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INSILICO SCREENING OF POTENT ANTICANCER AGENTS FROM LIVER EXTRACT OF MARINE PUFFER FISH *AROTHRON HISPIDUS*

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ABSTRACT

Cancer is one of the leading causes of death in both developed and developing countries and is therefore of worldwide concern. The Prorein – Ligand interaction plays a significant role in structural based drug designing. In our research work the liver extract of Puffer fish *Arothron hispidus* showed anticancer activity against COLO 320 cell line was employed for docking studies with the target Bcl2 PDB ID: 4LVT. Ligands were created and prepared for the docking procedure using Chemsketch. The ligands Heptadecanoic acid, Tetradecanoic acid, Uric acid, Pentadecanoic acid, Oleic acid, Semioxamazide, Tetradecanamide and n-Hexadecanoic acid with scoring values 38.4, 42.77, 16.61, 39.74, 46.88, 14.88, 35. 23 and 44.07 respectively. The H-bond distance between protein 4LVT and the ligand Oleic acid was found to be 2.962. The ligand interacts with amino acid Arg 143 with high docking score value 46.88.

INTRODUCTION

Cancer is a complex disease that is characterized by aberrant cell division. It is caused by genetic variations and many environmental factors. It can invade vital organs and is a major harbinger of imminent patient death throughout the world. [1] The dead list part of the cancer process, metastasis appears to rely on the help from macrophages, potent immune system cells that usually defend vigorously against this disease. Metastatic tumour growth is inhibited if these unusual macrophages are killed. In addition, metastatic disease is the major cause of cancer mortality. [2] Hence development of new anticancer drugs represents today's one of the most important research areas. An analysis of the number of chemotherapeutic agents and their sources indicate over 74.8% of the approved drugs are derived from natural compounds. [3] Chemoprevention entails the use of specific natural dietary or synthetic agents to thwart cancer development and progression. [4] Many potent natural products have display the effective anticancer activities from the marine environment. Nature derived pharmaceuticals including marine-derived bioactive products have been draw a great deal of attention from both the scientific community and the general public due to their demonstrated ability to suppress cancers. [5] Several anticancer agents are derived from marine sources, which are entered preclinical and clinical trials to display cytotoxic activity

against various tumour types. [6] A special attention is pay to this disease is based upon the fact that cancer ruins one of the chief causes of death in developing countries causing around two million deaths annually. [7] Therefore, the investigation of alternative treatments for this disease is much needed. Tetrodotoxin (TTX) is one of the most potent marine neurotoxins, named after the order of fish where it is most commonly associated, the Tetraodontiformes. The Tetraodontidae family has been mainly distributed in tropical and subtropical areas of Indian and Pacific regional waters. There are 189 species of puffer fishes and 28 genera in the family Tetraodontidae. [8] This fish is known to carry tetrodotoxin (TTX) [9,10,11] which is known a non-protein organic compound (amino perhydroquinazoline) and one of the strongest marine paralytic toxins today. Tetrodotoxin is a low molecular weight with 319 small molecules with a unique cage structure. The basic molecule for TTX consists of a positively charged Guanidium group. The amount of TTX in the puffer fish is species specific and varies among different organs in different seasons. [12]

Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way of drugs are designed. Rational Drug Design (RDD) helps to facilitate and speed up the drug designing process, which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor.[13] Molecular docking is protein – ligand interaction study and is a particularly vibrant research area because of its importance to structure-based drug design. The field of molecular docking has emerged during the last three decades and now is becoming an integral aspect in drug discovery and development area.

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule.[14] Molecular docking can be thought of as a problem of "lock – and –key", where one is interested in finding the correct relative orientation of the "key" which will open up the "lock". Here, the protein can be thought of as the "lock" and the ligand can be thought of as a "key". However, since both the ligand and the protein are flexible, a "hand – in – glove" analogy is more appropriate than "lock and key".[15]

Scoring functions are fast approximate mathematical methods used to predict the strength of the non-covalent interaction between two molecules after they have been docked. Most commonly one of the molecules is a small organic compound such as a drug and the second is the drug's biological target such as a protein receptor.[16] Scoring functions have also been developed to predict the strength of other types of intermolecular interactions, for example between two proteins [17] or between protein and DNA.[18, 19] Charifson *et al*., [20] reported a method for obtaining improved hit rates from docking databases of three – dimensional structures in to proteins. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function was analysed by.[21] Taylor *et al*., [22] reported a review of protein – small molecule docking methods. Microwave assisted synthesis of some novel Benzimidazole substituted Fluoroquinalones and their antimicrobial evaluation was reported by.[23] Kahraman *et al*., [24] investigated the shape and variation in protein binding pockets and their ligands. Reproducing the conformations of protein bound ligands, a critical evaluation of several popular conformational searching tools was reported by.[25] Pan Wang and Bao – Ting Zhu [26] carried out an investigation on usefulness of Molecular Modelling Approach in Characterizing the Ligand – Binding Sites of Protein. The present study focused on the molecular docking studies of potent anticancer agents from liver extract of marine puffer fish A*rothron hispidus*.

MATERIALS AND METHODS

For our present study we used ACD/ Chem Sketch Freeware, version 11.[27]
2.1. GOLD - Protein- Ligand Docking.
2.2 Target Selection - The X-ray Crystal Structure of Bcl2 (PDBID: 4LVT) complexed with an inhibitor [28] was retrieved from protein Data Bank.[29]
2.3 Ligand selection.[30]

A total of 8 compounds were selected based on the review of literature. The selected compounds were obtained by drawing their 2D Structures in ACD – Chemsketch (Version 12) (ACD/ Chem Sketch Freeware.[27]

2.4 Binding site prediction.[31]
2.5 Virtual Screening.[32]
2.6 Docking interactions.[33]
2.7 Protein Setup
2.8 Selecting Ligands
2.9 Selecting a Fitness Function
2.10 Specifying GA Setting
2.11 Run the Docking

RESULTS

The crystal structure of the Bcl2 protein derived from PDB ID: 4LVT (Figure 1) was used as a target for docking simulations. Ligands were created and prepared for the docking procedure using Chemsketch. The ligands Heptadecanoic acid, Tetradecanoic acid, Uric acid, Pentadecanoic acid, Oleic acid, Semioxamazide, Tetradecanamide and n-Hexadecanoic acid with scoring values 38.4, 42.77, 16.61, 39.74, 46.88, 14.88, 35.23 and 44.07 respectively (Table 1). The H-bond distance between protein 4LVT and the ligand oleic acid was found to be 2.962. The ligand interact with amino acid Arg 143 with high docking score value 46.88. The structures of the ligand obtained from the Chemsketch are shown in Figure 2 – 9.

DISCUSSION

Cancer is spreading in the whole world and it takes away more than 6 million lives each year.[34] Among the fishes, puffer fish showed strong anticancer activity. Although TTX is one of the most dangerous marine natural products, its distinctive action mode draws great attention from researchers. Anticancer molecules isolated from marine organisms belong to diverse structural classes including polypeptides, terpenes, steroids and peptides. [35] A large number of studies confirm that almost all apoptosis stimulating factors can cause structural damage and mitochondrial dysfunction.[36] The identification of oncogenes involved in the initiation and progression of tumors has generated targets for the development of new anticancer drugs. Molecular Docking study helps to predict the intermolecular complex formed between two constituent molecules. It is an extensively used computational technique for the study of molecular identification. In our study, the bioactive compounds present in the liver extract of marine Puffer fish *A.hispidus* showed potent anticancer activity against COLO 320 cell line. Hence the anticancer activity of the compounds present in the liver extract was further supported by molecular docking studies. The target protein Bcl2 was obtained from PDB ID: 4LVT using Chemsketch. In the present investigation the ligand Oleic acid showed best ligand binding interaction with score value of 46.88. The findings of [37,14,38,39] lended support to our results. The results clearly indicate that the toxins present in the fish having bioactive compounds that may be used for therapeutic needs.

Melanny *et al*., [37] developed a potential anticancer drug by synthesizing some of *p*-methoxycinnamoyl hidrazides. The compounds were synthesized from the ethyl *p*-methoxycinnamate (EPMC), isolated from rhizome of *Kaemferia galanga*. The structures of the compounds were confirmed by UV-vis spectrophotometry, 1H-NMR, 13C-NMR, FT-IR, and MS spectroscopic methods. The study was followed by anticancer activity evaluation of the compounds by *insilico* study using Molegro® ver. 5.5 and by *in vitro* assay against human breast cancer cells (T47D) by 3-(4,5-Dimethylthiazol-2-yl)-2-5-Diphenyltetrazolium Bromide (MTT) method. The result showed that 3- (4-methoxyphenyl)- N - (3- (4-methoxyphenyl) acryloyl) acrylohydrazide has the highest value of rerank score (-124.81).

Sanghani *et al*.,[14] performed *insilico* screening of natural polytriterpene phytochemical that are thought to have potential to inhibit mutated IG11. Out of the two triterpenes bosewellic acid and ursolic acid, boswellic acid showed inhibition activity with IG11. Igwe *et al*., [38] performed molecular docking using Patchdock and firedock online docking server. Molecular docking result showed the global energies and their ranks. The global binding energy value – 26.85 kcal/ mol was ranked first because it had the least energy. The most feasible position for anastrozole to inhibit promyelocytic leukemia protein was found to be -26.85 kcal/ mol. Rather *et al*., [39] revealed molecular dynamic simulation. The results indicated that complex 2 (N-ethyl-50-carboxamido adenosine and kiss2r protein) is better than complex 1 (2-(4-(2-Carboxyethyl) phenethylamino)-50-N-ethylcarboxamidoadenosine and kiss2r protein) and its implication in drug designing. Agu *et al*.,[40] reported that molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. The results specified that molecular docking is a useful tool for identifying the molecular targets of nutraceuticals in the management of diseases. It may offer information about how nutraceuticals work and support the creation of new therapeutics**.**

CONCLUSION

Molecular docking was applied to explore the binding mechanism and to correlate its docking score with the activity of anticancer compounds. The protein ligand interaction plays a significant role in structural based drug designing. From the molecular docking study, it was concluded that, among the ligands, Oleic acid are probable lead molecules than the rest of the ligands for anticancer owing to their high score values. This infers that the lead molecule is one with maximum interaction having high score value. Thus the concept of protein - ligand interaction helps in designing new drugs for cancer.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Table 1 GOLD Score for anti-cancer activity with Bcl2 of Human

Ligand	Atom in Ligand	Atom in Protein	H-Bond Distance	Score
Heptadecanoic acid	O	Asn140	2.914	38.4
	O	Arg143	2.566, 2.430	

Tetradecanoic acid	O	Arg104	3.018	42.77
Uric acid	N	Phe101	2.998	16.61
Pentadecanoic acid	-	-	-	39.74
Oleic acid	O	Arg143	2.962	46.88
Semioxamazide	-	-	-	14.88
Tetradecanamide	-	-	-	35.23
n-Hexadecanoic acid	-	-	-	44.07

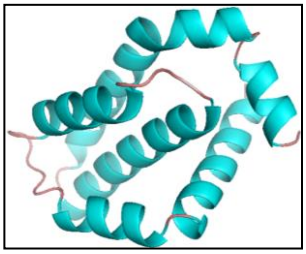

Fig 1

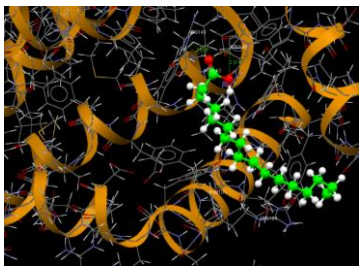

Fig 2

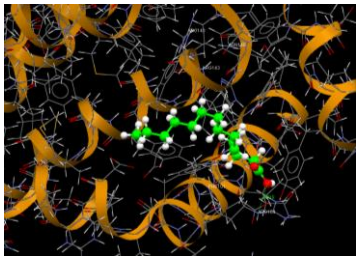

Fig 3

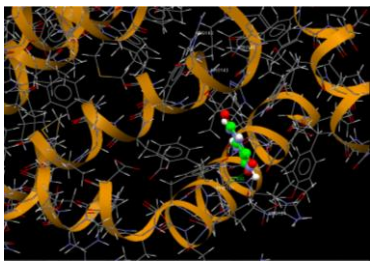

Fig 4

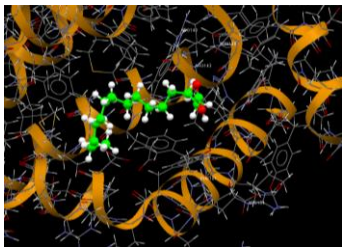

Fig 5

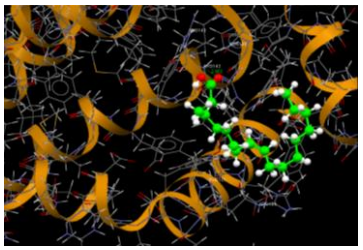

Fig 6

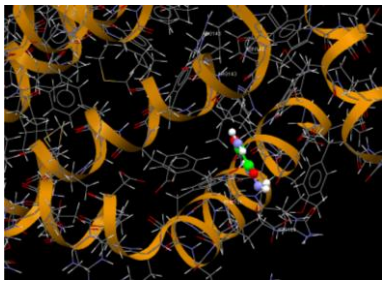

Fig 7

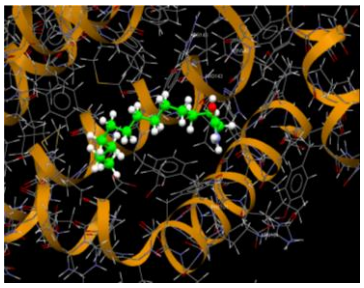

Fig 8

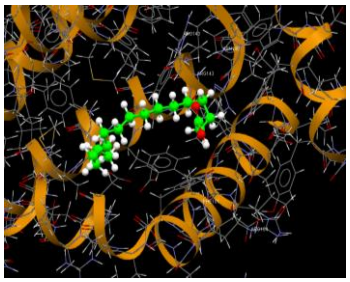

Fig 9

Fig 1 : Structure of Bcl2 (PDB ID: 4LVT)
Fig 2 : Interaction of Heptadecanoic acid with Bcl2
Fig 3 : Interaction of Tetradecanoic acid with Bcl2
Fig 4 : Interaction of Uric acid with Bcl2
Fig 5 : Interaction of Pentadecanoic acid with Bcl2
Fig 6 : Interaction of Oleic acid with Bcl2
Fig 7 : Interaction of Semioxamazide with Bcl2
Fig 8 : Interaction of Tetradecanamide with Bcl2
Fig 9 : Interaction of n-Hexadecanoic acid with Bcl2

REFERENCES

1. Dikmen M, Ozturk N, Ozturk Y. The Antioxidant Potency of *Punica granatum L*. Fruit Peel Reduces Cell Proliferation and Induces Apoptosis on Breast Cancer. Journal of Medicinal Food. 2011;14:1638–46. doi: 10.1089/jmf.2011.0062. *[DOI] [PubMed] [Google Scholar]*
2. Pollard JW. Metastatic Cancer and Macrophages: Cells Thought To Protect Against Cancer May Actually Promote It', Albert Einstein College of Medicine of Yeshiva University report, Ph.D. Thesis Yeshiva University 2009.
3. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of Natural Products 2012; 75: 311- 335.
4. Kong CS, Kim JA, Yoon NY, Kim SK. Induction of apoptosis by phloroglucinol derivative from Ecklonia Cava in MCF-7 human breast cancer cells. Food and Chemical Toxicology 2009; 47: 1653 - 1658.
5. Lordan S, Ross R P, Stanton C. Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic diseases. Marine Drugs 2011; 9:1056-1100.
6. da Rocha AB, Lopes RM, Schwartsmann G. Natural products in anticancer therapy. Current Opinion in Pharmacology 2001; 1: 364 - 369.
7. WHO. The World Health Report - Health systems: improving performance, World Health Organization. Geneva 2006.
8. Oliveira JS, Fernandes SCR, Schwartz CA, Bigues Pires JC, de Freitas O. Toxicity and toxin identification in *Colomesus asellus*, an Amazonian (Brazil) fresh water puffer fish. Toxicon 2006; 48: 55- 63.
9. Bilecenoglu M, Kaya M, Akalin S. Range expansion of silver stripe blassop, *Lagocephalus sceleratus*, (Gemelin, 1789) to the Northern Agean Sea. Aquat. Invasions 2006; 1: 289 - 291.
10. Kasapidis, Peristeraki, Tserpes, Magoulas. First record of the Lessepsian migrant *Lagocephalus sceleratus*, (Gmelin, 1789), (Osteichthyes: Tetraodontidae) in the Cretan Sea (Aegean, Greece). Aquatic Invasions 2007; 2: 71-73.
11. Sabrah MM, El-Ganainy A, Zaky MA. Biology and toxicity of the puffer fish *Lagocephalus sceleratus* (Ameline 1789) from the Gulf of Suez, Egyptian. Journal Aquaculture Research 2006;32: 283-297
12. Yu CF, Yu P H. The annual toxicological profiles of two common puffer fish, Takifugu niphobles (Jordan and Snyder) and Takifugu alboplumbeus (Richardson), collected along Hong Kong coastal waters. Toxicon, 2002; 40: 313-316.
13. Richon,AB. An Introduction to Molecular Modelling. <http://www.netsci.org/Science/Compchem/feature01.html> (accessed on 5 October 2009). Originally appeared in Mathematech 1994; 1: 83.
14. Sanghani HV, Ganatra SH, Pande R. Molecular Docking Studies of Potent Anticancer Agent. Journal of Computer Science Systems Biology2012; 5:12 -15.
15. Jorgensen WL. Rusting of the lock and key model for protein ligand binding Science 1991; 254: 954 - 955.
16. Jain, AN: Scoring functions for protein – ligand docking. Current Protein and Peptide Science 2006; 7: 407- 420.
17. Lensink MF, Mendez R, Wodak SJ. Docking and scoring protein complexes: CAPRI 3rd Edition', Proteins Structure Function and Bioinformatics 2007; 69: 704 -718.
18. Robertson TA, Varani G. An all-atom distance-depentent scoring function for the prediction of protein-DNA interactions from structure. Proteins: Structure, Function and Bioinformatics 2007; 66: 353 - 374.

19. http://en.wikipedia.org/wiki/scoring functions for docking Scoring function for docking 2011.
20. Charifson PS, Corkery JJ, Murcko MA, Walters WP. Consensus Acoring: A Method for Obtaining Improved Hit Rates from Docking Databases of Three-dimensional Structures into Proteins. Journal of Med Chem. 1999; 42: 5100 - 5109.
21. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. *Antitumour* and *cytotoxic compounds.* Journal of Computational Chemistry 1998; 19:1639-1662.
22. Taylor RD, Jewsbury PJ, Essexl JW. A review of protein – small molecule docking methods. Journal of Computer – Aided Molecular Design 2002; 16: 151-166.
23. Jubie S, Rajeshkumar R, Yellareddy B, Siddartha G, Sandeep N, Surendrareddy K, Dushyanth HS, Elango K. Microwave assisted synthesis of some novel benzimidazole substituted Fluoroquinalones and their antimicrobial evaluation. Journal of Pharmacological Science and Research 2010; 2: 69-76.
24. Kahraman A, Morris R J, Laskowski RA, Thornton, JM. Shape variation in protein binding pockets and their ligands. Journal of molecular Biology 2007; 368: 283- 301.
25. Bostrom J. Reproducing the conformations of protein-bound ligands: a critical evaluation of several popular conformational searching tools. Journal of Computer Aided Molecules 2001; 15:1137-1152.
26. Pan Wang & Bao-Ting Zhu. Usefulness of Molecular Modelling Approach in Characterizing the Ligand-Binding Sites of Proteins: Experience with Human PDI, PDIp and COX. Current Medical Chemistry 2013; 20: 1-15.
27. ACD/ChemSketch Freeware. Advanced Chemistry Development Inc, Toronto, ON, Canada. version 11; 2006. www.acdlabs.com.
28. Chamberlain S, AtkinsC, Deanda F, Dumble M, Groy A, Korenchuk S, Kumar R, Lei H, Mook R, Moorthy G, Redman A, Rowland J, Sabbatini P, Shewchuk L. Discovery of 4, 6-bis-anilino-1H-pyrrolo[2,3-d] pyrimidines: potent inhibitors of the IGF-1R receptor tyrosine kinase. Bioorg Med Chem. 2008; 19: 469 - 473.
29. <http://www.rcsb.org/pubmed>
30. Weininger D. SMILES, a Chemical Language and Information System. 1. Introduction to Methodology and Encoding Rules. Journal of Chemical Information and Computer Science 1988; 28: 31-36.
31. Rarey M, Kramer B, Lengauer T, Klebe G. A fast flexible docking method using an incremental construction algorithm. Journal of Molecular Biology 1996; 261: 470- 489.
32. Wolf LK. Digital briefs: New software and websites for the chemical enterprise. C & EN2009; 87: 32.
33. Stierand K, Maass PC, Rarey M. Molecular Complexes at a Glance: Automated Generation of two-dimensional Complex Diagrams. Bioinformatics 2006; 22: 1710 - 1716.
34. Abdullaev F1, Rivera - Luna R, Roitenburd – Belacortu V. Espinosa - Aguirre J. Pattern of Childhood Cancer Mortility in Mexico. Archives of Medical Res 2000; 31: 526 - 531.
35. Mayer AM and Gustafson KR. Marine pharmacology in 2001-2: Antitumour and Cytotoxic compounds. European Journal of Cancer 2005; 40: 2676 - 2704.
36. Marchetti B, Gallo F, Farinella Z, Romeo C, Morale MC. Luteinizing hormone-releasing hormone (LHRH) receptors in the neuroendocrine-immune network: Biochemical bases and implications for reproductive physiopathology Basis for Literature review in Annals of the New York Academy of Sciences 1996; 784: 209 - 236.
37. Melanny Ika Sulistyowaty, Nugroho AE, Putra GS, Ekowati J, Budiati T. Synthesis, Molecular Docking Study and Anticancer Activity Examination of p-

Methoxycinnamoyl Hydrazides. International Journal of Pharmaceutical and Clinical Research 2016; 8: 623- 627.

38. Igwe Kalu Kalu, Amaku Friday James, Otuokere Ifeanyi Edozie. Theoretical approach on the structural aspects of 2, 2' – [5-(1,2, 4-triazolidin-1-ylmethyl) benzene- 1,3-diyl] bis (2- methylpropaneitile) (Anastrozole) and its molecular docking studies with promyelocytic leukemia protein. International Journal of Innovative Science, Engineering and Technology 2015; 2: 324 - 331
39. Rather M A, Subhajit Dutta, Praveen Kumar Guttula, Bhushan C.Dhandare S. I, Yusufzai, Mehar Imran Zafar. Structural analysis, molecular docking and molecular dynamics simulations of G-proteincoupled receptor (kisspeptin) in fish. Journal of Biomolecular Structure and Dynamics 2019; 29:1-18.
40. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, Ugwuja EI, Aja PM. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. National Library of medicine. Pub Med 2023;13(1)13398. Doi10.1038/s415598 -023 – 40160-2.