Asian Journal of Biotechnology and Genetic Engineering

Volume 7, Issue 1, Page 1-7, 2024; Article no.AJBGE.110809

Computational Exploration of Adenylyl Cyclase Type 2 Inhibition by Oleandrin Glycosides via Molecular Docking Studies

Syed Aun Muhammad [a*], Syeda Tahira Qousain Naqvi [a], Tahir Naqqash [a] and Sadaf Noor [a]

[a] Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University Multan, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/110809

Original Research Article

Received: 15/10/2023
Accepted: 22/12/2023
Published: 04/01/2024

ABSTRACT

Adenylyl cyclase type 2 (ADCY2) is an enzyme linked to membranes that is implicated in the spread of many cancer types and tumors. As such, it may be a target for therapeutic intervention. Using molecular docking, we examined how these compounds bound to ADCY2. Cancer is treated with chemotherapy drugs; however, these drugs frequently have serious side effects. The current study aims to computationally assess the anticancer potential of natural glycosides, such as oleandrin. We used Phyre to evaluate the quality of the protein model after modeling the structure of adenylyl cyclase. For the binding affinity analysis, the Molecular Operating Environment (MOE) program was utilized. The ligand used to dock with the ADCY2 molecule was oleandrin. Oleandrin exhibited a strong binding affinity towards the ADCY2 target molecule, as demonstrated by its binding energy of -97.7513 kcal/mol. The anticancer potential of oleandrin is indicated by its strong binding affinity for the adenylyl cyclase protein target. The findings of this study suggest that oleandrin may have been

**Corresponding author: E-mail: aunmuhammad78@yahoo.com;*

used in cancer treatment as an ADCY2 inhibitor. This research would contribute to the development of novel anti-cancer drugs, expanding the range of available cancer treatments.

Keywords: ADCY2; lead compounds; oleandrin; anticancer molecules; computational analysis.

1. INTRODUCTION

The "cancer" describes aberrant cell division that results in alterations at the cellular and epigenetic levels. Multiple genetic alterations, such as point mutation, deletion, and translocation, are necessary for the expression of the cancer phenotype [1-3]. Carcinogenesis may arise from the inactivation of tumor suppressor genes that guard against malignant phenotype. Located on chromosome 5, ADCY2 belongs to the class-B family of adenylyl cyclase and is a potential cancer candidate gene [4]. Prostate and colorectal cancers are caused by abnormal ADCY2 DNA methylation. Since ADCY2 levels are upregulated in a variety of cancer types, inhibiting ADCY2 has been shown to produce therapeutic effects and may be a viable therapeutic target for the treatment of cancer [5-8]. This gene is not sensitive to Ca_2^+, and in the case of colorectal cancer, there is low gene expression in the calcium signaling pathways due to hypermethylation [4]. DNA hypomethylation can have short-term anticancer effects, but in cancer cells that have survived demethylation chemotherapy, it may accelerate tumor progression; therefore, a chemical inhibitor is needed to counteract the activity [6,9].

Traditional medicine has utilized oleandrin, a cardiac glycoside present in oleander plants, for its purported therapeutic benefits, including the treatment of cardiac insufficiency. It is extensively used in the treatment of many illnesses, including congestive heart failure. Oleandrin's potent antiviral and broad anticancer properties have garnered a lot of attention lately [10,11]. Clinical evidence supporting the safety or efficacy of oleander or any of its constituents, including oleandrin, is nonexistent. Due to its characteristics as a cardiac glycoside, oleandrin obstructs many vital cellular functions, the most significant of which is the inhibition of Na-K ATPase. Because oleandrin is known to be toxic, dosage and patient conditions may be taken into consideration when administering the molecule. However, oleandrin has a limited window of therapeutic efficacy and has several toxicities, particularly typical cardiotoxicity, which is frequently fatal. However, considering potential research strategies to reduce toxicity, the development of safe clinical applications of oleandrin might be feasible [12,13].

As a result, the progress of chemotherapeutic drugs to treat cancer is a drawn-out process due to high rates of mutation, an unclear genetic basis, and costly techniques. Therefore, through epigenetic research, particularly focusing on natural sources, new drug development strategies are needed to find new therapeutic drugs for the treatment of cancer. Compared to traditional drug discovery through ligand selection, in silico investigation is a more cost-effective and efficient method of designing targeted structural drugs [14,15]. These methods have evolved into an essential part of drug discovery and development research [16,17]. The projection of ADCY2 inhibitors is based on minimum binding energy and optimized binding affinity. Natural molecules called oleandrin have the ability to function as medicinal agents. The purpose of this analysis is to look into oleandrin's ability to inhibit ADCY2 and avert cancer.

2. MATERIALS AND METHODS

Target Protein Accession: The Phyre tool was used to model the three-dimensional structure of adenylyl cyclase type 2 (ADCY2) as described previously [18].

Ligand Selection: The chemical database PubChem was used to obtain the chemical structure of oleandrin. A two-dimensional structure was prepared using ChemBioDraw, and the MOE program was uploaded with the ligand's SMILE format.

Ligand and Target Optimization: Drug Discovery Studio version 3.0 was used to optimize the PDB coordinates of the target protein and ligand molecules by energy minimization, 3D protonation, and stable conformation parameters for docking analysis.

Analysis of target active binding sites: The MOE tools were used to analyze the target protein's active binding sites, which are the ligand's coordinates in the original target protein grids.

Molecular Docking Analysis: In order to comprehend the structural underpinnings of this protein target specificity, structural complexes of the ADCY2 target with particular compounds (ligands) were analyzed using a computational ligand-target docking approach [19]. Ultimately, MOE software handled the docking process. "Grid point" refers to the energy of ligand-target enzyme interaction. Atomic affinity potentials calculated on a grid were used to assess the energy of interaction between the ligand and protein at each stage of the simulation. The default setting is applied to the remaining parameters.

3. RESULTS

Molecular docking is widely 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 molecules. It also provides valuable information about drug-receptor interactions. Consequently, a deeper comprehension of the drug-receptor interaction was made possible by molecular docking studies of physiologically active molecules [20]. To investigate the structural interaction with the ADCY2 target molecule, oleandrin (Fig. 1) was utilized. The target enzyme, the cyclase protein, was successfully docked with ligand molecules, as indicated by the minimum binding energy (Table 1).

3.1 Protein Model Analysis

Forty percent of our protein models could be modeled with 70% confidence using multiple templates. Approximately 90% of the amino acid residues in the predicted model are located in the preferred region, while only 2% or fewer are found in the prohibited region, as per the Ramachandran plot analysis. This analysis also reveals the lower energy conformations for psi and phi, even though the target protein's backbone confirmation is shown graphically (Fig. 2).

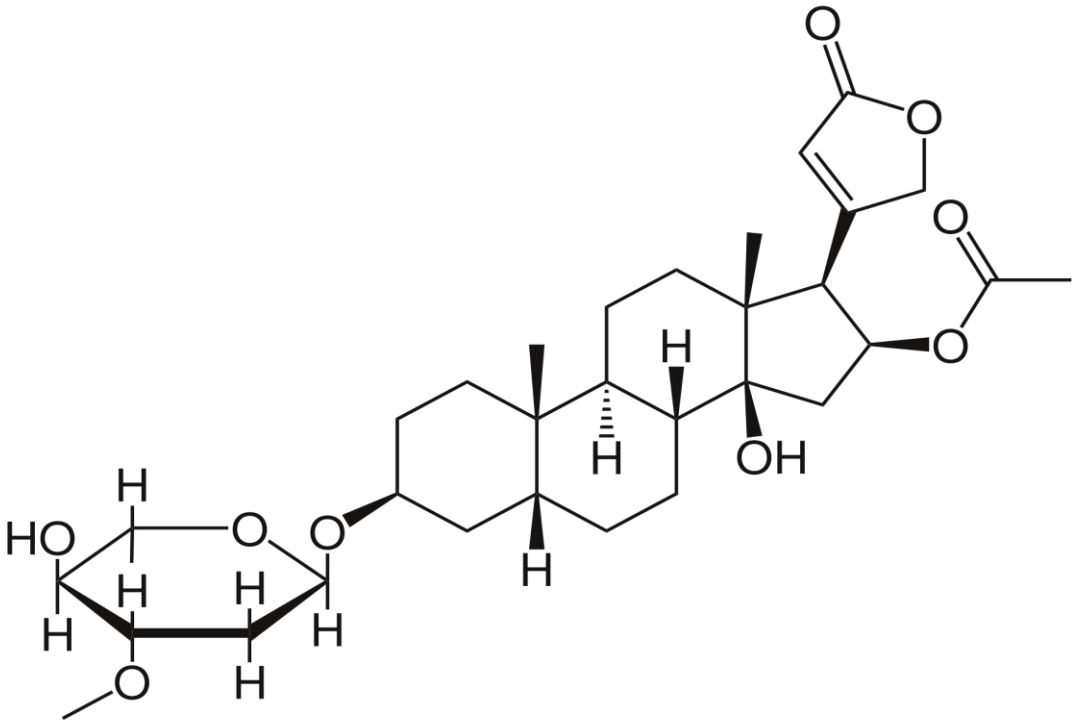

Fig. 1. 2-Dimensional structure of oleandrin molecule

Table 1. Molecular interaction and binding affinity analysis of oleandrin with adenylyl cyclase protein indicate significant binding energies

S. No.	RSEQ	MSEQ	S	MSD_REFINE	E_CONF	E_PLACE	E_SCORE1	E_REFINE	E_SCORE2
1	1	1	-97.75	6.7822	122.2464	3.7020	-14.7432	-840.6664	97.75
2	1	1	-96.78	1.4824	144.5431	-76.9624	-13.3140	-1549.398	-96.78
3	1	1	-76.55	2.7745	129.4949	-89.4457	-13.4239	-1126.785	-76.55
4	1	1	-73.45	3.3255	158.4738	-57.8128	-12.7721	-1193.312	-73.45
5	1	1	-66.55	3.0177	136.5870	28.2565	-13.0568	-1205.925	-66.55
6	1	1	-62.18	3.1998	150.4120	-41.5129	-14.9953	-1244.526	-62.18
7	1	1	-60.45	2.7413	125.6251	-65.3608	-12.2720	-632.8759	-60.45
8	1	1	-56.81	1.9094	135.7356	-77.8389	-12.7636	-686.370	-56.81
9	1	1	-21.64	1.4128	132.5431	-60.6173	-12.4701	-910.455	-21.64
10	1	1	-19.72	6.0641	133.4191	-72.1392	-13.9765	-777.8820	-19.72

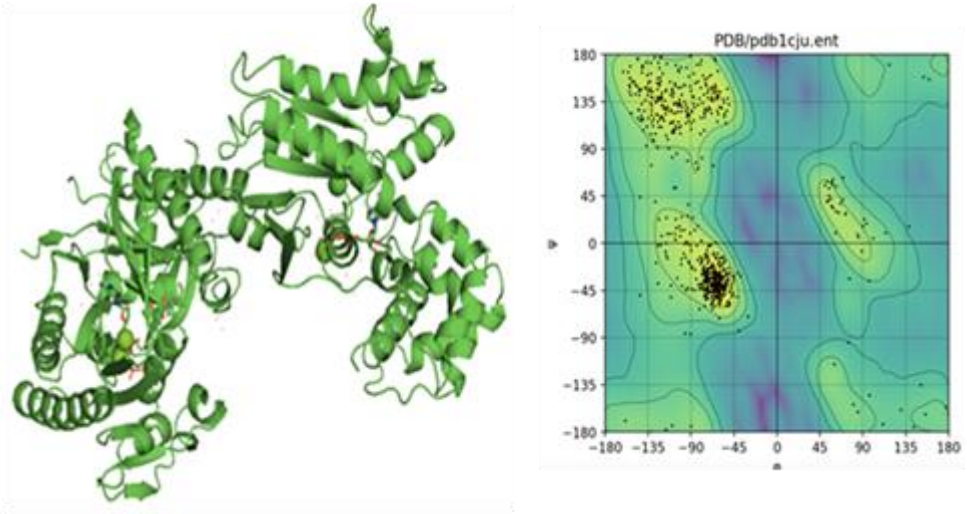

Fig. 2. The model of the protein adenylyl cyclase's conformation and helical structure. Drug Discovery Studio version 3.0's Ramachandran plot troponin verified the quality of the protein model by showing that the amino acid residues are located in the "favored region" of the plot

3.2 Analysis of Active Site

The active site of the adenylyl cyclase protein can be analyzed to determine its likely locations. Fig.3 depicts potential ligand binding mechanisms at aromatase active sites. Residues like VAL, ARG, PHE, MET, SER, TYR, ASN, GLU, PRO, LYS, GLN, ILE, and ALA of cyclase proteins formed an H-bond with ligand molecules (Fig. 3).

3.3 Docking Analysis

MOE docking results indicated that lower energy complexes are stabilized by hydrogen bonds, stacking interactions, or intermolecular interactions. The molecular docking and drug-target matching are ranked using these stacking interactions. A calculation of the molecular hydrophobicity potential was made taking into account the hydrophobic characteristics of these interacting molecules (the target and ligands of the aromatase protein) [14]. The cyclase target's lowest energy complexes, as predicted by computation, are all stabilized by stacking interactions and intermolecular hydrogen bonds, according to the docking procedure used to dock the ADCY2 target with oleandrin. The ligand atoms involved in docking with the enzyme are determined to be A, SA, OA, HD, and N. Comparing oleandrin to other ligands, it demonstrated a comparatively good binding affinity of -97.7 kcal/mol. The binding mode of oleandrin to cyclase that is most energetically favorable was presented by the AutoGrid model. After docking the ligand, oleandrin, into the created combined grids, evaluating the binding energy and RMSD from the native pose, it is found that the weight-averaged grids perform the best. Based on the RMSD values, the ligand exhibited the best interaction with the target proteins. These ligand molecules can precisely interact with the adenylyl protein target, according to docking results (Fig. 3).

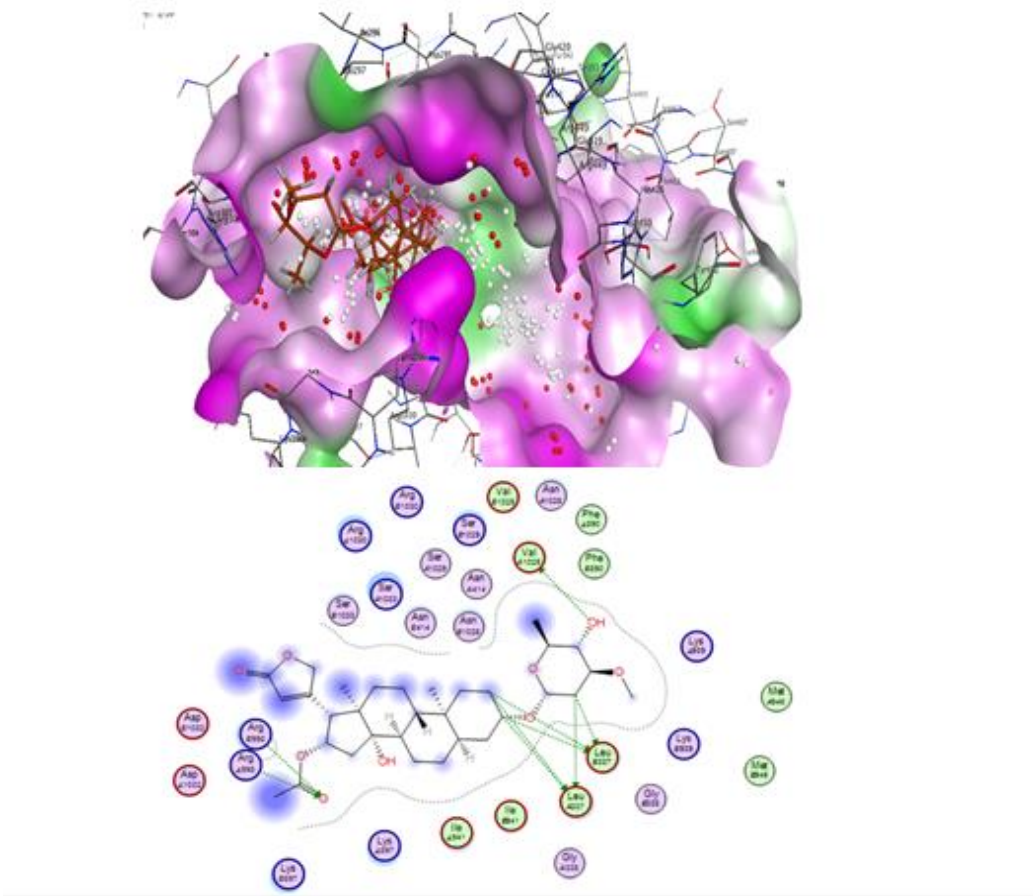

Fig. 3. Molecular docking and binding interaction of oleandrin with target protein

4. DISCUSSION

This work involved the docking analysis of the human ADCY2 molecule with an oleandrin glycoside molecule. It has been shown in earlier research that ADCY2 expression is dysregulated in a variety of cancer types, such as colorectal, bladder, oral, small cell lung, and breast carcinomas [21-24], which makes this enzyme a viable target for treatment. Owing to the lack of therapies for ADCY2 at the metastatic stage and its proliferation, the research makes important discoveries by investigating novel cytotoxic and chemotherapeutic agents and focusing on novel approaches to inhibit or stop ADCY2 activity. Previously, triazole compounds were explored as ADCY2 inhibitors via molecular docking analysis. The binding energy for two triazole compounds were found to be -4.4328 and -5.2686 kcal/mol. Which indicated the anticancer potential of such compounds by inhibiting ADCY2 [25]. However, natural compounds are more advantageous as compared to chemical compounds as they naturally contains several phytochemicals which can act effective anticancer agents. Exploration of natural molecules may lead to the development of anti-cancer agents. Because of its pharmacological properties, oleandrin is regarded as a significant active ingredient with antimicrobial and anti-cancer properties. As a result, the bioactivity of these substances is well known [26]. The primary source of oleandrin, a highly fat-soluble CG with a wide range of pharmacological effects, is N. oleander. In folk medicine, oleandrin is frequently used to treat a variety of illnesses, such as congestive heart failure, abscesses, asthma, dysmenorrhea, sores, eczema, epilepsy, herpes, leprosy, malaria, ringworm, scabies, indigestion, strokes, and neurodegenerative diseases. Oleandrin has both medical and toxicological significance [11,27]. However, oleandrin is extremely toxic to many organs, particularly the heart, and has a limited window of therapeutic use. Future studies must devise strategies to lessen oleandrin's toxicity based on its physicochemical

attributes, pharmacokinetic traits, and toxicological mechanisms to increase the drug's therapeutic use. Subsequent clinical trials ought to consider oleandrin formulations in addition to dosage. Enhancing the water solubility and looking for attenuation programs are essential steps in enhancing oleandrin's therapeutic potential. A comprehensive reference for the growth of oleandrin's clinical applications is the review of its pharmacokinetic behavior and toxicity. As the active component of multiple medications, oleandrin has been approved and used in Phase I and Phase II clinical trials to date. To achieve optimal therapeutic efficacy with minimal toxicity in clinical practice, it may become necessary and feasible in the future to require personalized medicine-directed use of oleandrin for drug safety [11,28].

These naturally occurring glycosides have been shown to be an effective cancer treatment due to their association with ADCY2 in cancer development and proliferation inhibition [29,30]. Thus, the present study employs in silico analysis to explore the inhibition of ADCY2 by oleandrin to control cancer development. The results indicated effective binding energies i.e., -97.7 kcal/mol of the ligand and target protein confirmed by the in-silico ligand-binding affinity for the target protein from ADCY2. As a result, the strong binding affinity of oleandrin with ADCY2 demonstrated their effective anti-cancerous properties. Further studies should be carried out to validate the effectiveness of oleandrin.

5. CONCLUSION

The remarkable method of in-silico screening and docking analysis of oleandrin glycoside molecules as drug ligands aids in the understanding of protein-ligand affinity. One potential anticancer treatment strategy is oleandrin's ability to inhibit the enzyme adenylyl cyclase type-2. The intermolecular interaction that can be explored for future experimental use stabilizes the energy values of these glycosides and target protein complexes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shillitoe E., et al. Genome-wide analysis of oral cancer—early results from the cancer genome anatomy project. Oral Oncology. 2000;36(1):8-16.
2. Bird A. DNA methylation patterns and epigenetic memory. Genes & Development. 2002;16(1):6-21.
3. Baylin SB, Jones PA. A decade of exploring the cancer epigenome—biological and translational implications. Nature Reviews Cancer,. 2011;11(10):726-734.
4. Li Y., et al. Molecular cloning, sequence identification, and gene expression analysis of bovine ADCY2 gene. Molecular Biology Reports. 2014. 41:3561-3568.
5. Herman JG., et al. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proceedings of the National Academy of Sciences. 1994;91(21): 9700-9704.
6. Herman JG., et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proceedings of the National Academy of Sciences. 1998;95(12):6870-6875.
7. Catto JW., et al. Promoter hypermethylation is associated with tumor location, stage, and subsequent progression in transitional cell carcinoma. Journal of Clinical Oncology, 2005;23(13):2903-2910.
8. Zheng M., et al. TRIO amplification and abundant mRNA expression is associated with invasive tumor growth and rapid tumor cell proliferation in urinary bladder cancer. The American Journal of Pathology. 2004;165(1):63-69.
9. Pfeifer GP. Defining driver DNA methylation changes in human cancer. International Journal of Molecular Sciences. 2018;19(4):1166.
10. Newman RA., et al. Antiviral effects of oleandrin. Journal of Experimental Pharmacology. 2020:503-515.
11. Zhai J., et al. Oleandrin: a systematic review of its natural sources, structural properties, detection methods, pharmacokinetics and toxicology. Frontiers in Pharmacology. 2022;13:822726.
12. Kanwal N., et al. Oleandrin: A bioactive phytochemical and potential cancer killer via multiple cellular signaling pathways. Food and Chemical Toxicology. 2020; 143:111570.

13. Langford SD, Boor P.J. Oleander toxicity: an examination of human and animal toxic exposures. Toxicology. 1996;109(1):1-13.
14. Aqeel MT., et al. In silico approach for the development of phenolic derivatives as potential anti-angiogenic agents against lysyl oxidase-like 2 enzyme. Future Journal of Pharmaceutical Sciences. 2022;8(1):1-11.
15. Kolla L., et al. The case for AI-driven cancer clinical trials–The efficacy arm in silico. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer.2021;1876(1): 188572.
16. Bajorath J. Integration of virtual and high-throughput screening. Nature Reviews Drug Discovery. 2002;1(11):882-894.
17. Varela-Rial AM, Majewski, G. De Fabritiis, Structure based virtual screening: Fast and slow. Wiley Interdisciplinary Reviews: Computational Molecular Science. 2022;12(2):e1544.
18. Kelley LA., et al. The Phyre2 web portal for protein modeling, prediction and analysis. Nature Protocols. 2015;10(6): 845-858.
19. Muhammad SA., et al. Insilico study of anti-carcinogenic lysyl oxidase-like 2 inhibitors. Computational Biology and Chemistry. 2014;51:71-82.
20. Muhammed MT, Aki-Yalcin E. Molecular docking: principles, advances, and its applications in drug discovery. Lett Drug Des Discov. 2022;19.
21. Abbas SZ, Qadir MI, Muhammad SA. Systems-level differential gene expression analysis reveals new genetic variants of oral cancer. Scientific Reports. 2020;10(1): 14667.
22. Chen SL., et al. Prognosis and regulation of an adenylyl cyclase network in acute myeloid leukemia. Aging (Albany NY). 2020;12(12):11864.
23. Yang J., et al., Identification of adenylate cyclase 2 methylation in bladder cancer with implications for prognosis and immunosuppressive microenvironment. Frontiers in Oncology. 2022;12: 1025195.
24. Zhao G., et al., Estrogen promotes cAMP production in mesenchymal stem cells by regulating ADCY2. International journal of stem cells. 2020;13(1):55-64.
25. Abbas SZ., et al., In silico Study of Adenylyl Cyclase Type 2 Inhibitors. GSJ. 2021;9(4).
26. Zaid H., et al., Physicochemical properties of natural based products versus synthetic chemicals. The Open Nutraceuticals Journal. 2010;3(1).
27. Francischini CRD., et al., Antitumor effects of oleandrin in different types of cancers: Systematic review. Toxicon. 2022;216:15-27.
28. Ni D., et al., Murine pharmacokinetics and metabolism of oleandrin, a cytotoxic component of Nerium oleander. Journal of Experimental Therapeutics and Oncology. 2002;2(5):278-285.
29. Majolo F., et al., Medicinal plants and bioactive natural compounds for cancer treatment: Important advances for drug discovery. Phytochemistry Letters. 2019;31:196-207.
30. Frye M., et al., Genomic gain of 5p15 leads to over-expression of Misu (NSUN2) in breast cancer. Cancer Letters 2010; 289(1):71-80.

© 2024 Muhammad et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/110809