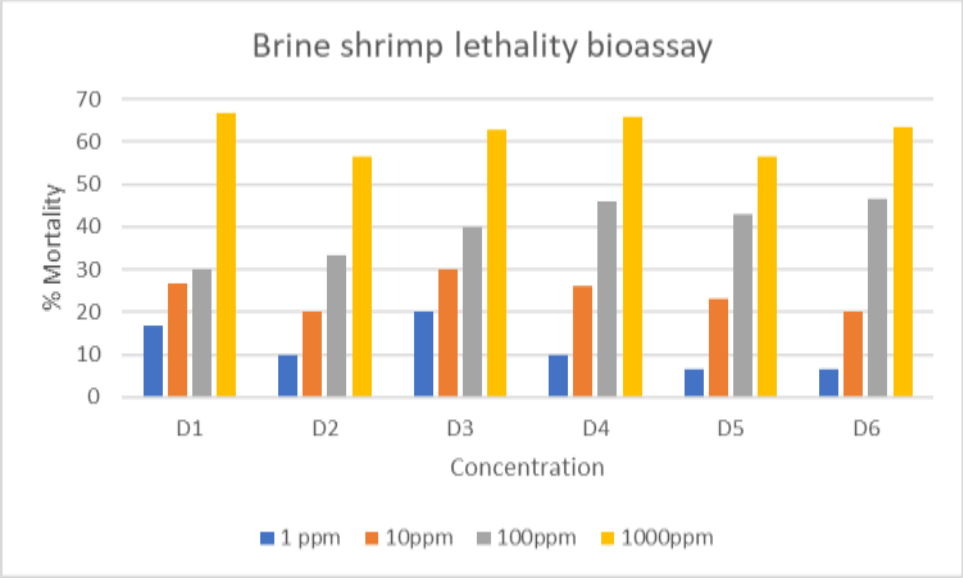


Figure 4: Graphical representation showing activity of derivatives with respect to cisplatin

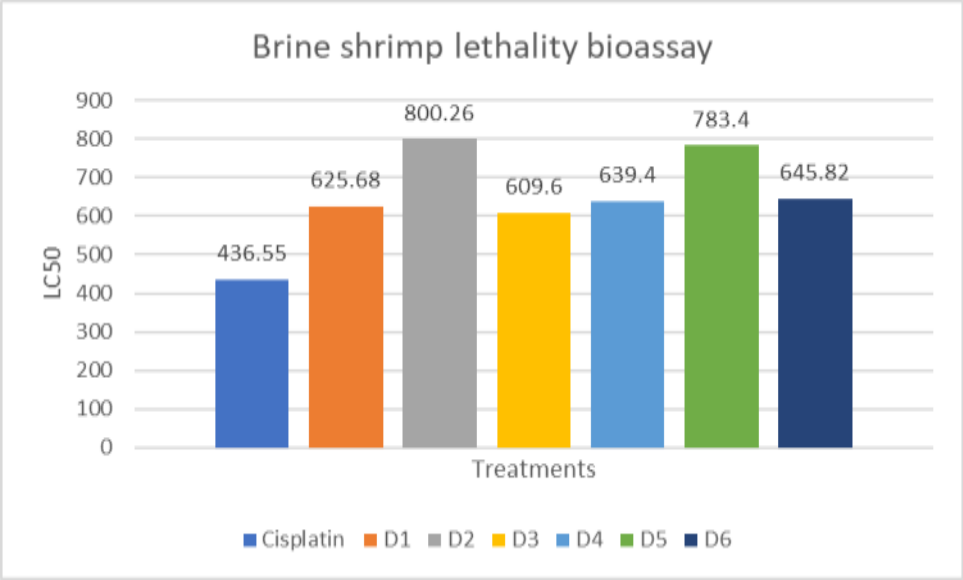


Figure 5: Graphical representation showing LC_{50} values of derivatives and cisplatin

4. 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-3 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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References

1. T. Gronning, (2015) History of Cancer, in Graham A, The SAGE Encyclopedia of cancer and Society, 2nd Edition, Thousand Oaks, CA: Sage Publications, pp, 49-Kumari M, Singh V. Breast cancer prediction system, Procedia computer science. 2018 Jan 1; 132:371-6.
2. D Yang, An B, W Wei, L Tian, B Huang, H Wang. *ACS Combinatorial Science*. 2015 Feb 9;17(2):113-9..
3. YK Yoon, MA Ali, AC Wei, AN Shirazi, K Parang, TS Choon. *Eur j of medicinal chemistry*. 2014 Aug 18;83:448-54.
4. H Debus, *Annalen der Chemie und Pharmacie.* 1858, 107(2), 199. https://doi.org/10.1002/jlac.18581070209
5. RG Ingle, DD Magar. *Int. J. Drug Res. Tech.* 2011;1(1):26-32.
6. M Correia, M Rodrigues, P Paiga, D. Delerue-Matos, Fungicides. in Encyclopedia of Food and Health. Academic Press, Cambridge, MA, 2016, p. 169. https://doi.org/10.1016/b978-0- 12-384947-2.00342-1
7. S. Tahlan, K Ramasamy, SM Lim, SA Shah, V Mani, B Narasimhan. *BMC chemistry.* 2019 Dec;13(1):1-6.
8. Mayhoub AS, Khaliq M, Botting C, Li Z, Kuhn RJ, Cushman M. *Bioorganic & medicinal chemistry*. 2011 Jun 15;19(12):3845-54.

9. K Starčević, M Kralj, K Ester, I Sabol, M Grce, K Pavelić, Karminski-Zamola G. *Bioorg & medi chemistry*. 2007 Jul 1;15(13):4419-26.

10. F Hoebrecker, Benzimidazole. Ber. 1872;5:920-6.

11. M Tonelli, M Simone, B Tasso, F Novelli, V Boido, F Sparatore, G Paglietti, S Pricl, G Giliberti, S Blois, C Ibba. *Bioorg & medil chemistry*. 2010 Apr 15;18(8):2937-53.

12. J.B Wright, 1951, *The chemistry of benzimidazoles*, chemical review, 48(3), pp, 397-541.

13. PN Preston, Benzimidazoles, Chemistry of Heterocyclic Compounds**:** Part 1. 1981 Jan 1; 40:1-285.

14. AY ChenA, Yu C, A Bodley, LF Peng, LF Liu. A new mammalian DNA topoisomerase I poison Hoechst 33342: cytotoxicity and drug resistance in human cell cultures. *Cancer research.* 1993 Mar 15;53(6):1332-7.

15. Rithe, RS Jagtap. and SS Ubharhande, 2015. *Rasayan J. Chem*, *8*, pp.213-217

16. JA Kumar, AK Tiwari, AZ Ali, K Madhusudhana, BS Reddy, S Ramakrishna,. *J of enz inhibition and medicinal chemistry*. 2010 Feb 1;25(1):80-6.

17. D Vásquez, CF Lagos, J Mella-Raipán, L González, R Ebensperger, MJ Alvarez-Figueroa, E Sáez, H Pessoa-Mahana, R Araya-Secchp, *J of the Chilean Chemical Society.* 2007;52(4):1281-7.

18. SK Gupta, N Kumar, D Pathak. *Indian drugs.* 2013 Jan;50(1):50-8.

19. RS Kankate, PS Gide, DP Belsare. *Arabian Journal of Chemistry*. 2019 Dec 1;12(8):2224-35.

20. M Bhosale, A Yadav, C Magdum, S Mohite.. *Int J Sci Res Sci & Technol.* 2020; 7 (5): 51. 2020 Sep;62.

21. HV Sanghani, SH Ganatra, R Pande. *J Comput Sci Syst Biol*. 2012;5(1):12-5.

22. M Bhosale, A Yadav, C Magdum, S Mohite. *Int. J. Curr. Adv. Res.* 2020; 09(08)(A): 22894-22899.

23. JL McLaughlin, LL Rogers, JE Anderson. *Drug information journal*. 1998 Apr;32(2):513-24.

24. S Nadeem, M Bolte, S Ahmad et.al., 2010.. *Inorganica Chimica Acta*, *363*(13), pp.3261-3269.