April 30, 2019

PharmacologyOnLine

Archives • 2019 • vol.1 • *301-318*

IN-SILICO STUDY OF MAPK INHIBITION BASED LEAD IDENTIFICATION FROM THE ISOLATED COMPOUNDS OF *CROTON OBLONGIFOLIUS* ROXB FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA

Sajon, S.R.*

1Department of Pharmacy, Faculty of Biological Science and Technology, Jashore University of Science and Technology, Jashore-7408, Bangladesh

sadiur.sajon@yahoo.com

Abstract

The aim of this study was to identify potential lead compounds from *Croton oblongifolius* Roxb for treatment of hepatocellular carcinoma by inhibition of MAPK by *insilico* strategy. We used Schrödinger Maestro v 10.1 for processing of retrieved ligands and protein molecule along with grid generation, docking analysis and physicochemical property analysis. In our study, we found that Oblongionoside B (-8.90 kcal/mol), Oblongionoside C (-9.54 kcal/mol), Oblongionoside D (-9.61 kcal/mol), Isohamnetin (-8.57 kcal/mol), Hyperoside (-8.53 kcal/mol) and clerspide A (-8.63 kcal/mol) are the six potential lead compounds that can be used in the MAPK inhibition mediated hepatocellular carcinoma treatment. We hope that further study will be conducted to evaluate the potentiality of these compound in both in-vitro and in-vivo method.

Keywords: *Croton oblongifolius; MAPK; Hepatocellular carcinoma; Molecular docking*

Introduction

Cancer is defined as the abnormal cells division without control and is able to produce immature cells. These cells can spread to other parts of the body through the blood and lymphatic systems. Cancer is not one disease but it is a very complex form of many diseases. The cancer agents (carcinogens) can be present in air, water, food, chemicals and sunlight that are exposed to the people [1]. Hepatocellular carcinoma is the fifth most common cancer in men (5,54,000 cases, 7.5% of the total) and the ninth in women (2,28,000 cases, 3.4% of the total), with 7,82,000 new cases occurring in 2012 and approximately 7,46,000 persons die each year from this [2,3]. Whereas, in Bangladesh, the prevalence of HCC in male is 1.5% and prevalence of HCC in female is 0.6% [4]. Several experiments suggests that HCC is a possibility of factors such as inactivation of tumour suppressor gene, activation of multiple oncogene and over production of growth factors. More than 20 genes are identified to play a role in HCC which includes Ras, c-myc, c-fos and c-jun, rho, transforming growth factor-α, hepatocyte growth factor and c-met, c- ErbB-2, u-plasminogen activator, MXR7, MDM2, MAGE, matrix metalloproteinase and many more [5, 6]. MEK-MAPK signal transduction pathway is the most frequently targeted in the downstream of receptor and non-receptor tyrosine kinase and ras family of GTP-binding protein and enhancement of MEK-MAPK is notably observed in carcinogenic cell lines [7-10]. Studies with small molecule inhibitors of MEK activity [19, 20] demonstrate a role for MEK in mediating expression of proteinases implicated in invasion and metastasis [11, 12], and disruption of normal epithelial morphology [13, 14]. Increased MAPK (ERK1/2) and MEK1/2 expression and p42 MAPK in 5 HCC samples has been reported [15]. Treatment of cells with various growth factors produces activation of MEK1/2 and its downstream target, MAPK, resulting in proliferation, differentiation and survival [16]. Another study conducted by Huynh *et al* suggests that MAPK is activated in all the experimental HCC samples which suggests that targeting MAPK for treatment of HCC is a great initiative in this field [17].

Croton oblongifolius Roxb belongs to the Euphorbiaceae family mostly found in Central, Western and Southern India, also eastwards to Bengal [18]. Seeds of *Croton oblongifolius* Roxb are reported to be used in diarrhea and oedema, whereas root bark and seeds are used as purgative, in liver disease and also in reducing high blood pressure. Moreover, it is used to treat gastric ulcer, dyspepsia and dysentery [19]. There are many compounds which are isolated from different parts of *Croton oblongifolius* Roxb which exhibits a variety of pharmacological activities such as nasimulan A isolated from leaves was reported to show cytotoxicity against MOLT-3 cell line and also showed anti-microbial effect against some microbes [19], Furanocembranoids from stem bark exhibited to have cytotoxic effect against BT474, CHAGO, Hep-G2, KATO-3, SW-620 cell lines in MTT colometry [20], (-)-ent-kuar-16-en-19-oic acid isolated from stem bark showed inhibition of Na+,K+-ATPase activity with cytotoxicity [21], croblongifolin from stem bark exhibited cytotoxicity against HEP-G2, KATO-3, BT-474, CHAGO cell lines [22], neocrotocembranal isolated from stem bark showed cytotoxicity against P-388 cell lines etc. There are also many compounds which were isolated from different parts of Croton obingifolius Roxb such as (+)-Cyclosativene, (e)-Caryophyllene, gamma-Muurolene, gamma-Terpinene, Germacrene D [23], (-)-hardwickic acid, 11-dehydro (-)-hardwickic acid [24], Clerspide A, Canthoside A, Glochidionioside D, Oblongionoside A_F [25], hyperoside, isohamnetin [26] etc. Among all the isolated compounds many were reported to be cytotoxic against hep-G2 cell lines which is cancerous immortal liver cell line [20, 22] and aerial part of the plant exhibited hepatoprotective activity in-vivo [26]. And we can see that this plant has a potential to be a good source of medicine for the treatment of hepatocellular carcinoma.

However, this in-silico study was performed to identify potential compounds isolated from *Croton oblongifolius* Roxb which can be used in-vitro and if proven to be useful then in-vivo study of hepatocarcinoma treatment by inhibiting the enzyme mitogen activated protein kinase (MAPK).

Methods

Retrieval and preparation of compounds

A total of 83 compounds which were reported in different articles [20, 21, 22, 23, 24, 25, 26, 28-39]

were retrieved from PubChem. Doxorubicin (InchKey: AOJJSUZBOXZQNB-TZSSRYMLSA-N; Pubchem CID: 31703) used as standard and was also retrieved from pubchem. All the compounds were downloaded in 3 dimentional state in .sdf format and assigned to LigPrep 3.1, (Schrödinger, LLC, New York, NY, 2015) for energy minimization by applying OPLS 2005 force field and where pH was maintained at 7.0±2 using Epic 3.1 module of Schrödinger-Maestro (Version 10.1). The output file was also saved in .sdf format. LigPrep assists to convert 2D to 3D structures efficiently and accurately for computer analysis. LigPrep optionally expands tautomeric and ionization states, ring conformations and removal of problematic structure, optimization of geometric structure and conversion of output file for varied types of application of this software.

Retrieval and preparation of protein

Crystal structure of mitogen activated protein kinase (MAPK) was retrieved from RCSB Protein Data Bank in pdb format. The retrieval id of MAPK crystal structure is 4lmn. Before processing of the protein unwanted molecules such as ANP and MG were removed from the 3D structure using Discovery studio version 4.5 and the refined structure was saved in .pdb format. Protein Preparation Wizard of Schrödinger-Maestro v 10.1 was used to make the protein compatible for Glide module. In this process, in primary stage, bond order was assigned, hydrogen molecules were added, zero-order bonds with metal were created, disulfide bonds were created and selenomethionines were converted to methionines. In protein refinement stage, hydrogen bonds were assigned using the PROPKA module at pH 7, water molecules with less than three hydrogen bonds to non-water molecules were removed and at last energy minimization was carried out using OPLS 2005 force field setting the maximum heavy atom RMSD to 30 Å.

Receptor Grid Generation

Glide version 6.6 was used for receptor grid generation. Glide is a module which is used for excluding the ligand from ligand-protein complex and defining a box where the desired ligands can be added as a compex. In this study, van der waals radius scaling was set to default of scaling factor 1.00 Å and charge cut-off 0.25 Å. A cubic box of specific dimensions centred on the centroid of the active site residues was generated for the receptor. The bounding box was set to 15 Å × 15 Å × 15 Å and x, y, z axis are set at 31.7, 30.02, -13.06 sequentially.

Glide molecular docking

After completing the preliminary steps, the glide 6.6 module of Schrödinger-Maestro v 10.1 was used for determining the docking scores of desired complex. Glide is designed to assist in high-throughput screening of potential ligands based on binding mode and affinity for a given receptor molecule. We can compare ligand scores with those of other test ligands, or compare ligand geometries with those of a reference ligand. Additionally, we can use Glide to generate one or more plausible binding modes for a newly designed ligand. In this study, Flexible ligand docking was performed with Glide of Schrödinger-Maestro (version 10.1) [40, 41] within which penalties were applied to non-cis/trans amide bonds. Glide standard precision docking was performed with these molecules, and hits above 4 kcal/mol based on docking score with MAPK enzyme in XP mode, keeping all docking parameters as default. No bonding constraints were given during docking calculations. Using Monte Carlo random search algorithm, ligand poses were generated for each input molecule, and binding affinity of these molecules to the MAPK enzyme was predicted regarding Glide docking score. Post-docking minimization was performed with OPLS 2005 force field, and one pose per ligand was saved.

Molecular Property and ADME/T analysis

QikProp 4.3 module Schrödinger-Maestro (version 10.1) is used to determine the physical property of the moecules under study. We used Lipinsky's rule of five [42] and Jorgensen's rule of three [43, 44] to describe the acceptability of the compounds. Also, admet SAR web server was used to identify selected pharmacokinetic parameters and toxicity of the best docked compounds.

Results

In our study, out of all the 83 compounds, six compounds expressed better docking score compared to standard drug doxorubicin (-8.31

kcal/mol). They are Oblongionoside B (-8.90 kcal/mol), Oblongionoside C (-9.54 kcal/mol), Oblongionoside D (-9.61 kcal/mol), Isohamnetin (-8.57 kcal/mol), Hyperoside (-8.53 kcal/mol) and clerspide A (-8.63 kcal/mol). They were further docked in extra precision mode to exclude false positive and in this mode Oblongionoside B showed best docking score which is -13.55 kcal/mol and Oblongionoside D showed docking score of -12.59 kcal/mol. Interacting amino acids were also identified using 2D mode of ligand-protein interaction. All these results are included in table-1.

Physical properties of the compounds are displayed in table 2 and 3. In table 2, physical properties according to Lipinsky's rule of five are expressed. And in table 3, physical properties of the molecules according to Jorgensen's rule of three are expressed.

Selected pharmacokinetic and toxicological parameters such as absorption through blood-brain barrier and human intestine, inhibition of P-glycoprotein, inhibition of CYP450 2C9, modification of human ether-a-go-go gene, acute oral toxicity and rat acute toxicity of the selected best docked compounds are generated in table-4.

Discussion

With rapid development of modern biological and chemical technologies, computer aided drug design is rapidly changing the nature of novel drug like candidate identification and also, this method widely accepted due to its ease of application and reduction of process cost [45]. And in the field of computer aided drug design, molecular docking is a competent tool for identification of novel drug like molecules [46]. Molecular docking is a computation based study which considers covalent association between a macromolecule such as receptor or enzyme and a micro molecule such as ligand efficiently. Prediction of binding energy of small molecules with receptors or enzyme have a huge impact on lead identification as it screens a huge database of drug like molecules to find the best fit for the receptor and further modify it for using it as a drug candidate [47]. Molecular docking study of protein structures involves different association between protein and ligand and the associations are verified on the basis of binding energy. The association with the least energy is considered the best pose and the complex is then further analyzed. One of the areas of molecular docking is protein-ligand docking which is gaining popularity due to its role in structure based drug design [47-52].

In our study, we docked 83 compounds isolated from different parts of *Croton oblongifolius* Roxb and docked it against mitogen activated protein kinase (4lmn) to identify potent compounds that can inhibit the protein more efficiently than the standard drug doxorubicin. Doxorubicin a anthracycline based chemotherapeutic agent which is isolated from Streptomyces peucetius and clinical studies showed that systemic administration of doxorubicin showed limited clinical benefits however, administration of doxorubicin through hepatic artery route, showed significant tumour shrinkage and partial responses were in 30-70% patients [53]. During our study, we found that some compounds such as Oblongionoside B (-8.90 kcal/mol), Oblongionoside C (-9.54 kcal/mol), Oblongionoside D (-9.61 kcal/mol), Isohamnetin (-8.57 kcal/mol), Hyperoside (-8.53 kcal/mol) and clerspide A (-8.63 kcal/mol) showed better docking score than the standard drug doxorubicin (-8.31 kcal/mol). By further docking these compounds in glide XP mode, we found that Oblongionoside B and Oblongionoside D showed best docking score which is -13.55 kcal/mol and -12.59 kcal/mol sequentially. Glide XP is a harder function that exacts severe penalties such as poses that violates established physical chemistry principles and useful in lead optimization and other studies where a limited number of compounds are considered [40]. 2D and 3D binding mode of Oblongionoside B and D along with their hydrophobicity of binding pocket is shown in figure 1-6. And figure 7-9 demonstrates the 2D and 3D binding mode of standard drug doxorubicin and the hydrophobicity of binding pocket.

In table 2, descriptors used in Lipinsky's rule of five are molecular weight of the molecule (mol_MW), estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution (donorHB), estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution (accptHB), predicted

octanol/water partition coefficient (QPlogPo/w) and number of non-hindered rotatable bonds (#rotor). On the other hand, in Jorgensen's rule of three, the denoted descriptors are predicted aqueous solubility, log S (QPlogS), predicted apparent Caco-2 cell permeability in nm/sec (QPPCaco) and number of likely metabolic reactions (#metab) which is expressed in table 3. All the acceptable values are crosschecked with software manual for reliability. And, we found that all these compounds are within the acceptable range to be used as potential lead compound.

In table 4, we considered the server based selected pharmacokinetic and toxicological parameters. Here, we can see that all six compounds except oblongionoside B and Clerspide A showed negative result in blood-brain barrier absorption. In case of human intestinal absorption, oblongionoside C and D showed negative results. Moreover, oblongionoside C and D has only inhibitory potential of P-glycoprotein whereas other four has both inhibitory and non-inhibitory potential. Among the six compounds only isohamnetin has inhibitory potential for CYP450 2C9. All the six compounds are weak inhibitors of human ether-a-go-go gene. Also, human oral toxicity and rat acute toxicity is also shown in table 4.

Conclusion

Computer aided drug design is a process where we can shorten the time for library search for finding out our desired compound for specific drug target. In our study, we used this method to identify lead compounds for MAPK inhibition based hepatocellular treatment among the 83 compounds isolated from *Croton oblongifolius* Roxb and identified six compounds which can act as potential lead compounds for the treatment of hepatocellular carcinoma. I hope to extend my experiment in future by evaluating these compounds for their desired activity by both in-vitro and in-vivo method and if possible we would like to modify the leads for better activity and selectivity.

Abbreviations

HCC: Hepato Cellular Carcinoma; MAPK: Mitogen Activated Protein Kinase; ERK: Extracellular signal-Regulated Kinases; mol_MW: molecular weight of the molecule; donorHB: estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution; accptHB: estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution; QPlogPo/w: predicted octanol/water partition coefficient; #rotor: number of non-hindered rotatable bonds; QPlogS: predicted aqueous solubility, log S; QPPCaco:), predicted apparent Caco-2 cell permeability in nm/sec; #metab: number of likely metabolic reactions; SP: Standard Precision; XP: Extra Precision; LD: Lethal Dose.

References

1. Alison, M.R. Cancer. Encyclopedia of life Sciences, Nature Publishing Group / www.els.net 2001; 1-8.

2. Flores, A., Marrero, J.A. Emerging trends in hepatocellular carcinoma: focus on diagnosis and therapeutics. Clin Med Insights Oncol 2014; 8: 71 -76 [PMID: 24899827. DOI: 10.4137/CMO. S9926]

3 Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., Bray, F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359 -E386 [PMID: 25220842 DOI: 10.1002/ijc.29210].

4. Hussain, S.M.A. Comprehensive update on cancer scenario of Bangladesh. South Asian J Can. 2013 Oct-Dec; 2(4): 279–284.

5. Zeng, J.Z., Wang, H.Y., Chen, Z.J., Ullrich, A., Wu, M.C. Molecular cloning and characterization of a novel gene which is highly expressed in hepatocellular carcinoma. Oncogene 2002, 21:4932-4943.

6. Huynh, H., Chow, P.K., Ooi, L.L., Soo, K.C. A possible role for insulin like growth factor-binding protein-3 autocrine/paracrine loops in controlling hepatocellular carcinoma cell proliferation. Cell Growth Differ 2002, 13:115-122.

7. Lewis, T.S., Shapiro, P.S., Ahn, N.G. Signal transduction through MAP kinase cascades. Adv Cancer Res 1998, 74:49-139.

8. Ballif, B.A., Blenis, J. Molecular mechanisms mediating mammalian mitogen-activated protein

kinase (MAPK) kinase (MEK)- MAPK cell survival signals. Cell Growth Differ 2001, 12:397-408.

9. Hoshino, R., Chatani, Y., Yamori, T., Tsuruo, T., Oka, H., Yoshida, O., Shimada, Y., Ari-I, S., Wada, H., Fujimoto, J., Kohno, M. Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors. Oncogene 1999, 18:813-822.

10. Amundadottir, L.T., Leder, P. Signal transduction pathways activated and required for mammary carcinogenesis in response to specific oncogenes. Oncogene 1998, 16:737-746.

11. Liu, E., Thant, A.A., Kikkawa, F., Kurata, H., Tanaka, S., Nawa, A., Mizutani, S., Matsuda, S., Hanafusa, H., Hamaguchi, M. The Ras-mitogen-activated protein kinase pathway is critical for the activation of matrix metalloproteinase secretion and the invasiveness in v-crktransformed 3Y1. Cancer Res 2000, 60:2361-2364.

12. Reddy, K.B., Krueger, J.S., Kondapaka, S.B., Diglio, C.A. Mitogen-activated protein kinase (MAPK) regulates the expression of progelatinase B (MMP-9) in breast epithelial cells. Int J Cancer 1999, 82:268-273.

13. Chen, Y., Lu, Q., Schneeberger, E.E., Goodenough, D.A. Restoration of tight junction structure and barrier function by down-regulation of the mitogen-activated protein kinase pathway in ras- transformed Madin-Darby canine kidney cells. Mol Biol Cell 2000, 11:849-862.

14. Lu, Q., Paredes, M., Zhang, J., Kosik, K.S. Basal extracellular signalregulated kinase activity modulates cell-cell and cell-matrix interactions. Mol Cell Biol 1998, 18:3257-3265.

15. Schmidt, C.M., McKillop, I.H., Cahill, P.A., Sitzmann, J.V. Increased MAPK expression and activity in primary human hepatocellular carcinoma. Biochem Biophys Res Commun 1997, 236:54-58.

16. Ballif, B.A., Blenis, J. Molecular mechanisms mediating mammalian mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals. Cell Growth Differ 2001, 12:397-408.

17. Huynh, H., Nguyen, T.T.T., Chiw, K.P., Tan, P.H. *et al*. Over-expression of the mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK in hepatocellular carcinoma: Its role in tumor progression and apoptosis. BMC Gastroentero 2003, 3:19.

18. Khare, C.P. Indian Medicinal Plants: An illustrated Dictionary. Springer Reference. 2nd edition. Page: 180.

19. Sommit, D.; Petsom, A.; Ishikawa, J.; Roengsumran, S. Cytotoxic activity of Natural Labdanes and their semi-synthetic modified derivatives from *Croton oblongifolius*. Planta Med. 2003, 69, 167–170.

20. Roengsumran, S.; Petsom, A.; Kuptipyanuwat, N.; Vilaivan, T.; Ngamrojnavanich, N.; Chaichantipyuth, C.; Phuthong, S. Cytotoxic labdane diterpenoids from Croton oblongifolius. Phytochemistry 2001, 56, 103–107.

21. Ngamrojnavanich, N.; Sirimongkon, S.; Roengsumran, S.; Petsom, A.; Kamimura, H. Inhibition of Na+,K+-ATPase activity by (-)-ent-kaar-16-en-19-oic acid and its derivatives. Planta Med. 2003, 69, 555–556.

22. Roengsumran, S.; Petsom, A.; Sommit, D.; Vilaivan, T. Labdane diterpenoids from *Croton oblongifolius*. Phytochemistry 1999, 50, 449–453.

23. Athikomkulchai, S., Tadtong, S., Ruangrungsi, N. and Hongratanaworakit, T. (2015) Chemical Composition of the Essential Oil from *Croton oblongifolius* and its Antibacterial Activity against Propionibacterium acnes. Natural product communications, 10, 1459-1460.

24. Aiyar, V. and Seshadri, T. (1972) 11-dehydro (—)-hardwickic acid from croton oblongifolius. Phytochemistry, 11, 1473-1476.

25. Takeshige, Y., Kawakami, S., Matsunami, K., Otsuka, H., Lhieochaiphant, D. and Lhieochaiphant, S. (2012) Oblongionosides A–F, megastigmane glycosides from the leaves of *Croton oblongifolius* Roxburgh. Phytochemistry, 80, 132-136.

26. Subramanian, S.S., Nagarajan, S. and Sulochana, N. (1971) Flavonoids of some euphorbiaceous plants. Phytochemistry, 10, 2548-2549.

27. Ahmed, B.; Alam, T.; Varshney, M.; Khan, S.A. Hepatoprotective activity of two plants belonging

to the Apiaceae and the Euphorbiaceae family. J. Ethnopharmacol. 2002, 79, 313–316.

28. Mandal, L. and Bose, S. (2011) Pharmacognostic Standardization and Quantitative Estimation of some Isolated Phytoconstituents from Roxb. *Croton oblongifolius*. Journal of PharmaSciTech, 1, 10-15.

29. Roengsumran, S., Pornpakakul, S., Muangsin, N., Sangvanich, P., Nhujak, T., Singtothong, P., Chaichit, N., Puthong, S. and Petsom, A. (2004) New halimane diterpenoids from *Croton oblongifolius*. Planta medica, 70, 87-89.

30. Ahmed, B., Alam, T., Najam, L. and Khan, S.A. (2016) Oblongiside: A New Steroidal Glycoside from Aerial Parts of *Croton oblongifolius* Roxb. Asian Journal of Chemistry, 28, 517.

31. Aiyar, V. and Seshadri, T. (1970) Components of *Croton oblongifolius*—III: Constitution of oblongifolic acid. Tetrahedron, 26, 5275-5279.

32. Roegsumran, S., Jaiboon, N., Chaichit, N., Sommit, D., Pattamadilok, D., Chaichantipyuth, C. and Petsom, A. (2002) Hydrogen bonding in labdane diterpenoids, labda-7, 12 (E), 14-triene-17-oic acid and labda-12 (Z), 14, 17-triene-18-oic acid. Journal of chemical crystallography, 32, 511-517.

33. Roengsumran, S., Achayindee, S., Petsom, A., Pudhom, K., Singtothong, P., Surachetapan, C. and Vilaivan, T. (1998) Two new cembranoids from *Croton oblongifolius*. Journal of natural products, 61, 652-654.

34. Roengsumran, S., Singtothong, P., Pudhom, K., Ngamrochanavanich, N., Petsom, A. and Chaichantipyuth, C. (1999) Neocrotocembranal from Croton o blongifolius. Journal of natural products, 62, 1163-1164.

35. Roengsumran, S., Musikul, K., Petsom, A., Vilaivan, T., Sangvanich, P., Pornpakakul, S., Puthong, S., Chaichantipyuth, C., Jaiboon, N. and Chaichit, N. (2002) Croblongifolin, a new anticancer clerodane from *Croton oblongifolius*. Planta Medica, 68, 274-277.

36. Roengsumran, S., Pata, P., Ruengraweewat, N., Tummatorn, J., Pornpakakul, S., Sangvanich, P., Puthong, S. and Petsom, A. (2009) New cleistanthane diterpenoids and 3, 4-seco-cleistanthane diterpenoids from *Croton oblongifolius*. Chemistry of natural compounds, 45, 641.

37. Youngsa-ad, W., Ngamrojanavanich, N., Mahidol, C., Ruchirawat, S., Prawat, H. and Kittakoop, P. (2007) Diterpenoids from the roots of *Croton oblongifolius*. Planta medica, 73, 1491.

38. Pudhom, K. and Sommit, D. (2011) Clerodane diterpenoids and a trisubstituted furan from *Croton oblongifolius*. Phytochemistry Letters, 4, 147-150.

39. Suwancharoen, S., Chonvanich, O., Roengsumran, S. and Pornpakakul, S. (2012) seco-Kaurane skeleton diterpenoids from *Croton oblongifolius*. Chemistry of natural compounds, 48, 583-586.

40. Friesner, R.A., Banks, J.L., Murphy, R.B., Halgren, T.A., Klicic, J.J., Mainz, D.T. et al. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem ACS Publications. 2004;47: 1739–49.

41. Halgren, T.A., Murphy, R.B., Friesner, R.A., Beard, H.S., Frye, L.L., Pollard, W.T. *et al*. Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. J Med Chem. ACS Publications. 2004;47:1750–9.

42. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Delivery Rev. 2001, 46, 3-26.

43. Jorgensen, W. L.; Duffy, E. M. Prediction of Drug Solubility from Monte Carlo Simulations.Bioorg. Med. Chem. Lett. 2000, 10, 1155-1158.

44. Jorgensen, W. L.; Duffy, E. M. Prediction of Drug Solubility from Structure. Adv. Drug Delivery Rev. 2002, 54, 355-366.

45. Pozzan, A. Molecular descriptors and methods for ligand based virtual high throughput screening in drug discovery. CurrentPharmaceutical Design, 12(17), 2006,pp 2099–2110.

46. Wang, J., Kollman, P.A., Kuntz, I.D. Flexible ligand docking: a multistep strategy approach. Protien,36(1),1999,pp1-19.

47. Kitchen, D.B., Decornez, H., Furr, J.R., Bajorath, J. Docking and scoring in virtual screening for drug discovery: Methods and applications. NatureReviewsDrug Discovery, 3, 2004, pp 935-948.

48. Muegge, I., Rarey, M. Small molecule docking and scoring.Review in Computational Chemistry, 17, 2001,pp1-60.

49. Shoichet, B.K., McGovern, S.L., Wei, B., Irwin, J.J. Lead discovery using molecular docking. InternationalJournal of Molecular Sciences, 6(4),2002, pp 439-46.

49. Halperin, I., Ma, B., Wolfson, H., Nussinov, R. Principles of docking: An overviewof search algorithms and a guide to scoring functions. Proteins, 47,2002,pp409-443.

50. Brooijimans, N. and Kuntz, I.D. Molecular recognition and docking algorithms. Annual Review of Biophysics and Bimolecular Structure, 32,2003,pp335-373.

51. Sousa, S.F., Fernandes, P.A., Ramos, M.J. Protein-ligand docking: Current status and future challenges. Proteins, 65, 2006, pp 15-26.

52. Kolb, P., Ferreira, R.S., Irwin, J.J., Shoichet, B.K., Docking and chemoinformatic screens for new ligands and targets. Current Opinionin Biotechnology, 20, 2009, pp 429-436.

53. Tam, K. The Roles of Doxorubicin in Hepatocellular Carcinoma. ADMET & DMPK 1(3) (2013) 29-44; doi: 10.5599/admet.1.3.7.

Table 1: Docking score of best docked compounds

Compound	Protein	Interacting amino acids	Docking score (kcal/mol) (SP)	Docking score (kcal/mol) (XP)
Doxorubicin	4lmn	ASN78, GLY77, LYS192	-8.31	-8.32
Hyperoside	4lmn	GLY77, ARG189, ARG234, ASN221	-8.53	-11.67
Isorhamnetin	4lmn	PHE209, VAL211, SER212	-8.57	-9.81
Oblongionoside B	4lmn	GLY77, ASP190. LYS192, ASN195, ASP208, ARG234	-8.90	-13.55
Oblongionoside C	4lmn	GLY77, ASP190, LYS192, ASN195, ASP208, PHE209	-9.54	-12.46
Oblongionoside D	4lmn	GLY77, ASP190, LYS192, ASN195, ASP208, PHE209, ARG234	-9.61	-12.59
Clerspide A	4lmn	GLY77, ASN78, ASP190, LYS192, ASP208	-8.63	-11.68

Table 2: Molecular property according to Lipinsky's Rule of five of the best docked compounds

Compounds	mol MW	DonorHB	accptHB	QPlogPo/w	#rotor	Number of violations	Acceptable number of violations [42]
Doxorubucin	543.526	5	14.8	-0.411	11	3	Maximum 4
Hyperoside	464.382	7	13.75	-1.381	11	2	Maximum 4
Isohamnetin	316.267	3	5.25	1.208	5	0	Maximum 4
Oblongionoside B	522.589	8	18.5	-1.021	16	3	Maximum 4
Oblongionoside C	508.605	7	17.75	-0.761	16	3	Maximum 4
Oblongionoside D	508.605	7	17.75	-0.773	16	3	Maximum 4
Clerspide A	446.407	6	17.1	-1.255	13	2	Maximum 4

Acceptable values- mol MW: 130.0 – 725.0, donorHB: 0.0 – 6.0, accptHB: 2.0 – 20.0, QPlogPo/w: –2.0 – 6.5, #rotor: 0-15 [Qikprop 4.3 User manual]

Table 3: Molecular property according to Jorgensens's Rule of three of the best docked compounds

Compounds	QPlogS	QPPCaco	#metab	Number of violations	Acceptable number of violatin [43, 44]
Doxorubucin	-2.353	2.466	9	2	Maximum 3
Hyperoside	-2.596	2.605	8	2	Maximum 3
Isohamnetin	-3.344	60.509	5	0	Maximum 3
Oblongionoside B	-2.617	28.149	8	1	Maximum 3
Oblongionoside C	-2.383	18.171	7	2	Maximum 3
Oblongionoside D	-2.355	17.874	7	2	Maximum 3
Clerspide A	-1.637	104.185	6	0	Maximum 3

Acceptable value- QPlogS: –6.5 – 0.5; QPPCaco: <25 poor,>500 great; #metab:1-8 [Qikprop 4.3 user manual]

Table 4: Selected pharmacokinetic parameters of best docked compounds

Parameters	Oblongionoside B	Oblongionoside C	Oblongionoside D	Hyperoside	Isohamnetin	Clerspide A
Blood-Brain Barrier	+	-	-	-	-	+
Human Intestinal absorption	+	-	-	+	+	+
P-glycoprotein Inhibitor	I/NI	I	I	NI	NI/I	I/NI
CYP450 2C9 Inhibitor	NI	NI	NI	NI	I	NI
Human Ether-a-go-go-Related Gene	WI	WI	WI	WI	WI	WI
Acute Oral Toxicity	iii	i	i	iii	iii	iii
Rat Acute Toxicity (LD50, mol/kg)	2.88	3.41	3.41	2.39	2.72	2.64

+ = Positive, - =Negative, I = Inhibitor, NI = Non-Inhibitor, i =Category I contains compounds with LD50 values less than or equal to 50mg/kg, iii = Category III includes compounds with LD50 values greater than 500mg/kg but less than 5000mg/kg.

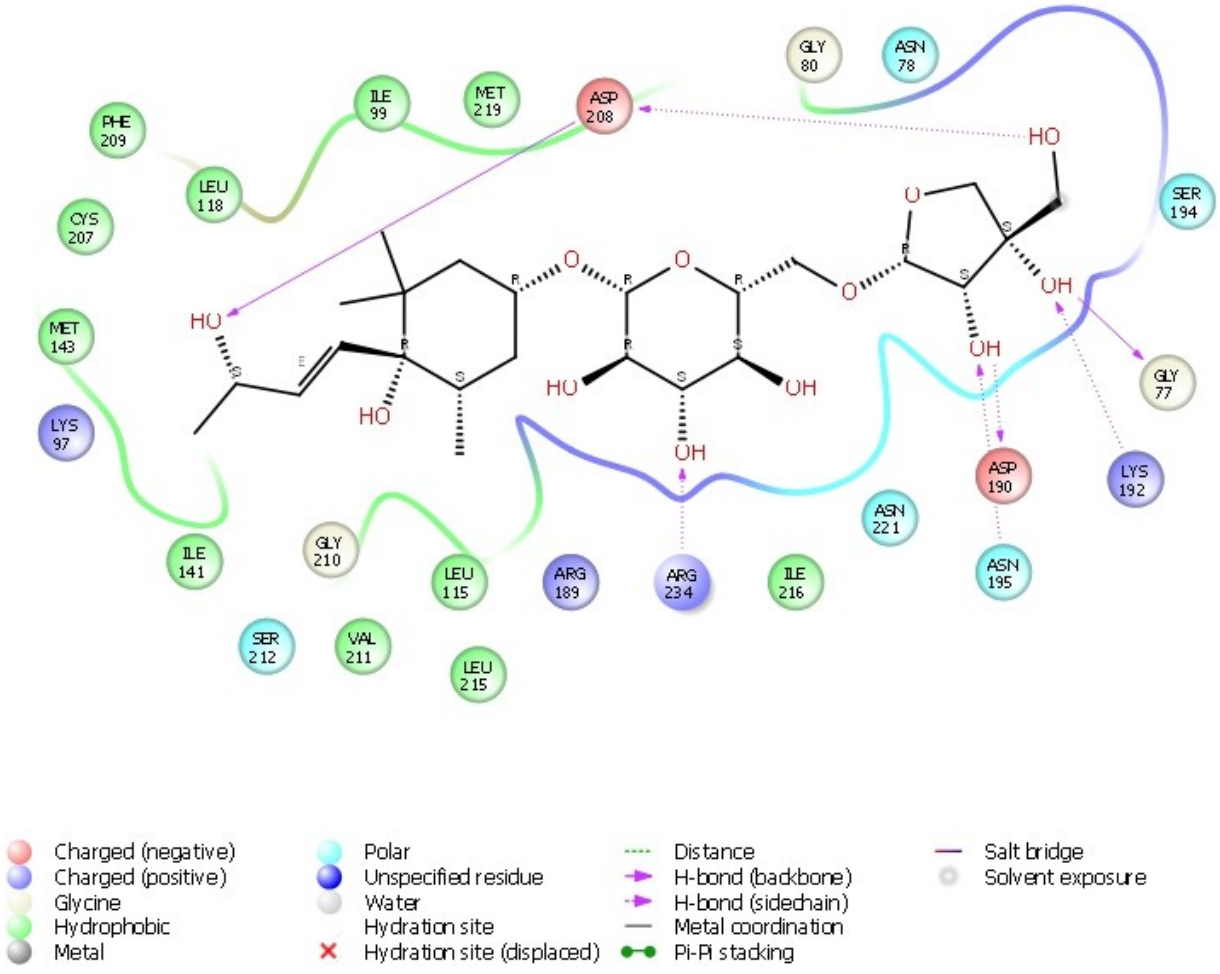

Figure 1: 2D presentation of interacting amino-acids of 4LMN with Oblongionoside B.

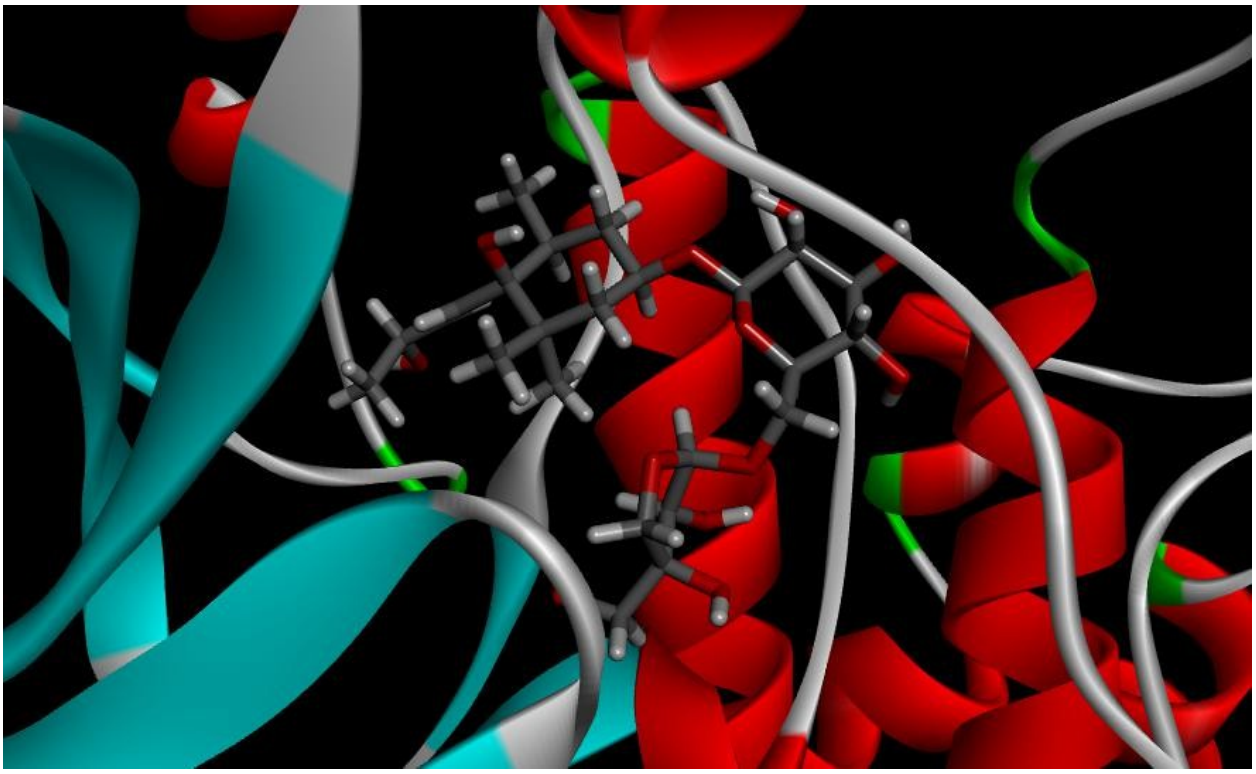

Figure 2: 3D presentation of Oblongionoside B in its binding site of 4LMN.

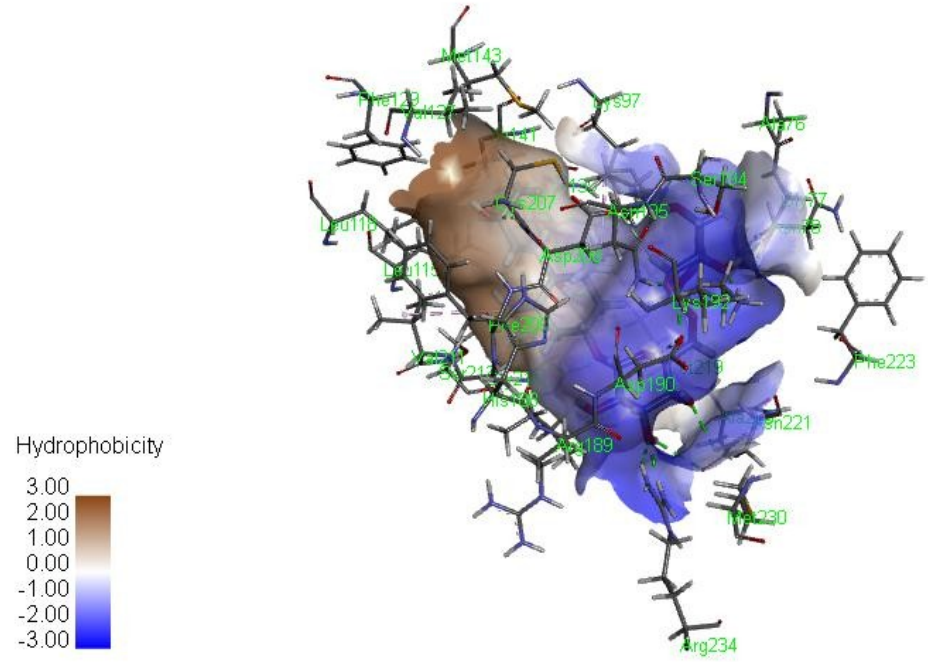

Figure 3: Hydrophobicity of the binding pocket of 4LMN with Oblongionoside B.

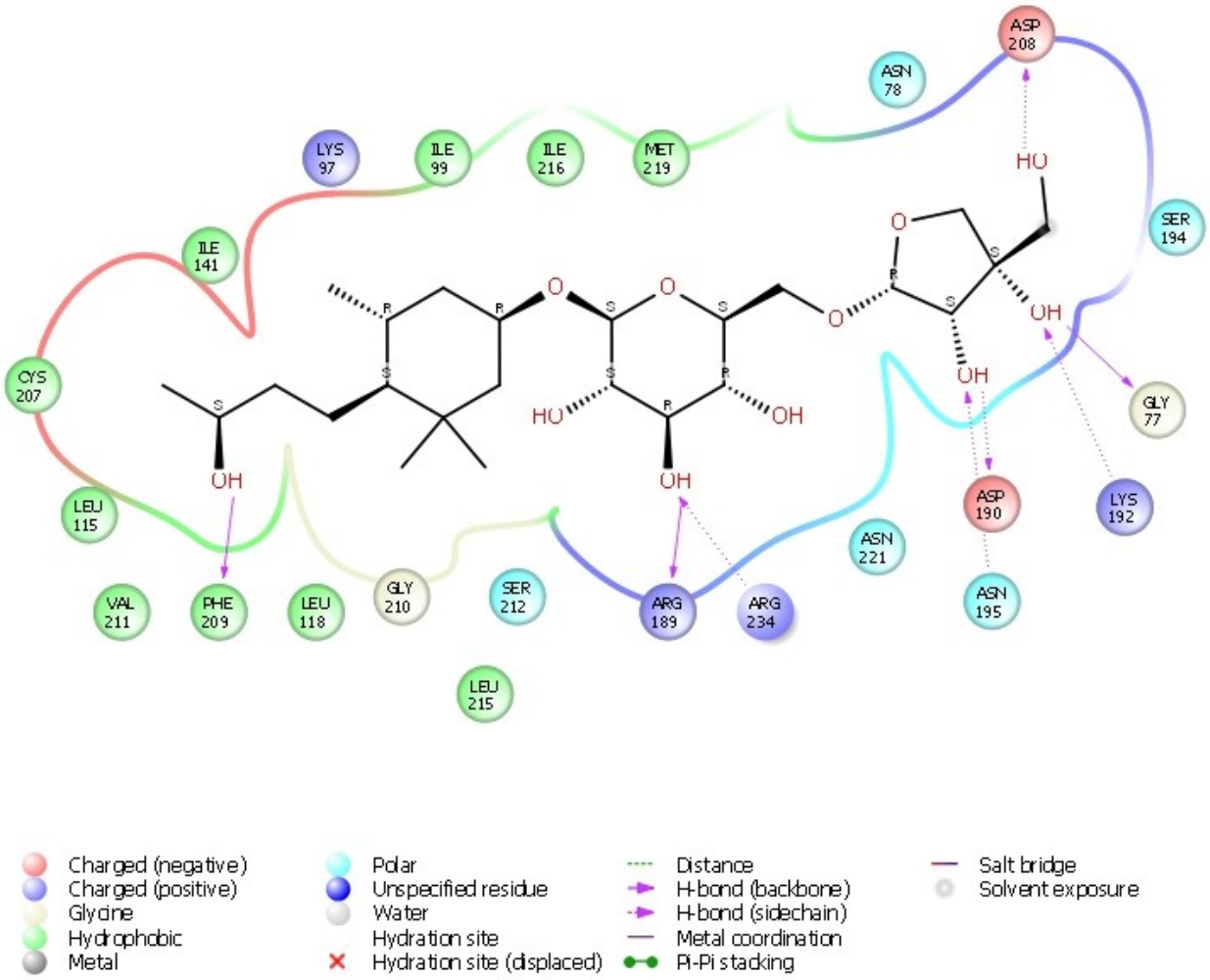

Figure 4: 2D presentation of interacting amino-acids of 4LMN with Oblongionoside D.

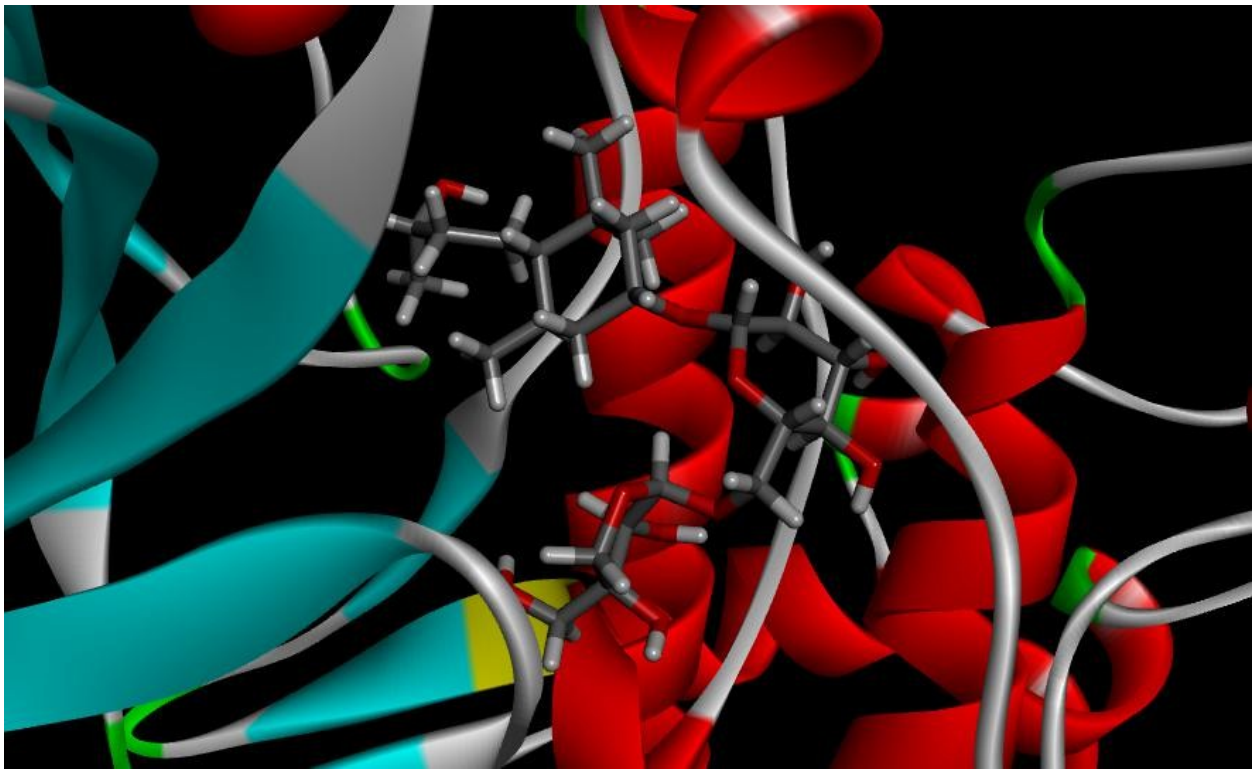

Figure 5: 3D presentation of Oblongionoside D in its binding site of 4LMN.

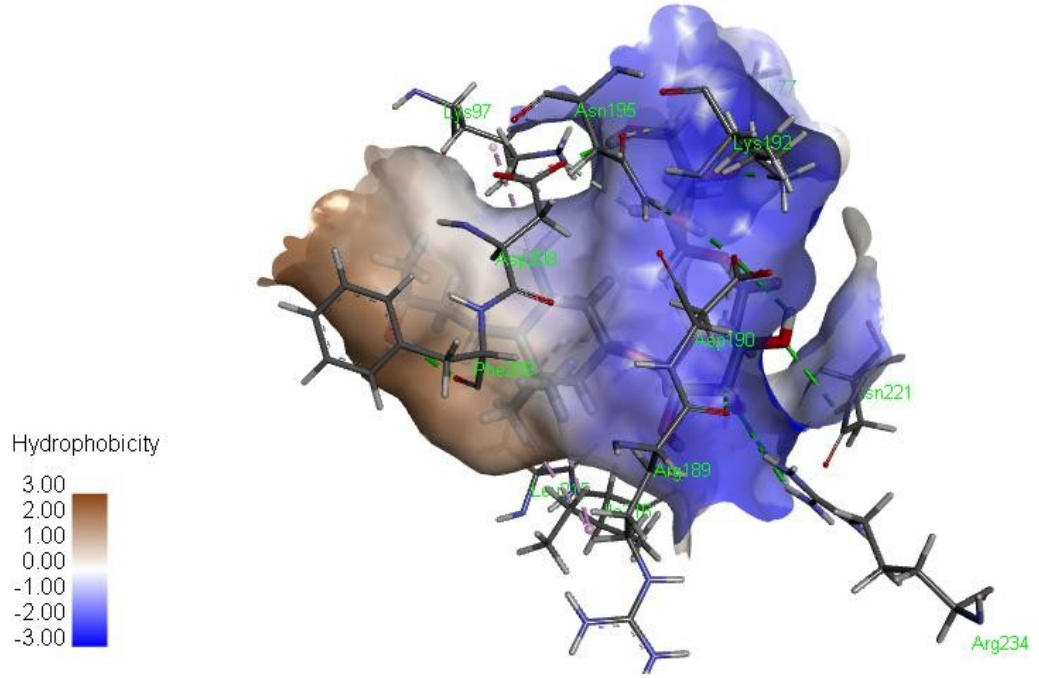

Figure 6: Hydrophobicity of the binding pocket of 4LMN with Oblongionoside D.

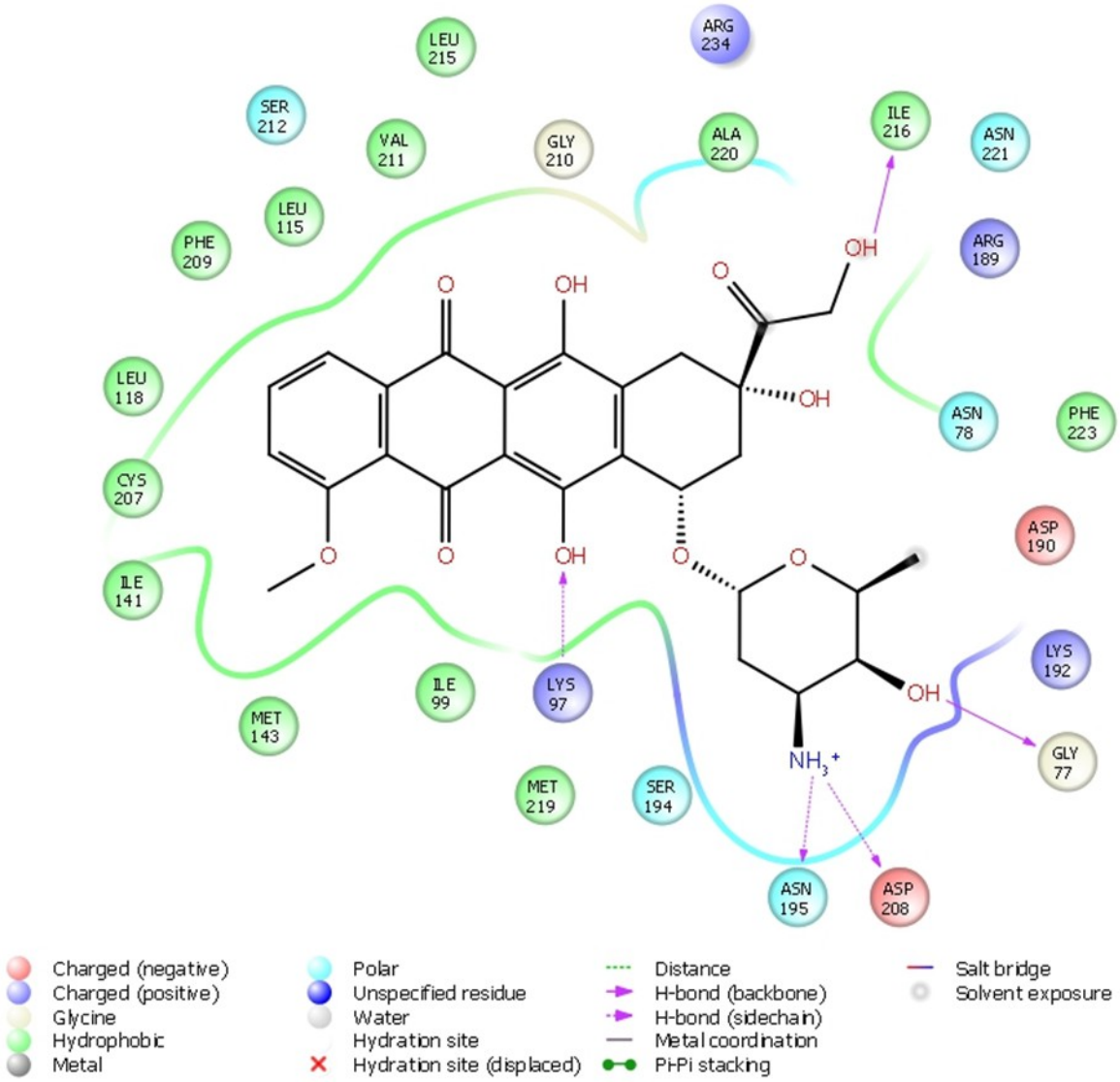

Figure 7: 2D presentation of interacting amino-acids of 4LMN with Doxorubicin.

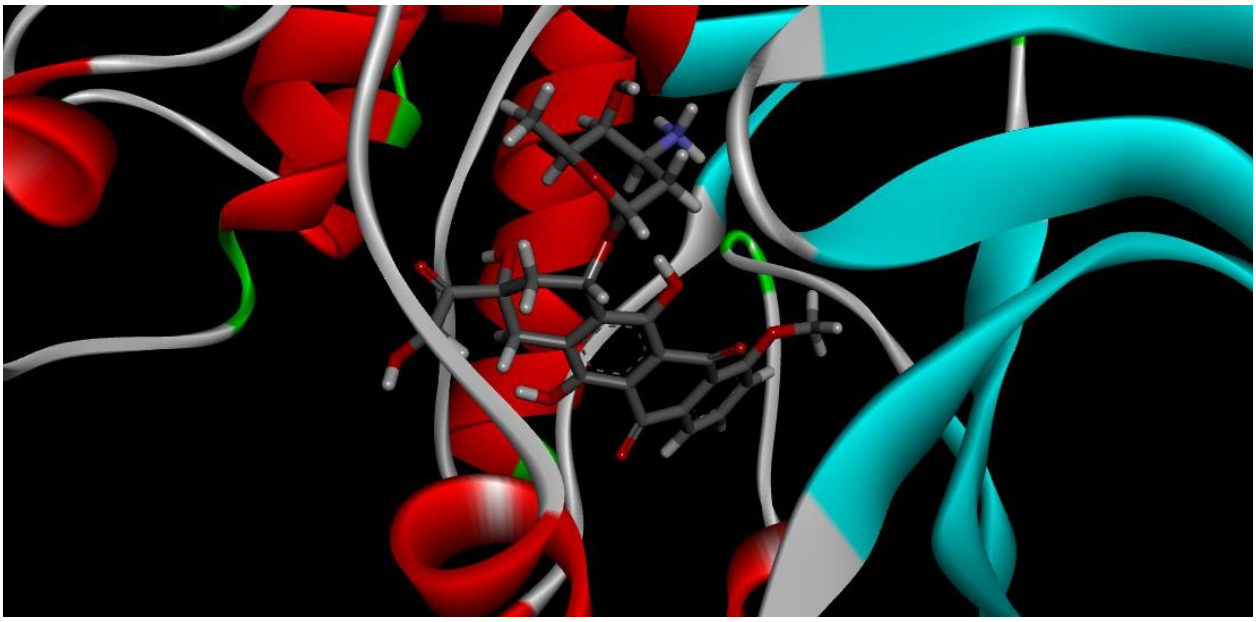

Figure 8: 3D presentation of Doxorubicin in its binding site of 4LMN.

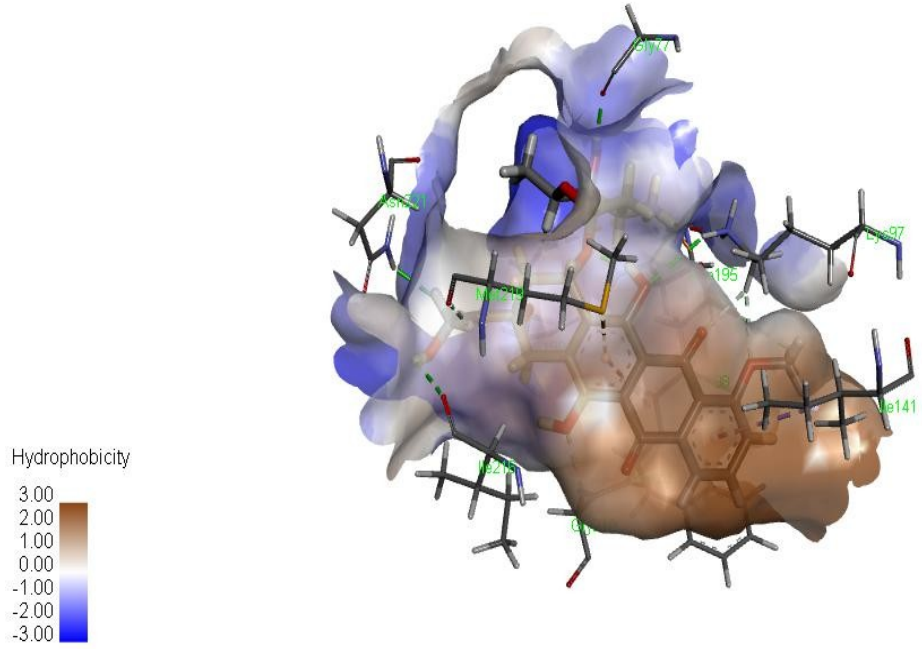

Figure 9: Hydrophobicity of the binding pocket of 4LMN with Doxorubicin.