STRUCTURE-BASED VIRTUAL SCREENING AND MM-GBSA CALCULATION TO PREDICT POTENT INHIBITORS FROM NATURAL PRODUCTS FOR DNMT-ASSOCIATED CANCER

R. Dushanan[1], S. Weerasinghe[2], D. P. Dissanayake[2], R. Senthilnithy[1*]

[1]Department of Chemistry, The Open University of Sri Lanka, Sri Lanka
[2]Department of Chemistry, University of Colombo, Sri Lanka

INTRODUCTION

DNA methylation is one of the most stable epigenetic marks in human beings (Reik, 2007). Generally, DNA methylation occurs at the 5' of the cytosine base, mainly at the CpG (cytosine-phosphate-guanine) dinucleotide environment. The C5-DNA methyltransferases (DNMTs) are the key factors that catalyze the methylation process in DNA (Jurkowska et al., 2011). The CpG dinucleotides are primarily situated in CpG islands and on CpG island shores (Weber et al., 2007). If the promoter CpG island is methylated, then the gene responsible is suppressed by transcription factors (Jones et al., 1998). DNA methylation is essential for maintaining chromosomal stability and protecting from mutations (Jurkowska et al., 2011). Accordingly, failure to maintain the DNA methylation and the formation of abnormal DNA methylations accompany the over-expression of specific proteins or enzymes, eventually leading to cancer (Sharma et al., 2009). However, unlike genetic mutations, epigenetic modifications can reverse (Issa & Kantarjian, 2009).

Many recent studies have proven that inhibition of DNMT significantly contributes to cancer growth control. Therefore DNMT inhibitors have been considered promising anticancer agents. Also, research in the inhibition of DNMT is a rapidly growing and up-and-coming area for cancer chemotherapy (Issa & Kantarjian, 2009). This work is focused on studying the binding affinity of some selected natural products obtained from the Sri Lanka Flora database with the DNMT enzyme through computational techniques. Also, this study investigates the atomic level description of inhibitor binding sites of the DNMT enzyme and calculates the relative docking score of the compounds with DNMT enzymes. These outcomes will explore how these natural products change the active site of the DNMT enzyme and their interaction strength with the DNMT enzyme. The result of this study could be used to discover new inhibitors for clinical research.

METHODOLOGY

Database preparation and virtual screening

The Sri Lanka Flora database contains two-hundred structures of natural products extracted from Sri Lanka-oriented plants. The first twenty structures extracted from Apocynaceae family plants were imported for this study. The imported structures were first energy minimized using the macro model module of the Schrödinger suite. Then the various tautomeric and ionization states were generated at the physiological pH of 7 ± 2 using the ligprep module (Sherin & Manojkumar, 2021). A compound may act as an efficient inhibitor for a particular target but may not show drug-like properties. Therefore, initial filtering is needed to assess the drug-likeness of the database. The drug-likeness of the database was checked by the *in silico* approach of ADMET screening (absorption, distribution, metabolism, excretion, and toxicity) using the QikProp module. QikProp produces forty-four physically relevant descriptors and thus predicts which property is violated by the compound. Lipinski's rule of five (Lipinski, 2004) and Pfizer's rule of 3/75 (Lionta et al., 2014) were applied to filter the drug-like molecules from the database. The crystal structure of the DNMT enzyme was downloaded (PDB ID: 3SWR) from the protein data bank and prepared using the Protein Preparation wizard workflow in the Schrödinger program. Initially, the DNA fragments and

the solvent molecules were removed from the system. The Zn(II) ions and the inhibitor were intact.

The prepared natural product database was then screened over the prepared structure of the DNMT enzyme using the virtual screening workflow of the Glide module (Sherin & Manojkumar, 2021). The receptor grid of size 30 Å × 30 Å × 30 Å was generated at the centroid of the inhibitor (zebularine) in the prepared DNMT enzyme. The docking methodology was validated by removing the bound inhibitor in the crystal structure, redocking it, and superimposing on its original pose.

Virtual screening in glide is a three-step process with increasing precision, namely high-throughput virtual screening (HTVS), standard precision (SP) docking, and extra precision (XP) docking. Initially, all the molecules were subjected to HTVS, and the top ten hits were screened by the SP method. Then, the top five hits of the SP output were subjected to the more efficient XP docking calculations. Finally, the top three hits of XP were selected for further analysis.

MM-GBSA calculations

The shortlisted three hits were subjected to binding free energy calculations. The Gibbs binding free energies of the shortlisted DNMT-compound complexes were calculated using the prime MM-GBSA method, considering the VSGB solvation model and the minimization sampling method (Wang et al., 2021). The total Gibbs energy of binding was calculated as the difference between the free energies of the DNMT-compound complex and the free energy of the DNMT enzyme and compounds in the free state.

RESULTS AND DISCUSSION

Screening of the database

The drug-likeness of the twenty natural products was analyzed by ADMET screening, resulting in four as toxic compounds due to higher molecular weight and the high number of hydrogen bond acceptors. Therefore, four compounds were rejected, and the rest sixteen compounds were screened over the DNMT receptor using the virtual screening workflow of the Glide module. These compounds were screened first by using the HTVS mode. The HTVS protocol filters out the inactive compounds. Next, the ten compounds with the highest docking score from the HTVS were selected for the SP docking calculations. This was followed by XP docking of the top five compounds from the SP docking output. The docking score measures DNMT-compound interactions, with a more negative number indicating stronger interactions between the DNMT enzyme and the compound. Finally, the top three compounds, fluorocarpamine, oxobuxifoline, and lanuginosine, with the XP docking scores of -4.70, -6.02, and -4.70 were chosen for further investigation. The binding interactions of these top three compounds with the DNMT enzyme are shown in Figure 1.

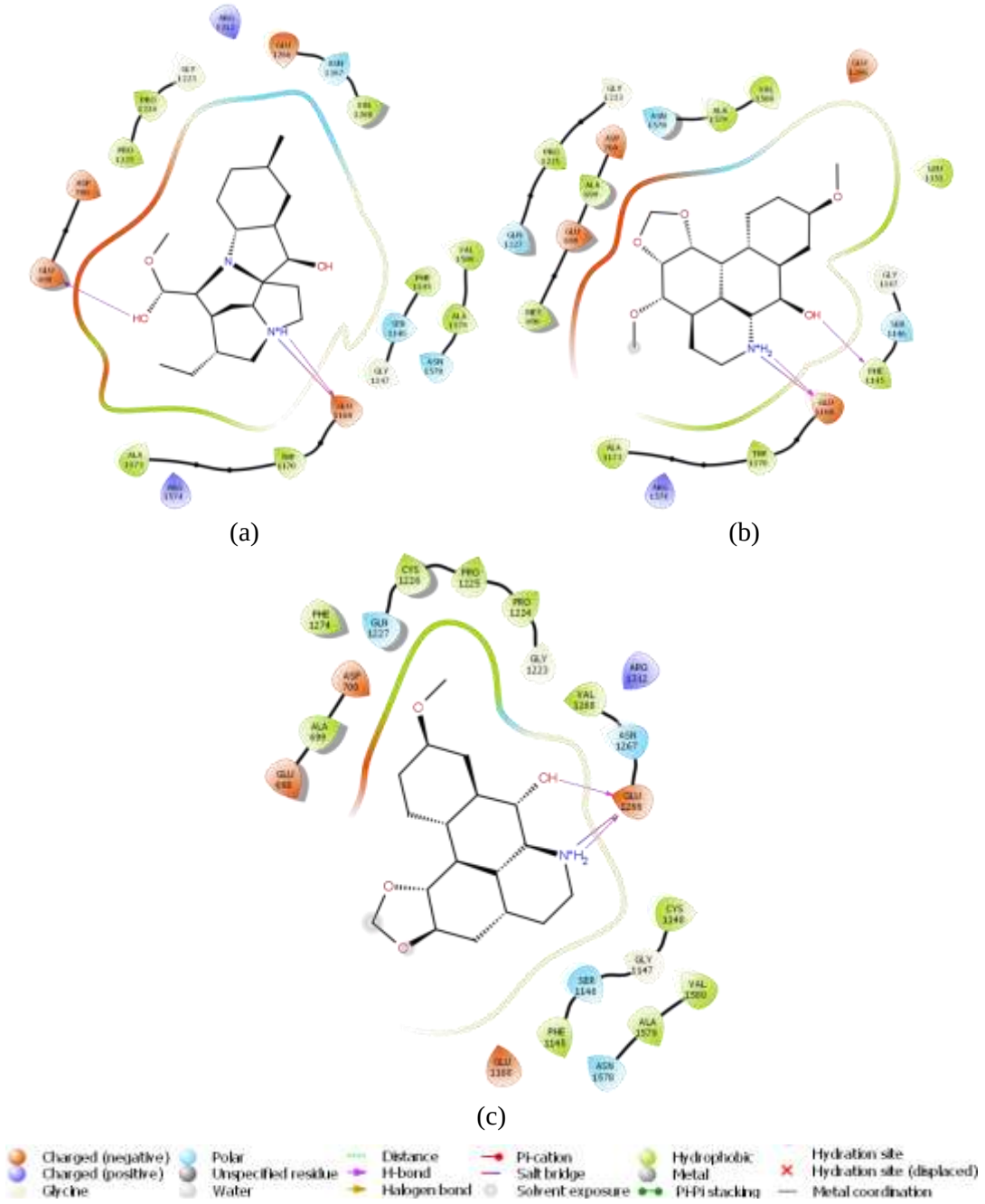

Figure 1. DNMT-compound interaction diagrams of (a) fluorocarpamine, (b) oxobuxifoline, and (c) lanuginosine in complexation with DNMT enzyme.

MM-GBSA calculations

Molecular docking is a vital tool for assessing the DNMT-compound interactions. However, it is not always true that the relative binding affinities of the DNMT-compound complexes predicted by molecular docking are accurate. The discrepancies are due to inadequate solvation treatment. Therefore, the solvation effect was accounted for by performing MM-GBSA calculations.

Several previously reported studies highlighted that the free energies of binding of the protein-ligand complexes predicted by MM-GBSA correlate well with their experimentally reported activities (Barreiro et al., 2007; Du et al., 2011; Lyne et al., 2006). Additionally, the MM-GBSA method has been better at handling structurally different compounds than the

other reported methods for calculating binding free energies. Therefore, the binding modes of the shortlisted compounds were rescored, and their Gibbs energies of binding (ΔG_{bind}) were calculated. The ΔG_{bind} is a direct measure of the stability of the DNMT-compound complex. The more negative the ΔGbind, the greater the DNMT-compound complex's stability. The ΔG_{bind} values for fluorocarpamine, oxobuxifoline, and lanuginosine are -19.7, -36.78, and -40.56 kcal mol^{-1}, respectively.

Further, to fetch compounds that can act as potent DNMT inhibitors, the bound inhibitor, zebularine, was taken as the reference molecule. Its binding energy was computed (-30.70 kcal mol^{-1}) and compared with the shortlisted compounds. Out of the three compounds, fluorocarpamine which had less negative binding free energies than zebularine was rejected. The Glide-XP docking and MM-GBSA calculations of zebularine, fluorocarpamine, oxobuxifoline, and lanuginosine are summarized in Table 1.

Table 1. Docking parameters and Gibbs energies of binding (kcal mol^{-1}) of the potent DNMT inhibitors.

Compound name	DS	E_{vdW}	E_{coul}	E_{model}	Glide energy	hbond	lipo	ΔG_{bind}
Zebularine	-5.40	-23.39	-13.32	-41.90	-36.71	-2.62	-14.60	-35.72
Fluorocarpamine	-4.70	-27.79	-12.66	-48.18	-38.33	-2.05	-18.33	-19.70
Oxobuxifoline	-6.02	-23.39	-18.64	-41.90	-36.71	-2.83	-15.65	-40.56
Lanuginosine	-4.70	-27.70	-18.82	-39.45	-35.62	-2.10	-14.28	-36.78

Thus, two compounds were identified as hits from the twenty natural products and will be considered for further analysis.

CONCLUSIONS

Out of twenty natural products from the Sri Lanka Flora database, only two survived the selectivity condition after the virtual screening and MM-GBSA filters imposed on them. The selectivity of the hits was found based on the strong binding interaction of the compound with the active site of the DNMT enzyme. Thus, According to these methods, two molecules oxobuxifoline and lanuginosine were proposed as potent DNMT inhibitors. Both compound structures contain a quinoline scaffold in their structure. Thus, a combination of various *in-silico* tools can help develop DNMT inhibitors, and the quinoline scaffold can be considered a promising template for discovering these inhibitors. Further, these compounds can be taken for *in-silico* and *in-vitro* studies and subsequently used as a possible therapeutic treatment for various cancer types.

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REFERENCES

Barreiro, G., Guimarães, C. R. W., Tubert-Brohman, I., Lyons, T. M., Tirado-Rives, J., & Jorgensen, W. L. (2007). Search for non-nucleoside inhibitors of HIV-1 reverse transcriptase using chemical similarity, molecular docking, and MM-GB/SA scoring. *Journal of Chemical Information and Modeling*, *47*(6), 2416–2428. https://doi.org/10.1021/ci700271z

Du, J., Sun, H., Xi, L., Li, J., Yang, Y., Liu, H., & Yao, X. (2011). Molecular modeling study

of checkpoint kinase 1 inhibitors by multiple docking strategies and prime/MM-GBSA calculation. *Journal of Computational Chemistry*, *32*(13), 2800–2809. https://doi.org/10.1002/jcc.21859

Issa, J. P. J., & Kantarjian, H. M. (2009). Targeting DNA methylation. *Clinical Cancer Research*, *15*(12), 3938–3946. https://doi.org/10.1158/1078-0432.CCR-08-2783

Jones, P. L., Veenstra, G. J., Wade, P. A., Vermaak, D., Kass, S. U., Landsberger, N., Strouboulis, J., & Wolffe, A. P. (1998). Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nature Genetics*, *19*(2), 187–191. http://doi/10.1038/561

Jurkowska, R. Z., Jurkowski, T. P., & Jeltsch, A. (2011). Structure and Function of Mammalian DNA Methyltransferases. *ChemBioChem*, *12*(2), 206–222. https://doi.org/10.1002/cbic.201000195

Lionta, E., Spyrou, G., Vassilatis, D., & Cournia, Z. (2014). Structure-Based Virtual Screening for Drug Discovery: Principles, Applications and Recent Advances. *Current Topics in Medicinal Chemistry*, *14*(16), 1923–1938. https://doi.org/10.2174/1568026614666140929124445

Lipinski, C. A. (2004). Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discovery Today: Technologies*, *1*(4), 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007

Lyne, P. D., Lamb, M. L., & Saeh, J. C. (2006). Accurate prediction of the relative potencies of members of a series of kinase inhibitors using molecular docking and MM-GBSA scoring. *Journal of Medicinal Chemistry*, *49*(16), 4805–4808. https://doi.org/10.1021/jm060522a

Reik, W. (2007). Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature*, *447*(7143), 425–432. https://doi.org/10.1038/nature05918

Sharma, S., Kelly, T. K., & Jones, P. A. (2009). Epigenetics in cancer. *Carcinogenesis*, *31*(1), 27–36. https://doi.org/10.1093/carcin/bgp220

Sherin, D. R., & Manojkumar, T. K. (2021). Exploring the selectivity of guanine scaffold in anticancer drug development by computational repurposing approach. *Scientific Reports*, *11*(1), 1–11. https://doi.org/10.1038/s41598-021-95507-4

Wang, E., Fu, W., Jiang, D., Sun, H., Wang, J., Zhang, X., Weng, G., Liu, H., Tao, P., & Hou, T. (2021). VAD-MM/GBSA: A Variable Atomic Dielectric MM/GBSA Model for Improved Accuracy in Protein-Ligand Binding Free Energy Calculations. *Journal of Chemical Information and Modeling*, *61*(6), 2844–2856. https://doi.org/10.1021/acs.jcim.1c00091

Weber, M., Hellmann, I., Stadler, M. B., Ramos, L., Pääbo, S., Rebhan, M., & Schübeler, D. (2007). Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nature Genetics*, *39*(4), 457–466. https://doi.org/10.1038/ng1990